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Characterization of Dissolved Organic Matter in Industrial Wastewaters

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A thesis submitted in fulfillment of the requirement for the degree of
Doctor of Philosophy

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Dedicated with much love and gratitude

to my

parents, husband and baby son

Errata

Page iv Section 4.3	Title in upper case should be in title case
Page xx Paragraph 3 Line 1	"organic mill effluents" should be "organic effluents"
Line 7	"…conducted." should be "…conducted;"
	CHAPTER 1
Page 1-2 Paragraph 1 Line 4	"Bonsoi et al." should be "Bonsor et al."
Paragraph 2 Line 5	"Bennet et al." should be "Bennett et al."
Page 1-3 Paragraph 2 Line 2	"This…is…" should be "They…was…"
Line 3	"found the" should be "found that the"
Paragraph 3 Line 2	"(…Daton)" should be "(…Dalton)"
Page 1-4 Paragraph 2 Line 6	"Neasi" should be "Ncasi"
Page 1-5 Paragraph 2 Line 4	"…process." should be "…process has not been considered."
Paragraph 3 Line 7	"studied" should be "characterized"
Page 1-6 Paragraph 1 Line 14	"digestion, lagoon" should be "digestion in a lagoon,"
Page 1-11 Paragraph 3 Line 1	"readily involved" should be "efficient"
Line 2	"readily involved" should be "efficient"
	CHAPTER 3
Page 3-1 Paragraph 2 Line 1	"pond…" should be "pond,…"
Line 2	"shifted" should be "increased"
Line 7	"piped" should be "pipe"
Page 3-2 Paragraph 1 Line 8-9	"(Figs.1, 2 and 3)" should be "(Fig.3.1, 3.2 and 3.3)"
Page 3-12 Paragraph 2 Line 6	"total flow" should be "total input flow that merge with AMCOR discharge"
Page 3-17 Paragraph 1 Line 10	"0.99" should be "0.98"
Page 3-20 Paragraph 2 Line 2	"Fig.10" should be "Fig.3.10"
Table 3.6 Column 6	Heading should contain "(Mean value of the frequency function)"
Page 3-24 Table 3.8 Column 1	"Figs.11, 12, 13" should be "Figs.3.11, 3.12, 3.13"
Page 3-26 Paragraph 1 Line 1	"except additional inputs" should be included after "trigger"
Page 3-28 Paragraph 3 Line 1	"1600-6500" should be "2600-5500"
Page 3-29 Table 3.9 Column 4	Heading should contain "(Polydispersity)"
Page 3-30 Table 3.10 Column 1	"Figs.14, 15" should be "Figs.3.14, 3.15"
Page 3-32 Paragraph 4 Line 2	"…weights." should be "…weights, compared to LVOS receiving water and treated wastewater in DD treatment plant."
	CHAPTER 4
Page 4-2 Paragraph 1 Line 1	"major organic contributor to" should be "major contributor of organic matter to"
Page 4-10 Paragraph 1 Line 6	"significantly" should be "substantially"
Paragraph 2 Line 1	"It seems the…" should be "According to the absolute concentrations of fulvic acids, it seems the…"
Page 4-11 Paragraph 1 Line 1	"The results show…" should be "The Fig.4.2 show..."
Line 2	"least contribution is from the humic acid fraction. " should be "least fraction of DOC is due to the humic acid."
Page 4-13 Paragraph 2 Line 1	"using dilution fold of mill effluent (Table 3.4 in chapter 3)" should be included after the word "estimated"
Line 5	"since and the fact that" should be "as"
Page 4-17 Table 4.1 Title	"…substances in the DD…" should be "…substances in the LVOS wastewater, after treatment in the DD…"
Page 4-28 Paragraph 4 Line 1	"(154 mgC/L)." should be "(154 mgC/L), compared to other organic fractions."

CHAPTER 5

Page 5-1 Paragraph 2 Lines 2-5	"removed" should be "degraded"
Line 2	"by microbes." should be "by microbial action."
Page 5-9 Paragraph 1 Line 10	"(Fig.5.1)." should be "(Table 5.1)"

CHAPTER 6

Page 6-1 Paragraph 1 Line 1	"done" should be "carried out" "impact" should be "effects"
Page 6-6 Paragraph 2 Line 3	"...in the previous chapter." should be "...below."

CHAPTER 7

Page 7-1 Paragraph 1 Line 1	"Untreated mill effluent..." should be "The effect of biological processes on the major organic fractions of untreated mill effluent from the AMCOR pulp and paper mill plant was investigated."

CHAPTER 8

Page 8-6 Fig 8.1(a), (b)	Regression is excluded. It should be one way ANOVA.
Page 8-7 Fig 8.2(a), (b)	Regression is excluded. It should be one way ANOVA.
Page 8-8 Fig 8.3(a), (b)	Regression is excluded. It should be one way ANOVA.
Page 8-9 Fig 8.4(a), (b)	Regression is excluded. It should be one way ANOVA.

CHAPTER 9

Page 9-3 Paragraph 3 Line 5	"biological" should be "microbial"
Page 9-27 Paragraph 1 Line 6	"However, the raw..." should be "The raw..."
Page 9-32 Paragraph 2 Line 4	"in that respect" should be "in terms of DOC"

CHAPTER 10

Page 10-5 Paragraph 2 Line 7	"fulvic acid reduced" should be "fulvic acid concentration was reduced"
Paragraph 3 Line 1	"It seems that..." should be "The..."
Page 10-10 Paragraph 2 Line 5	"Some how" should be "Somehow"
Page 10-17 Paragraph 1 Line 8	"the low molecular weight range fulvic acids" should be "fulvic acids in the low molecular weight range"
Paragraph 2 Line 6	"As far as humic acids are concerned,..." should be "For the humic acids,..."
Page 10-19 Paragraph 3 Line 1	"skew value,..." should be "skew value of the molecular weight distribution peaks,..."
Page 10-21 Paragraph 1 Line 3	"exhausted" should be "removed"

CHAPTER 11

Page 11-1 Paragraph 2 Line 1	"three microbially treated coloured fractions" should be "microbially treated three coloured fractions"
Line 6	"condition" should be "conditions"
Page 11-2 Paragraph 1 Line 4	"...major..." should be "...isolated ..."
Page 11-2 Paragraph 2 Line 13	"which process..., is more responsible..." should be "which microbial process..., is responsible..."

CHAPTER 12

Page 12-1 Title	"Conclusions" should be "Summary of Results"

Addendum

Page 1-11 Paragraph 2 Hypothesis 3 should be replaced by
The DOC that is more efficiently removed by microbes contains mainly smaller molecules whereas refractory DOC contains mainly the lager molecules.

Page 3-3 Paragraph 1 Line 1 should be replaced by
The objectives of the experiments undertaken to characterize effluents and receiving wastewaters by molecular weight studies are:

Page 3-14 Paragraph 2 Line 7 The following sentence should be included before the sentence "Within 24 hours of sampling....."
The samples were collected and placed in ice (coolers containing ice) till transport to the laboratory.

Page 5-1 Abstract Paragraph 1 should be replaced by
The AMCOR mill effluent and wastewaters collected from the LVOS channel and treated waters collected from the DD treatment plant were subjected to flow FFF analysis to establish the variation in average molecular weights and molecular weight distributions of organic matter in the solution. The hydrophobic acids (macromolecular substances) in the media were isolated by XAD-8 resin fractionation. These hydrophobic acids (fulvic and humic) were subjected to further study by using flow FFF to analyze the variation of average molecular weights and molecular weight distributions.

Page 5-6 Section 5.2.4 should be included
5.2.4 Statistical Accuracy and Precision
Two sets of samples were subjected to investigation. Each set contained 10 samples collected over 10 sampling sites and an effluent discharged from the mill.

Flow FFF analysis was carried out in triplicate with each sample in both sets. The relative standard deviation of average molecular weights in raw effluent was 5%. It was 3% in wastewaters in the LVOS channel and was only 1-2% in treated waters in the DD ponds.

Isolation of hydrophobic acid fractions was carried out (by XAD-8 resin fractionation) in duplicate with each sample in both sets. Flow FFF analysis was carried out in triplicate with each hydrophobic acid fraction (fulvic and humic). The fulvic and humic acid fractions of both sets were subjected to FFF analysis. The relative standard deviation of average molecular weights of fulvic acid was 2-3% and of humic acid was 5% in effluent and all waters.

Page 5-13 Paragraph 2 Line 5 The sentence ending ".....weights." should be
.....weights, since resultant high average molecular weights can be due to either increase in high molecular weight fractions or decrease in low molecular weight fractions.

Page 5-14 Paragraph 1 Line 6 The sentence "But comparison....." should be
But comparison of molecular weight distribution of a given isolated fraction from two sampling sites could be valid, since the concentrating factor in the isolation procedure remains the same with each sample.

Page 6-2 Paragraph 3 should be replaced by
The main hypotheses to be tested were:
(1) Aerobes are more efficient in degradation of DOC than anaerobes
(2) Anaerobes are more efficient in conversion of refractory DOC into degradable DOC than aerobes
(3) Anaerobes are more efficient in conversion of POM into DOC than aerobes

Page 6-10 Table 6.1 The following footnote should be included.
The average molecular weights reported here are mean values of triplicate analyses. For INLET 41 ML sample, the mean values were with ±150 Dalton in aerobic experiments and ±250 Dalton in anaerobic experiments. For EXIT POND C sample, these values were with ±50 Dalton in aerobic experiments and ±80 Dalton in anaerobic experiments.

Page 9-4 Paragraph 3 Line 4 The sentence "The inlet and....." should be replaced by
The samples collected from the primary treatment plant were inlet and outlet streams of the Dorr-Oliver Clarifier, and they were labelled as follows:

Page 9-10 Section 9.3.3 should be included
9.3.3 Statistical Accuracy and Precision
Two sample batches were analyzed. The laboratory microbial experiments were carried out in triplicate and were repeated twice.

The DOC was measured in duplicate with each sample and the reproducibility of DOC analysis was checked, by repeating the analyses on three successive days. The relative standard deviation of DOC analyses was within 0.5-1.5% range. The instrumental error (TOC-5000) was less than 0.1% (recalibrated each time with freshly prepared standards).

The molecular weights were measured in triplicate with each sample. The relative standard deviation of the average molecular weight analyzed was 2-3%. The precision of the flow FFF instrument was checked with Okefenokee Swamp and Mattole soil humic standards.

Page 10-11 Tables 10.1, 10.2 and 10.3 The following footnote should be included.in common to the above three tables.
The molecular weight distributions (frequency function vs molecular weight) were often asymmetric and showed some peak spreading (from the peak maxima) towards high retention times only and is given by the skewness (sk) of the peak.

Page 10-20 Paragraph 2 should be reconstructed as follows
Line 5 .....consists of the fulvic acids (Figs.10.2-10.4).
Line 9 .....acids. The high molecular weight.....

Page 10-22 Paragraph 2 Line 2 should be included
The amount of hydrophobic substances removed at 60°C degrees was higher (40%) than that at 37°C degrees. Physico-chemical treatment also removed hydrophobic substances but to a lesser extent (20%).

Page 11-2 Paragraph 2 Objective 2 should be
Whether chemical transformations occur in XAD-8 fractions subjected to microbial treatment

AMENDMENTS TO FIGURE HEADINGS

CHAPTER 3

Page 3-6 Fig.3.3 Title Line 3 "The ponds marked as 1,2,3,4 and 5 are stands for pond A, pond B, pond C, pond D and pond E respectively." should be included.

Page 3-9 Fig.3.5 Title Line 2 "Sampling points are given as follows; SCC (Sandy Creek Crossing), RD (Rosedale Dissolver), RM (Rosedale Meter), P83 (Peg83), P133 (Peg133), IN2 (Inlet No.2)" should be included.

CHAPTER 9

Page 9-20 Fig.9.6 Title "...colour value with..." should be "...colour value in raw solution with..."

Page 9-22 Fig.9.8(a) The title should be "CAUSTIC SEWER – Mw distributions of humic acid solutions with degradation time under aerobic conditions"

Fig.9.8(b) The title should be "CAUSTIC SEWER – Mw distributions of humic acid solutions with degradation time under anaerobic conditions"

Page 9-27 "Fig.13(a) and Fig.13(b)" should be "Fig.9.13(a) and Fig.9.13(b)"

Page 9-30 "Fig.15(a) and Fig.15(b)" should be "Fig.9.15(a) and Fig.9.15(b)"

Fig.9.15(a) The title should be "CAUSTIC SEWER –Variation of colour value of raw, humic acid and fulvic acid solutions with degradation time under aerobic conditions"

Fig.9.15(b) The title should be "CAUSTIC SEWER –Variation of colour value of raw, humic acid and fulvic acid solutions with degradation time under anaerobic conditions"

Page 9-32 "Fig.17(a) and Fig.17(b)" should be "Fig.9.17(a) and Fig.9.17(b)"

Fig.9.17(a) The title should be "PULP MILL SEWER – Variation of colour value of raw, humic acid and fulvic acid solutions with degradation time under aerobic conditions"

Fig.9.17(b) The title should be "PULP MILL SEWER – Variation of colour value of raw, humic acid and fulvic acid solutions with degradation time under anaerobic conditions"

Exclusions

(a) Paragraphs and Sections

Page 4-3	Fig.4.1
Page 5-1	Paragraph 1
Page 5-4	Paragraph 2
Page 5-5	Lines 1-4
Page 6-2	Paragraph 3
Page 6-5	Section 6.2.4
Page 7-2	Paragraph 1
Page 7-5	Section 7.2.3
Page 7-6	Section 7.2.4
Page 7-7	Section 7.2.5

(b) Sentences

Page 4-20	**Paragraph 3 Line 9**	The fulvic acids being.....
Page 9-27	**Paragraph 1 Line 8**	Therefore, the average.....
Page 10-21	**Paragraph 1 Line 6-13**	Due to lack...

(c) Phrases and words

Page 1-5	**Paragraph 2 Line 3-4**	these researchers have not considered
	Paragraph 3 Line 7	characterization of
Page 3-3	**Paragraph 1 Line 2**	weight
	Line 10	weight
Page 4-10	**Paragraph 1 Line 2**	gradually
	Line 3	(Fig.4.2)
Page 5-2	**Paragraph 1 Line 3**	above
Page 5-14	**Paragraph 2 Line 6**	than all the other samples
Page 6-1	**Paragraphs 2 Line 2**	proper
	Paragraphs 3 Line 2	proper
Page 6-18	**Paragraph 2 Line 1**	proper
Page 9-33	**Paragraph 2 Line 4**	even
Page 10-2	**Paragraph 3 Line 2**	effectively
Page 10-17	**Paragraph 1 Line 8**	the removed
Page 10-23	**Paragraphs 1, 2, 3**	As far as.....concerned,
Page 11-18	**Paragraph 3 Line 8**	somewhat

General corrections to be made throughout the thesis

Recipient water should be **receiving** water
Fig.4.1 referred in text should be **Fig.2.5**
MilliQ water stands for **deionized water**
Doc should be **DOC**
Carbon sterilized should be **precleaned**

List of Abbreviations

TOC	- Total Organic Carbon
TC	- Total Carbon
TIC	- Total Inorganic Carbon
DOC	- Dissolved Organic Carbon
DOM	- Dissolved Organic Matter
POC	- Particulate Organic Carbon
POM	- Particulate Organic Matter
IC	- Inorganic Carbon
NPOC	- Nonpergeable Organic Carbon
AMCOR	-
CSR	-
SECV	- State Electricity Commission of Victoria
LVOS	- Latrobe Valley Outfall Sewer
DD	- Dutson Downs
HTD	- hydro thermal drying
COD	- Chemical Oxygen Demand
BOD	- Biological Oxygen Demand
DO	- dissolved oxygen
ML	- mega Litre(s)
FFF	- Field Flow Fractionation
FlFFF	- Flow Field Flow Fractionation
EPA	- Environment Protection Authority
SCC	- Sandy Creek Crossing
RD	- Rosedale Dissolver
RM	- Rosedale Meter
P83	- Peg 83
P133	- Peg 133
IN2	- Inlet point of No.2 storage
EX A	- Exit point of pond A
EX B	- Exit point of pond B
EX C	- Exit point of pond C
EX D	- Exit point of pond D
EX 2	- Exit point of pond E (Exit point of No.2 storage)
UV	- Ultra Violet
KHP	- potassium hydrogen pthalate
HA	- humic acid
HFi	- hydrophilic acids
HFoA	- hydrophobic acids
HFoN	- hydrophobic neutrals
HFoT	- total hydrophobic substances
HFiT	- total hydrophilic substances

mL	- milli Litre(s)
L	- Litre(s)
cm	- centi meter(s)
nm	- nano meter(s)
mg	- milli gramme(s)
min.	- minute(s)
sec.	- second(s)
M	- molarity in mol dm^{-3}
dm.	- decimeter(s)
R	- retention ratio
V°	- void volume
V_r	- retention volume
t°	- void time
t_r	- retention time
V	- channel flow rate
V_c	- cross-flow rate (field flow rate)
l	- mean thickness of a sample cloud
λ	- Retention parameter
D	- diffusion coefficient
U	- cross-flow velocity
w	- channel thickness
M	- molecular weight
Mi	- molecular weight of i^{th} fraction
Mw	- weight average molecular weight
Mn	- number average molecular weight
A	- constant for a given sample, carrier liquid at a constant temperature
b	- constant for a given sample, carrier liquid at a constant temperature
PSS	- poly(styrenesulfonate) standard(s)
sk	- skew
ρ	- Polydispersity

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Abstract

Information on the nature of the industrial effluents is needed to assess the best treatment option, which will generate organic waste effluents with least harm to the environment. Most organic matter in industrial effluents contain coloured moieties with molecular weights ranging from 400-150,000 Dalton. This study concentrates on determining the molecular weight and hydrophobic-hydrophilic nature of several effluents.

The main objective of the current project was to investigate the use of biological treatment for organic mill effluents and the effect of this on the composition of these effluents. One of the main questions addressed was which organic fraction is more prone to biological attack under given conditions, and which molecular weight fractions are responsible for the variation of effluent colour during biological treatment.

The organic mill effluents investigated in this work are as follows. An effluent from the AMCOR mill in Victoria (Australia) and the recipient water of the Latrobe Valley Outfall Sewer (LVOS) system, and the treatment complex at Dutson Downs; several effluent streams from the Tasman pulp and paper mill in Tarawera (New Zealand) and the recipient water (Tarawera River); An effluent from CSR sugar mills in Queensland (Australia) was fractionated into major organic fractions and laboratory biological experiments were conducted. coal washings left after hydro thermal drying of Latrobe Valley brown coal by the State Electricity Commision of Victoria (SECV). The effect of primary treatment due to in-house processes such as anaerobic digester process and physico-chemical process (coagulation, flocculation and filtration) on coal washings was studied.

Since field studies did not provide complete information on the biological effect on mill effluents, laboratory biological experiments were carried out. The primary objective of these experiments was to determine the relative significance of aerobic and anaerobic reactions as a biological treatment for effluents. Attention was given to DOC, colour and average molecular weight of the effluent medium after aerobic or anaerobic treatment.

Flow field-flow fractionation was used to characterize the molecular weight of the effluents. XAD-8 resin separation was employed to fractionate dissolved organic matter into broad chemical groups, depending on the hydrophobic nature of the molecules.

Both fractionation techniques were successfully used for characterization of mill effluents before and after biological treatment. Aerobic degradation was more efficient than anaerobic degradation. Aerobes readily removed low molecular weight matter while anaerobes readily degraded high molecular weight matter from the dissolved organics. In the presence of particulate matter the anaerobes preferentially reacted on solids and released dissolved organics. The most vulnerable organic fraction was the hydrophilics, but microbe refractory hydrophilics were also present. Microbes then preferentially reacted on fulvic acids rather than on humic acids. However, in the absence of fulvic acids microbes reacted on humic acids. The effluent colour was mainly associated with low molecular weight fulvic acids and high molecular weight humic acids. Some biochemical transformations have occurred between the major organic fractions during microbial treatment.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person or persons, except where due reference is made.

K. C. M. Fonseka

Acknowledgment

It is a great pleasure to express my deep sense of gratitude to my main research supervisor Dr Ronald Beckett (Reader in Chemistry), Department of Chemistry, Water Studies Centre, Monash University (Melbourne, Australia) for his invaluable advice, guidance, constructive suggestions and helpful discussions during this research. I would like to acknowledge Dr David Dixon, CSIRO Division of Chemicals and Polymers (Melbourne, Australia) and Dr James Ranville, Colorado School of Mines (USA) for their contribution for the success of this project.

My special thanks should go to Melbourne Water for the Industrial Waste Postgraduate Award, the scholarship awarded to me for my PhD project. Also, my thanks should go to Jenny Gilbert (Gippsland water), Steve Hodges (SECV), Ron Hutchins (CSR) for providing me regarding information on samples collected for this research.

Also, my pleasure to Elizabeth Flanagan for, proof reading this thesis and Mara Pavlidis for, spending much time on grammatical constructions throughout.

I wish to express my deep sense of thanks to Pamela Herman (Manager, Monash Research Graduate School) and Helen Garretty (Research and Training support Branch, Gippsland Campus, Monash University), who contributed immensely for the completion and submission of my thesis for the award of PhD degree. I also wish to acknowledge PhD and Scholarship Committee and Student Steering Committee for driving me through the completion of this thesis to submit for examination.

I deeply appreciate Jenny Reeder and Sarah Lawson (Monash Postgraduate Association) for their immense contribution and continuous support for the completion of my PhD thesis. In that respect, I must give my deep-hearted gratitude to Prof. Brian Parmenter and Assoc.Prof. Micheal Morgan (Student Steering Committee).

I take this opportunity to thank the staff at Water Studies Centre, especially Pam Dickinson (Administrative Officer) and Sandra Sdraulig, Ashley Liang and Dr. Nicola Porter in the Analytical Laboratory for their supportive and friendly atmosphere throughout the project. Colleagues who worked as a research group at FFF Laboratory are also gratefully acknowledged for their generous assistance.

Finally, I deeply appreciate my husband, Gayan Perera for his invaluable contribution at the preparation of my PhD thesis for the submission to examiners. Indebtedness must be acknowledged for his understanding and patience throughout the years I devoted to the academic career. My baby Mihin must be gratefully acknowledged for being a soothing baby without interrupting at my final phase of the PhD.

Chapter 1

Introduction

Industrial wastewater being discharged to public sewers must be characterized in order to assess the treatment options and thus minimize the detrimental effects due to pollutants in these discharges. Industries which involve large-scale usage of water are required to recycle wastewater back to designated stages of their processes, or to treat effluents before discharging to public waterways. The pulp and paper manufacturing industry is well renowned for using large volumes of water in the bleaching and pulping processes and as a consequence, generates large volumes of wastewater. The paper industry is currently one of the largest water users (Yulke et al., 1981).

The quantity of the effluent and as well as its quality generally depends on the type of processes being used in a particular industry. The effluents from the pulp and paper industry are chemically more complex than that of other industries, and vary from mill to mill. Fluctuations in the composition of pulp and paper mill effluents are mainly due to fluctuations in the wood raw materials used in the pulping process but are also due to the type of process used - pulping technology (Heimburger et al., 1988; Rapson, 1989 and Chung et al., 1990). The type of bleaching process used also contributes if the mill output is a combined effluent from both the pulp and bleach plants. These effluents are environmentally significant and industries are increasingly being required to develop manufacturing processes that generate minimum amounts of organic waste effluents. This is in order to minimize their harm to the environment.

Not all mill effluents cause detrimental effects in recipient waters. According to some researchers there are no definite effects in recipient waters due to industrial discharges (Mehrle et al., 1989; McLeay et al., 1987). Some previous researchers even claim some beneficial effects of pulp and paper mill discharges (McLeay et al., 1987; Bonsoi et al., 1988). Despite these claims beneficial effects of pulp and paper mill effluents, Kringstad et al. (1984) found that some of the organochlorines present in the mill effluents have the potential to bioaccumulate. Lehtinen et al. (1984) reported that some effluents significantly harmed biological populations in aquatic environments.

The effluents being discharged to recipient waters add some toxicity, biological oxygen demand (BOD), colour and turbidity to the recipient water systems. The chlorinated lignins originate from the chlorination and caustic extraction stages (Sjorstrom, 1981; Walden, 1976). The caustic extraction stage in pulp bleaching of the paper industry is responsible for 70-75% of the colour in the effluent (Bennet et al., 1971; Nasr et al., 1978). The major sources of colour may vary from industry to industry, but usually originates from lignins and tannins in most industries.

The chromophore groups in pulp and paper mill effluents generally come from lignin degradation. These chromophore groups are often quinone groups within molecules containing conjugated double bonds (Luner et al., 1970). The functional groups commonly found are phenolic, hydroxyl, weak acidic groups, and unsaturation. According to Schmidt and Joyce (1980), the major functional groups contributing to colour of pulp bleaching effluents are aromatic and quinonoid moieties. They pointed out

that these functional groups could be conjugated with carbonyl and ethylene groups. They also mentioned that these coloured molecules vary in size with molecular weights ranging from 400-150,000 Dalton. The studies on molecular weight reported in this thesis could be used to help select the best treatment option.

Dixon et al. (1991) studied the characterization and treatability of pulp and paper mill effluents. This showed that colour removal is associated with the removal of hydrophobics rather than hydrophilics substances. They found the pulp liquor had an average molecular weight of about 5100 Dalton but lower molecular weights were observed for washings from the paper plant (2300 Dalton) and treated effluent after the alum clarification process plus biological treatment (1000 Dalton). Dixon et al.'s overall findings were that physicochemical plus biological treatments selectively remove higher molecular weight material. They also found that colour removal is associated with hydrophobic substances. According to their studies the noncolured hydrophilic substances are more resistant to the physicochemical treatments.

The size and chemical characterization of pulp and paper mill effluents carried out by Beckett et al. (1992) reported a high molecular weight (11000 Daton) for an effluent from a wood pulping process. They reported that the pulping process generates high molecular weight material whilst bleaching and paper manufacturing processes generate much lower molecular weight material. They also mentioned that the treated effluent from the stream which undergoes both flocculation with alum and polyelectrolyte plus biological treatment contained only low molecular weight components.

The work undertaken by Beckett et al. (1992) on the characterization of pulp mill effluents before and after physicochemical treatment showed that coagulation based treatment methods are more successful in removing higher molecular weight organics as well as hydrophobic organic matter. They observed that these treatment methods have only little effect on low molecular weight organic matter and hydrophilic substances. They found activated carbon removes most of the hydrophobic compounds and even more of the hydrophilics. Adsorption by activated carbon was a selective removal method.

Beckett et al. also found that, even if some effluents have similar gross parameters such as colour, turbidity and TOC, they can still have significantly different molecular weight distributions. These authors proved that effluents of similar colour and TOC values could contain different organic matter composition, which in turn may require different ways of treatment. Earlier researchers have also suggested the need for physicochemical treatment to achieve removal of high colour and COD (Neasi, 1968; Gehm et al., 1968).

Most industries have taken the necessary steps to improve inhouse processes in order to reduce effluent volumes and the pollutant nature of the effluent before discharge. Such inhouse processes may include an extended delignification process, an improved washing process, the staged addition of bleaching agents, or use of alternative bleaching agents. Even though the above processes are now being used in most industries, still the resultant effluents usually require further treatment before discharge into public waterways. In the past, preference has been given to biological treatment methods with aerated lagoons and

activated sludge plants often being selected as an external treatment option (Frostell et al., 1991).

Several biological treatment methods have been established. The activated sludge process is widely used for effluent treatment in Kraft pulp and paper mills (Gehm et al., 1968; Ayers et al., 1971; Chen et al., 1974; Welch, 1979; Buckley, 1981). However, these researchers have not considered the behavior of Kraft lignins during the sludge process. Obiaga (1974) and Touzel et al. (1977) found condensation of dissolved Kraft lignin during wastewater aeration. Sikes (1976) suggested the addition of nutrients and prolonged aeration for treatments of these effluents by aerated lagoon systems or by activated sludge systems. Ayers et al. (1971) and Hakulinen et al. (1982) have studied alternative treatment methods for pulp and paper mill industry wastewater.

A batch study of the aereobic/anaerobic removal of chlorinated organic compounds in an aerated lagoon was studied by Chernysh et al. (1992). Bryant et al. (1987,1988) and Amy et al. (1988) suggested biosorption in the aerobic zone followed by anaerobic degradation and dehalogenation within the anaerobic zone in a lagoon, as a major pathway for the removal of adsorbable organic halogens. The fate of high and low molecular weight organochlorine compounds in a laboratory scale lagoon has been studied by Collins in 1991. The characterization of microbiology of the lagoon has also been studied by Collins et al. (1991); Liss et al. (1992) and Fulthorpe et al. (1993).

A literature survey has revealed that certain bacteria and fungi are capable of biodegrading organic chlorine compounds formed during the chlorine bleaching process of pulp effluents. Usually the biodegradation of high molar mass compounds in bleaching wastewaters, which make up most of the organic content of a bleaching wastewater, is slower than for low molar mass compounds. Also, bacteria are slower than fungi in biodegrading bleaching effluents (Zurer (1987); Pellinen et al., 1990 and Lankinen et al., 1990). According to their studies biodegradation occurs under both aerobic and anaerobic conditions. During the first stage of degradation, they observed chlorine removal, which did not necessarily require the complete breakdown of the skeleton of these complex molecules. Saunamaki et al. (1991) also reported that microbiological degradation is the major cause for the removal of organic chlorine compounds in kraft effluent in activated sludge treatment plants. They observed most of the chlorinated organics removed by microbiological action were low molar mass compounds, such as chlorinated phenols. In their studies on anaerobic digestion, lagoon before or after the activated sludge process did not improve the removal of such low molar mass chlorinated organics. A pilot-study undertaken by Gergov et al. (1988) also found similar results.

At present, it is not clear which molecular weight fractions are preferentially removed by bacteria under aerobic or anaerobic condition, and which organic chemical fractions are more prone to biological attack under given conditions. One of the main questions to be addressed in this thesis is whether the colour of an effluent is altered as a consequence of biological treatment and, if so, which molecular weight fractions and which organic chemical fractions are involved. The other issue is to assess the feasibility of using

molecular weight measurements and changes in the hydrophobic-hydrophilic nature of the effluents as a tool for selecting the best treatment option.

The main objective of the current project was to investigate the biological treatment of organic mill effluents and the effect of this on the composition of these effluents. The investigations involved some studies of the efficiency of biological treatment for removal of specific organic fractions from industrial effluents. Gross parameters, such as colour, turbidity and TOC, are not sufficient for this purpose due to their lack of specificity. These parameters alone do not provide accurate guidelines for assessing treatment options. In the current project the total dissolved organic content of the effluent was fractionated on the basis of molecular weight and hydrophobic nature. The changes in the amount of hydrophobic and hydrophilic fractions present and changes in weight and number average molecular weights and molecular weight distribution patterns were monitored during treatment. It is hoped that such information may assist in the selection of the optimum treatment method for a given wastewater system.

One of the major aims of the current study was to compare biological processes to physico-chemical treatment methods. Thus, this study has concentrated on the microbiological degradation of the highly concentrated organic effluents from pulp and paper mill plants and recipient waters containing mill effluent discharges. The results were compared with the trends observed for physico-chemical treatments, such as those based on adsorption and coagulation.

The pulp and paper mill effluent from AMCOR mill at Maryvale in Gippsland (Victoria, Australia), and its recipient water in the Latrobe Valley Outfall Sewer (LVOS) system was subjected to extended investigation. The factors influencing the treatment of pulp mill effluent based on the AMCOR mill pulp and paper mill effluent was outlined by Duncan et al (1992). The mill discharges in excess of 14 ML/day on average into the Latrobe Valley Outfall Sewer (LVOS) channel. The recipient water containing the AMCOR mill effluent passes down the LVOS channel to reach the Dutson Downs treatment complex. The mill effluent is mixed with domestic sewage along the LVOS drain and subsequently passes into aerobic and anaerobic treatment ponds at the Dutson Downs disposal area.

In this work a series of sites along the LVOS system have been studied, as the effluent was thought to be altered by biological degradation. The main part of the study has focussed on the Latrobe Valley outfall drain and the Dutson Downs treatment complex.

Mill effluents from Tasman's pulp and paper mill situated in Tarawera, New Zealand and Tasman's mill recipient waters at the Tarawera River lower reaches were also investigated. Different effluent streams released by different plant processes were characterized to deduce which plant process is the best suited to which treatment option before discharge. The Tasman mill discharges in excess of 206 million litres per day on average into the primary treatment plant (clarifier and sludge lagoon) and then to the secondary treatment plant (Tasman's treatment ponds), which eventually discharges to

the Tarawera River. A series of sites along the Tarawera River have also been analyzed for biological degradation.

Since field studies on the industrial effluents of the AMCOR and Tasman mills did not provide complete information on the biological effect on mill effluents, further experiments were undertaken in the laboratory. The field studies have been interrupted by assuming the effect of dilution can be estimated using the average flows and inputs in the LVOS canal. Tarawera River was investigated by the monitoring data available.

Raw pulp effluent from the Tasman pulp milling plant and combined effluent from the AMCOR pulp and paper mill plant were chosen for the laboratory studies. Three wastewater sites from the LVOS recipient waters and an effluent from the caustic bleaching plant of the Tasman mill were also selected for further laboratory study.

The primary objective of the laboratory scale biological experiments was to determine the relative significance of aerobic and anaerobic reactions on DOC removal from an effluent during biological treatment. The effect on specific molecular weight fractions and colour was determined.

Several other organic effluents were investigated. Coal washings from the State Electricity Commission of Victoria (SECV) was sampled before and after treatment in an anaerobic digester. Also, the filtrate left after physicochemical processes (coagulation, flocculation and filtration) of coal washings was sampled. The effluents from the

anaerobic digester at two temperatures and the filtrate from an alum treatment plant were studied. Emphasis was given to the molecular weight changes and also to the biological effect on the major organic fractions.

An effluent from the CSR sugar mill at Plane Creek, Queensland (Australia), was investigated. This was the disposal wastewater removed after molasses fermentation. The effluent was fractionated into major organic fractions depending on the hydrophobic nature of the molecules. Laboratory biological experiments were conducted with these major organic fractions to study the relative significance of aerobic and anaerobic conditions on biochemical transformations. The molecular weight was monitored to determine which molecular weight fractions preferentially undergo biochemical transformations during the biological treatment.

Two fractionation techniques have been extensively used in this current project. The relatively new technique of flow field-flow fractionation was employed to record the molecular weight distributions in order to characterize mill effluents before and after a given treatment method. An XAD-8 resin adsorption/desorption separation process was employed to fractionate the dissolved organic matter in effluents into broad chemical groups, depending on the hydrophobic nature of the molecules. These two techniques were used on designated samples withdrawn during aerobic or anaerobic treatment to monitor which organic fraction and which molecular weight fraction was being degraded by microbes under the given conditions.

The initial working hypothesis was that the lower molecular weight organic fractions, particularly the hydrophilic components, are more susceptible to microbiological degradation. Conversely, the higher molecular weight fractions are considered to be more resistant to microbiological degradation. In contrast, physicochemical treatment based on coagulation selectively removes a greater proportion of the higher molecular weight and more hydrophobic molecules.

Various hypotheses were tested during the conduct of this research project. These are:

- aerobes are more readily involved in DOC removal than anaerobes
- anaerobes are more readily involved in microbial conversion of refractory DOC or suspended POM into readily utilizable DOC. The aerobes are capable of removing this biologically produced DOC
- more readily removable DOC by microbes should be comprised of the smaller molecules, and refractory DOC should be comprised of the larger molecules
- aerobes preferentially remove lower molecular weight DOC while anaerobes preferentially remove higher molecular weight DOC
- aerobes preferentially remove colourless DOC and anaerobes preferentially remove coloured DOC.

Chapter 2

Techniques And Principles

2.1 INTRODUCTION

The physical characterization of organic matter in mill effluents and wastewaters was carried out by molecular weight studies. The flow FFF technique has been used to determine the average molecular weights and molecular weight distributions of effluents and wastewaters and their extracted fulvic and humic acids fractions. Previous workers have used other techniques for studies on the molecular size of paper mill effluents (Forss et al 1969, Connors et al 1980, Faix et al 1981, Marchessault et al 1982, Lewis et al 1983). Since the other techniques such as membrane ultrafiltration and size exclusion chromatography suffer from serious problems; such as, sample adsorption, pore blockage, repulsion from membrane/gel (Buffle et al 1978, Hine et al 1984, Stockner et al 1990, Chin et al 1991), flow FFF was used in this study, as it may be less susceptible to these problems. The reason is the separation in flow FFF is carried out in an open, unpacked channel, which is described as a one-phase separation.

The chemical characterization of organic matter in mill effluents and wastewaters was carried out by selective adsorption of different organic compounds onto a nonpolar resin (Amberlite XAD-8) to separate dissolved organic matter into major chemical groups. The separation scheme devised by Leenheer (1981) has been used to determine three major organic fractions; i.e. hydrophilic substances, fulvic acids and humic acids, in raw effluents and wastewaters. Previous workers have used this technique to obtain two major

fractions i.e; hydrophilic and hydrophobic, from paper mill effluents (Beckett et al 1992, Dixon et al 1991, Dixon et al 1992).

2.2 FIELD-FLOW FRACTIONATION TECHNIQUE

Field-flow fractionation (FFF) is a high resolution technique that is capable of separating macromolecules, colloidal material and particulate material (Giddings 1988, Giddings et.al. 1978, Cadwell 1988, Janca 1988). FFF has been described as a one-phase separation technique (Giddings J.C. 1969). The samples are partitioned into different regions having different carrier velocities in an open, thin, ribbon-like channel (Fig.2.1).

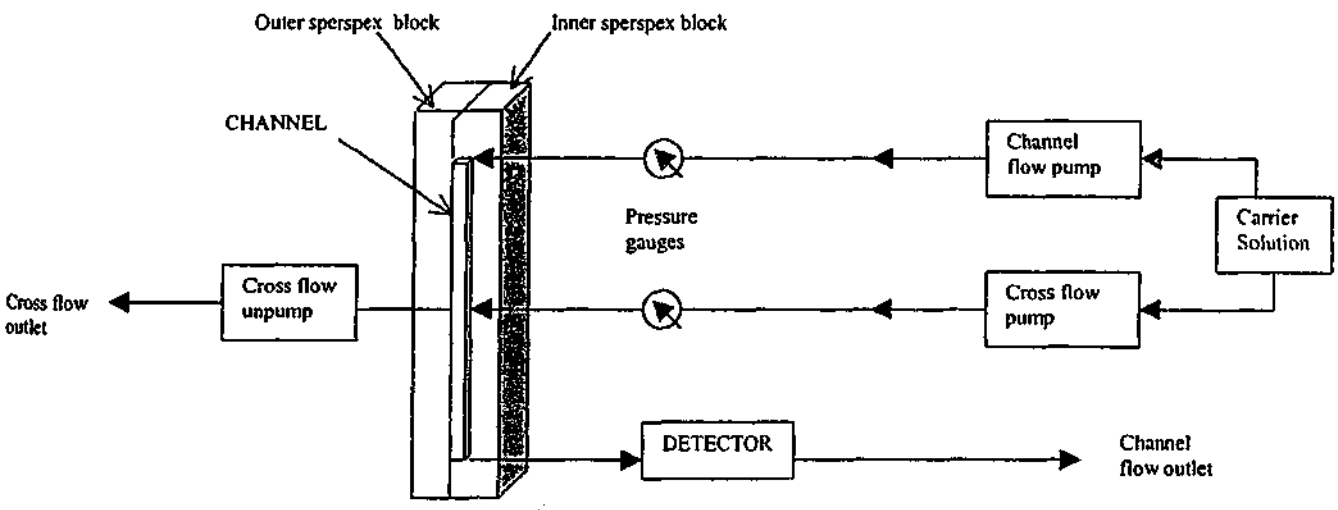


Fig. 2.1 Schematic diagram of flow-FFF system

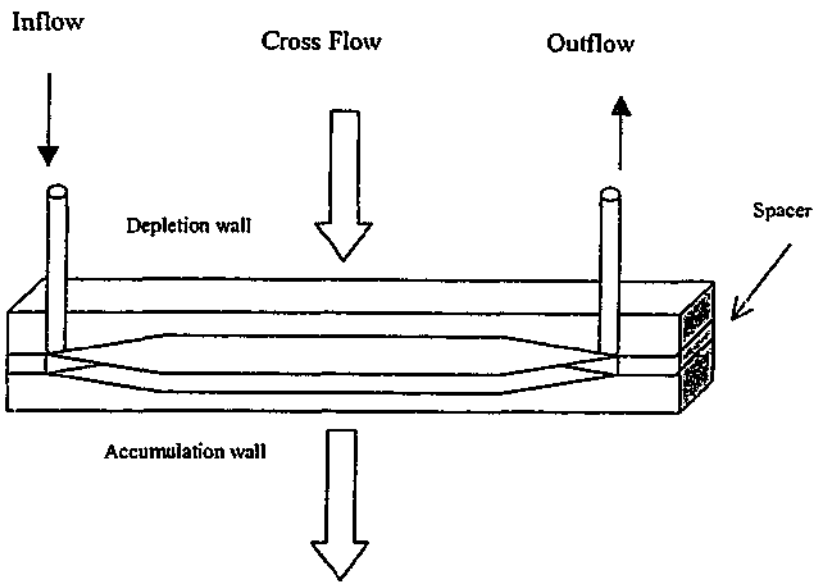


Fig. 2.2 Schematic diagram of flow-FFF channel

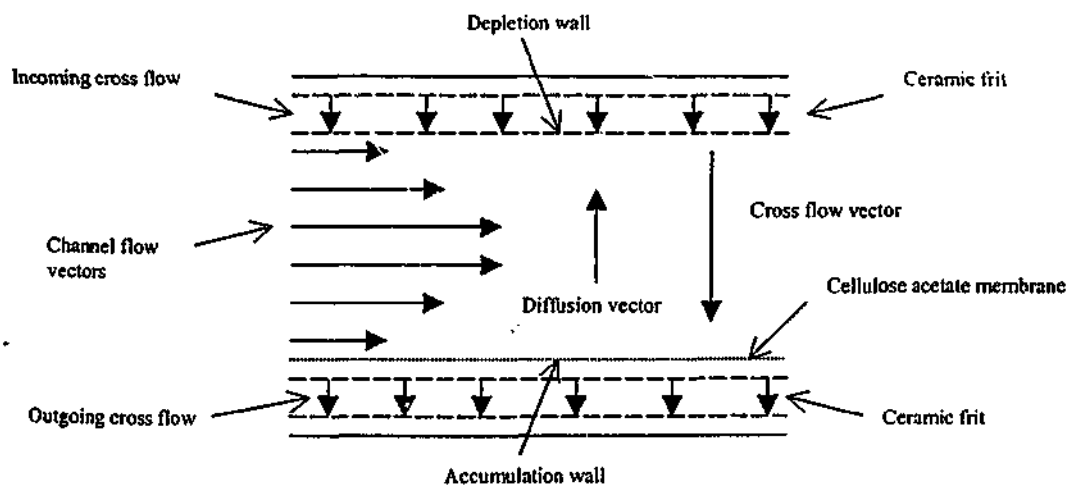


Fig. 2.3 Schematic diagram of the vertical section of flow-FFF channel

The so-called channel consists of a single phase, which is a moving fluid, while chromatography is a two-phase separation technique where samples in the liquid phase are partitioned into some stationary phase so that two phase separation occurs. FFF operates like chromatography, however it differs in one fundamental respect: in FFF the separation is induced by an external field or gradient, such as electrical, thermal, magnetic, flow etc. (Fig.2.2); in chromatography the separation is induced by interaction with a stationary phase.

The partitioning of samples in FFF is accomplished by the externally applied field, which acts in a direction perpendicular to the moving single phase. This moving single phase within the channel has laminar flow and the fluid velocity profile is parabolic (Fig.2.3).

All FFF methods essentially require the following two conditions;

(1) A difference in flow velocities of a flowing fluid laminar flow of a fluid within a tube or channel fulfils this requirement. Because the flow velocity of the fluid at the wall is negligible while it is maximum at the centre or core. Thus a gradient of flow velocities is created across the dimension of the tube or channel. The flow of a fluid under this situation is called a differential flow or shear flow. The so-called differential flow is a requirement of all FFF methods.

(2) An external force that is capable of driving particles or molecules across the tube or channel perpendicular to the direction of fluid flow. This perpendicular external force is considered as the applied field in field-flow fractionation (Fig.2.2). The particles or molecules are moved to different regions across the tube or channel due to the applied

field. Since velocities across the dimension of the channel are different, the particles or molecules at different positions are swept down at different velocities. They are separated eventually as a result of two perpendicular vectors, which are called flow vector and field vector.

FFF is carried out in an open, thin, ribbon-shaped, unpacked channel with two parallel walls firmly sandwiched between a spacer (Figs.2.1 and 2.2). A uniform flow of carrier liquid is introduced at one end of the channel and is allowed to travel along the channel. A small volume of sample containing particles or macromolecules is injected at the inlet point of the channel, which is the inflow point of the stream of carrier liquid as it enters the channel.

A field is applied across the channel from one face (perpendicular to the carrier stream) and is called the depletion wall (Fig.2.3). The applied field forces the sample particles or molecules towards the opposite wall of the channel, which is called the accumulation wall. The movement of particles or molecules towards the accumulation wall is counteracted by a diffusive transport away from the accumulation wall. Thus, a steady-state distribution is rapidly achieved the particles form equilibrium layers of sample clouds having a thickness of only a few micrometers. The largest particles accumulate nearest the wall and are highly retained in the channel. They have a thin sample cloud thickness due to low diffusivity. These thin sample clouds containing the larger particles or molecules are caught up by low velocity flow vectors near the accumulation wall. As a consequence they migrate down the channel at a slower rate. In contrast, the smaller

particles or molecules form thicker sample clouds due to high diffusivity and they are occupied in the faster flow laminae away from the wall. As a consequence they migrate down the channel at a faster rate and elute earlier than larger components. An injected pulse of particles or molecules will consequently emerge from the channel at a retention time that can be calculated. If properties of these components are unknown, this calculation can be used to deduce the specific property such as mass, diameter, etc.

2.3 FLOW FIELD-FLOW FRACTIONATION

Flow FFF is probably the most widely applicable among FFF methods for fractionation and characterization of macromolecules. The applied field or driving force in flow FFF is a 'cross flow' perpendicular to the channel. The flow FFF requires the following conditions:

(1) The sample must be soluble or dispersible in the moving liquid phase.

(2) Use of a semipermeable membrane at the accumulation wall which can retain all sample molecules, while only carrier solution can pass through it.

The schematic diagram of the flow FFF experimental set up is illustrated in Fig. 2.1. The channel in flow FFF is constructed with two parallel semipermeable walls which allows a uniform flow of carrier stream to be pumped across the channel (Fig. 2.2). They are called the depletion wall and accumulation wall. The sample is injected from the channel inflow. The injected sample is pushed down to the accumulation wall by the applied cross flow and is concentrated at the semipermeable membrane near the accumulation wall.

The cross flow enters the channel from the depletion wall and travels across the channel and exits from the accumulation wall. As a result it displaces the sample molecules into various flow vectors. The molecules displaced due to cross flow vectors are then counteracted with diffusion vectors, depending on their diffusion coefficients. The separated molecules are eventually eluted from the channel at various retention times. The relationship between diffusion coefficient and molecular weight must be determined because the separation is based on differences in diffusion coefficient. The expected elution sequence is lower molecular weights first because the smaller the molecule the higher the diffusivity, so that they are caught up by faster flow vectors further away from the accumulation wall (i.e. faster laminae nearer the axial center of the channel).

The theory applicable to flow FFF has been extensively developed by Giddings et.al. (1980). The fundamental equation for the retention ratio R in FFF is given by the following general expression:

$$R = V^{\circ}/V_r = t^{\circ}/t_r = 6\lambda \{\coth 1/2\lambda - 2\lambda\} \qquad (1)$$

Where V° is the channel void volume, V_r is sample retention volume, t° is the void time, t_r is the corresponding retention time and λ is the retention parameter. When $\lambda \rightarrow 0$, the limiting expression leads to following equation:

$$R = 6\lambda \qquad (2)$$

The retention parameter *(λ)* is directly obtained from equation (1) and for flow FFF the following relationship holds (Giddings 1976):

$$\lambda = D / Uw = DV^{\circ} / V_c w^2 \qquad (3)$$

In the above equation *D* is the diffusion coefficient, *w* is the channel thickness, *U* is the fluid cross-flow velocity. To obtain *D* values equation (3) can be combined with equation (1) and (2). The value of *D* can be expressed by the following equation:

$$D = \{V_c w^2 R\} / \{6 V^{\circ}\} = \{V_c w^2 l\} / \{6 V t_r\} \qquad (4)$$

Where V_c is the cross (field) flow rate, which is the volumetric flow rate across the channel, *V* is the channel flow rate, *l* is the mean thickness of sample cloud, *R* is the retention ratio.

Differences in *D* values lead to separate components having different retention. The relationship between *D* and molecular weight *M* for macromolecules and particles is given by the simple power law equation below (Tanford et.al. 1961):

$$D = A / M^b \qquad (5)$$

Here, *A* and *b* are constants for a given sample, carrier liquid at a constant temperature and the value of *b* varies with the configuration of the particles, such as whether they are random coil or globular or rod-like. Exponent *b* generally lies in the range between 1.0

and 0.33 depending on the particle configuration. A plot of log D vs log M is a linear relationship and it can be used as the calibration line to determine the molecular weight of unknown samples.

$$\log D = \log A - b \log M \qquad (6)$$

2.4 XAD-8 RESIN ADSORPTION TECHNIQUE

A column chromatographic method using a macroporous sorbent was used for fractionation of dissolved organic solutes according to their sorption characteristics. The chemical nature of dissolved organic matter (DOM) can be studied by Amberlite XAD-8 resin adsorption techniques, by which dissolved organic matter is fractionated into various components depending on their hydrophobic character (Mantoura and Riley 1975, Malcolm et al 1977, Thurman and Malcolm 1981, Leenheer 1981, Leenheer 1985, Aitken 1985). The 'hydrophobic effect' is the principal driving force for sorption of dissolved organic molecules onto the resin sorbent. The degree of hydrophobicity is governed by several major factors including, molecular weight, aromaticity, aliphaticity and the number of polar and ionic functional groups present per molecule. Thurman et al. (1978) found sorption of organic solutes such as humic substances is correlated with their solubility in aqueous media, which in turn is related to the pH of the solution. At low pH, weak acids are protonated and adsorbed on to the resin, and at high pH weak acids are ionized and desorption is favored. Therefore, solute samples are acidified with mineral acid such as HCl and passed through a column of XAD-8 resin (Fig.2.4). The adsorbed

organic solutes are recovered by elution with a basic solution, usually 0.1N NaOH or NH_4OH solution.

2.4.1 Dissolved Organic Carbon fractionation by XAD-8 resin adsorption

XAD-8 resin is a nonionic macroporous copolymer of acrylic-ester called methyl methacrylate. The sorption on these resins is mainly governed by the hydrophobic effect of the solute. Thurman et al., in 1978, have shown the pH dependence of the distribution coefficient of fulvic acid on XAD-8 resin. According to their study, the distribution coefficient of fulvic acid on XAD-8 resin is sufficiently high at pH < 3.0 to use adsorption as a means of isolating fulvic acids. The weak acids tend to protonate at low pH and ionize at high pH, so that adsorption on the resin is favored at low pH and desorption is favored at high pH. Fulvic acid is efficiently eluted from the XAD-8 resin by NaOH, but it should be noted that NaOH does not bleed TOC from the XAD-8 resin. Thus NaOH was used to recover the adsorbed fulvic acid by eluting the XAD-8 column.

Since XAD-8 resin is an acrylic resin it is more hydrophilic than other XAD resins. Therefore, it is wet easily and adsorbs more water compared to other XAD resins. Its sorption kinetics are much faster, thus equilibrium is attained more rapidly (Aiken et al.1979). For these reasons XAD-8 resin was used over other XAD resins (e.g.XAD-4 or XAD-7) for the isolation of fulvic acid in this study. The properties of XAD-8 resin are given below as outlined by Aitken (1985).

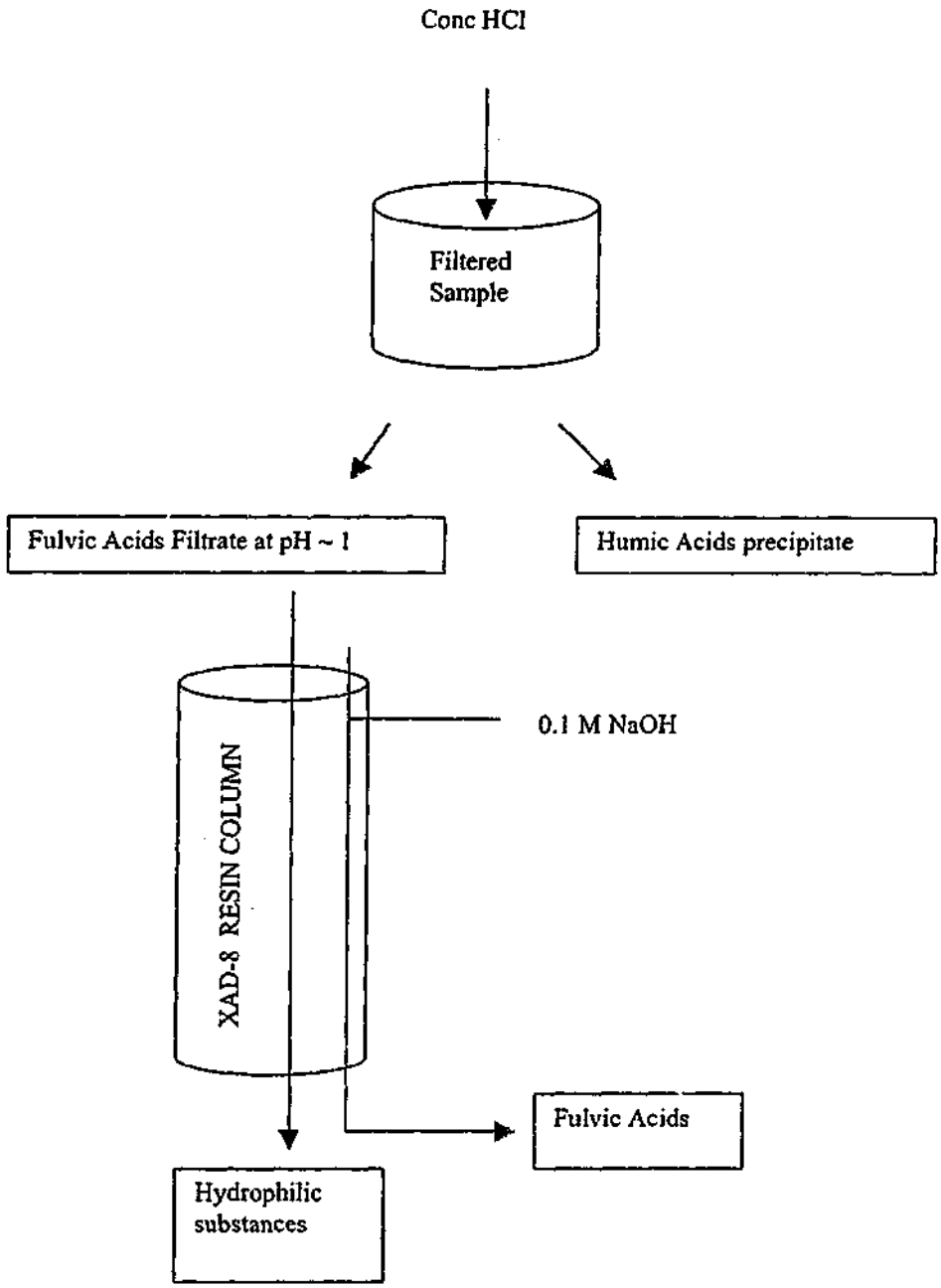


Fig 2.4 Schematic diagram of XAD-8 resin fractionation scheme

Table 2.1 Properties of XAD-8 resin outlined by Aitken (1985)

Average Pore Diameter	250 $A°$
Specific Surface Area	140 (m^2/g)
Specific Pore Volume	0.82 (cm^3/g)
Solvent Uptake (water)	1.31 - 1.36 (g/g of Dry Resin)
Distribution Coefficient (K_D)	604
Elution Efficiency (non retain by resin)	98

The scheme devised by Leenheer (1981) for the concentration, fractionation and isolation of organics was modified in this work to achieve preisolation of humic acids. The modification made was to separate the humic acid from the raw solution before passing it through the XAD-8 column. In this way only fulvic acid was adsorbed and recovered by XAD-8 column. The scheme used in this current work is illustrated in the schematic diagram in Fig.2.5.

XAD-8 resin is a good sorbent for hydrophobic substances such as humic acids. Leenheer (1981) has developed a fractionation scheme that quantitatively classifies organic solutes into hydrophobic-base, -acid, -neutral fractions and hydrophilic-base, -acid, -neutral fractions. Usually groups of organic components fractioned by XAD-8 fractionation are classified as hydrophobic substances (humic substances) and hydrophilic substances. In addition, humic substances can be separated into humic acids and fulvic acids. Humic acid in water is defined as dissolved organic matter which precipitates at pH = 1 and fulvic acid is soluble at pH = 1. Therefore, the humic acid can separate from the fulvic acid as a precipitate at the beginning of XAD-8 resin fractionation, so that the fulvic acid fraction is the hydrophobic fraction adsorbing onto a macroporous nonpolar resin at pH <

2 followed by desorption at pH = 13 during the XAD-8 resin fractionation. The portion of dissolved organic matter which passes through the XAD-8 resin at pH < 2 is called the hydrophilic substances. In fact the so-called hydrophilic fraction contains three fractions (acids, bases and neutrals) plus the hydrophobic bases of the Leenheer scheme. According to the Leenheer scheme, the hydrophobic neutral fraction is retained by the resin under these conditions.

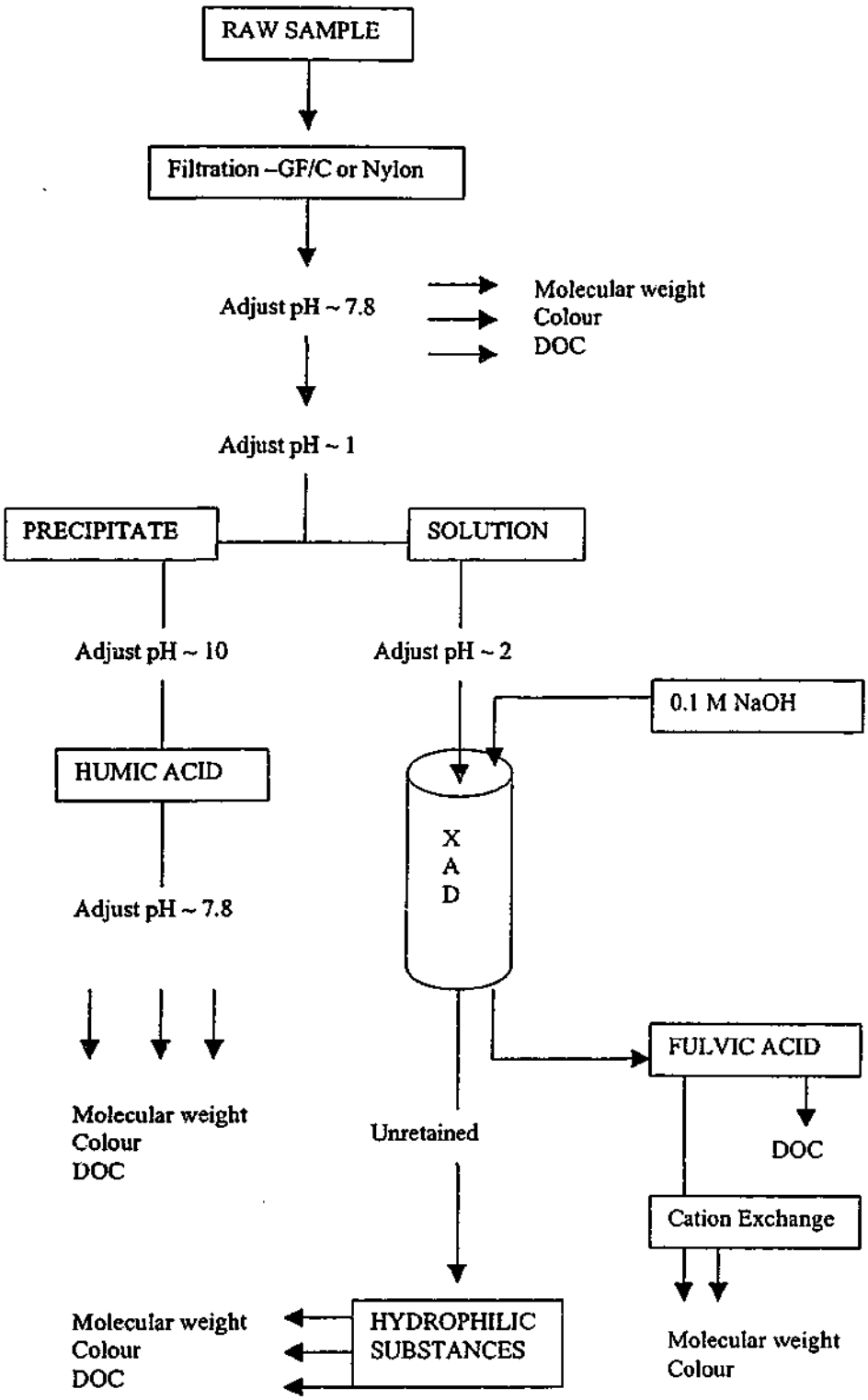


Fig.2.5 Schematic Diagram of Experimental Scheme

Addendum to Chapter 2

2.5 EFFLUENTS AND WASTEWATERS SUBJECTED TO FRACTIONATION

The main part of the study has focussed on characterization of dissolved organic matter in several industrial effluents. Two selected downstream receiving wastewater systems of two pulp and paper mills (in Australia and in New Zealand) were also studied. Further studies were undertaken with some selected industrial effluents and some receiving wastewaters by carrying out microbiological experiments in the laboratory.

2.5.1 Effluents and wastewaters related to field studies

2.5.1.1 AMCOR effluent and Latrobe Valley Outfall Sewer System

The pulp and paper mill effluent from AMCOR mill at Maryvale in Gippsland (Victoria, Australia), and its recipient water in the Latrobe Valley Outfall Sewer (LVOS) system were subjected to extended investigation. In this work a series of sites along the LVOS channel have been extensively studied, as the effluent was being subjected to alterations due to biological degradation along the sewer.

The mill effluent carrying wastewater in the LVOS channel is mixed with domestic sewage while transported along the channel. The Dutson Downs treatment complex, to which wastewater from the LVOS channel is subsequently released for further treatment, was also extensively studied. The factors influencing the treatment of pulp and paper mill effluent in the AMCOR pulp and paper mill were outlined by Duncan et al (1992).

2.5.1.2 TASMAN effluent and Tarawera river lower reaches

Mill effluents from Tasman's pulp and paper mill situated in Tarawera, New Zealand and Tasman's mill recipient waters at the Tarawera River lower reaches were also investigated. Different effluent streams released by different plant processes were characterized to deduce which plant process effluent is the best suited to which treatment option before discharge. The effluents from the primary treatment plant (clarifier and sludge lagoon) and the secondary treatment plant (Tasman's treatment ponds) were also studied. A series of sites along the Tarawera River have also been analyzed to study the effects of biological degradation.

2.5.1.3 SECV effluent from HTD process of brown coal

Coal washings left after hydrothermal drying (HTD) of brown coal, at the State Electricity Commission of Victoria (SECV), Australia was studied. Further studies were undertaken with coal washing effluents collected after in-house treatment in an anaerobic digester at two set temperatures. Also, the HTD filtrate left after physicochemical processes in an alum treatment plant (coagulation, flocculation and filtration) was also studied. Emphasis was given to the molecular weight changes and also to the biological effect on the major organic fractions.

2.5.1.4 CSR effluent from molasses fermentation process

An effluent called Dunder, which is left after the fermentation process of molasses in the CSR sugar mill at Plane Creek, Queensland (Australia), was investigated. This was the disposal wastewater removed after molasses fermentation.

2.5.2 Effluents and wastewaters chosen to laboratory studies

The primary objective of the laboratory scale biological experiments was to determine the relative significance of aerobic and anaerobic reactions on DOC removal from an effluent during biological treatment The effect on specific molecular weight fractions and colour was determined. Microbiological experiments were carried out in the laboratory with the following chosen effluents and wstewaters.

(1) AMCOR effluent collected from the inlet stream which enters a storage tank (41 megalitre tank which stores the AMCOR effluent for about 2-3 days and is subsequently discharged into the LVOS channel). This is a combined output of pulp and paper milling plants of AMCOR mill in Gippsland, Victoria, Australia

(2) Raw effluent collected from the outlet stream exits from the above storage tank, just before discharge into the LVOS channel

(3) Wastewater collected from a piped section at the LVOS channel which contains oxygen injectors

(4) Wastewater collected from the end point of a piped canal along the LVOS channel carrying the mixed effluent-sewage discharge

(5) Wastewater collected from an open sewer site along the LVOS channel

(6) Treated wastewater collected from the DD treatment plant

(7) Raw effluent collected from the discharge of pulp milling plant of the Tasman mill at Tarawera, New Zealand

(8) Raw effluent collected after caustic extraction stage at caustic bleaching plant of the Tasman mill at Tarawera, New Zealand

(9) Dunder effluent left after molasses fermentation at the CSR sugar mill at Plane Creek, Queensland, Australia

Chapter 3

Molecular Weight Characterization Of A Paper Mill Effluent And Recipient Water By Flow FFF

Abstract

The variation of the molecular weight and the molecular weight distribution in a pulp and paper mill effluent and its recipient water was studied. Effluent from the AMCOR mill at Maryvale in Gippsland (Victoria, Australia) which is a bleached Kraft mill was investigated. The Latrobe Valley outfall sewer (LVOS) system to which the mill effluent is discharged was also subjected to investigation. The AMCOR mill effluent and the recipient waters were physically characterized by use of flow field-flow fractionation. The study has been extended to investigate the molecular weight changes occurring within the anaerobic and aerobic treatment ponds in the Dutson Downs (DD) treatment complex which receives effluent from the LVOS.

During the storage of the mill effluent in a '41 Megalitre storage' pond the average molecular weight in the effluent shifted to higher molecular weight. It was found that the high molecular weight fractions were removed and low molecular weight fractions were produced within the oxygenated piped sections. In the open sewers, low molecular weight fractions were removed and high molecular weight fractions were formed. The microbial assimilation of low molecular weight fractions is most likely to occur in open sewers, while microbial oxidation (partial degradation) of high molecular weight fractions is most likely to occur in piped lines containing oxygen injectors. Within the treatment complex, low molecular weight fractions were removed during the initial phase (aerobic) and high molecular weight fractions were removed in the latter phase (anaerobic).

3.1 INTRODUCTION

The pulp and paper mill effluent from the AMCOR bleached kraft mill situated at Maryvale in Gippsland (Victoria, Australia) was investigated. The Latrobe Valley Outfall Sewer (LVOS) system is the recipient wastewater system of the AMCOR mill effluent. Therefore, the LVOS channel was subjected to extended investigation. Pulp and papermill effluent generated from the AMCOR pulp and paper mill factory is stored in a storage having a capacity of 41 megalitres and is discharged into the LVOS channel in excess of 14 megalitres per day on average. The mill effluent carrying recipient wastewaters is transported down the LVOS channel (Figs.1 and 2) to reach the Dutson Downs treatment complex (Fig.3), which is approximately 75 km from the AMCOR mill. The mill effluent containing wastewater is mixed with domestic sewage while it is transported down the LVOS channel. The wastewater in the LVOS channel subsequently enters into the anaerobic and aerobic treatment ponds at the Dutson Downs disposal area.

A series of sites along the LVOS system, where the major input is the AMCOR Maryvale pulp and paper mill effluent, have been studied, as the molecular weights of organic matter in sewer was thought to be affected by biological degradation and/or physical adsorption/settling effects. The current study has focussed on the changes in molecular weights and molecular weight distributions along the LVOS channel. The study has been extended to investigate the molecular weight changes occurring within the Dutson Downs treatment ponds.

The objectives of the work undertaken in this section are:

- an investigation of the changes in weight average molecular weight of a pulp and paper mill effluent as a result of holding the effluent in a storage and then discharging it into a major recipient wastewater system
- to study the changes in molecular weight distributions of the mill effluent recipient wastewater as it is transported down the sewer channel
- to study which molecular weight fraction is affected by installing the oxygenated pipelines and using open non-oxygenated sewer channels
- to study which molecular weight fraction is affected by treatment ponds and the variation of weight average molecular weight as a result of pond treatment.

3.2 STUDY SITE

The study has focussed primarily on the LVOS and the DD treatment complex. The AMCOR Maryvale Pulp and Paper Mill plant is situated in South Gippsland about 10 km east of the town of Traralgon. The Latrobe River Catchment and layout of major water supply and wastewater disposal works are illustrated in Fig.3.1. These treatment plants are controlled by the Latrobe Valley Water and Sewerage Board. The effluent generated by the AMCOR mill meets the Environment Protection Authority (EPA) standards for various parameters, so that it is legally discharged into the LVOS channel for treatment at the Dutson Downs wastewater treatment complex.

Fig.1 LATROBE VALLEY WATER AND SEWERAGE BOARD
LATROBE RIVER CATCHMENT AND LAYOUT OF MAJOR
WATER SUPPLY AND WASTE WATER DISPOSAL WORKS

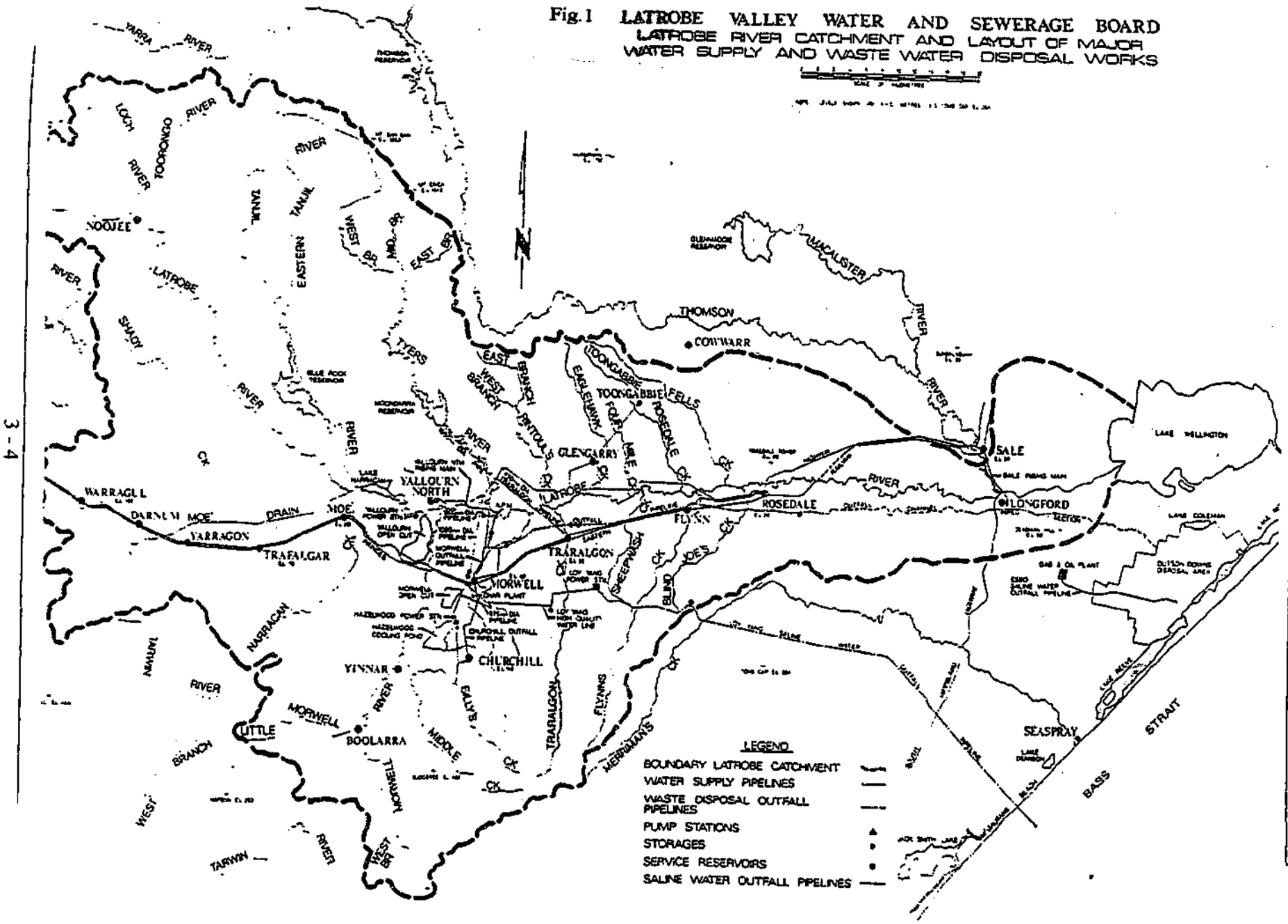
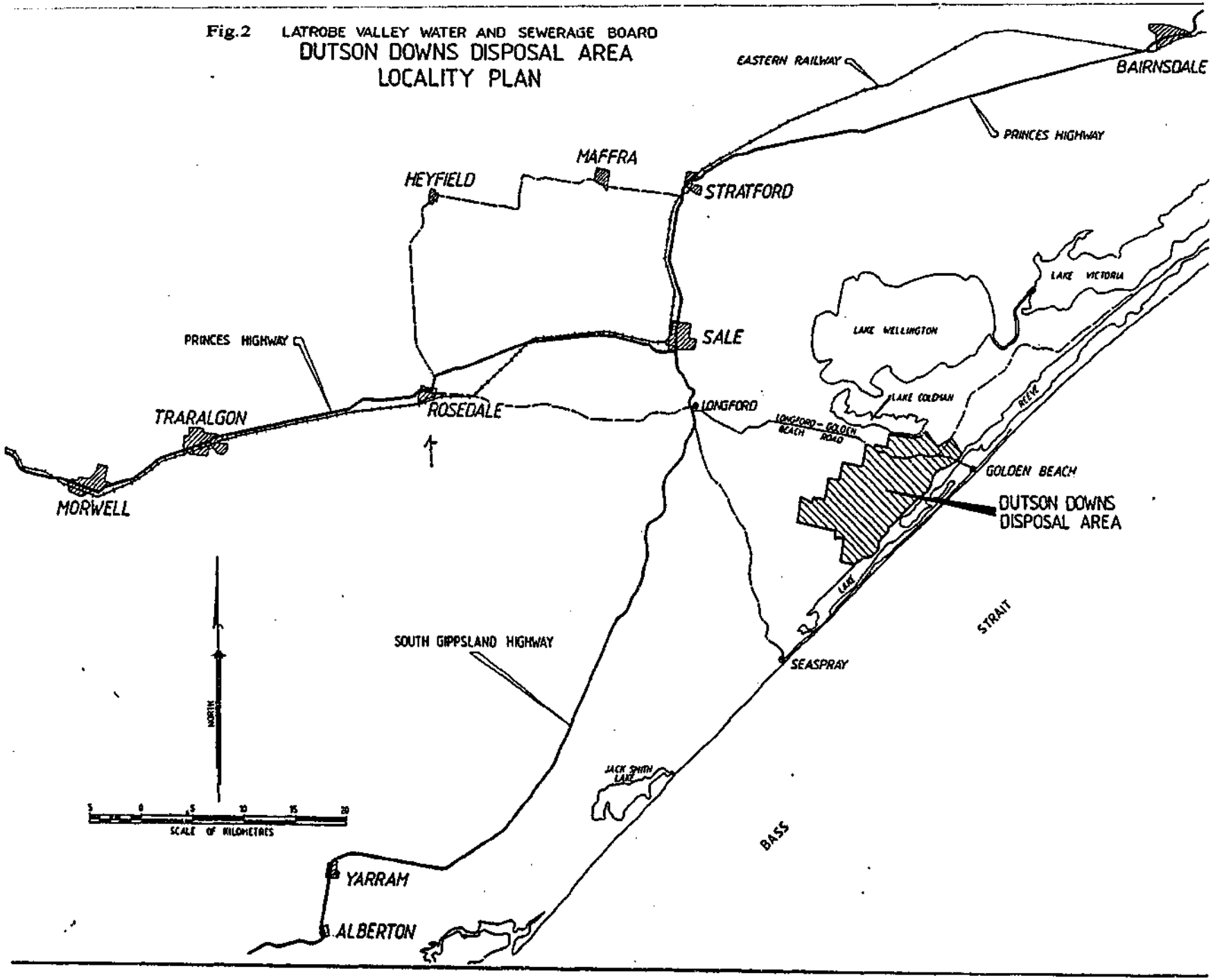


Fig.2 LATROBE VALLEY WATER AND SEWERAGE BOARD
DUTSON DOWNS DISPOSAL AREA
LOCALITY PLAN



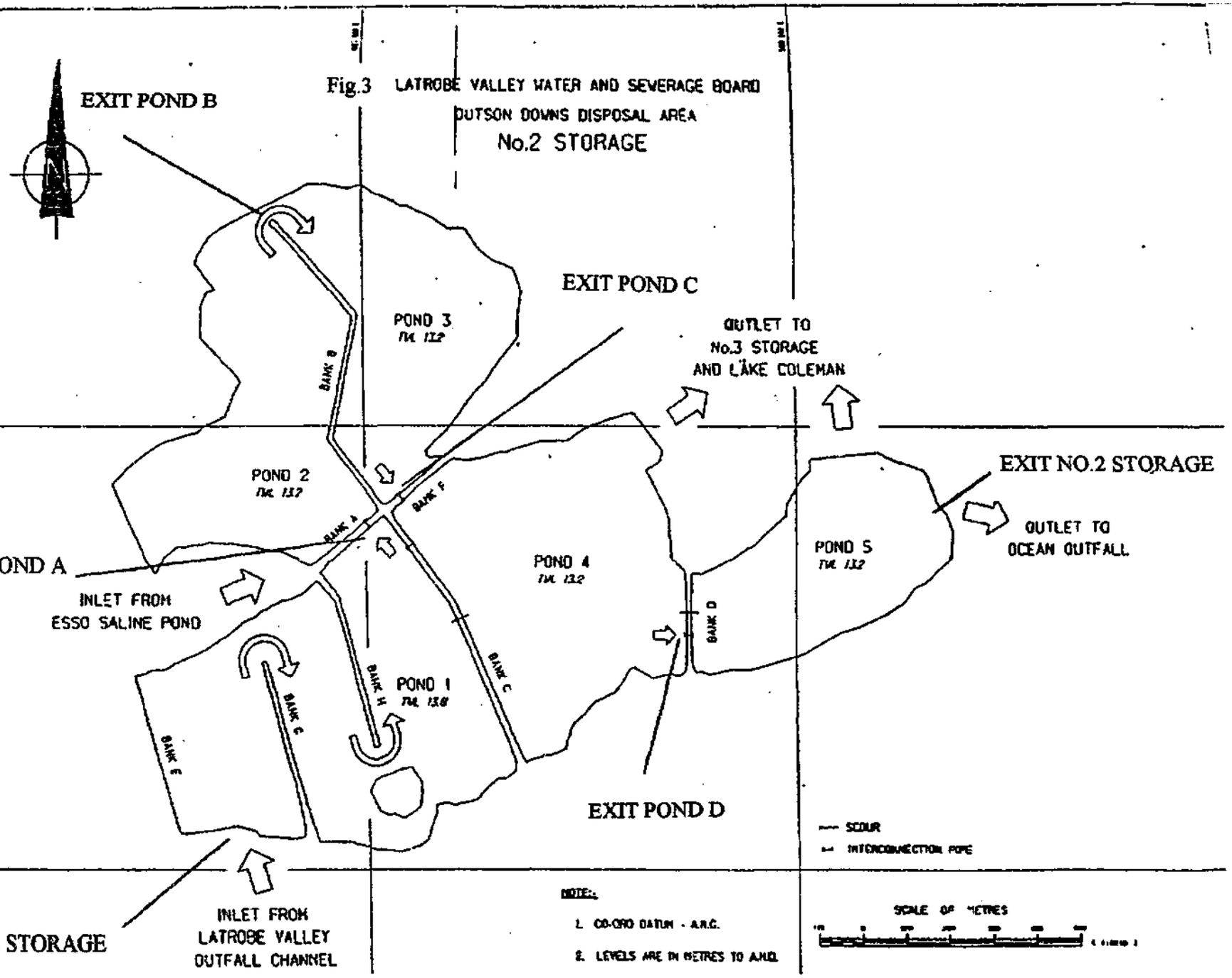


Fig.3 LATROBE VALLEY WATER AND SEWERAGE BOARD
DUTSON DOWNS DISPOSAL AREA
No.2 STORAGE

The 'Dutson Downs Disposal Area Locality Plan' is illustrated in Fig.3.2. The AMCOR mill effluent joins the LVOS channel, where it is transported down the closed and open canals and is conveyed to pond A of No.2 storage of the DD treatment complex (Fig.3.2). The saline wastewaters from the Esso-BHP oil and gas plant are also conveyed to the DD treatment ponds (between pond A and pond B). The pondages in No.2 storage of the DD treatment complex is illustrated in Fig.3.3. The wastewaters conveyed to the DD treatment plant are subjected to a series of biological treatment and settling phases over a period of about three months. An extra 1.5 km pipeline, called the Ocean Outfall Pipeline was installed in June 1992 to effect the disposal of treated effluent. The treated wastewater is discharged from DD plant through this Ocean Outfall Pipeline and deposited directly off Delray Beach into Bass Strait where dilution and rapid dispersion occurs.

A total of four sample sets have been collected during the period of 1992-1994 under this project. The site locations in each sample set are summarized in Tables 3.1(a) and (b).

Table 3.1(a) Wastewater sampling sites selected over the LVOS channel

Sampling sites in LVOS channel	Set 1 (October 1992)	Set 2 (March 1993)	Set 3 (August 1993)	Set 4 (November 1994)
Sandy Creek Crossing		√		
Rosedale Dissolver		√		√
Rosedale Meter	√		√	√
Peg 83	√	√	√	√
Peg 133	√	√		
Inlet No.2		√		√

Table 3.1(b) Wastewater sampling sites selected over the DD treatment plant

Sampling sites in No.2 storage	Set 1 (October 1992)	Set 2 (March 1993)	Set 3 (August 1993)	Set 4 (November 1994)
Exit pond A	√	√		
Exit pond B	√	√		
Exit pond C	√	√	√	√
Exit pond D	√	√		
Exit No.2	√	√		

The LVOS system downstream of the AMCOR mill consists of two major channels, which are labeled as LVOS channel 1 and LVOS channel 2. Each containing a piped section and an unpiped section (Fig.3.4) which are labelled as "piped section 1", "open sewer 1", "piped section 2" and "open sewer 2" respectively.

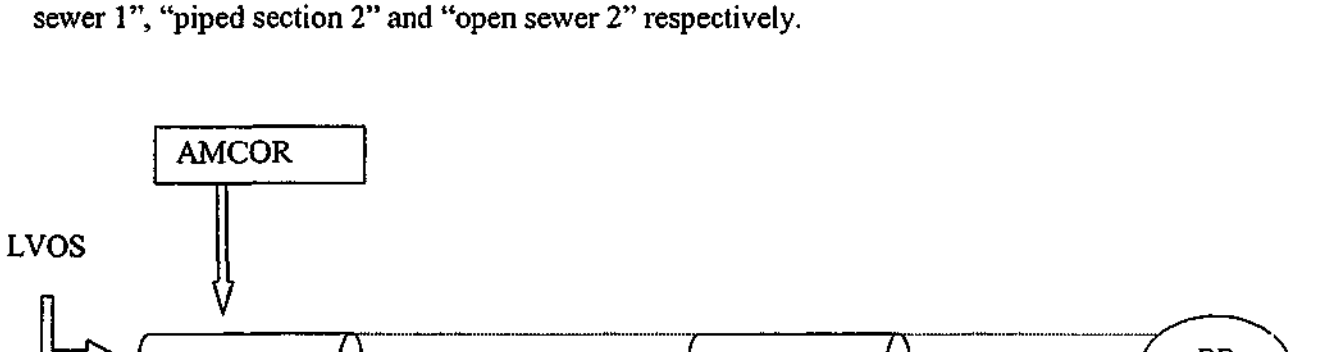


Fig.3.4 SCEMATIC DIAGRAM - An illustration of basic channels in Latrobe Valley Outfall Sewer System at AMCOR Mill downstream

The major input to this outfall is the Maryvale pulp and paper mill (AMCOR) effluent. The AMCOR mill is a major contributor of water, dissolved organic carbon (DOC) and colour to the system. Additional inputs of water, DOC and colour occur from sewage

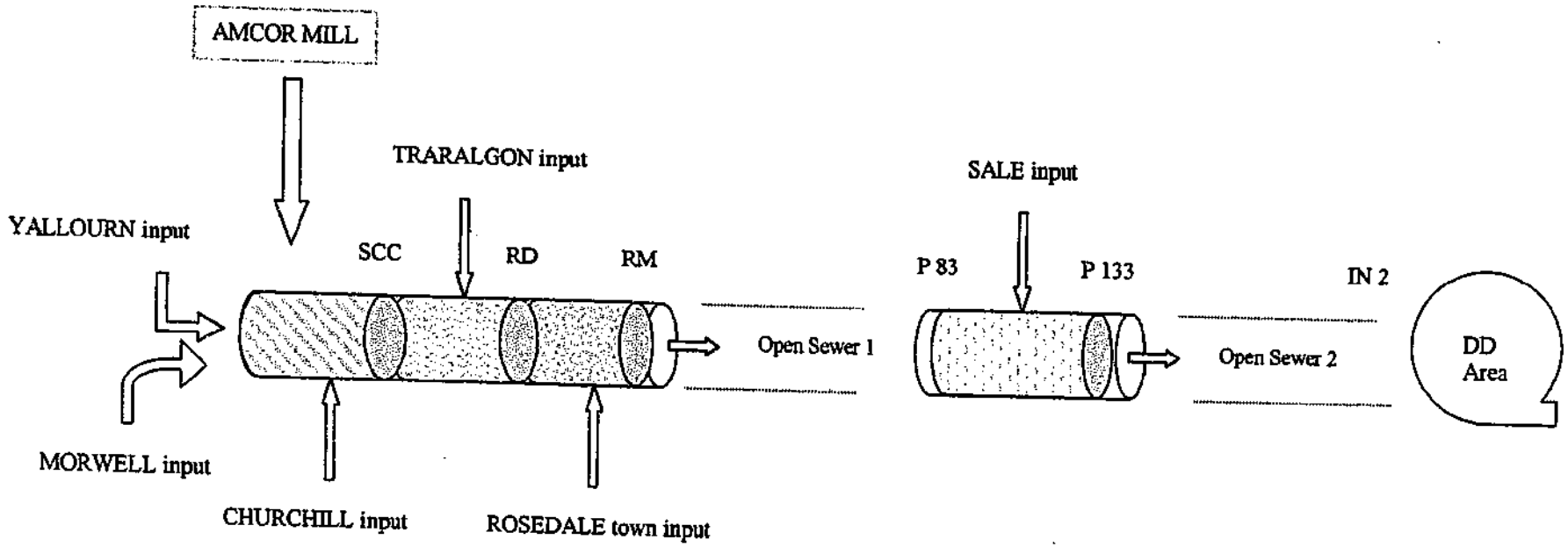


Fig.3.5 SCEMATIC DIAGRAM - An illustration of sampling sites with major organic inputs along the channels in Latrobe Valley Outfall Sewer System

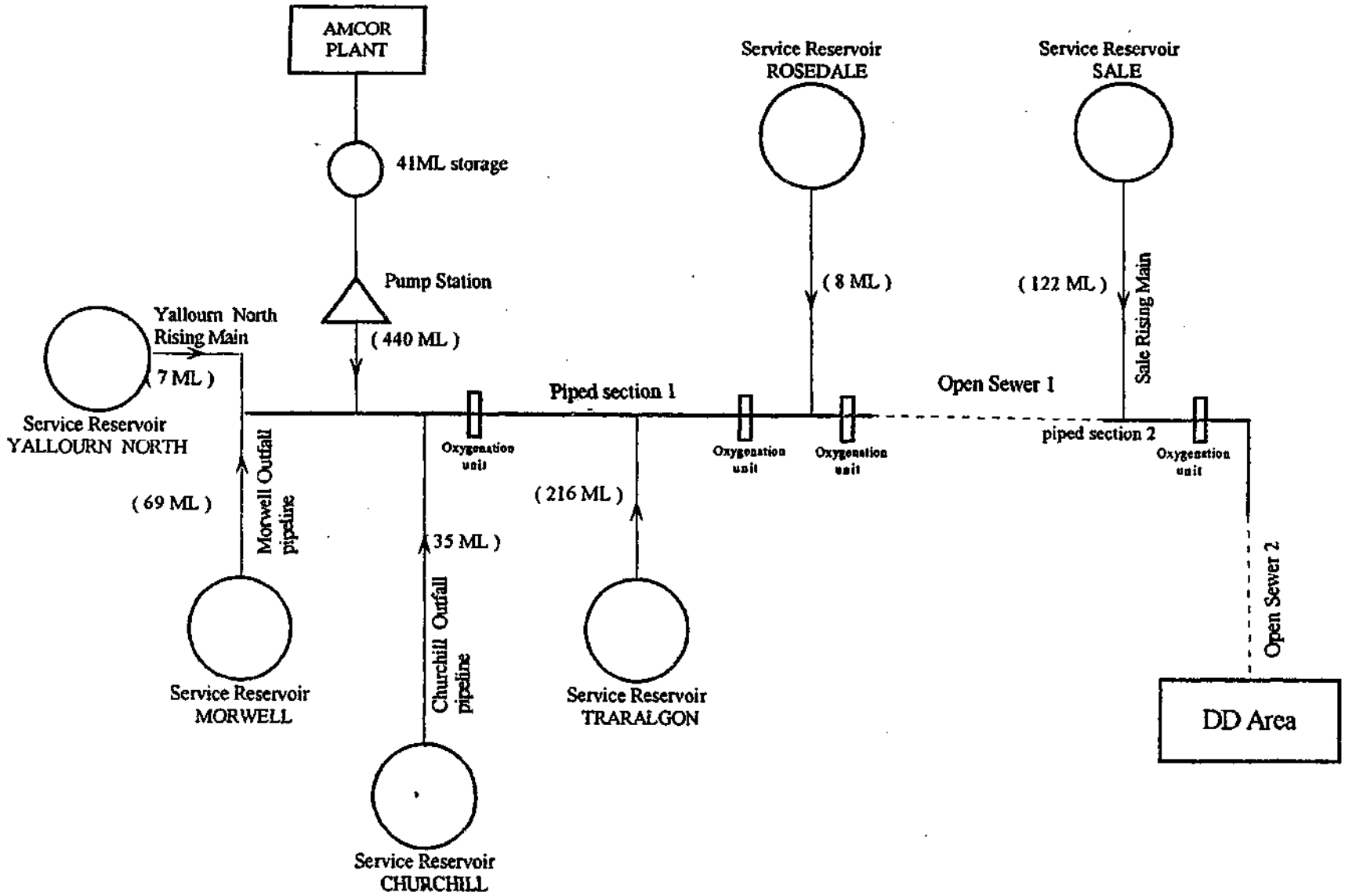


Fig.3.6 Schematic diagram of major inputs to LVOS channel from various sources and their monthly average estimates

treatment plants at Morwell, Churchill, Traralgon, Rosedale and Sale. These additional inputs are illustrated in Fig.3.5 and Fig.3.6. Also shown on the schematic diagram in Fig.3.6 are estimates of the major inputs of water/wastewater from various sources in the system. These estimates are given by Latrobe Valley Water and Sewerage Board.

Pulp and papermill effluent generated from the AMCOR Maryvale pulp and papermill factory is accumulated in a storage, which has a capacity of 41 ML and is discharged into the LVOS drain in excess of 14 ML/day on average. Sampling has been undertaken as the inlet stream enters the storage (inlet 41 ML) and as the outlet stream exits the storage (outlet 41 ML) as summarized in Table 3.2.

Table 3.2 Effluent streams of AMCOR mill 41 megalitre storage

Sampling site	Description
inlet 41 ML	Inlet stream of 41 megalitre storage enters the storage from AMCOR plant
outlet 41 ML	Outlet stream exits from 41 megalitre storage discharges to LVOS system

3.2.1 The LVOS channel

Table 3.3 Sampling sites of the LVOS channel

Sampling site	Description
Sandy Creek Crossing Rosedale Dissolver	Two points along the waste disposal outfall pipelines (closed pipeline section 1)
Rosedale Meter	End point of closed pipeline section 1
Peg 83	End point of open channel section 1
Peg 133	End point of closed pipeline section 2
IN 2	Inlet point where LVOS channel enters the No.2 storage of Dutson Downs disposal area

The effluent from the AMCOR mill is discharged into the LVOS pipeline directly from the 41 megalitre storage, having been stored there for 3-4 days. The outlet stream enters the LVOS system and is transported down the pipeline. It is oxygenated at three oxygen dissolvers set up at three points, which are located in same distance within the piped section 1.

The mill effluent from the 41 megalitre storage discharges on average 440 ML/month into the LVOS system (Fig.3.6). According to the flow summary of Latrobe Valley Water and Sewerage Board (Victoria, Australia) in 1992, it is combined with 7 ML/month on average from Yallourn North Rising Mains, 69 ML/month on average from Morwell outfall pipelines, and 140 ML/month on average from flushing water from the Morwell service reservoir. The total flow in the LVOS system is up to 216 ML/month on average. These inputs to the system dilute the mill effluent concentration (Table 3.4). As the combined effluent is transported down the pipeline, the outfall pipeline from Churchill joins the LVOS system, which introduces an input of 35 ML/month on average.

Table 3.4 Major inputs to the LVOS channel in 1992 as specified by Latrobe Valley Water and Sewerage Board (Victoria, Australia) and estimated dilution folds of mill effluent at major input intersections

Intersection of LVOS flow	Average monthly input (ML)	Dilution fold of mill effluent
Yallourn North Mains Morwell outfall pipelines	216	1.5
Churchill outfall pipelines	35	1.6
Traralgon Mains	216	2.06
Rosedale town	8	2.1
Sale Rising Mains	122	2.4

Immediately after the intersection of the Churchill outfall pipelines, the LVOS flow is oxygenated by the first oxygen injector. Subsequently, Traralgon Mains also joins the

LVOS system contributing 216 ML/month on average to the LVOS system. The second oxygen injector is encountered after the Traralgon Mains intersection. A subsequent input into the LVOS system is from Rosedale township, which contributes 8 ML/month on average. The third oxygen injector is encountered immediately after the intersection of Rosedale town Mains input into the LVOS system.

These additional mains and outfall pipelines mainly consist of domestic sewage as they contain only 140 ML on average of flushing water from the Morwell outfall pipelines and 2 ML on average of other industrial wastewater from the Churchill outfall pipelines. As a result of these four inputs into the LVOS system, the total wastewater reaching the Rosedale Meter totals 915 ML/month on average. A subsequent input of 122 ML/month of domestic sewage is released to the LVOS system at Sale from the Sale Rising Mains. The LVOS system is oxygenated (within piped section 2) also before reaching the site Peg 133.

3.2.2 Dutson Downs treatment complex

The Dutson Downs treatment complex consists of storages with anaerobic and aerobic ponds. The No.2 storage at which the LVOS channel enters consists of five ponds, which are interconnected by pipes through existing banks A, B, C, D, E and F (Fig.3.3). Samples have been collected from the exit points of each pond within the waste treatment complex (Table 3.5). The exit point at which No.2 storage discharges its treated wastewaters from the DD treatment complex is called exit No.2. The exit point of No.2

storage (exit No.2) is the outlet to the ocean outfall, which disposes the treated wastewater directly into Bass Strait.

Table 3.5 Sampling points of No.2 storage at Dutson Downs Treatment Complex

Sampling site	Description
EX A	Exit point of Pond A at interconnection pipe on Bank A
EX B	Exit point of Pond B at terminus of Bank B
EX C	Exit point of Pond C at interconnection pipe on Bank F
EX D	Exit point of Pond D at interconnection pipe on Bank D
EX 2	Exit point of the No.2 storage (outlet to ocean outfall from Pond E)

3.3 EXPERIMENTAL

3.3.1 Sampling and pre-treatment

Sampling of raw unmodified 'direct mill effluents' from the AMCOR Maryvale pulp and paper mill and raw unmodified 'total wastewaters' along the LVOS system, together with raw 'treated wastewaters' from the DD treatment complex was performed by Gippsland Water. The samples were collected at a depth of 0.5 m from the surface of the wastewater by submerging 1L polyethylene bottles, which had been previously rinsed 3 times with double-distilled water and finally rinsed with sample wastewater or sample effluent. Within 24 hrs of sampling, the samples were transported under refrigerated conditions (<4°C) from the Latrobe Valley region to the Melbourne laboratory of Monash University. Samples were then sterilized by adding sodium azide to achieve a final concentration of 0.03% and refrigerated at 4°C until analyzed. Samples were ultrasonicated for 10 minutes in a bath sonicator in order an attempt to disaggregate the particles. These ultrasonicated samples were vacuum filtered through 1.2 μm GF/C filters

which had been prewashed with MilliQ water and 10 mL of sample wastewater or sample effluent.

3.3.2 FFF Technique

Flow field-flow fractionation was used for molecular weight determinations. The method of analysis was similar to that outlined elsewhere (Beckett et al., 1987). The equipment and techniques used during this study were described in the previous chapter, and have been described by Beckett et al. (1992).

3.3.3 Channel dimensions and flow FFF parameters

The channel void volume (V°) was 1.28 mL and the dead volume from channel outlet to the detector was 0.25 mL. The channel dimensions were 22.4 cm in length from channel inlet to channel outlet and 2.0 cm in breadth. The triangular end pieces were 2.4 cm in height from triangle base to tip at both ends of the channel. The channel thickness was 0.0254 cm. The pre-channel dead volume from the injection loop to the channel inlet was 0.05 mL so that the injection delay time was set to 1 sec for a channel flow rate of 5 mL/min. The relaxation time was set to 16 sec by assuming the cross flow was 4.9 mL/min. The elution time is affected by the post channel dead time, which was calculated for each individual run by measuring the channel flow rate. In these experiments the dead time was always 3 sec.

The flow rates used in this study were maintained at 5 mL/min. for both channel flow and field flow. The carrier solution was 0.05 M tris(hydroxymethyl)aminomethane

(TRISMA, Aldrich), 0.00308 M NaN_3 and 0.0268 M HNO_3 and the pH was 7.9 (Beckett et.al., 1987). A fixed-wavelength (254 nm) BAS UV flow-through detector in conjunction with an ICl DP600 chart recorder was used to record fractograms. Detector response was recorded on a chart recorder at 12 mm/min. and data were collected on a computer by FFF Data Collection software package. Molecular weights were computed using Field-Flow Fractionation data software version 2.0 (FFFractionation LLC, SaltLake City, Utah, USA).

Flow FFF runs were carried out in triplicate so that the repeatability of measurements could be established. Triplicate measurements of molecular weight gave relative standard deviation values of 2-3%. To evaluate the channel reproducibility and recovery Okefenokee Swamp standard (GA, USA) was run and the relative standard deviation of the retention time was 2-3%.

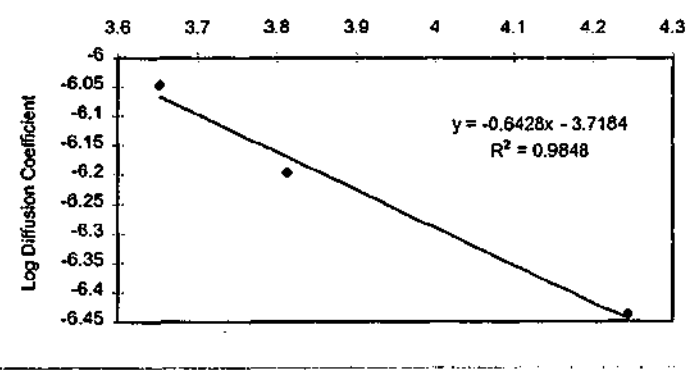
The flow FFF instrument was calibrated with poly(styrenesulfonate) standards (Polysciences, Inc.) by preparing them in 0.05 M tris buffer to give a concentration of 1 mg/mL. A Calibration curve was constructed by fractionating sodium poly(styrenesulfonate) standards of molecular weights 4500 (Mw=4500, Mn=4100), 6500 (Mw=6500, Mn=5900) and 17500 (Mw=17500, Mn=16000).

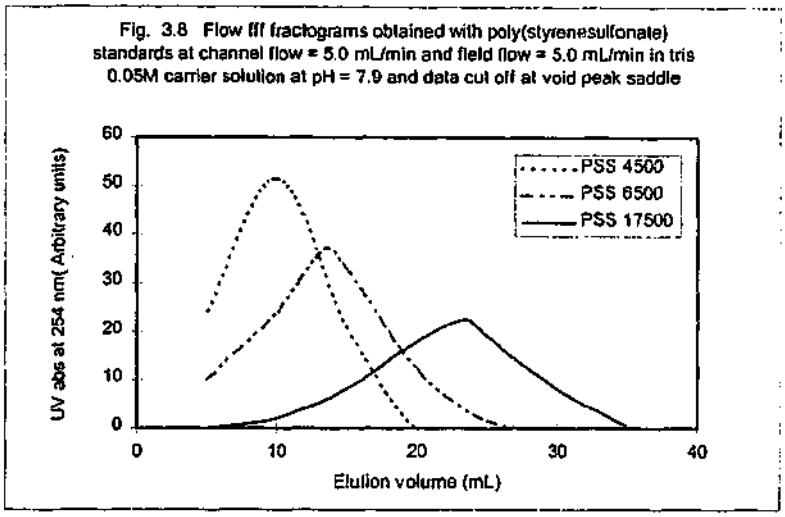
3.4 RESULTS AND DISCUSSION

3.4.1 Molecular weight calibration

Calibration was achieved with three sodium poly((styrenesufonate) standards of molecular weights of 4500, 6500 and 17500 Dalton, as shown in Fig.3.7. The calibration constants (ϕ and n) in this experiment found were ϕ=0.000191 and n=0.643. These ϕ and n values are constants for a given sample and carrier liquid at a constant temperature, and the value of n varies with the configuration of the macromolecules e.g. random coil, globular or rod-like. These constants are A and b respectively in the equation (6) (refer to Chapter 2, Section 2.3) for the relationship between diffusion coefficient (D) and molecular weight (M) in the plot of log D vs log M for macromolecules, derived from the simple power law equation (Tanford et al., 1961). A least-square regression line was obtained with R^2=0.99 value as the calibration line.

Fig 3.7 Calibration line constructed with PSS [poly(styrene sulfonate)] molecular weight standards using measured retention time



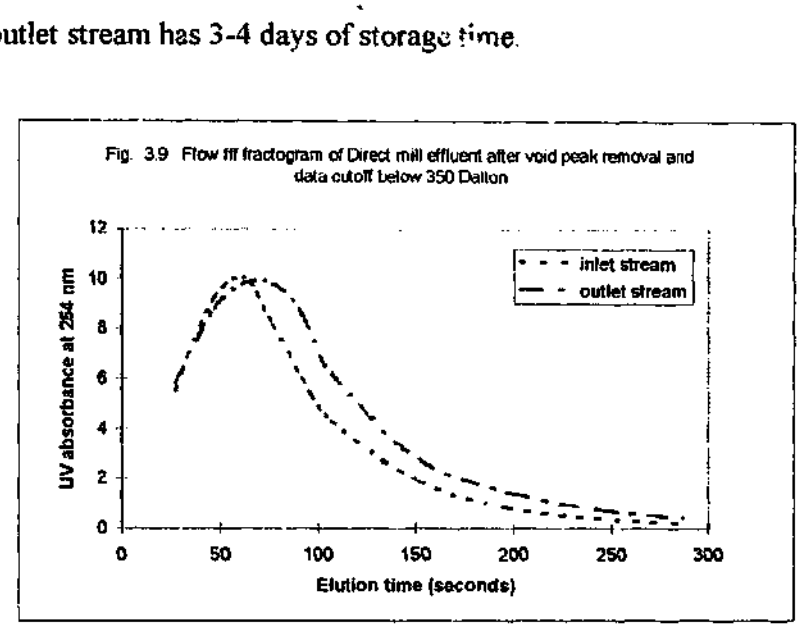


The fractograms obtained with these three standards are shown in Fig.3.8. The resulting fractograms and their retention times were recorded and the calibration was monitored with a secondary reference humic substance sample isolated from Okefeenokee swamp, GA, USA (USGS, Denver, CO). To evaluate the channel reproducibility and recovery, the differences in elution profiles were monitored by performing a series of runs over several days, with the above secondary reference sample. The molecular weights obtained were Mw=3400(100) and Mn=2200(50) with standard deviations given in brackets, where Mw and Mn are the weight average molecular weight and the number average molecular weight respectively.

3.4.2 Raw mill effluents

Two effluent streams were chosen from the AMCOR Maryvale pulp and paper mill plant, the inlet and outlet streams of the 41 megalitre storage. The inlet stream is a combined output of the pulp mill and the paper mill, which incorporates different processes and

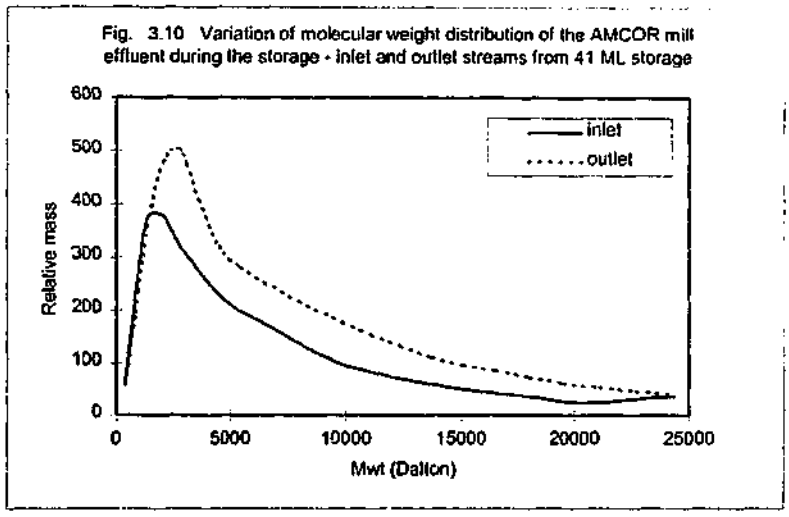
different stages producing various types of effluent cocktails. The only difference between these two samples is the storage time, so that the inlet stream has nil storage time and the outlet stream has 3-4 days of storage time.



These two effluent samples were analyzed for their molecular weights and molecular weight distributions by the flow FFF technique. The sample concentration in the eluent was measured by UV absorbance at 254 nm. The flow FFF fractograms (after removal of void peak) are shown in Fig.3.9. As retention volume is approximately inversely proportional to the diffusion coefficient, which in turn is a nonlinear function of molecular weight, an increase in retention time reflects an increase in the molecular weight.

The flow FFF fractograms were used to obtain molecular weight distributions, by using FFF Data Analysis Software version 2.0. The mass concentration of each fraction at the ith molecular weight increment (eluted at ith retention volume increment) is divided by

the molecular weight increment and plotted on the Y-axis. The resultant molecular weight distributions for the inlet and outlet stream samples are shown in Fig.3.10.



It is clearly seen that none of the effluent samples are monodispersed. The shapes of the molecular weight distributions are asymmetric. Fig.10 gives the effect of storage on molecular weight. The highly skewed distribution curves of raw mill effluents are due to the presence of much higher molecular weight molecules, which take much time to elute within the elution-time profile.

Table 3.6 Molecular weight characteristics of raw AMCOR mill effluents

Effluent sample	Mw	Mn	Mw/Mn	M_{max} (Relative mass at $Peak_{max}$)	$\bar{X}$
Inlet stream	7100	3400	2.09	1800	5700
Outlet stream	10500	4500	2.33	2400	7900

Table 3.6 summarizes the characterization of raw mill effluents by molecular weight analysis based on flow FFF.

It can be seen from the fractograms and the molecular weight distribution curves of the inlet and outlet streams of the AMCOR mill effluent storage pond that there is a considerable tailing of the molecular weight distribution at the higher molecular weight end. These long tails contribute significantly to the uncertainity of the Mw values due to their low mass concentration levels which can result in unreliable measurements on the detector response. This makes establishing the baseline of the fractogram quite difficult and will result in errors in the calculated Mw and Mn values. Therefore, the data was truncated at 18000 Dalton.

Table 3.6 shows that all molecular weight parameters have increased due to storage. The peak maximum of the molecular weight distribution curve has shifted from 1800 Dalton to 2400 Dalton. A higher Mw value (10500 Dalton) was obtained with the stored sample than with the unstored sample (7100 Dalton). Two suggestions for this increase are:

(1) possible removal of low molecular weight material from the system by microbial degradation, or

(2) possible condensation reactions within the system.

The molecular weight distributions were analyzed to evaluate whether these processes could have occurred and whether it is the low molecular weight fraction or the high molecular weight fraction, which has been affected due to storage. The results of previous studies related to molecular weight distributions of humic acids undertaken by Thurman et al., (1985) have shown that both degradation of higher molecular weight plant material occurs as well as polymerization, condensation and aggregation reactions.

Since ultrasonication was performed just prior to sample injection, the presence of large aggregates should have been minimized. The above two phenomena could have occurred to a different extent and at different rates, and which process preferentially occurs is uncertain.

3.4.3 Wastewater sites in the LVOS channel

Three wastewater sites were investigated. They were selected at three end points of three alternative piped and unpiped sections along the LVOS channel. The three samples investigated are as follows, and their locations are referred on the schematic diagram in Fig.3.5:

(1) Rosedale Meter

(2) Peg 83

(3) Peg 133

The above three samples were analyzed for molecular weights and molecular weight distributions with flow FFF. The molecular weight characteristics of these three total wastewater samples are summarized in Table 3.7.

Table 3.7 Molecular weight characteristics of wastewater sites in the LVOS channel

	Mw	Mn	Mw/Mn	M_{max} (Rel. mass)	$\bar{X}$
R Meter	3400	2100	1.62	1800	2400
Peg 83	5800	3100	1.87	2250	5100
Peg 133	4500	2300	1.96	1450	3800

Fig. 3.11 Variation of molecular weight distribution in recipient water within the piped section 1 of the LVOS channel

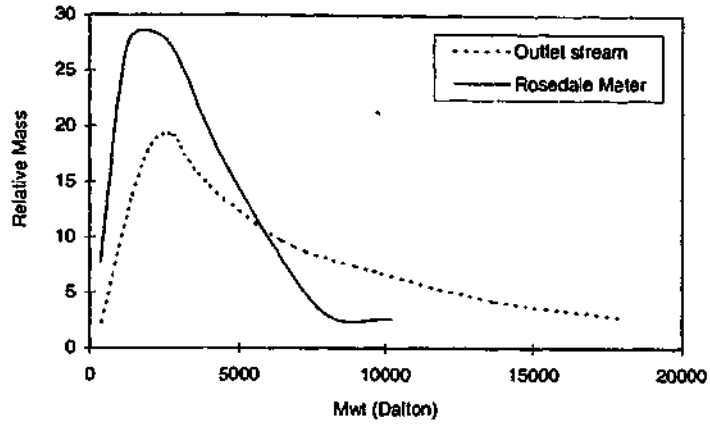


Fig. 3.12 Variation of molecular weight distribution in wastewater within the open sewer 1 of the LVOS channel

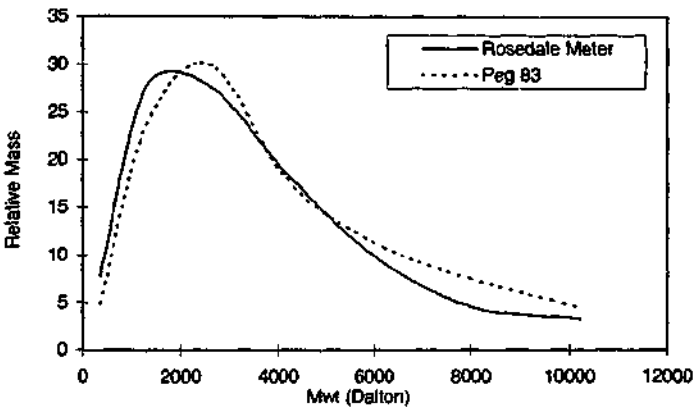
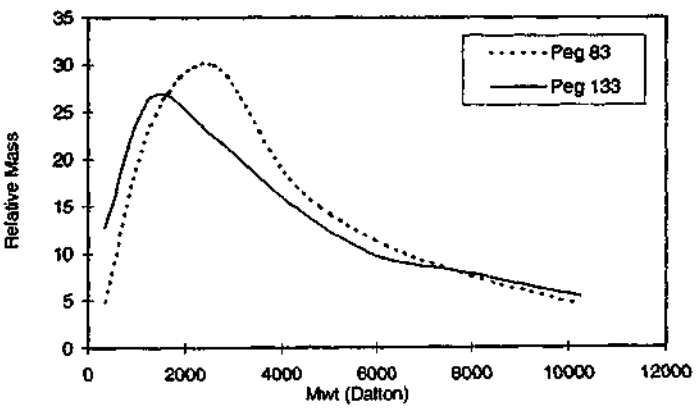


Fig. 3.13 Variation of molecular weight distribution in wastewater within piped section 2 of the LVOS channel



The changes in the molecular weight distributions between the sampling sites are shown in Figs. 3.11, 3.12 and 3.13. The experimental findings are summarized in Table 3.8.

Table 3.8 The effect of alternating oxygenated piped sections and anoxic open sewer channels on the molecular weight fractions (Mi)

	Effect on Mi (Dalton)	Possible mechanism
41ML storage →Rosedale Meter (piped section 1) Fig.11	< 2600 ↑, 2600 - 5500 ↑, > 5500 ↓	partial degradation of high molecular weight soluble organics
Rosedale Meter →Peg 83 (open channel 1) Fig.12	< 2000 ↓, > 5500 ↑	small extent of microbial assimilation of low molecular weight organics, polymerisation or condensation reactions, leaching soluble organics due to partial degradation of residual particulates
Peg 83 →Peg 133 (piped section 2) Fig.13	< 1600 ↑,1600-7000 ↓	partial degradation of high molecular weight soluble organics.

Both piped sections 1 and 2 have oxygenation units and the effluent-sewage pool is oxygenated as it is transported down the pipeline. Within the piped sections partial degradation of high molecular weight soluble organics seems to be prevalent. This process could be facilitated by oxygenation as similar results were found with both oxygenated piped sections (section 1 and 2). In contrast, microbial removal of low molecular weight soluble organic molecules, condensation reactions of low molecular weight soluble organics, or both of these processes could have occurred within the open sewer channel. In addition, partial degradation of residual particulates such as degraded

products of leaf litter, may result in leaching of high molecular weight soluble organics into the organic pool in the open sewer channel.

3.4.3.1 Piped section 1 in the LVOS channel

The molecular weight distribution curve of Rosedale Meter is more symmetric than the raw mill effluent curves (Fig.3.11). This is evident by the fact that the Rosedale Meter molecular weight distribution curve is skewed (sk = 0.67) to a lesser extent than the raw mill effluents (sk = 1.94). This reflects the absence of large molecules with much higher molecular weights in the Rosedale Meter site. The Rosedale Meter sample is less polydispersed and molecular weight distribution has a narrower high peak compared to that of the raw mill effluent.

The raw mill effluent that enters the LVOS system reaches the Rosedale Meter point in about 24 hrs. As the raw mill effluent reached the Rosedale Meter site, the following changes were observed. The low molecular weight fractions (i.e. Mi < 2600 Dalton) increased by 100%, which was calculated by peak area. The low-medium molecular weight fractions (i.e. Mi 2600-5500 Dalton) seemed to have increased only by 14%, that is, preferential formation of nominally low molecular weights (Mi < 2600 Dalton) rather than medium molecular weights (Mi ~ 2600-5500 Dalton). The high molecular weight fractions (i.e. Mi > 5500 Dalton) decreased by 79% (calculated by peak area) compared to the original mill effluent.

Since oxygenation within the pipe (piped section 1) is the only external trigger which is likely to have a major effect on the fate of the raw mill effluent, the variations occurring within the piped section 1 could have occurred due to three possible reasons:

(1) microbial degradation of high molecular weight soluble organic matter. These microorganisms may not be capable of removing the resultant low to medium molecular weight products from the environment

(2) partial degradation of the particulate organic matter in the raw mill effluent after entering the LVOS outfall pipeline to yield low molecular weight soluble byproducts

(3) adsorption and subsequent settling of high molecular weight components and removing them from the soluble organic carbon pool.

Since major inputs to the LVOS flow from Traralgon, Churchill, etc. also contain unknown composition, the increase in low molecular weight material could be due to low molecular weight organic inputs. However, whether the predominant mechanism of high molecular weights ((Mi >5500 Dalton) removal is due to either degradation by microbes or due to adsorption/physical settling is still uncertain.

3.4.3.2 Open Sewer 1 in the LVOS channel

Peg 83 is the end point of open sewer 1, which is approximately 14 km away from the Rosedale Meter. There is no domestic or industrial input to the system between the Peg 83 and the Rosedale Meter sites. As it is an open sewer canal, there is no oxygenation unit for this section although some dissolution of atmospheric oxygen would occur.

The molecular weight distribution curve of Peg 83 (Fig.3.12) is slightly more asymmetrical and skewed (sk = 1.37) than that of Rosedale Meter (sk = 0.67). The wastewater at Peg 83 contains some fractions of much higher molecular weight material than at the Rosedale Dissolver. This clearly illustrates that the molecular weight distribution curve of Peg 83 has been shifted slightly towards the higher molecular weight range. The molecular weight distribution curve also shows a small decrease (12%) in the low molecular weight fractions (Mi < 2000 Dalton) according to the peak area. This may suggest microbial assimilation of low molecular weight substances (Mi < 2000 Dalton) from soluble organic matter in the effluent-sewage pool as it is transported down the open sewer. Fig.3.12 demonstrates an increase of 39% (by peak area) in the high molecular weight fractions (Mi > 5000 Dalton) from the Rosedale Meter to Peg 83.

The Peg 83 site show higher average molecular weights (Mw=5800 Dalton and Mn=3100 Dalton) relative to the Rosedale Meter site (Mw=3400 Dalton and Mn=2100 Dalton). The increase in high molecular weight organic fractions in Peg 83 could be due to partial degradation of soluble leaf organic matter. As Peg 83 is in an open section of the sewer system, decomposing leaf litter may be an additional source of soluble organic matter and its associated high molecular weight organic fractions. This may be occurring as organic matter leaches from the bank into the canal and/or microbial reaction on particulate organic matter within the canal. Other researchers have found high average molecular weights for decomposing leaves (Hall et al., 1972). Similar findings for natural water organics, aqueous extracts of wood and soil organic matter were reported by Christman et al. (1966). Thus, there is a possibility of the presence of natural organics derived from

several allochthonous sources in Peg 83. This would be an external contributor to the in situ soluble organics of effluent-sewage pool of the LVOS system, which was so far treated as a closed piped system.

3.4.3.3 Piped section 2 in the LVOS channel

Peg 133 is the end point of piped section 2, which is approximately 7 km away from Peg 83. The molecular weight distribution curve (Fig.3.13) of Peg 133 is more symmetrical than Peg 83 and it is skewed (sk = 1.18) to a lesser extent than Peg 83 (sk = 1.37). This indicates a lack of higher molecular weight organics in Peg 133, compared to that found in Peg 83. Perhaps one could expect the average molecular weight would be lower in Peg 133 than in Peg 83, having been oxygenated during its transport down the piped section 2. This is because lower average molecular weights were observed in the effluent-sewage pool which had been transported down the piped section 1 under oxygenated conditions.

The medium molecular weight fraction (Mi ~ 1600-6500 Dalton) has decreased by 16% in Peg 133 compared to Peg 83. As previously described, this observation may be attributed to partial degradation of the medium molecular fraction (Mi ~ 1600-6500 Dalton) by microorganisms rather than microbial assimilation. The low molecular weight fractions (Mi <1600 Dalton) have increased by 28% in Peg 133 compared to Peg 83. Apparently these microorganisms are not capable of removing the residual molecules after partial degradation. The microorganisms involved in this process may selectively react with certain molecular sites of the organic molecule rather than assimilating the entire molecule (Tabeck et al., 1964). Adsorption/physical settling which usually affects

higher molecular weight components does not seem to be significant in Peg 133 because there is a similarity in the distribution of higher molecular weight fractions (Mi > 6500 Dalton).

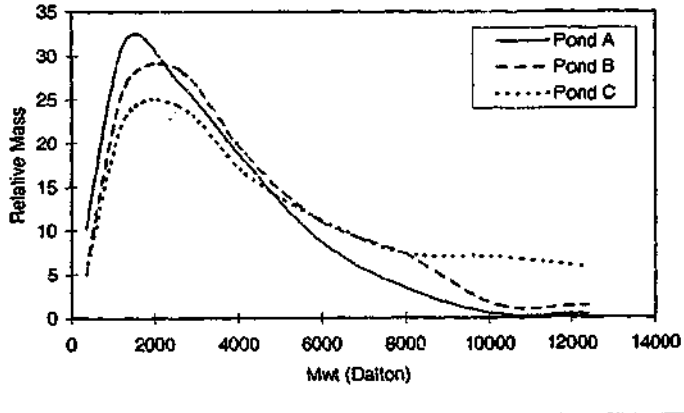
3.4.4 Treated wastewater sites in the DD treatment complex

The effluent-sewage pool is transported down the open sewer 2 and enters the DD treatment complex at pond A, which is the first pondage of a series of five. The molecular weight distributions of the pondage sites are illustrated in Figs.3.14 and 3.15. The molecular weight characteristics of treated wastewater sites in the DD treatment complex are summarized in the Table 3.9.

Table 3.9 Molecular weight characteristics of treated wastewater sites in the DD treatment ponds

Sampling site	Mw	Mn	Mw/Mn	M_{max} (Rel mass)
exit pond A	3150	1950	1.62	1550
exit pond B	3950	2400	1.66	1900
exit pond C	8200	3500	2.34	1900
exit pond D	5100	2700	1.89	2000
exit No.2	4200	2500	1.68	2000

Fig. 3.14 Variation of molecular weight distribution in wastewater within the initial phase (aerobic phase) of the DD treatment ponds



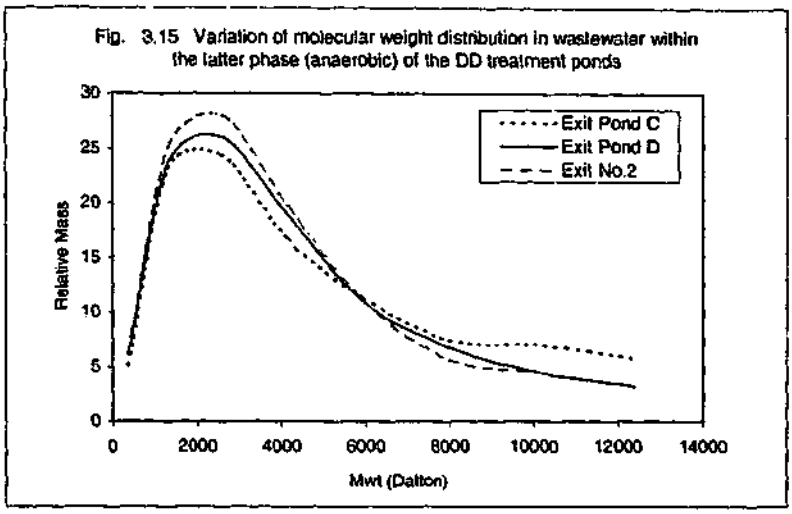


Fig. 3.15 Variation of molecular weight distribution in wastewater within the latter phase (anaerobic) of the DD treatment ponds

The general trends are summarized in the Table 3.10.

Table 3.10 The effect of treatment on molecular weight fractions (Mi) in the treatment ponds

	Effect on Mi (Dalton)	Possible mechanism
pond A → pond B Fig.14	< 2000 ↓ , > 2000 ↑	microbial assimilation of low molecular weight molecules, polymerisation or condensation reactions or leaching soluble organics due to partial degradation of residual particulates
pond B → pond C Fig.14	< 5500 ↓ , > 8000 ↑	physical settling of medium sized molecules, leaching soluble organics due to partial degradation of residual particulates
pond C → pond D Fig.15	1600-5500 ↑ , > 6000 ↓	partial degradation of high mwt soluble organics
pond D → pond E2 Fig.15	little variation	steady state

Table 3.9 shows that the weight average molecular weight has markedly increased from pond A to pond C. Correspondingly, the number average molecular weight also has increased. The increase in weight average molecular weight in the effluent-sewage pool in these pondages could be due to:

(1) decay of low molecular weight (Mi < 1600 Dalton) soluble organic matter due to microbial assimilation

(2) generation of higher molecular weight (Mi > 5500 Dalton) soluble organic matter due to condensation reactions at the expense of smaller to medium molecular weight molecules within the system

(3) partial degradation of residual particulate organic matter by microorganisms, releasing high molecular weight (Mi > 5500 Dalton) soluble organic matter.

The general trends (see Table 3.10), which emerge from this study are:

(1) Within the treatment complex, the microbial assimilation of low molecular weights could be achieved in the early (i.e. primary) stages of the treatment ponds, probably within the initial pondage. Also, condensation reactions are possible at this primary stage as higher proportions of low molecular weight molecules are found. Leaching of high molecular weight soluble organics to the effluent-sewage pool from residual particulates is also possible.

(2) As the wastewater proceeds to the secondary stages of the pondage (pond C), it is likely that removal of nominally medium molecular weight molecules will occur, as degradable low molecular weight molecules have been exhausted at the primary stage.

Leaching of high molecular weight soluble organics to the effluent-sewage pool from residual particulates is also possible.

(3) As treatment in the pondages proceeds towards the tertiary stage (pond D), the microbial partial degradation of high molecular weight soluble organics may occur. However, these are not generally assimiable by microbes, even though they are capable of partially degrading them. This partial microbial degradation of high molecular weight soluble organics starts to occur after the removal of degradable medium molecular weight molecules (Table 3.10).

(4) Beyond the tertiary stage (pond E) a steady state may occur. Possible partial degradation of particulate organics may occur to a certain level.

3.5 CONCLUSIONS

Effluents from a bleached Kraft pulp and paper mill (AMCOR) situated in Gippsland (Victoria, Australia) contained high average molecular weights. Their molecular weights were distributed from 1000 Dalton to 25,000 Dalton. The combined effluent from the pulp and paper mill possesses an approximate weight average molecular weight of 7000 Dalton, and is highly polydispersed. This effluent is retained for about 3-4 days in the 41 megalitre storage.

Analysis of samples taken from the inlet stream and the outlet stream of the storage showed that the average molecular weight and polydispersity of the inlet stream was

slightly lower than that of the outlet stream. Removal of lower molecular weight fractions (Mi < 2000 Dalton) by microbial degradation or condensation to form larger molecules and/or microbial processing of suspended solids could be suggested as the reasons for the higher average molecular weight after storage.

Approximately 35% reduction of Mi < 2000 Dalton organic material and 22% increase of Mi > 2000 Dalton organic material was observed. Both fractions, ie. low molecular weights and high molecular weights, are affected in stored wastewater. These phenomena may occur by different processes; to a different extent; at different rates, and by different microbial populations.

The molecular weights within the LVOS system behaved in different ways depending on whether oxygenated piped sections or open sewers were involved.

- Within the piped sections high molecular weights were removed and an increase of low molecular weight fractions were observed. The microbial oxidation of large molecules may result in production of small molecules, which are resistant to further microbial attack. The microbial oxidation of high molecular weight fractions appears to preferentially occur within the piped sections. The actual processes governing the increase of low molecular weights and decaying of high molecular weights could not be deduced from these analyses and more detailed laboratory experimentation is required. However, preferential formation of low molecular weights and depletion of high molecular weights was evident within the piped sections.

- In open sewers low molecular weight components decreased while high molecular weight material increased. The natural soluble organic matter derived from allochthonous sources and leached from partial degradation of particulate organic matter may contribute in open sewers. The microbial assimilation of low molecular weight molecules or their removal due to polymerization/condensation was evident. The end point of the open sewer showed a higher average molecular weight than at the starting point. The microbes seem to preferentially react/degrade lower molecular weight organic matter within the open sewers.

- Within the DD treatment ponds molecular weights during the initial aerobic phase from pond A to pond C behaved similarly to the open sewer. The molecular weights behaved as in oxygenated piped sections during the latter anaerobic phase from pond D to the exit point. That is the weight average molecular weights increased during the initial phase of treatment ponds while they decreased in the latter treatment ponds.

Chapter 4

Chemical Characterization Of A Paper Mill Effluent And Recipient Water Using XAD-8 Resin Fractionation

Abstract

The pulp and paper mill effluent of the AMCOR mill, Latrobe Valley Outfall Sewer (LVOS) channel and the Dutson Downs (DD) treatment complex were investigated in order to establish the physical and/or biological effects on the dissolved organic carbon (DOC). A series of sites along the LVOS system have been studied, as the DOC in the sewer was thought to be affected by biological degradation and/or physical settling/adsorption effects. The current study has focussed on the changes in DOC and major organic fractions along the LVOS channel, and in the DD treatment ponds. AMCOR mill effluent and receiving wastewaters were chemically characterized by XAD-8 resin fractionation.

The results show that the major constituent of the total humic acids in the LVOS flow that enters the DD treatment complex is from sources other than the AMCOR plant. About 77% of the total humic acids appear to have been removed during the pond treatment at DD. The fulvic acids in the LVOS flow have been degraded within the LVOS channel 2, and the degraded fulvic acids were also most likely derived from sources other than the AMCOR mill. Almost 30% of the total fulvic acids in the LVOS flow that enter the DD treatment ponds have been removed during the pond treatment. The mill effluent hydrophilic substances were considerably degraded along the LVOS channel. There was not a large reduction in total hydrophilic substances within the treatment ponds. The relative percentage of residual hydrophilic substances in the treatment ponds is somewhat higher, indicating that they are quite refractory.

4.1 INTRODUCTION

The pulp and paper mill effluent of the AMCOR mill plant is the major organic contributor to the Latrobe Valley Outfall Sewer (LVOS) channel, and the Dutson Downs (DD) treatment complex. The current study has focussed on changes in the total DOC and the major organic fractions along the LVOS channel, and in the DD treatment ponds. The AMCOR mill effluent and the receiving wastewaters in the LVOS channel were characterized by XAD-8 resin fractionation in order to study the biological and/or physical effects on the major organic fractions. The study has extended to DD treatment ponds as well. Attempts were made to determine whether any biological and/or physical processes are involved in DOC removal in addition to dilution effects. Therefore, the contributions from the AMCOR mill effluent and other major inputs were estimated with respect to the total DOC and the major organic groups.

4.2 EXPERIMENTAL

Fractionation of dissolved organic carbon was done for chemical characterization of Maryvale pulp and paper mill effluent and Latrobe Valley Outfall Sewer system. Attempts were made to differentiate and identify different organic groups depending on their lipophilicity. Amberlite XAD-8 resin was chosen for dissolved organic carbon resin fractionation, which is available from Rohm and Haas as an industrial-grade product, in 40-60 mesh beads. The experimental method is outlined in Fig.4.1.

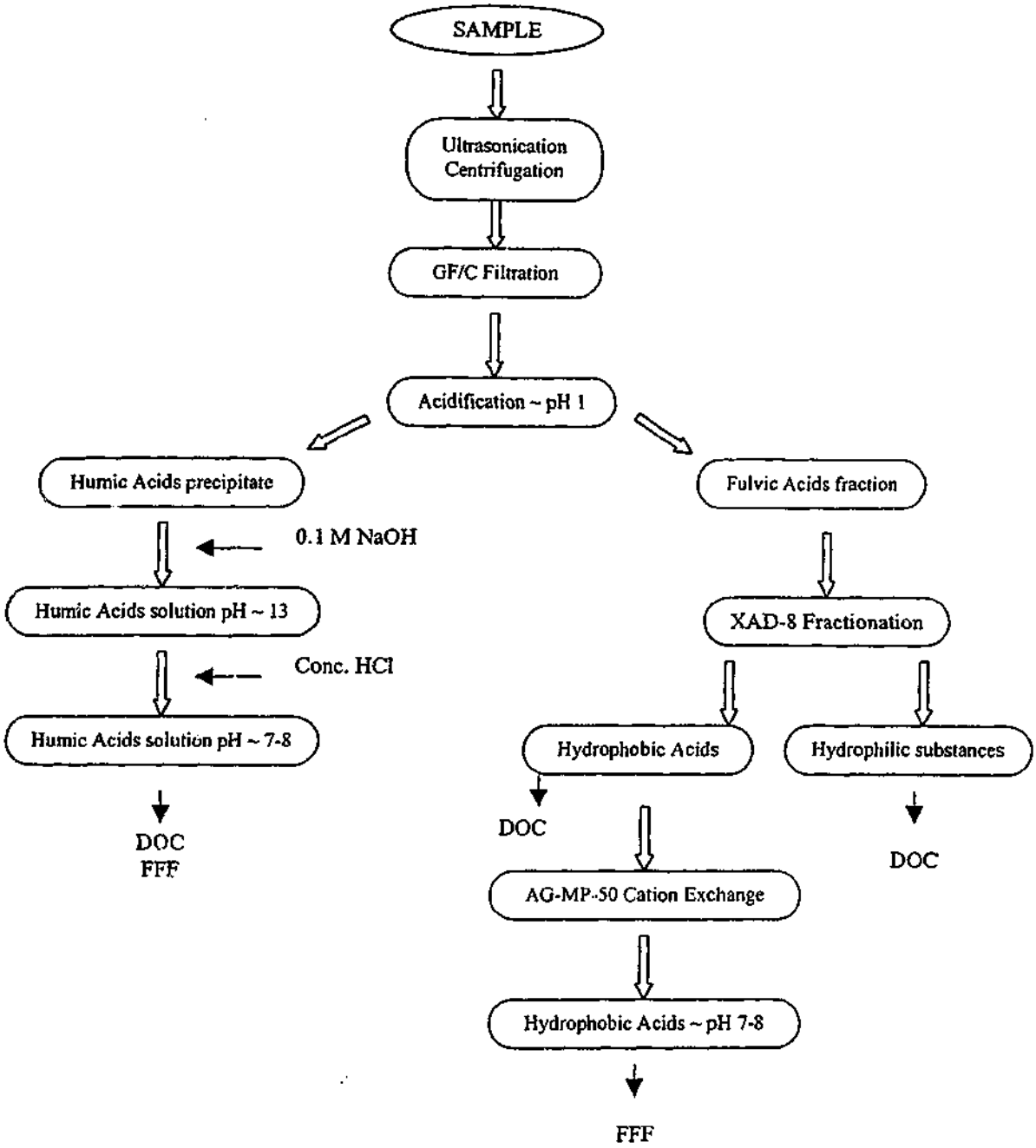


Fig. 4.1 SCHEMATIC DIAGRAM
XAD-8 Resin Fractionation Scheme devised for fractionation and isolation of major organic chemical groups

4.2.1 Amberlite XAD-8 Resin cleaning by Soxhlet extraction

The resin was slurried in 0.1 M NaOH and the fines were decanted after stirring for one hour and the remainder was stored in 0.1 M NaOH. This was repeated over three days and the resin was rinsed with milliQ water, 0.1 M HCl and again with milliQ water and finally with methanol. It was then packed into a cellulose chamber (cup) and placed in a Soxhlet extractor and fitted into a round bottom flask, containing methanol. The resin was Soxhlet-extracted sequentially for 24 hrs with methanol, then 24 hrs with acetonitrile, and again for 24 hrs with methanol. This Soxhlet-extracted resin was stored in methanol until used.

4.2.2 Amberlite XAD-8 Resin column preparation

The column was constructed of glass, 30 cm in length and 1.6 cm internal diameter. It was plugged with a small 0.25 cm plug of glass wool, and backwashed to remove fine glass wool particles. The glass column was then packed with 20 ml Amberlite XAD-8 resin in 1:1 methanol-water slurry. The connecting tubing used in this study was teflon with glass end caps. The peristaltic pump tubing was made of silicone rubber.

4.2.3 Amberlite XAD-8 Resin regeneration

The packed column containing the methanol-water slurry was then pumped with milliQ water and thoroughly rinsed until free of methanol by passing 50 bed volumes of milliQ water through it. The milliQ water pumped through the column was checked for carbon until the DOC of the effluent reached a value less than 1 mgC/L. Subsequently it was rinsed with 0.1 M HCl and 0.1 M NaOH by alternatively passing through 10 bed volumes of acid

or base at a time. The acid-base washing cycle was repeated three times and ended with acid passing through the column.

4.2.4 Sampling and sample pre-treatment

Sites downstream of the AMCOR mill were chosen for this study. Samples were also collected from the LVOS channel and the DD treatment complex. The sampling procedure and pretreatment was outlined in Chapter 3.

4.2.5 Fractionation of raw samples into broad organic chemical groups

The scheme used in this experimental phase is illustrated in the schematic diagram in Fig.4.1.

4.2.5.1 Isolation and pre-fractionation of humic acids

The nondiluted, sterilized and filtered wastewater samples to be fractionated were adjusted to pH < 2 with concentrated HCl and were kept in the refrigerator for 72 hrs for humic acid precipitation. The precipitated humic acids were centrifuged at 3750 rpm for ½ hr and separated by decanting the supernatant liquid into glass vessels, cleaned by heating in an oven at 500° C overnight. The supernatant liquid left was the fulvic acid fraction. The precipitated and settled humic acid was mixed with 10 mL 0.1 M HCl and centrifuged again at 3750 rpm for 10 minutes. The supernatant HCl liquid was then decanted and added to the fulvic acid fraction. The precipitates of humic acid were then transferred to glass vials with the aid of milliQ water and the final volume was made up to 20 mL with 0.1 M NaOH solution, and the pH was immediately adjusted to 7.6 with 1 M HCl.

4.2.5.2 Preparation of humic acid fractions for DOC analysis

The pH of the dissolved humic acid solution was brought to pH 3-4 just prior to DOC analysis, as there is a possibility of precipitation with longer times at low pH. The dilutions were made where necessary and the measured values of DOC were corrected for final concentrations by using their dilution factors.

4.2.5.3 XAD-8 resin fractionation of the fulvic acid fraction

The effluent waters after humic acid removal were then used to isolate the fulvic acid using an XAD-8 resin. The desired DOC level for XAD-8 fractionation was achieved by diluting the sample concentration to 10% of the original. So that, 60 mL of sample was made up to 600 mL final volume with milliQ water and this was used as column eluent. The processed water at pH < 2 was pumped through the resin column at 2.5 mL/min with a Gilson peristaltic pump. The column void volume was ~ 13.0 mL as the volume of XAD-8 resin was ~ 65% of the bulk column volume (Leenheer 1981). One void volume was displaced before collecting any effluent passing through the resin. The fraction collected through the XAD-8 column is known as the hydrophilic substance fraction, although it includes hydrophobic bases as well (Leenheer 1981). The nonsorbed portion of the influent within the column was displaced by one void volume of 0.01 M HCl solution. This washing liquid was combined with the fractions of hydrophilic substances collected through XAD-8 resin.

The column was then eluted with 50 mL 0.1 M NaOH at half the rate used for the sample introduction, i.e. 1.25 mL/min. One void volume was displaced and was discarded before collecting the fulvic acid fraction. The fraction collected with 0.1 M NaOH elution is

known as fulvic acid. At the end of the desorption one void volume was displaced by 0.1 M NaOH and was combined with the fulvic acid fraction collected. The hydrophobic neutral fraction is that retained in the XAD-8 column under these experimental conditions.

4.2.5.4 Preparation of fulvic acid fraction for DOC analysis

The fulvic acid fractions were divided into two sub-samples for DOC analysis and were adjusted to pH 3-4 immediately with concentrated HCl. The freshly prepared solutions were analyzed for DOC. The remaining solutions were kept in the refrigerator for future analysis. Dilutions were made where necessary and the measured DOC values were corrected using their dilution factors to obtain the final concentrations.

4.2.6 DOC Analysis

4.2.6.1 Sample Preparation for DOC analysis

Samples were sterilized with sodium azide (0.03% final concentration), vortex mixed for 1-2 minutes and ultrasonicated for 10 minutes in a bath sonicator. They were vacuum filtered through 1.2 μm GF/C filters, which have been kept at 500°C overnight in an oven. All glassware was kept at 500°C overnight in an oven prior to use. The processed samples were diluted with milliQ water depending on the visual colour. Dilution factors were used to calculate the final concentrations.

4.2.6.2 Dissolved Organic Carbon analysis

DOC measurements were made using a Schimadzu TOC-5000 total organic carbon analyzer. The processed diluted samples were used for DOC measurements. The samples

were placed in an autosampler provided with glass vials. The autosampler was also provided with an acid vial filled with 1 M HCl for sample acidification, so that each sample was acidified with 1 M HCl acid automatically before each measurement. The instrument was set to achieve sparging for 1 minute with zero grade air before sample injection. The injections were performed in triplicate and the averaged values and standard deviations were automatically recorded. The instrument was instructed to perform a maximum of five injections when lack of reproducibility with the first three injections was indicated.

4.2.6.3 Instrumental setup and calibration of the instrument

The instrument was allowed to heat up to 680-700°C under a constant flow of zero grade air. When the temperature was stabilized the instrument calibration was started. The autosampler is provided with a series of large glass vials (kept at 500°C overnight in an oven) for standard solutions to which standard carbon solutions are placed.

For the above calibration a series of standard total carbon solutions were freshly prepared from a potassium hydrogen pthalate (KHP) stock solution. The instrument was calibrated for two calibration ranges and during the sample analysis the instrument was automatically switched to the appropriate carbon range with each individual sample. A series of inorganic carbon standard solutions were made with a stock solution of a sodium bicarbonate and sodium carbonate ($NaHCO_3$+Na_2CO_3) mixture. The instrument was calibrated to a higher and lower carbon range using these IC standard solutions. The instrument calculated the DOC value by subtracting the IC value from the TC value.

4.2.6.4 Preparation of calibration line

The calibration was made with three points using the TC standard solutions in the higher carbon strength range (100 mgC/L, 50 mgC/L and 10 mgC/L). The instrument was instructed to make a three point calibration by introducing the above three carbon standards. The instrument was instructed to record the peak area for each injection and automatically averaged three peak areas of three successive injections with each standard. The averaged peak area was tabulated with the corresponding carbon strength of the standard introduced and the peak area vs carbon strength was plotted. Similarly another three-point calibration line was constructed at lower carbon range by using three standard solutions of lower carbon strength (10 mgC/L, 5 mgC/L and 1 mgC/L). Three point calibration plots were also made with the IC standard solutions. The least square fit was obtained with all calibration plots and these least square regression straight lines were used as calibration lines. The correlation coefficient (r^2) of linear regression was recorded and the coefficient of variation (cv) of the peak areas for the standard solutions was recorded each time to evaluate the reproducibility.

4.2.6.5 Statistical Accuracy and Precision

Two sets of samples were collected from 11 sampling sites over the LVOS channel and DD treatment complex including AMCOR mill. XAD-8 resin fractionation was carried out six times with each sample. DOC was analyzed on each XAD-8 fraction in triplicate. Two sub-samples were taken from each sample for one DOC analysis. The accuracy of the instrument and reproducibility of the DOC analysis were checked by doing three sets of analyses on successive days. Each day the instrument was recalibrated.

4.3 RESULTS AND DISCUSSION

4.3.1 Distribution of major organic fractions at AMCOR mill downstream sites

DOC values recorded at different sites in the LVOS and DD system are given in Fig 4.2. The total DOC gradually decreased along the LVOS channel (except at Peg 83) and also in the DD treatment ponds. The XAD-8 resin fractionation has revealed that the hydrophilic fraction has followed the total DOC trend within the LVOS channel while humic acids fraction has only followed the trend of DOC within the treatment ponds (Fig. 4.2). The hydrophilics fraction does not vary significantly within the treatment ponds; the hydrophilics being preferentially removed within the channel while humic acids are preferentially removed within the treatment ponds. These trends can be explained by the hypothesis that the hydrophilic substances would be more prone to microbiological attack within the channel, whilst the humic substances are more prone to aggregation and adsorption within the treatment ponds.

It seems the fulvic acid fraction has also decreased along the LVOS channel but to a lesser extent than the hydrophilic fraction since the fulvic acid fraction in the mill effluent has a considerably lower concentration than the hydrophilic fraction (Fig. 4.2). Within the DD treatment ponds the decrease in fulvic acids is evident but to a lesser extent than humic acids. This is consistent with the observations that humic acids are less soluble than fulvic acids and hence tend to precipitate and adsorb more readily, leading to removal by settling.

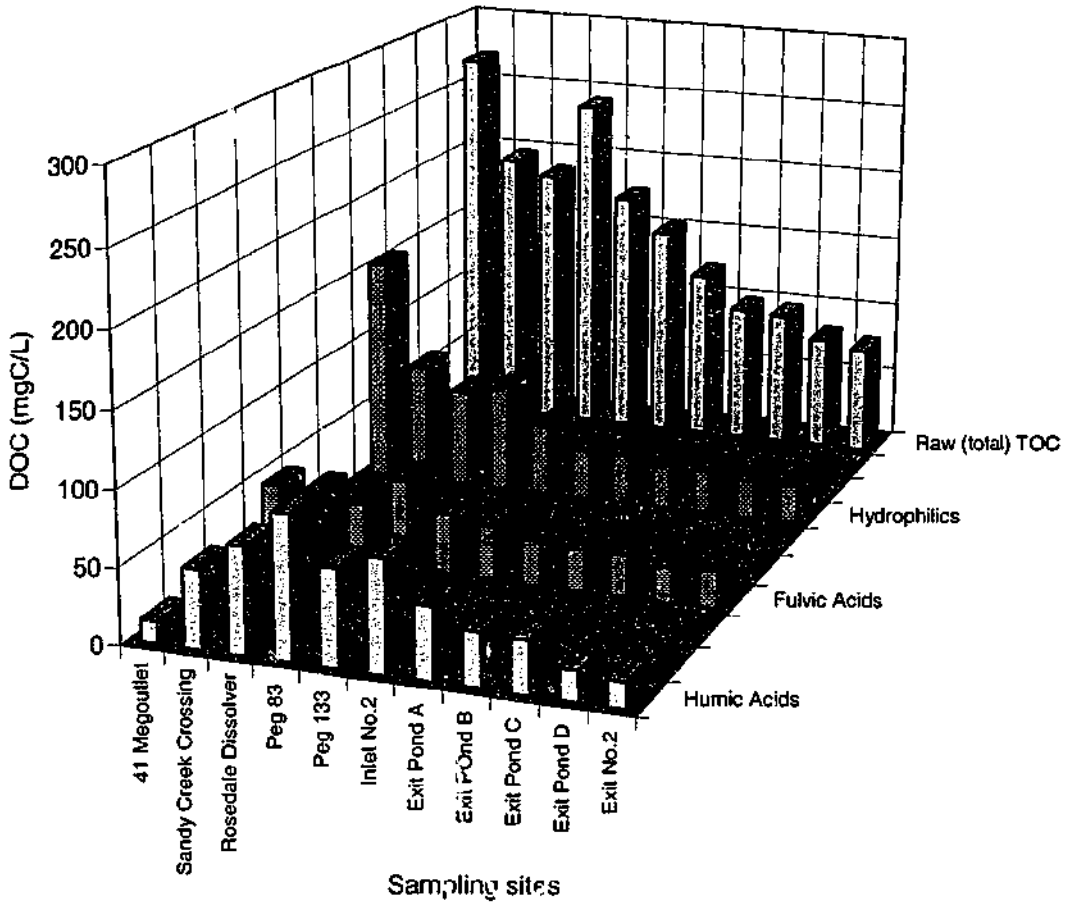


Fig.4.2 The effect on major organic fractions – LVOS channel and DD treatment ponds
DOC OF XAD-8 FRACTIONS vs SAMPLING SITES

The results show the major fraction of DOC in mill effluent consists of hydrophilics while the least contribution is from the humic acid fraction. The DOC concentration trend along the LVOS channel represents the combined effect of dilution plus degradation of the residual mill effluent and major DOC inputs entering the channel. The distribution of each organic fraction can be differentiated into mill DOC and other DOC by considering dilution factors (refer to Chapter 3) due to major inputs if no degradation of mill DOC is assumed.

The major inputs from the Morwell outfall pipelines and the Churchill outfall pipelines are merged upstream at Sandy Creek Crossing. As far as the Morwell input is concerned, only 32% (refer to Chapter 3, section 3.2) of the monthly average discharge (ML/month) originates from Morwell city and the remainder consists of flushing water from the Morwell service reservoir. However, the Churchill input consists of ~10% (refer to Chapter 3, section 3.2) from other industrial inputs such as APM Wood and Difabrizo, Australian Char Plant, and the remainder is from the Churchill Grit Chamber of the domestic sewage line. In fact, the Churchill input is only 35 ML per month on average compared to the total flow of 656 ML/month in the LVOS channel (mill effluent of 440 ML/month and inputs of 216 ML/month), so that there is no appreciable effect due to its input.

Similarly, despite the AMCOR mill effluent, the increase of humic acids at the Rosedale Dissolver site is most likely to be caused by the merging input from Traralgon Mains, which consists mainly of domestic sewage. Both the quantity of the input, as well as the strength of the input, from other sources plays a major role in determining the LVOS composition.

A reasonable assumption would be that the DOC originating in the AMCOR mill may be fairly resistant to biological degradation as mill effluents usually contain lignin byproducts belonging to a poorly defined group of compounds known as lignosulphates. These consist of molecules with relatively high molecular weights. In contrast, merging township organic inputs are domestic sewage, whose composition is mainly simple aliphatic organic

substances; such as, carbohydrates, amino acids, fatty acids, esters, amino sugars, which are more easily degradable (Painter et al.1959). These simple aliphatic degradable organic molecules are readily removable due to active uptake or biodegradation, while large aromatic refractory organic molecules may be physically removable due to adsorption and settling.

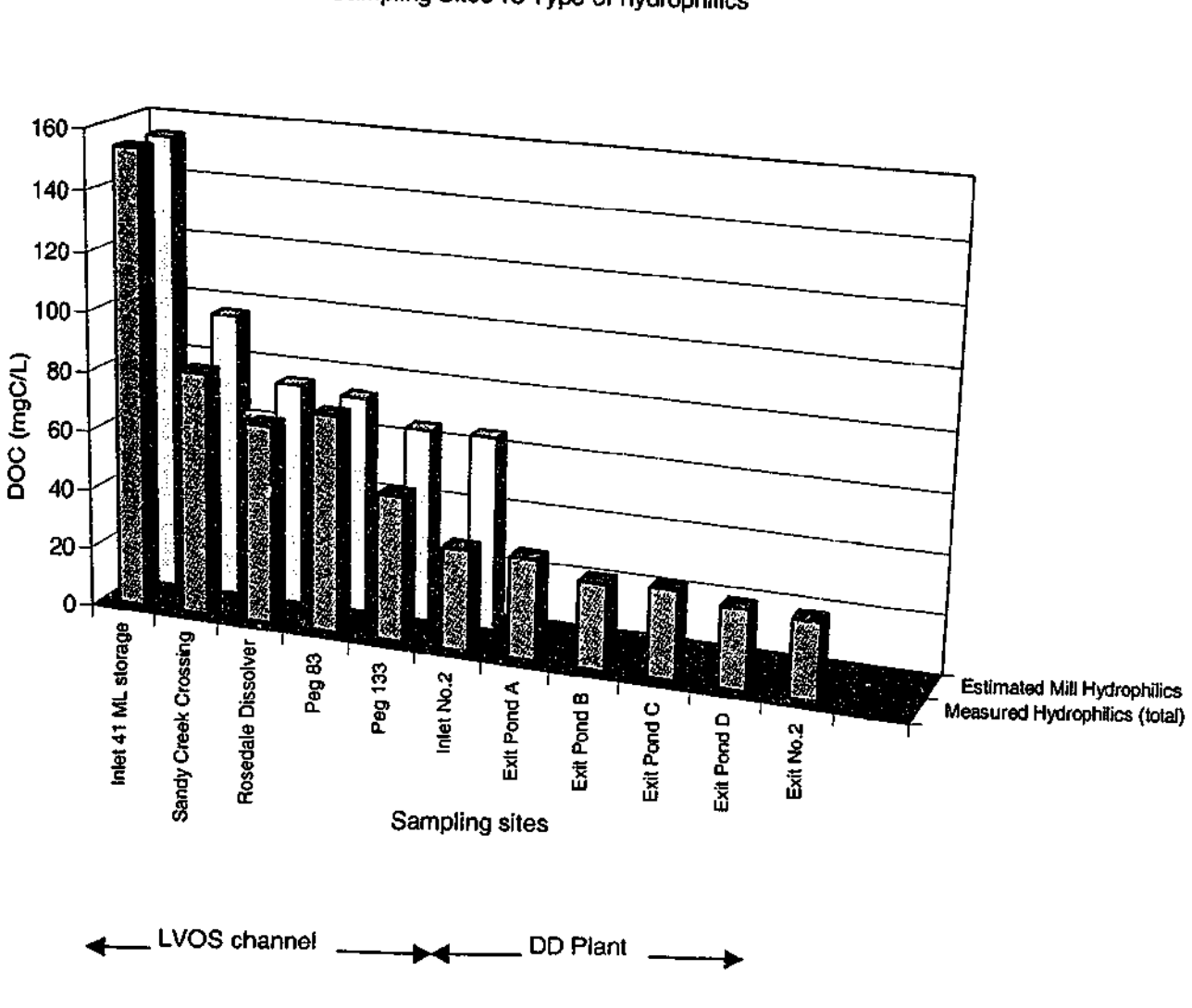
4.3.2 Distribution of hydrophilic substances at AMCOR mill downstream sites

The concentrations of the total hydrophilics and the estimated amounts of hydrophilics derived from the AMCOR mill present in the LVOS flow at sites downstream of AMCOR mill are illustrated in Fig. 4.3. Assuming no degradation of AMCOR hydrophilic substances occurs, their contribution due to the AMCOR mill effluent at various downstream sites were able to be estimated. This is possible since and the fact that the load of hydrophilic substances in the AMCOR mill effluent discharge to the LVOS drain and the dilution factors (refer to Chapter 3) at downstream sites due to discharges from other sources are known. These loads were taken from the mean values from historical records of Latrobe Valley Water and Sewerage Board, Victoria, Australia. The effect of dilution was determined at downstream sites to evaluate the effect of other possible changes due to biological and/or physical processes.

The concentration of hydrophilic substances in the AMCOR mill effluent seems to be very high (154 mgC/L) compared to sites downstream of the AMCOR mill along the LVOS channel (Fig. 4.3). It shows that the measured concentrations of the total hydrophilics at all downstream sites are always lower than the estimated values for the maximum possible

contributions of the AMCOR mill hydrophilics. The exception to this trend was the Peg 83 site. The measured concentrations reveal that degradation of hydrophilic substances from mill effluent has occurred.

Fig. 4.3 The effect on hydrophilics - LVOS channel and DD treatment ponds



Since the total values of the hydrophilic substances concentrations were lower than the expected value, the minimum removal of AMCOR mill discharge hydrophilic substances

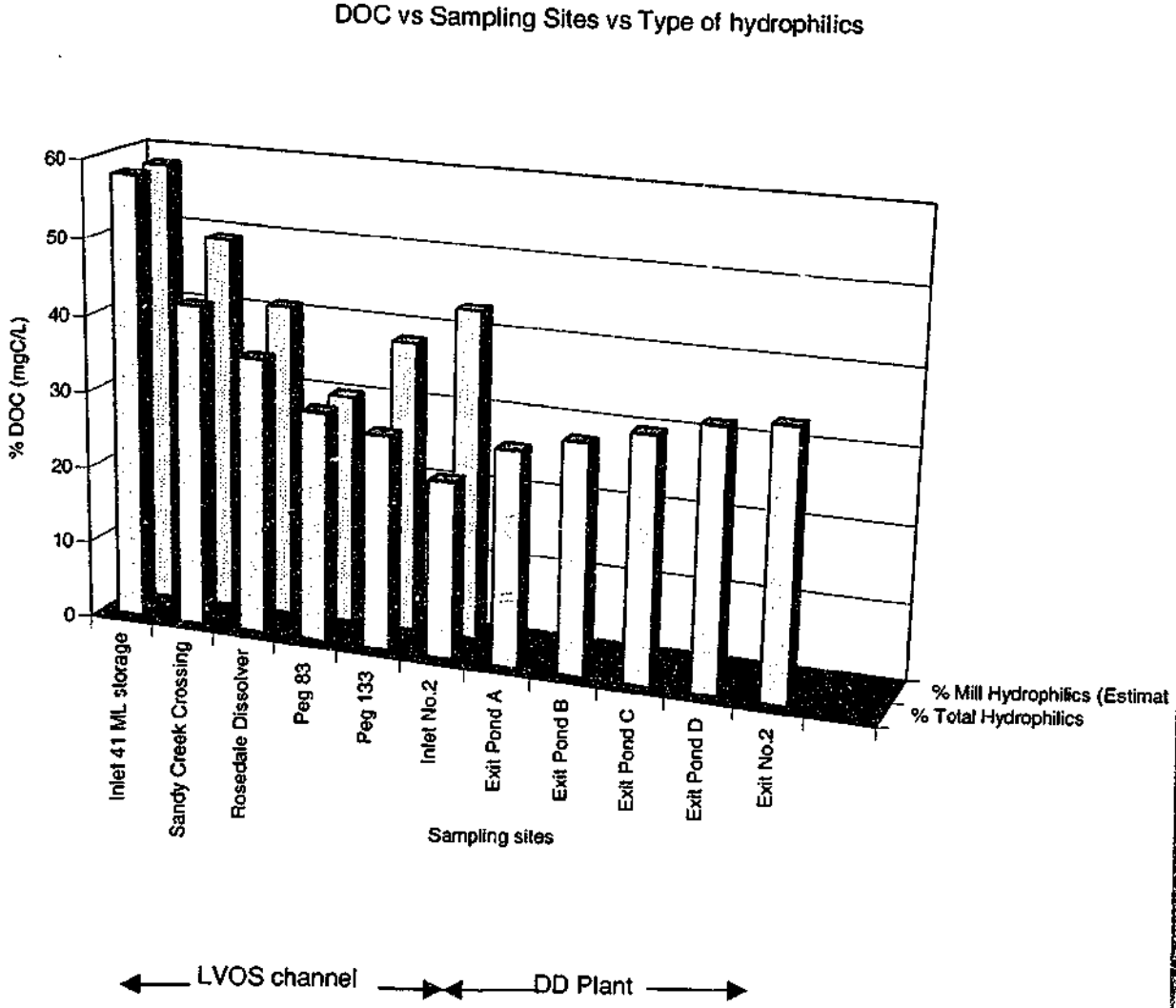
could be evaluated. In fact, the degree of reduction is the minimum possible removal of AMCOR mill origin hydrophilic substances, since the measured total hydrophilic substances will contain hydrophilic substances from other sources as well. Since merging inputs are mainly domestic sewage, their hydrophilics may be expected to be dominated by simple aliphatic organic substances, so that they are more likely to be degraded effectively than are AMCOR mill hydrophilics. Therefore, the measured total hydrophilics would most likely be the remaining AMCOR hydrophilics.

The experimental results show that the total hydrophilic substances at Peg 83 are almost equivalent to the expected estimated maximum possible AMCOR mill hydrophilic substances content, which is 73 mgC/L (Fig. 4.3). These hydrophilic substances account for 30% of the total DOC concentration in the medium at Peg 83 (Fig. 4.4). Thus if we assume contributions of other hydrophilics from Rosedale township Mains are readily removed, then hardly any removal of hydrophilic substances from the AMCOR mill discharge has occurred in the LVOS flow down to Peg 83. This assumption may be expected since the inputs are domestic sewage.

The removal of AMCOR mill hydrophilic substances from LVOS flow after Peg 83 seems to increase as it travels to Peg 133 and Inlet No.2 (Fig. 4.3). There is no merging input to LVOS flow after Peg 133, so that any decrease in hydrophilic substances beyond this point must be due to microbial degradation or removal to particulate forms. Degradation of hydrophilics within channel 2 is probably due to mill hydrophilics, since the more

degradable sewage hydrophilics from other sources appear to be removed before reaching the Peg 83 site.

Fig. 4.4 The effect on hydrophilics percentage - LVOS channel and DD treatment ponds



The total hydrophilic substance concentration in the LVOS influent to the DD treatment ponds (Inlet No.2) was 34 mgC/L (Fig. 4.3). The total hydrophilic substances decreased only marginally within the treatment ponds. Their concentration at the exit stage was 26

mgC/L representing a small removal of total hydrophilic substances from the LVOS influent within the treatment ponds of only ~8 mgC/L. In fact, the residual hydrophilics concentration in the treatment ponds seems to be rather higher than expected, as it is generally considered that hydrophilic substances are not very refractory. Apparently most of the degradable hydrophilic compounds have already been removed in the LVOS drain.

At each sampling location the total hydrophilic substances as a percentage of the total DOC and the estimated AMCOR mill hydrophilic substances were computed and the trends are illustrated in Fig. 4.4. The hydrophilic substances in the AMCOR mill discharge were about ~58% of the total DOC at its release stage (Fig. 4.4). As it reaches Sandy Creek Crossing, the estimated contribution of AMCOR hydrophilic substances was estimated to be 49%. As hydrophilics are usually non-refractory, the apparent decrease could be attributed to degradation or active uptake of mill hydrophilics by live biomass.

Table 4.1 Extent of removal of total hydrophilic substances in the DD treatment ponds

Sampling site	Removal of total hydrophilics (mgC/L)	% total hydrophilics removed
Exit Pond A	0	0
Exit Pond B	5	15
Exit Pond C	4	12
Exit Pond D	7	21
Exit No.2	8	24

The fraction of total hydrophilic substances increases within the treatment ponds. That is, it was 23% (Fig. 4.4) as the LVOS influent enters the DD treatment ponds (at Inlet No.2) and 35% in the effluent exits at Exit No.2. When LVOS flow is about to exit from No.2 storage, 83% of the AMCOR mill hydrophilic substances are already exhausted. Only 24% of total

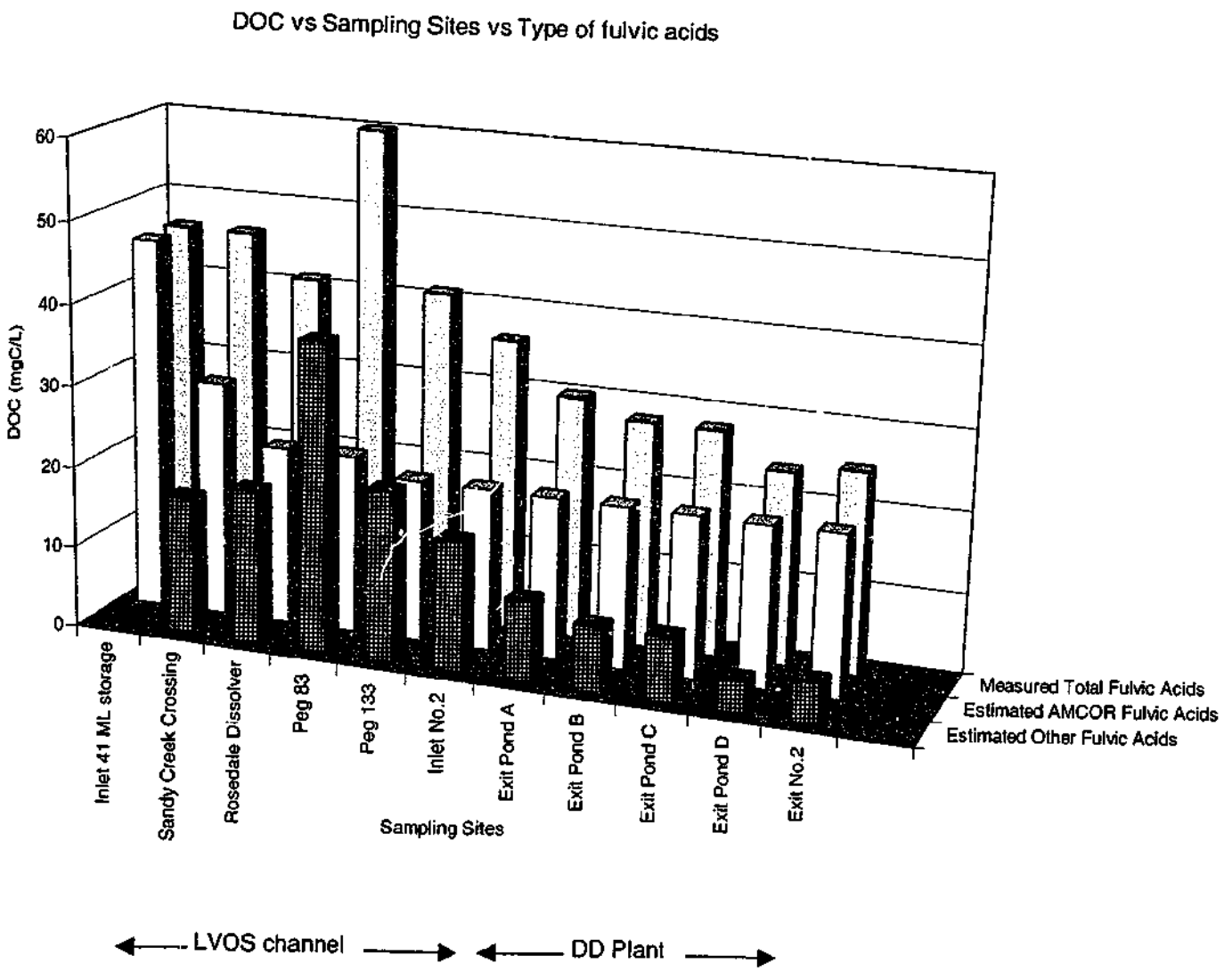
hydrophilic substances present in the LVOS influent entering the DD treatment ponds were removed within the treatment ponds (Table 4.1), whilst 78% of the mill hydrophilics have been removed within the LVOS channel.

The experimental results support the idea that the majority of AMCOR mill hydrophilic substances are non-refractory and are readily removable from the medium by microbial reaction during their transport down the LVOS channel.

4.3.3 Distribution of fulvic acids at AMCOR mill downstream sites

The fulvic acids due to AMCOR mill effluent and the total fulvic acids at downstream sites are illustrated in Fig. 4.5. The total fulvic acids in this study obviously represent both the total concentration due to the mill effluent and those from other input sources. Since dilution at each sampling site can be estimated from the mean flow data of the main channel and its inputs, the minimum contribution of fulvic acids from other inputs to the LVOS flow could be evaluated by assuming no removal or transformation of AMCOR fulvic acids other than dilution. Even though the fulvic acids concentration due to AMCOR mill discharge at downstream sites diminishes due to dilution it appears to be compensated by fulvic acids from merging inputs or from in situ production. This resulted in hardly any observable concentration change of total fulvic acids down the channel. The one notable exception being Peg 83 which shows a large increase due to an external input of allochthonous sources or microbial reaction on particulate matter. Overall the fulvic acids from other sources have increased within channel 1 and then decreased along channel 2 until reaching the DD treatment area.

Fig. 4.5 The effect on fulvic acids - LVOS channel and DD treatment ponds



Three major inputs (Yallourn plus Morwell and Churchill) probably all contribute to the merging fulvic acids at Sandy Creek Crossing. The concentration of these merging fulvic acids should not be less than 17 mgC/L to compensate for the dilution of the AMCOR fulvic acids. Likewise, the Traralgon input upstream of the Rosedale Dissolver site and Rosedale town input upstream of Peg 83 would introduce more fulvic acids to the LVOS

flow. These contributions should not be less than 19 mgC/L and 38 mgC/L respectively. However, whether these additional fulvic acids are from merging inputs or from in situ production is still uncertain. Furthermore, these fulvic acid concentrations are likely to be higher than their estimated values due to removal or transformation of some AMCOR fulvic acids in addition to dilution.

The influence of fulvic acids from merging inputs or from in situ production on the total fulvic acids concentration in the LVOS flow is uncertain since the degradability of AMCOR fulvic acids is unknown. However, a considerable increase in fulvic acid concentration was observed at Peg 83, which would require a high input of fulvic acids into the LVOS flow from allochthonous and/or autochthonous material within the open sewer 1, since there are no inputs between RD and Peg 83.

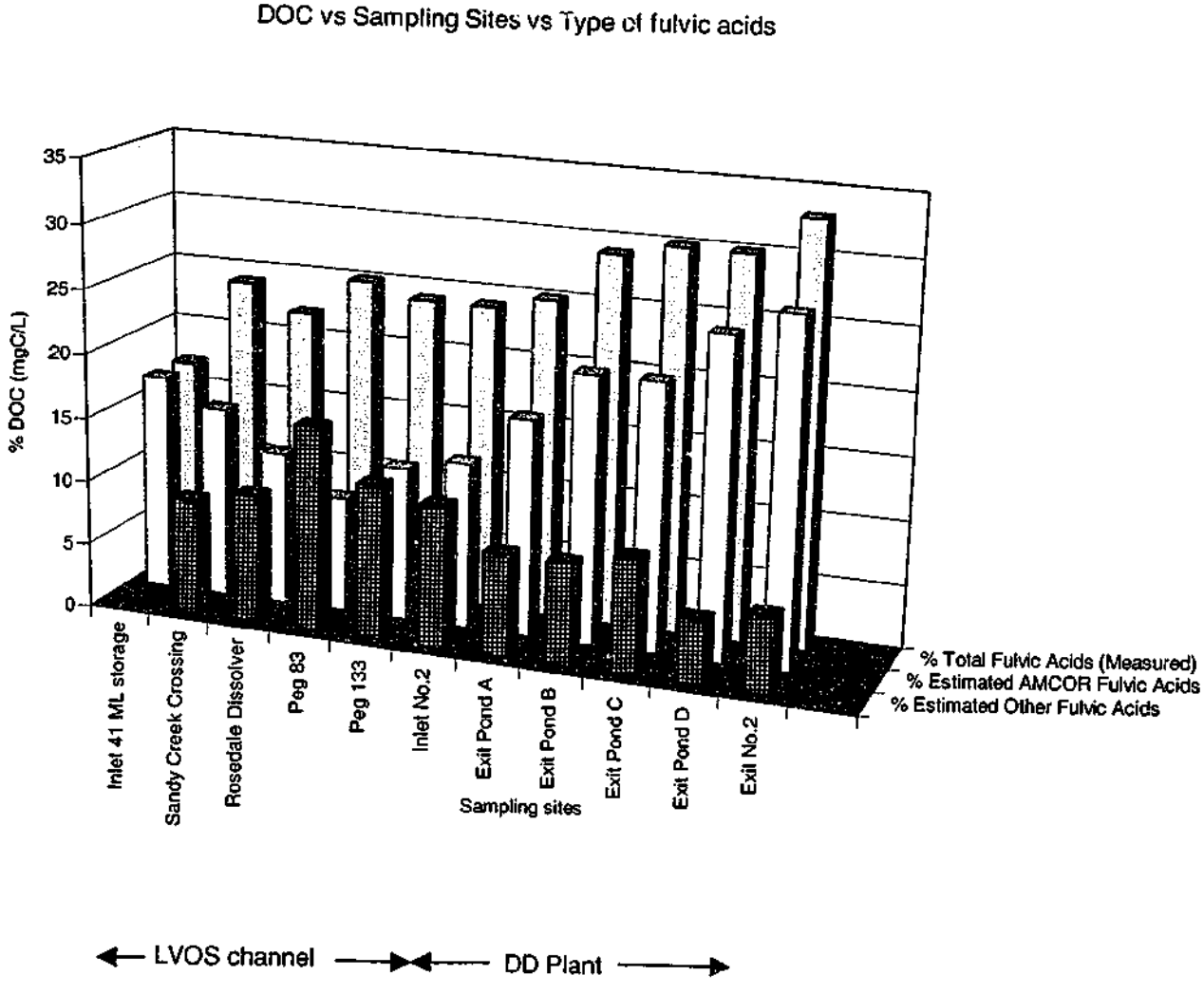
The sites downstream of Peg 83 show a decreasing trend for total fulvic acids until they reach the DD treatment ponds. Between Peg 83 and Peg 133, the Sale Rising Mains merge with the LVOS channel. According to the observed results, it seems the merging input does not increase the total fulvic acids content at Peg 133. In fact, even though this is a major input, the total fulvic acids have decreased between these two sites. It is clear that either the fulvic acids from AMCOR or from other sources in the LVOS flow have started to be removed or degraded between these two sites. Also, it is clear that the fulvic acids from either or both of these sources have degraded between Peg 133 and Inlet No.2, because there are no other inputs diluting the system in this section. The fulvic acids being removed

or degraded are most likely to have originated from merging inputs or from in situ production rather than from the AMCOR effluent.

The total fulvic acid concentration in the LVOS influent that enters the DD treatment ponds through Inlet No.2 is 36 mgC/L (Fig. 4.5). For the DD treatment ponds, the total fulvic acid content and its composition based on the original source are illustrated in Fig. 4.5. However, whether AMCOR fulvic acids are stable and fulvic acids from other sources are decomposing is still uncertain. Since the total fulvic acid concentration seems to be reduced only by a small amount within the treatment ponds, one can conclude that most of them are fairly refractory. Perhaps removal of fulvic acids within the treatment ponds could be due to larger molecules than those molecules removed within channel 2, since adsorption and settling is more favorable in treatment ponds where the water velocity is less.

The percentage of the fulvic acids in the total DOC from both AMCOR and other inputs to the LVOS system were computed and illustrated in Fig. 4.6. It shows the percentage of total fulvic acids is higher within the LVOS channel than in the mill effluent. It seems that after Sandy Creek Crossing the total fulvic acid fraction remains unchanged while the water is transported down the LVOS channel. In fact, the percentage of the fulvic acids gradually increases within the treatment ponds. The percentage of AMCOR fulvic acids could be higher or lower, depending on whether they are refractory or not. If AMCOR fulvic acids were refractory, the fraction of AMCOR fulvic acids in the LVOS effluent at the exit stage (Exit No.2) is twice (27%) that of the LVOS influent (13%) entering the DD complex (Inlet No.2).

Fig. 4.6 The effect on fulvic acids percentage - LVOS channel and DD treatment ponds



Since the concentration of total fulvic acids in the LVOS influent entering the DD treatment ponds and their remaining (residual) concentrations are known, the extent of removal within the treatment ponds can be evaluated (Table 4.2). The results show that 31% of the

total fulvic acids in LVOS influent to the treatment ponds have been removed from the medium when it reaches the exit stage.

Table 4.2 Extent of removal of total fulvic acids in the DD treatment ponds:

Sampling site	Removal of total fulvic acids (mgC/L)	% total fulvic acids removed
Exit Pond A	6	17
Exit Pond B	8	22
Exit Pond C	8	22
Exit Pond D	12	33
Exit No.2	11	31

4.3.4 Distribution of humic acids at AMCOR mill downstream sites

The humic acids were concentrated and precipitated by acidifying the GF/C filtered samples with concentrated HCl acid until pH < 2.0. As acidification proceeds with Peg 83 and Inlet No.2, cloud formation was observed before even achieving pH 2.0. Subsequently a brown amorphous substance settled to the bottom. On completion of the acidification it was noticed that these two samples generated a large amount of precipitate. A similar occurrence, but to a lesser extent, was observed with the Sandy Creek Crossing sample. The rest of the samples do not show instant precipitation of humic acids with acidification. However, somewhat similar brown cloudy precipitates were observed after 24 hours with Rosedale Dissolver, Peg 133 and Exit Ponds A, B and C. The extent of precipitation based on these visual observations was recorded after each 24 hours for 3 days while those acidified samples were stored at 4°C in the refrigerator. The results observed are summarized in Table 4.3.

Table 4.3 The extent of Humic Acids precipitation at pH < 2.0 with AMCOR mill effluent and downstream sites along the LVOS channel and Dutson Downs treatment ponds:

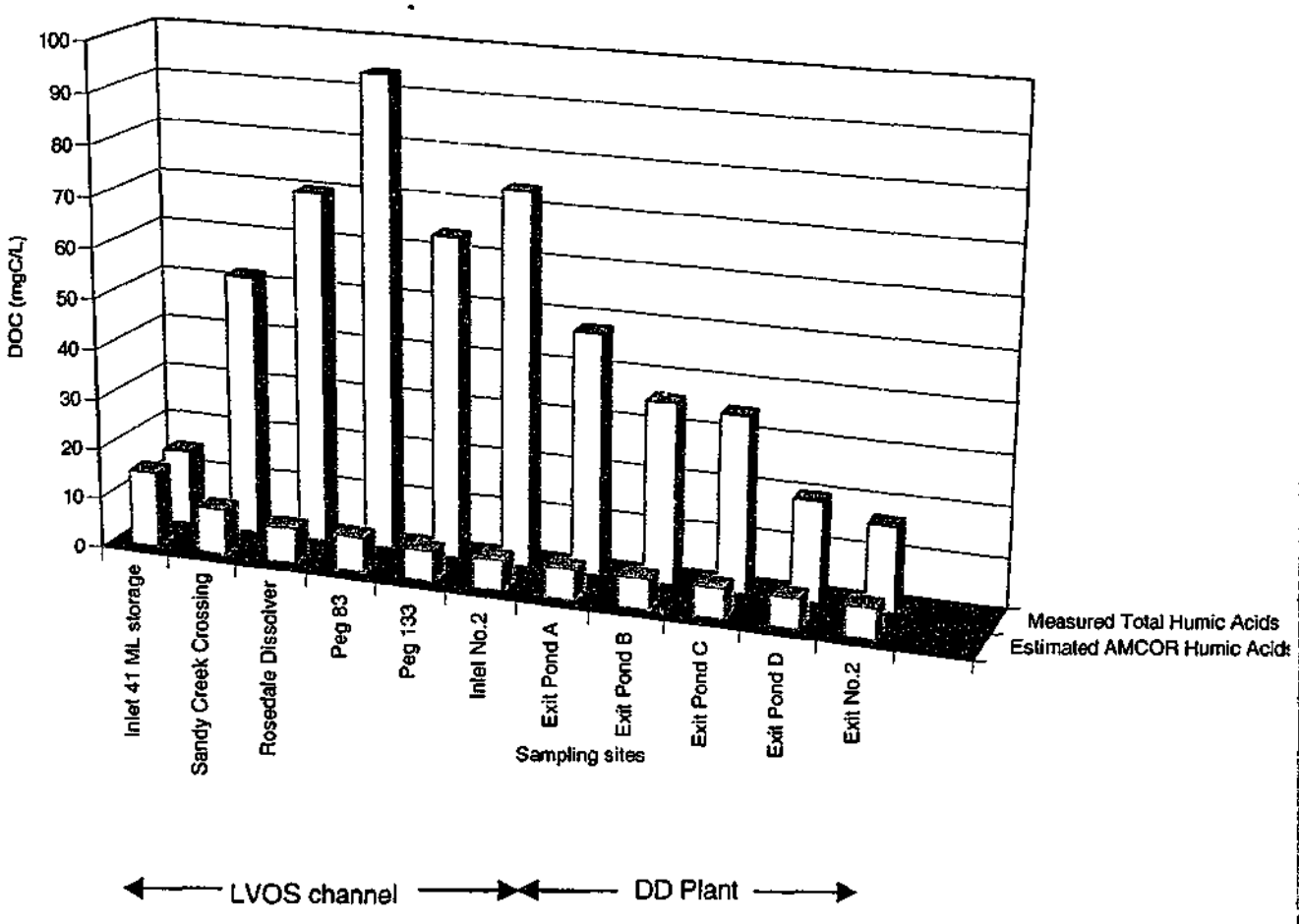
Direct Mill effluent	0 hours	24 hours	48 hours	72 hours
Inlet 41 ML storage	-	-	-	+

Downstream sites at LVOS	0 hours	24 hours	48 hours	72 hours
Sandy Creek Crossing	+	++	+++	+++
Rosedale Dissolver	-	++	+++	++++
Peg 83	++	+++	++++	+++++
Peg 133	-	+	++	+++
Inlet No. 2	++	+++	++++	++++
Exit Pond A	-	+++	+++	++++
Exit Pond B	-	++	++	+++
Exit Pond C	-	++	++	+++
Exit Pond D	-	-	+	++
Exit No. 2	-	-	+	+

The AMCOR mill effluent did not produce a precipitate within the first 48 hours. However, if left for an additional period of up to 72 hours, it started to precipitate very faintly. Hardly any precipitation was observed with the Exit No.2 sample. Concentrations of the total humic acids and contributions from mill effluent and from other sources (calculated assuming AMCOR humic acid behaves conservatively) were evaluated and illustrated in Fig. 4.7.

The concentration of humic acids in AMCOR mill effluent is low (15 mgC/L) compared to that of the AMCOR mill downstream sites. The humic acids due to the AMCOR mill effluent obviously decreases along the channel (towards DD treatment plant) as a result of dilution caused by merging inputs. This dilution effect, should reduce the concentration of

Fig. 4.7 The effect on humic acids - LVOS channel and DD treatment ponds



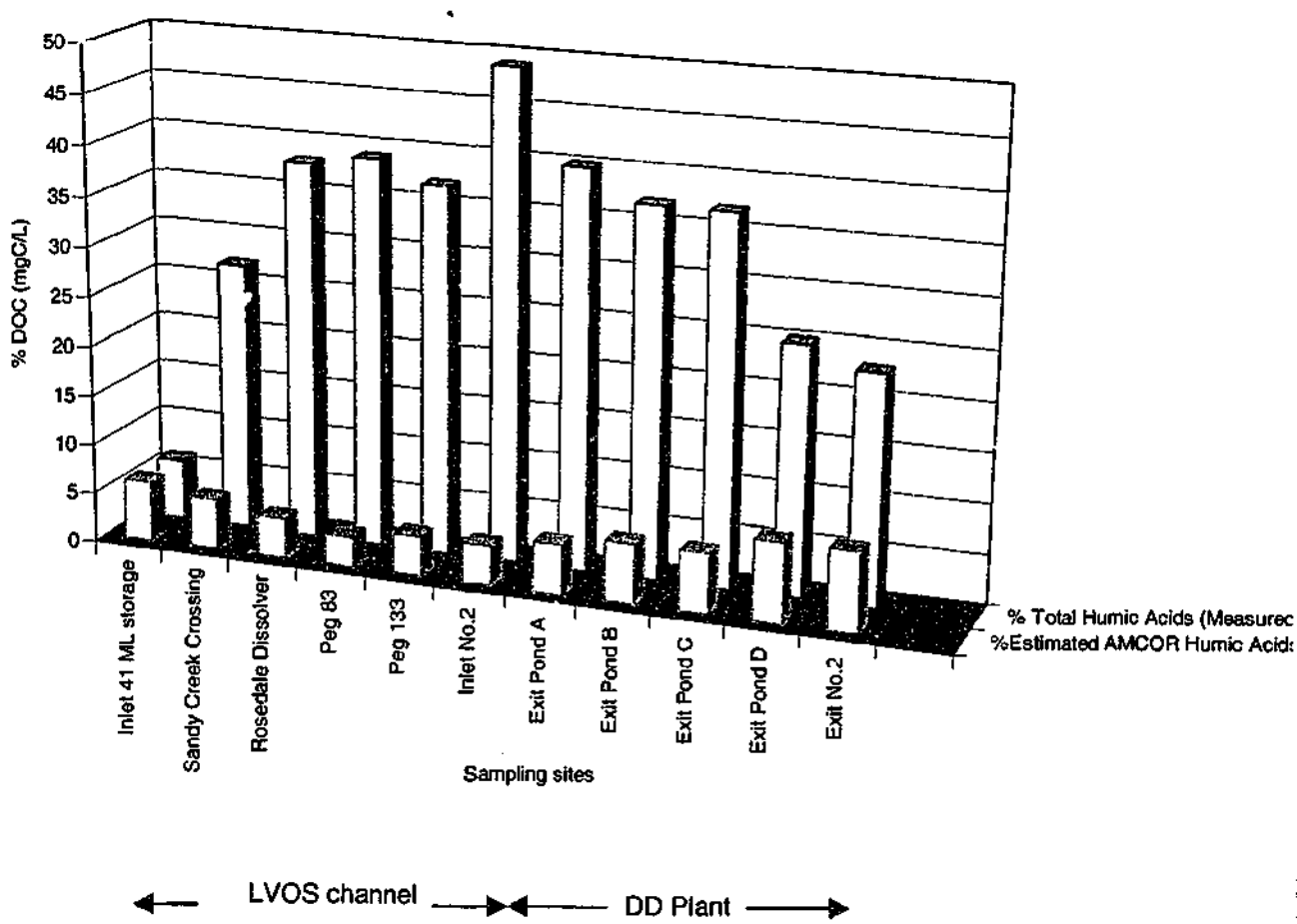
humic acids from AMCOR mill discharge to about 5 - 10 mgC/L, assuming that there is no removal by other processes. This amount is small compared to the total humic acid concentration measured along the channel, suggesting inputs or generation of humic acids in the LVOS drain.

The total humic acid concentration increased along the LVOS channel 1 and remained high within the LVOS channel 2. Fig. 4.7 shows a high concentration of total humic acids at Sandy Creek Crossing and at Rosedale Dissolver, even though humic acids concentration in the AMCOR mill discharge is as low as 15 mgC/L. The rest of the humic acid concentration observed at these two sites must be due to merging inputs or in situ production of humic acids by biological reaction on dissolved and particulate organic matter. Since the merging inputs are mainly domestic sewage, the higher concentrations of other humic acids at downstream sites may be due to humic-like materials in sewage.

The highest concentration of the total humic acids were observed at Peg 83, which was ~94 mgC/L (Fig. 4.7). The estimated contribution from merging inputs or from in situ production is ~87 mgC/L, since the AMCOR humic acids concentration should be only ~7 mgC/L after accounting for the dilution factor (refer to Chapter 3). The higher concentration of other humic acids at Peg 83 may be due to allochthonous and/or autochthonous inputs.

In the DD treatment ponds, the total humic acid content and the estimated contributions from the AMCOR mill discharge and other humic acids are illustrated in Fig. 4.7. The total humic acid concentration in DD ponds decreases towards the exit point of No.2 storage, where its concentration has reduced markedly to 17 mgC/L. Since the estimated mill effluent humic acids concentration is very small (~6 mgC/L) by this stage, the removal of the other humic acids is evident.

Fig. 4.8 The effect on humic acids percentage - LVOS channel and DD treatment ponds



The fraction of total humic acids as a percentage of the total DOC present at each sampling site, and the fractional contributions due to AMCOR mill discharge and other sources, are illustrated in Fig. 4.8. It shows that the percentage of total humic acids in AMCOR mill discharge is 6% of the total DOC value. The fraction of the total humic acids is higher at

the downstream sites and is ~50% as the LVOS flow reaches the inlet point of the DD treatment complex (Fig. 4.8).

The fraction of total humic acids in the total DOC (Fig. 4.8) was 40% at the exit point of pond A (exit pond A) and 23% at the exit point of No.2 storage (exit No.2). The removal of total humic acid during treatment in the ponds (from pond A to pond E), accounts for 77% (Table 4.4).

Table 4.4 Extent of removal of total Humic Acids in the DD treatment ponds:

Sampling site	Removal of total humic acids (mgC/L)	% total humic acids removed
Exit Pond A	26	35
Exit Pond B	38	51
Exit Pond C	39	53
Exit Pond D	54	73
Exit No.2	57	77

4.4 CONCLUSIONS

DOC concentration due to the AMCOR mill discharge gradually decreases along the LVOS channel due to dilution caused by merging township influxes. DOC due to merging inputs increases along the LVOS channel 1. However, DOC concentration at the inlet point of the DD treatment ponds proved that DOC removal occurred within the open sewer of the LVOS channel 2.

The concentration of hydrophilic substances in AMCOR mill effluent is high (154 mgC/L). The mill effluent hydrophilic substances were degraded within channel 2. However,

contributions, if any, from merging inputs were unable to be evaluated since observed concentrations were lower than the estimated AMCOR hydrophilics level. There is no remarkable reduction in hydrophilic substances within the treatment ponds. The residual hydrophilic substances content in the treatment ponds did not change much, and hence they appear to be quite refractory.

The fulvic acids content from other sources such as merging inputs or in situ production increases within channel 1 (from mill to Peg 83). The fulvic acids in the LVOS flow seem to degrade within channel 2. These degrading fulvic acids are most likely from sources other than the AMCOR mill. About 30% of the total fulvic acids in the LVOS flow were removed during the pond treatment.

The humic acid content in mill effluent is as low as 15 mgC/L, but much higher in the merging inputs. Regardless of the AMCOR mill effluent discharge, the volumetric discharge and concentration of the merging township inputs do play a major role in determining the LVOS composition. The major source of the total humic acids entering the DD treatment complex seems to be from other sources, such as in situ production and/or inputs. Almost 77% of the total humic acids was removed during the pond treatment.

Chapter 5

Molecular Weight Characterization Of Chemical Fractions Of A Paper Mill Effluent And Recipient Water

Abstract

The concentrations of hydrophobic organic substances along the LVOS channel and in DD treatment ponds were evaluated using a mass balance approach (Chapter3, Fig.3.6). It increases from the AMCOR mill outlet to the Peg 83 site as inputs merge with the channel. Then it decreases up to the inlet point of the DD treatment plant and through the DD treatment ponds as treatment proceeds. The changes in concentration of total hydrophobic substances along the LVOS channel can be attributed to the variability of merging inputs and/or in situ production. In the treatment complex it can be attributed to treatment effects. These hydrophobic substances were subjected to further study by the analysis of the molecular weight distributions using flow field-flow fractionation.

The merging inputs at piped section 1 contain low molecular weight fulvic acids. Within the piped section 1, these low molecular weight fulvic acids were removed initially, probably by microbes. Later microbial reactions on solids were resulted in high molecular weight fulvic acids. Eventually these high molecular weight fulvic acids were biologically degraded to low molecular weight fulvic acids in the open sewer 1, which were then subsequently removed in the piped section 2, probably by microbes. Transport through the open sewer 2 did not affect any of the fulvic acids.

Both the low and high molecular weight humic acids were introduced into the LVOS flow along the piped section 1 due to the merging inputs. Only the low molecular weight humic acids were preferentially produced by in situ production in the open sewer 1. Both types of humic acids (low and high molecular weights) were removed in the piped section 2, perhaps due to degradation. Transport through the open sewer 2 did not affect any of the humic acids.

Perhaps due to microbial reaction on solids, the high molecular weight fulvic acids were produced in the initial ponds (ponds A and B). The biological processes in pond C did not affect the molecular weight fractions of fulvic acids. The above high molecular weight fulvic acids were then degraded in pond D. The remaining low molecular weight fulvic acids perhaps started to condense at pond E, to form high molecular weight fulvic acids.

The biological processes in ponds initially removed the low molecular weight humic acids (pond A), but later (pond B) removed the high molecular weight humic acids also. Subsequent treatment up to pond C were produced humic acids possessing medium sized molecular weights, perhaps due to microbial reaction on solids. These humic acids were removed at pond D, probably due to adsorption and settling. The high molecular weight humic acids started to degrade into low molecular weight humic acids at pond E.

5.1 INTRODUCTION

The current study has focussed on changes in the molecular weight distribution of two major colored organic fractions along the LVOS channel and in the DD treatment ponds. Molecular weight analyses were undertaken by flow FFF after chemical characterization by XAD-8 resin fractionation. Attempts were made to study which molecular weight fractions of which major organic fractions had been affected by biological/physical processes occurring in the LVOS channel and DD treatment ponds.

5.2 EXPERIMENTAL

Samples from the Latrobe Valley Outfall Sewer System were fractionated into four major dissolved organic carbon groups, depending on the hydrophobic nature of their chemical structure. The substances known as humic acids were isolated by precipitation on increasing the hydrogen ion concentration in the medium. The substances known as fulvic acids were isolated by adsorption onto a polymer of methylmethacrylate called XAD-8 resin and subsequent elution with a base. The non-retained substances at low pH are known as hydrophilic substances. The substances retained on the column after base elution were estimated by difference and known as hydrophobic neutrals. The procedure was outlined in Chapter 4. The unfractionated solutions and coloured fractions isolated in Chapter 4 were subjected to molecular weight analysis by flow FFF.

5.2.1 Molecular weight analysis

Flow FFF was used for molecular weight studies. The method of analysis and theory of the technique was outlined in Chapter 2 (Beckett et.al.,1992). The flow FFF analysis and

instrument calibration was described in Chapter 3. The channel dimensions and flow FFF experimental conditions used in this chapter are given below:

Flow FFF experimental conditions:

Channel flow rate = 5.0 mL / min

Cross flow rate = 5.0 mL / min

Carrier solution = 0.05 M tris + 0.00308 M NaN_3 + 0.0268 M HNO_3

Carrier solution pH = 7.8

Injection delay = 1 sec

Relaxation time = 15 sec

Dead time = 3 sec

Channel void volume = 1.24 mL

Pre-channel dead volume = 0.05 mL

Post-channel dead volume = 0.25 mL

Channel dimensions :

Channel thickness = 0.0246 cm

Channel Length = 22.34 cm

Channel Breadth = 2.04 cm

Height from triangle base to tip = 2.43 cm

Triangle base = 2.0 cm

Calibration parameters :

$\phi = 0.000263$

$n = 0.609$

Calibration Standards :

Poly(styrenesulphonate) of molecular weights having 4500, 6500 and 17500 Dalton

Concentration of standards = 1 mg / mL

The flow FFF fractograms of processed raw solutions (unfractionated) and their precipitated acidic fractions, and XAD-8 adsorbed coloured fractions were recorded. Samples were brought to room temperature and vortex mixed for 1 minute before each injection. The molecular weights and molecular weight distributions of processed raw solutions and their two coloured fractions were computed using flow FFF data software version 2.0 (FFFractionation LLC, SaltLake City, Utah, USA). Flow FFF runs were undertaken in triplicate and relative standard deviations were always less than 5%.

5.2.2 Molecular weight distributions of fulvic and humic acids

The molecular weight distributions of macromolecules present in the raw solutions and in the coloured fractions were measured using flow FFF. The fractions were differentiated based upon solubility and adsorption to XAD-8 resin column at high hydrogen ion concentration. They were prepared for flow FFF analysis as described in Chapter 4 (Section 4.2). The unfractionated samples were comprised of a mixture of four major organic components. These are equivalent to humic acids, fulvic acids, hydrophobic neutrals and hydrophilic substances. Two coloured fractions isolated were humic and fulvic acids. The molecular weight analysis was not performed on the hydrophobic neutral or colourless hydrophilic fractions. The coloured hydrophobic neutral fraction could not be recovered from the XAD-8 column. The molecular weight distribution

profiles of macromolecules present in the system were recorded as the relative mass as a function of molecular weight *Mi*.

5.2.3 Effect of degradation on fulvic and humic acids

The molecular weight distributions and dissolved organic carbon concentrations of the raw samples and fractionated coloured organic material were measured. DOC values corresponding to the total hydrophobic substances were computed by subtracting the DOC concentration of hydrophilics from the DOC concentration of the raw total solution. Since the DOC values corresponding to humic and fulvic acids have been experimentally measured, DOC values corresponding to the hydrophobic neutrals retained by column could be computed. The percentages of humic, fulvic and hydrophobic neutrals in the total hydrophobic substances were estimated.

The measured molecular weight distributions, which are based on UV detection, represent the molecular weight distributions of the hydrophobic substances, since the hydrophilic substances usually do not absorb at 254 nm. The peak areas of the molecular weight distributions should be proportional to DOC concentration of the samples, since no sample dilutions were involved and the same volumes were injected.

The molecular weight distributions of the raw solutions (the mixture of four organic groups) plus the isolated coloured fractions of hydrophobic acids revealed which molecular weight fractions of which major organic groups were affected under the prevailing condition in each section of the LVOS and DD systems.

5.3 RESULTS AND DISCUSSION

5.3.1 Trends in abundance of hydrophobic substances

Since DOC concentrations of the parent solutions and of XAD-8 fractions equivalent to hydrophilic substances are known, the concentration of the total hydrophobic substances were evaluated by mass balance as outlined below:

$$\text{Total DOC of parent sample} = \sum \text{DOC of isolated fractions}$$

Since humic acids have been precipitated first, i.e. before XAD-8 fractionation,

$$[\,DOC_{total}\,] = [\,DOC_{precipitated\ fraction}\,] + [\,DOC_{XAD\text{-}8\ effluent}\,] + [\,DOC_{XAD\text{-}8\ adsorbed\ then\ desorbed\ fraction}\,] + [\,DOC_{XAD\text{-}8\ retained\ fraction}\,]$$

$$= [HA] + [HFi] + [HFoA] + [HFoN]$$

$$[\,DOC_{hydrophobic\ total}\,] = [\,DOC_{precipitated\ fraction}\,] + [\,DOC_{XAD\text{-}8\ adsorbed\ then\ desorbed\ fraction}\,] + [\,DOC_{XAD\text{-}8\ retained\ fraction}\,]$$

$$\text{i.e. } [HFoT] = [HA] + [HFoA] + [HFoN]$$

$$[\,DOC_{hydrophilic\ total}\,] = [HFiT] = [DOC_{XAD\text{-}8\ effluent}]$$

$$[\,DOC_{hydrophobic\ total}\,] = [DOC_{total}\,] - [DOC_{hydrophilic\ total}\,]$$

$$\text{i.e. } [HFoT] = [DOC_{total}] - [HFi]$$

where, HA = humic acid

[HFi] = hydrophilic substances

[HFoA] = hydrophobic acids

[HFoN] = hydrophobic neutrals

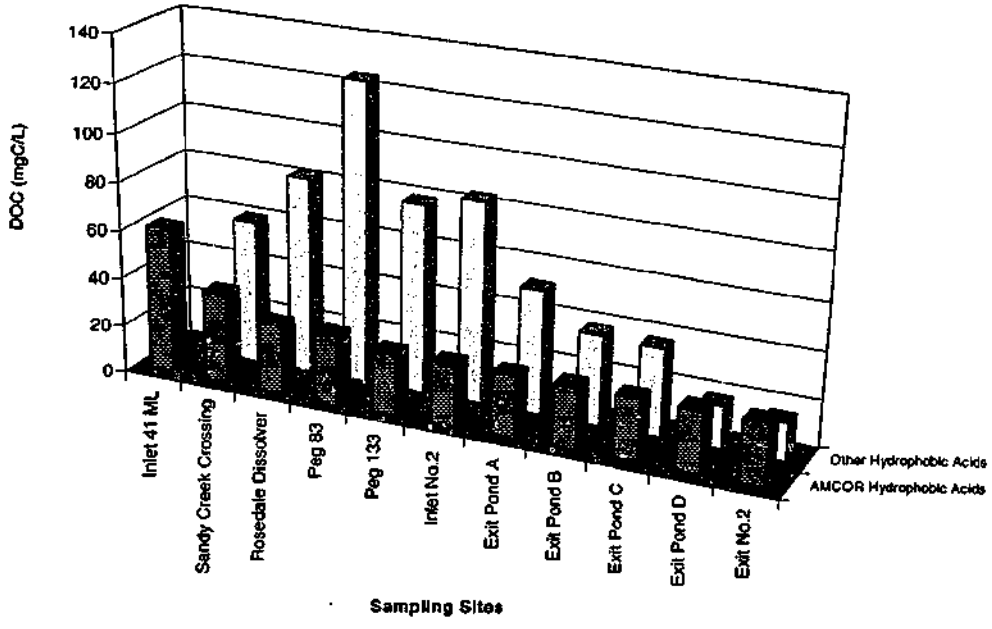
Table 5.1 summarizes the concentrations of hydrophobic substances present in the LVOS channel at different localities. It shows AMCOR mill discharge has 114 mgC/L of total hydrophobic substances (Table 5.1).

Table 5.1 Concentrations of hydrophobic substances and its composition in the LVOS flow at AMCOR mill downstream sites

SAMPLING SITE	TOTAL HYDROPHOBIC SUBSTANCES (mgC/L)	TOTAL HYDROPHOBIC ACIDS (mgC/L)	TOTAL HYDROPHOBIC NEUTRALS (mgC/L)
Inlet 41 ML	114	61	53
Sandy Creek Crossing	113	98	15
Rosedale Dissolver	118	111	7
Peg 83	167	154	13
Peg 133	124	105	19
Inlet No.2	116	110	6
Exit Pond A	86	78	8
Exit Pond B	68	64	4
Exit Pond C	65	63	2
Exit Pond D	53	44	9
Exit No. 2	49	42	7

Though the mill effluent is being diluted due to merging inputs, the concentration of the hydrophobic substances in *channel 1* remains unchanged at early stages (i.e. piped section 1), and then increases at later stages (i.e. open sewer 1). Peg 83 shows a remarkably high content (167 mgC/L) of hydrophobic substances. That is, regardless of the dilution caused by merging inputs the concentration of total hydrophobic substances in the LVOS flow at Peg 83 is higher than the amount of hydrophobic substances in the upstream sections (Table 5.1). In fact, the variations in the open sewer 1 seem to be due to in situ production of hydrophobic substances within the system. The rest of the sites within *channel 2* and within the treatment ponds (from pond A to pond E) show a decreasing trend for the concentrations of hydrophobic substances (Fig.5.1).

Fig. 5.1 DOC concentration of hydrophobic acids at LVOS/DD sampling sites originating from the AMCOR mill or other inputs



The variations observed within *channel 2* can be attributed to subsequent removal of hydrophobic substances by either of the two hypotheses, ie. biodegradation or physical adsorption/settling. The variations observed within the treatment ponds can be attributed to changes in hydrophobic substances due to the effect of biological treatment.

5.3.2 Trends in abundance of fulvic and humic acids

The concentrations of fulvic acids and humic acids present in LVOS flow originating from the AMCOR mill and other merging inputs are known as described in Chapter 4. Fig. 5.1 illustrates the trends in the absolute concentrations of total hydrophobic acids (fulvic acids + humic acids) originating from AMCOR mill or other inputs in samples collected from LVOS channel at various sites. The major assumption was that AMCOR humic and fulvic acids do not degrade or transform along the LVOS channel (Chapter 7, Fig.7.1). Thus, changes in fulvic and humic acid concentrations from the mill effluent are only due to dilution. The calculations based on dilution factors of merging inputs (Chapter 3) revealed that the Peg 83 site contains a large amount of hydrophobic acids originating from other sources than from AMCOR mill discharge (Fig. 5.1). The data shows that these two acids originating from sources other than the AMCOR mill tend to be removed in the later stages of the LVOS channel, i.e. *channel 2* (P83→inlet No.2) and in the DD treatment complex. The calculations are based on the following definitions:

$$[\text{ Substances equivalent to Humic Acids }] = [\text{ DOC}_{\text{precipitated fraction}}]$$

$$[\text{ Substances equivalent to Fulvic Acids }] = [\text{ DOC}_{\text{XAD-8 adsorbed and desorbed fraction}}]$$

$$[\text{DOC}_{\text{hydrophobic acids total}}] = [\text{ DOC}_{\text{precipitated fraction}}] + [\text{ DOC}_{\text{XAD-8 adsorbed and desorbed fraction}}]$$

Note: Humic acids have been precipitated before XAD-8 fractionation.

5.3.3 Molecular weight distributions of unfractionated samples and fulvic and humic acid fractions

The flow FFF technique is capable of high resolution separation of the macromolecules present in the wastewaters. The molecular weight distributions were obtained for the parent (raw) solutions (mixture of all organic groups) as well as for the two major coloured organic groups isolated. The weight (Mw) and number (Mn) average molecular weights with the polydispersity values (Mw/Mn) obtained for unfractionated samples (raw solutions) are given in Table 5.2. All samples were polydispersed with broad molecular weight peaks. There were no peaks due to individual components in the samples.

Table 5.2 Weight average and Number average molecular weights of total organics present in RAW TOTAL SOLUTION – AMCOR mill effluent, downstream sites and Dutson Downs treatment ponds

SAMPLING SITE	Weight av. Mwt. (Mw) (Dalton)	Number av. Mwt. (Mn) (Dalton)	Polydispersity (Mw/Mn)
Inlet 41 ML	7300	3250	2.25
Sandy Creek Crossing	4300	2450	1.76
Rosedale Dissolver	4900	2600	1.89
Peg 83	7050	3200	2.20
Peg 133	5100	2600	1.96
Inlet No.2	5650	2900	1.95
Exit Pond A	5100	2700	1.91
Exit Pond B	5450	3000	1.89
Exit Pond C	6050	3200	1.89
Exit Pond D	6350	3400	1.87
Exit No.2	5800	3200	1.81

These parent solutions were extensively fractionated into four major organic groups by means of solubility at pH <2 and XAD-8 adsorption. The fractions were expected to have reduced polydispersity compared to the raw samples. The weight and number average molecular weights with their polydispersity values obtained for isolated two fractions of coloured major organic groups are given in the Tables 5.3(a) and 5.3(b).

Table 5.3(a) Weight average and Number average molecular weights of FULVIC ACIDS – AMCOR mill effluent and downstream sites plus Dutson Downs treatment ponds

SAMPLING SITE	Weight av. Mwt. (Mw) (Dalton)	Number av. Mwt. (Mn) (Dalton)	Polydispersity (Mw/Mn)
Inlet 41 ML	3650	2200	1.66
Sandy Creek Crossing	4000	2450	1.63
Rosedale Dissolver	7950	4200	1.89
Peg 83	5100	2300	2.22
Peg 133	4850	2600	1.87
Inlet No.2	4000	2300	1.74
Exit Pond A	4750	2800	1.70
Exit Pond B	5800	3250	1.78
Exit Pond C	5900	3250	1.82
Exit Pond D	4700	2800	1.68
Exit No.2	5350	3000	1.78

Both fractions seemed to have lower polydispersity than their parent (raw) solutions (Table 5.2). The fulvic acids are less polydisperse than the humic acids in piped sections (eg. SCC, RD and P133), but have higher polydispersity than the humic acids in open sewers (eg. P83, IN2 and treatment ponds). The humic acid peaks are more skewed than the raw solution peaks. The reason may be due to the isolation by precipitation, which in

fact is supported by conclusions made by Thurman et al. (1982) on work undertaken on aquatic humic substances. That is, the isolation and separation process may change the molecular aggregation state even though the samples were redispersed at pH 8 before FFF analysis in an attempt to generate molecular dispersion.

Table 5.3(b) Weight average and Number average molecular weights of HUMIC ACIDS– AMCOR mill effluent and downstream sites plus Dutson Downs treatment ponds

SAMPLING SITE	Weight av. Mwt. (Mw) (Dalton)	Number av. Mwt. (Mn) (Dalton)	Polydispersity (Mw/Mn)
Inlet 41 ML	7200	4300	1.67
Sandy Creek Crossing	6300	3650	1.73
Rosedale Dissolver	7950	3850	2.07
Peg 83	6550	3450	1.90
Peg 133	7400	3600	2.06
Inlet No.2	6200	3600	1.72
Exit Pond A	8500	4450	1.91
Exit Pond B	7300	4150	1.76
Exit Pond C	7050	4300	1.64
Exit Pond D	6200	3900	1.59
Exit No.2	5500	3700	1.49

The molecular weight distribution peaks of humic acid fractions (Figs. 5.3(c) – 5.12(c)) obtained from the AMCOR mill effluent and the wastewater samples collected from LVOS sites were highly skewed. The weight average molecular weights (Table 5.3(b)) of humic acids are much higher compared to fulvic acids. In such a situation, the molecular weight distributions are of more practical value than the average molecular weights.

However, the molecular weight distribution curves obtained for fulvic acid and humic acid do not correspond quantitatively to that of the raw total wastewaters. One of the reasons was that the concentration factor of humic acid in the isolation of the precipitate from the solution was different to that of fulvic acid in the elution from the XAD-8 column. Also, the above two fractions were not diluted prior to FFF analysis in order to achieve the original concentration in the raw solution. But comparisons of the molecular weight distributions of those two fractions between sampling sites were accepted, since the concentrating factor remains the same for each sampling site.

5.3.4 Wastewater sites within LVOS channel 1

5.3.4.1 Initial phase of piped section 1 (AMCOR mill → SCC)

Figure 5.3 illustrates the molecular weight distributions of raw solution and two isolated coloured fractions (fulvic and humic acids) in the AMCOR mill effluent and a sample collected from a downstream site of the LVOS channel at SCC before oxygenation of the flow. The AMCOR mill effluent has a broader molecular weight distribution than the raw wastewater collected from the SCC site. Also, the molecular weight distribution of AMCOR mill effluent shows a more asymmetric peak with a significant tail than all the other samples. Similar results were observed with the AMCOR mill effluent sample collected in Set 1, six months before (Chapter 3). Also shown is the molecular weight distribution curve of the downstream sample collected from Sandy Creek Crossing, which is less polydisperse compared to the mill effluent.

In 1992 Beckett et al. conducted chemical fractionation and molecular weight distribution analysis of pulp mill effluents from different process streams. They found molecular weight distribution of the total hydrophobic acid fraction (humic acid plus fulvic acid) was almost identical to that of the total effluent. This similarity in molecular weight distribution was considered to be due to the low absorbance at 254 nm of the hydrophilic substances.

In this current study, even though it is assumed that the molecular weight distributions of the total raw solutions are mainly due to hydrophobic substances, the XAD-8 fractionated hydrophilics had some absorbance at 254 nm (Table 5.4).

Table 5.4 The maximum absorbances in the UV range observed with hydrophilic fractions isolated from AMCOR mill effluent and downstream sites along the LVOS channel plus treatment ponds in the DD treatment complex

SAMPLING SITE	λ_{max} (nm)	Absorbance at λ_{max} (nm)
Inlet 41 ML	302	2.36
Sandy Creek Crossing	302	2.65
Rosedale Dissolver	302	0.62
Peg 83	303	2.49
Peg 133	301	0.68
Inlet No.2	305-296	3.70
Exit Pond A	301	0.62
Exit Pond B	302	2.88
Exit Pond C	310-290	3.70
Exit Pond D	301	3.65
Exit No.2	302	2.70

Figure 5.3(a) reveals that the high molecular weight (> 6000 Dalton) hydrophobic component in the raw AMCOR effluent is being removed from the medium as the effluent is transported to the SCC site. An explanation for the above observation (Fig.5.3(a)) is that the high molecular weight (> 6000 Dalton) hydrophobic neutrals in the raw AMCOR effluent (Table 5.1) were being removed from the medium by means of biological degradation as the effluent is transported to the SCC site. However, the low molecular weight (< 6000 Dalton) material increased during the same time. The explanation for the increase in lower molecular weight (< 6000 Dalton) material is that the merging inputs from the Yallourn North plus Morwell and Churchill pipelines may carry hydrophobic substances with a higher proportion of molecular weights < 6000 Dalton. An alternative explanation for this observation is that formation of low molecular weight hydrophobic material occurs due to in situ production.

Whether these variations have occurred in the humic acid and/or fulvic acid is addressed by molecular weight distributions (Figs. 5.3(b) and 5.3(c)) of these two isolated coloured fractions. This reveals that fulvic acids and humic acids from other sources possess a higher proportion of low molecular weights (< 6000 Dalton). The other sources are either inputs merging from townships carrying domestic sewage or transformations from other organic groups such as hydrophobic neutrals. The low average molecular weight in the raw wastewater at SCC is most probably due to low molecular weight fulvic and humic acids contributed from other sources.

Figure 5.3(c) shows that even though the molecular weight distribution of humic acids at Sandy Creek Crossing is skewed to 25,000 Dalton, the bulk of the humic acid molecules are of lower molecular weights (< 10000 Dalton). The unfractionated parent solution of Sandy Creek Crossing (Fig. 5.3(a)) does not show the presence of any high molecular weights (> 15000 Dalton) material as in the humic acid distribution. The reason may be the precipitation, which was used to isolate humic acids from the parent solutions. In order to precipitate the humic acids, it must aggregate. If some of these aggregates were not completely disaggregated when the humic acid sample was redissolved, a polydisperse molecular weight distribution could result.

Thurman et al. (1982) also suggested the method of isolation of humic acids (i.e. precipitation) as a reason for the high polydispersity in humic acids due to aggregation. They observed more than one radii of gyration with soil humic acids isolated by precipitation and also found very polydispersed systems. They noted that this was not the case in aquatic fulvic acids where more monodispersed systems were obtained which had a single radius of gyration.

Previous research by Cameron et al. (1972) on the molecular weight of humic acids by sedimentation and diffusion measurements indicated that although tris buffer greatly reduced gel-solute interaction with Sepharose gels as proposed by Swift and Posner (1971), it did not completely eliminate this effect. Similarly the broadening of the retention volumes obtained in the flow FFF fractograms for humic acids in this sample,

as well as in other site samples, could possibly be due to membrane-solute interaction rather than real sample polydispersity.

Table 5.5 Relative abundance of organic substances equivalent to four major organic groups isolated from AMCOR mill effluent and downstream sites plus Dutson Downs treatment ponds

SAMPLING SITE	% Humic Acids	% Hydrophobic Neutrals	% Fulvic Acids	% Hydrophilics
Inlet 41 ML	6	19	17	58
Sandy Creek Crossing	27	7	24	42
Rosedale Dissolver	38	4	22	36
Peg 83	39	6	25	30
Peg 133	37	11	24	28
Inlet No.2	49	24	24	23
Exit Pond A	40	7	25	28
Exit Pond B	37	4	29	30
Exit Pond C	37	1	30	32
Exit Pond D	25	11	30	34
Exit No.2	23	9	33	35

In the current project the mill effluent collected from the AMCOR plant was found to contain 58% of the total DOC in the hydrophilic fraction and 42% in the hydrophobic fraction (Table 5.5). It was noted that 19% of the total DOC in the mill effluent was not recovered with NaOH elution. Sjöström et al. (1990) has conducted studies using pulp extract leached from Norway Spruce by water (pH 8) at 80°C for 6 hours. They found 50% of the TOC was in the hydrophilic fraction and that only about 40% of the TOC was retained by the XAD-8 resin at pH 2. They also noted ~30% of the hydrophobic material

adsorbed to XAD-8 resin was not recovered ' NaOH elution. According to Leenheer's scheme (1981) this non-recovered fraction corresponds to hydrophobic neutrals.

The percent abundances given in Table 5.5 show that the hydrophobic neutrals were reduced from 19% to 7% between Inlet 41 ML (mill effluent) and SCC. The disappearance of high molecular weights in mill effluent as it reaches the SCC site (Fig. 5.3(a)) could be attributable to a reduction of the hydrophobic neutrals. However, whether they have been degraded to the observed extent or transformed to another organic fraction/s is questionable at this stage.

The eluate collected after passing through XAD-8 resin at pH 1 in fact probably contains some hydrophobic substances which are ionized at pH < 2.0, which would be defined as hydrophobic bases (Sjöström et al., 1990). They observed the size exclusion chromatogram of the neutral hydrophilic fraction had two major peaks. They suggested from IR spectroscopic evidence that the second of these two peaks was due to neutral polysaccharides and their SEC retention time corresponded to an apparent molecular mass range from 3000 to 200,000 Dalton. According to the Leenheer scheme (1981), the hydrophilic substances are defined as those not retained by XAD-8 resin at pH 2 and this effluent actually contains all three hydrophilic fractions (acids, bases and neutrals) plus the hydrophobic bases. The presence of hydrophilic neutrals in the hydrophilic fraction could be responsible for the higher molecular weight distribution observed with raw mill effluent because it contains all three hydrophilic fractions including the hydrophilic neutrals.

Aquatic hydrophilic substances contain a fairly high proportion of small molecules, which are usually assumed to be UV non-absorbing materials. These substances are expected to be composed of simple molecules of sugars, amino acids, etc. It was found that the AMCOR mill effluent hydrophilic substances contain UV absorbing character (Table 5.4). Perhaps some of these may be structurally complex molecules with high molecular weights. These high molecular weight UV absorbing hydrophilic substances could give rise to a higher average molecular weight in the raw solutions.

Since 47% of the total hydrophobic substances were hydrophobic neutrals (19% of the total DOC), the presence of hydrophobic neutrals also could be responsible for the higher molecular weight distribution observed with raw mill effluent.

5.3.4.2 Latter phase of Piped Section 1 : Two wastewater sites before and after oxygenation (Sandy Creek Crossing → Rosedale Dissolver)

The variation of molecular weight distribution from SCC to RD in the raw solutions and the fulvic/humic acid fractions is illustrated in the Fig. 5.4. Some of the material in the lower molecular weight range (Mi < 10000 Dalton) in the raw solution has been diminished from the medium. Most of the removed material was < 4000 Dalton fulvic acid. The high molecular weight fractions in fulvic/humic acids curves (Figs 5.4(b) and 5.4(c)) increased in the RD sample and their weight average molecular weights were increased (Tables 5.3(a) and 5.3(b)). Since there was no removal of DOC from the hydrophobic acids (Chapter 4) between these two sites, Fig.5.4(b) may suggest molecular condensation of fulvic acids.

5.3.4.3 Open Sewer 1 : (Rosedale Dissolver → Peg 83)

The molecular weight distributions of raw (i.e. unfractionated) solution and two isolated fractions (fulvic and humic) at the end point of the Open Sewer 1 (Peg 83) are compared with a site within the Piped Section 1 (Rosedale Dissolver) in Fig. 5.5. The molecular weight fractions over the entire molecular weight range have markedly increased in the raw solution at Peg 83 (Fig.5.5(a)). The concentration of hydrophobic substances at Peg 83 was significantly higher compared to the upstream site (Table 5.1). There is a close similarity in the molecular weight distributions for the raw sample and the humic acid. Thus it seems that the molecular weight distribution of the raw total solution at Peg 83 has been mainly affected by the humic acid (Fig.5.5(c)) rather than by fulvic acid.

The molecular weight distribution of fulvic acid (Fig.5.5(b)) shows that the mass of material in molecular weight classes whose $Mi < 4000$ Dalton has increased at Peg 83 and the mass with $Mi > 4000$ Dalton has decreased. Thus the fulvic acids originating from other sources must have a higher proportion of smaller molecules with $Mi < 4000$ Dalton than the original AMCOR fulvic acids. Perhaps, some of the high molecular weight fulvic acids were biologically degraded to low molecular weight fulvic acid or simply dissociated into smaller molecules rather than being removed from the medium because there was no removal of fulvic acid DOC between these two sites (Chapter 4).

5.3.5 Wastewater sites within LVOS channel 2

5.3.5.1 Piped Section 2 (Peg 83 → Peg 133)

The molecular weight distribution of raw solution (unfractionated) and two coloured fractions at the start point and end point of the Piped Section 2 (Peg 83 and Peg 133) is illustrated in Fig. 5.6. There is a general reduction in organic matter over the entire molecular weight range (Fig.5.6(a)). The high molecular weight range (Mi > 6000 Dalton) in the raw solution was mainly affected by humic acids, whereas the low molecular weight range (Mi < 6000 Dalton) was affected by both humic and fulvic acids. The decreased hydrophobic material (Table 5.1) in the raw solution can be attributed to the removal of low molecular weight fulvic acid and both low and high molecular weight humic acids (Fig.5.6(b) and Fig.5.6(c)).

The reduction in low molecular weight (< 6000 Dalton) fulvic acids in the piped section 2 were perhaps due to biological degradation. The removed low molecular weight fulvic acids may be from other sources (Chapter 4, Fig.4.5), since experiments reported in Chapter 7 showed that AMCOR fulvic acids did not change due to aerobes (Chapter 7, Fig.7.1).

5.3.5.2 Open Sewer 2 (Peg 133 → Inlet No.2)

The raw solutions (ie. unfractionated) and fulvic/humic acid fractions of wastewater samples collected from two end points of the open sewer 2 analyzed for molecular weight distributions are illustrated in Fig. 5.7. Generally there is little change in the molecular weight distributions. The molecular weight fraction > 2600 Dalton has increased slightly

in the raw wastewater and in the humic acid fraction. This observation probably does not represent a significant difference. There were no merging inputs between these two sites and in situ production. Certainly these changes were marginal compared to the changes observed in the upstream sections, as discussed above. There were no differences in the molecular weight distributions of fulvic acids.

5.3.6 Wastewater sites within treatment ponds

5.3.6.1 Pond A Treatment (Inlet No.2 → Exit Pond A)

The molecular weight distributions of raw (unfractionated) wastewater taken from the inlet point of the No.2 storage and exit point of the treatment pond A are illustrated in Fig. 5.8(a). It shows all the molecular weight fractions diminish over the entire molecular weight range from 1000 to 20,000 Dalton (Fig. 5.8(a)) which would be attributable to the removal of hydrophobic substances, as these are detected by UV absorbance and DOC was removed from this fraction (Table. 5.1). Probably a higher proportion of the removed hydrophobic substances may be due to humic acid (Fig.5.8(c)) with molecular weights < 10,000 Dalton. Also, Fig. 5.8(c) suggests that it was not simply due to dilution since the high molecular weight fraction (> 10,000 Dalton) of the humic acid remains unchanged.

It is apparent that the molecular weight distribution of the raw total wastewater has been mainly affected by the removal of humic acid fraction and not by fulvic acid fraction. The biological treatment in pond A does not seem to remove any large fulvic acid molecules (Fig. 5.8(b)). Instead, an increase in the high molecular weight fractions was observed. However, the removed fulvic acids (Chapter 4, Fig.4.5) in pond A may be

smaller molecules with *Mi* < 500 Dalton. The apparent high molecular weight fractions in fulvic acid fraction at Exit Pond A (Fig.5.8(b)) may suggest some molecular associations during the treatment in pond A.

5.3.6.2 Pond B Treatment (Exit Pond A → Exit Pond B)

The molecular weight distribution of the raw wastewater taken from exit points of pond A and pond B (Fig. 5.9(a)) has revealed that molecular weight fractions with *Mi* < 2000 Dalton have diminished while fractions with *Mi* ~ 2000-17000 Dalton have increased during the treatment in pond B. Figs. 5.9(b) and 5.9(c) show that the changes in the molecular weight distributions of the raw solution was mainly due to fulvic acids and not due to humic acids. The relative abundance of fulvic acid increases while humic acid decreases during the treatment in pond B (Table 5.4). It seems that high molecular weight humic acid is removed in pond B

5.3.6.3 Pond C Treatment (Exit Pond B → Exit Pond C)

The molecular weight distributions of the raw wastewater taken before and after pond C treatment (exit points of pond B and pond C) and their fulvic/humic acid fractions are illustrated in Fig. 5.10. The masses over all molecular weight fractions in raw watewater appear to have increased slightly (Fig. 5.10(a)). The molecular weight distribution of fulvic acid (Fig. 5.10(b)) did not change. However, the molecular weight distribution of humic acid shows that the mass of molecular weight fractions ~ 2000-15000 Dalton (Fig. 5.10(c)) increases slightly. The raw solution seems to be affected by humic acid rather

than fulvic acid during the treatment in pond C. However, the above changes did not seem to be very significant.

5.3.6.4 Pond D Treatment (Exit Pond C → Exit Pond D)

The molecular weight distributions of raw wastewater and the hydrophobic acid fractions before and after Pond D treatment are illustrated in Fig. 5.11. The molecular weight distribution of fulvic acid (Fig. 5.11(b)) shows some decrease of material with molecular weights > 2000 Dalton. The decrease of humic acid (Fig. 5.11(c)) was quite noticeable over the entire molecular weight range with molecules of Mi > 18000 Dalton almost disappearing from the medium. However, this observation does not occur in the raw solution (Fig. 5.11(a)) as a result of treatment in the pond D. In fact the molecular weight distribution curves of the raw solutions before and after pond D treatment are almost superimposable, except for a marginal decrease for Mi < 6000 Dalton. Since the Mi > 6000 Dalton fraction of both samples are so similar, it can be inferred that the probable reduction of hydrophobic acids (Fulvic + Humic) is compensated by an increment of total hydrophobic neutrals. Table 5.4 shows that there is an increase in the proportion of hydrophobic neutrals from 1% in pond C to 11% in pond D.

5.3.6.5 Pond E Treatment (Exit Pond D → Exit Pond E)

The molecular weight distribution of raw solution and fulvic/humic acid fractions before and after pond E treatment is shown in Fig. 5.12. The molecular weight distribution peaks for the two raw samples are almost superimposed. The fulvic acid (Fig. 5.12(b)) shows that there is no significant difference except in the range of molecular weight fractions,

which is *Mi* < 6000 Dalton. The humic acid (Fig. 5.12(c)) shows that the molecular weight fractions between 1000-10000 Dalton have significantly increased. The raw solutions seem to be affected by both fulvic and humic acids with the opposing trends canceling each other out in the raw solutions.

5.4 CONCLUSIONS

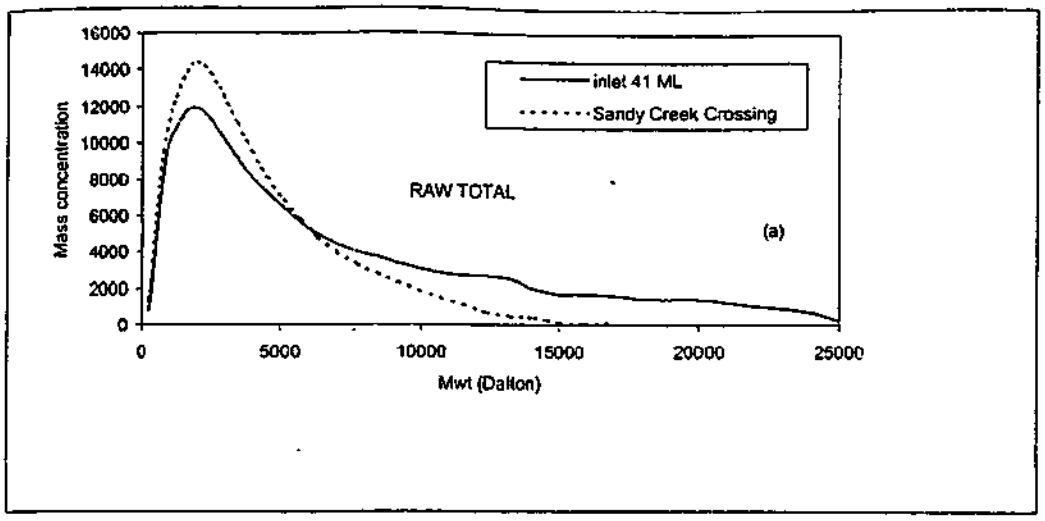
The molecular weight distribution of the AMCOR mill effluent is broader than the mill effluent receiving water. The mill effluent possesses a higher proportion of molecular weights >6000 Dalton, while those from merging inputs (Yallourn North plus Morwell and Churchill pipelines) possess a higher proportion of molecular weights <6000 Dalton. The increased low molecular weight (< 6000 Dalton) hydrophobic substances in the initial phase of the LVOS piped section 1 (AMCOR mill → SCC) could be due to both fulvic and humic acids of merging inputs or in situ production. The high molecular weight hydrophobic substances were preferentially removed in the initial phase of the LVOS piped section 1 while low molecular weight hydrophobic substances were removed in the latter phase (SCC → RD) of the same piped section. However, neither fulvic acid nor humic acid of molecular weights > 6000 Dalton were removed during the initial phase of the LVOS piped section 1. Therefore, the removed high molecular weight fractions (*Mi* > 6000 Dalton) may be hydrophobic neutrals in the mill effluent, since the hydrophobic neutrals make up a considerable proportion (47%) of the total hydrophobic substances in the AMCOR effluent.

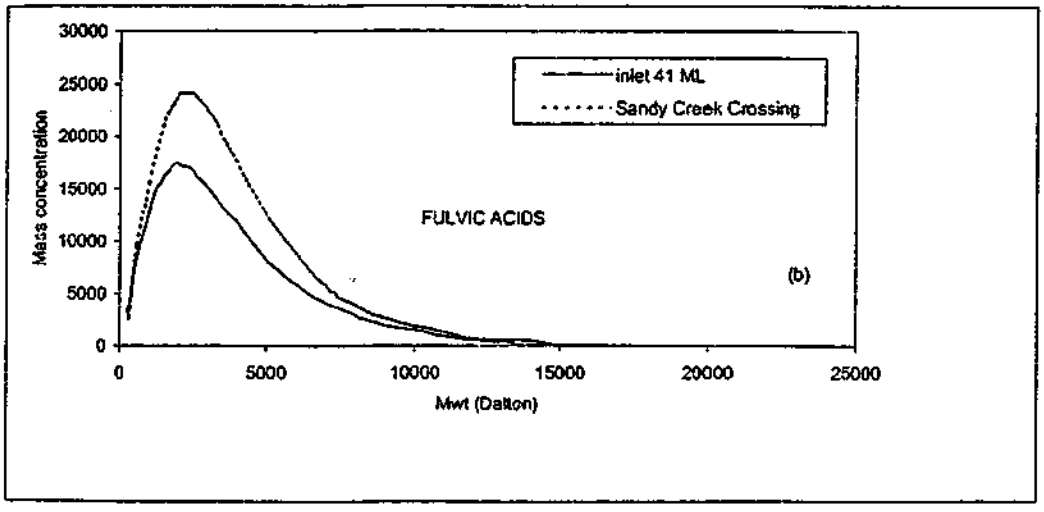
Most of the increase in low molecular weight (<4000 Dalton) hydrophobic substances occurring in the open sewer 1 (Peg 83) was due to fulvic acids, whereas the increase in high molecular weight (>4000 Dalton) hydrophobic substances was due to humic acid. The removal of low molecular weight hydrophobic substances in the piped section 2 (P83 → P133) was due to both fulvic and humic acids, while the removal of high molecular weight hydrophobic substances were humic acid. Transport through the open sewer 2 section (P133 → IN2) of the LVOS channel did not seem to affect the hydrophobic substances significantly.

The low molecular weight fulvic acid (<4000 Dalton) fraction increase within the initial phase of the LVOS piped section 1 may be from other sources, such as in situ production and/or merging inputs. These low molecular weight fulvic acid (<4000 Dalton) fractions degraded during the latter phase of the LVOS piped section 1; where as the high molecular weight fulvic acid (>4000 Dalton) fractions increased due to merging inputs and/or in situ production. These high molecular weight (>4000 Dalton) fulvic acid fractions were removed within the open sewer 1; whereas the low molecular weight (<4000 Dalton) fulvic acid fractions increase again may be due to in situ production, since there were no merging inputs at open sewer 1. These low molecular weight fulvic acid (< 4000 Dalton) fractions were perhaps biologically degraded within the piped section 2 of the LVOS channel, where the high molecular weight fulvic acids only slightly increased and seemed to be removed at open sewer 2.

The AMCOR humic acid possesses molecular weights up to about 20000 Dalton; whereas the bulk of the humic acids originating from the various other sources within the initial phase of the LVOS piped section 1 have molecular weights < 10000 Dalton, although some material >10000 Dalton were also present. The increase in the total molecular weight distribution by hydrophobic substances at open sewer 1 was mainly due to in situ production of humic acids. The molecular weight distribution of hydrophobic substances within the LVOS piped section 2 remains the same, which could simply be due to dilution of humic acid as a result of merging water from Sale Rising Mains. There were no significant variations observed in the molecular weight distributions of the total hydrophobic substances within the open sewer 2.

During the pond A treatment, the low molecular weight humic acids were removed; whereas the high molecular weight humic acids were removed during the pond B treatment. However, the high molecular weight fulvic acids were formed during the treatments in ponds A and B. The slight increase in molecular weight distribution observed during the treatment in pond C was due to in situ production of humic acid. The fulvic acid molecular weight distribution was not changed by the treatment in pond C. During the treatment in pond D, high molecular weight fulvic and humic acids were removed, but the molecular weight distribution of the total raw wastewater did not change during the treatment in pond D due to the formation of hydrophobic neutrals. There were hardly any changes to the hydrophobic substances due to pond E treatment.





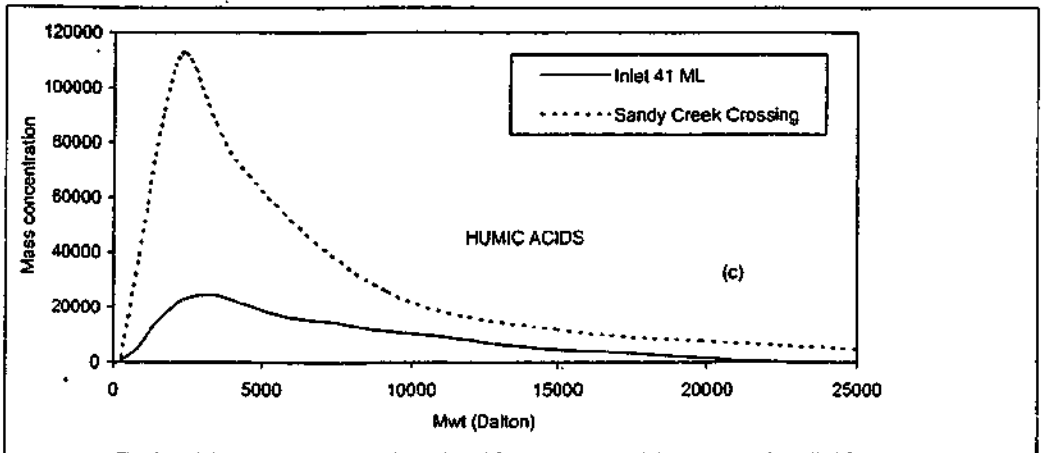
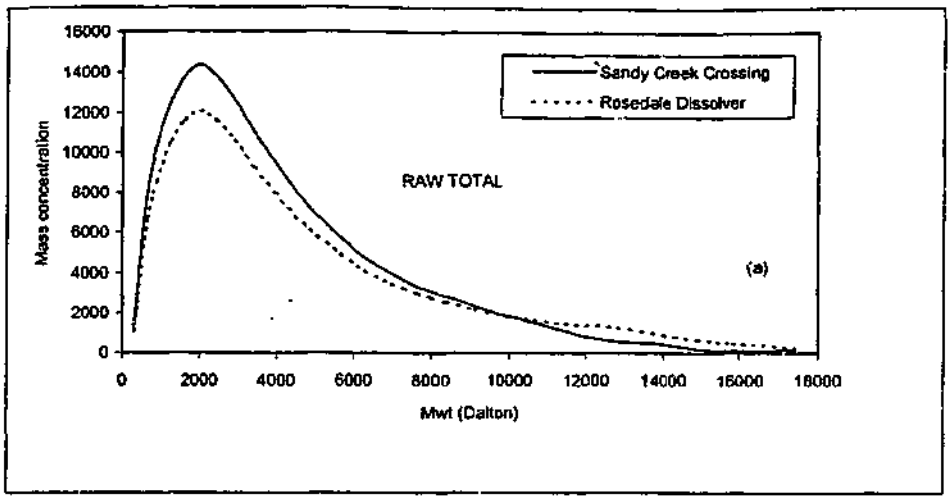
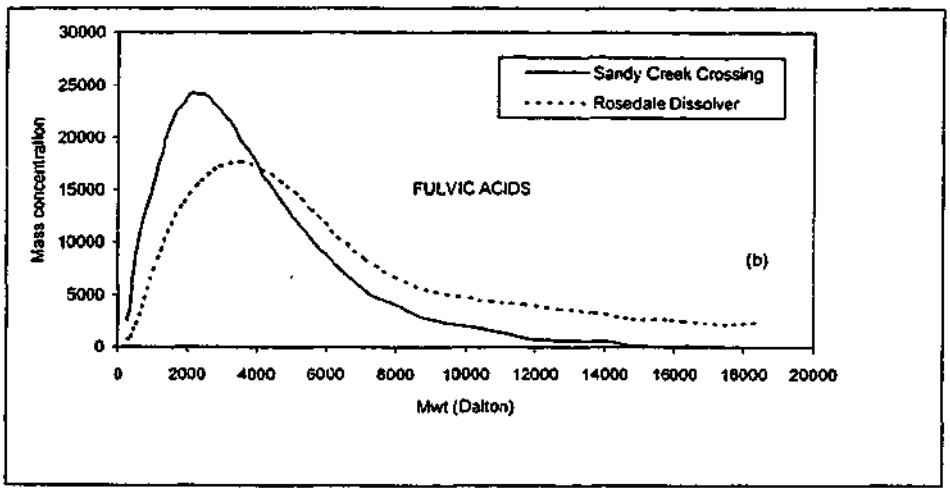


Fig. 5.3 Molecular weight distributions of AMCOR mill effluent and downstream site called Sandy Creek Crossing





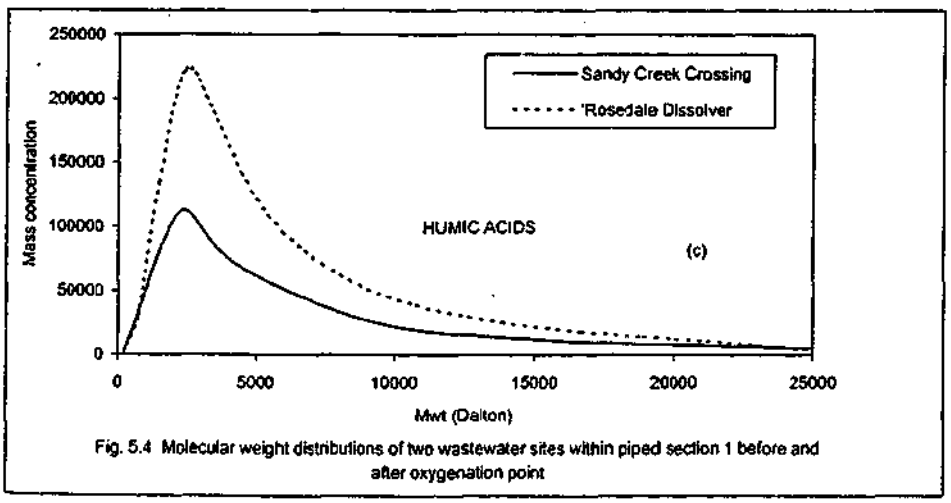
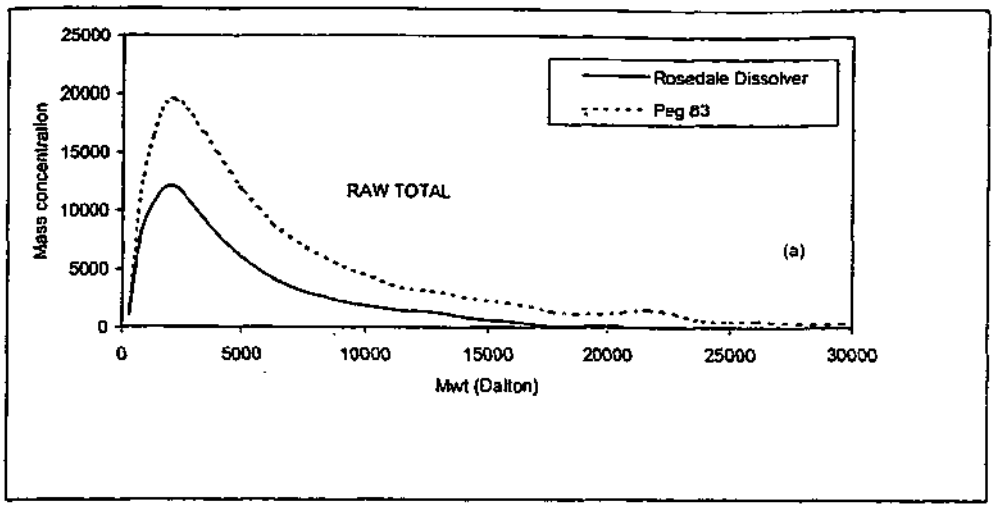
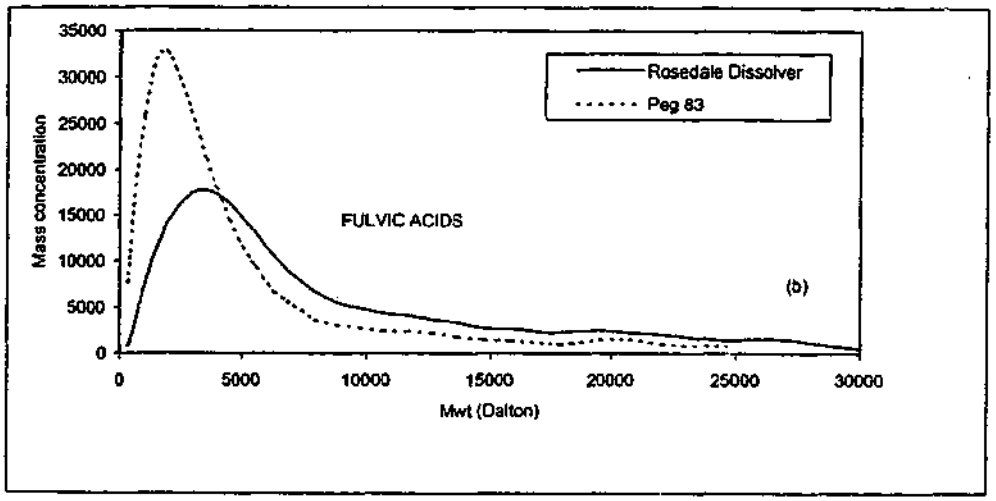


Fig. 5.4 Molecular weight distributions of two wastewater sites within piped section 1 before and after oxygenation point





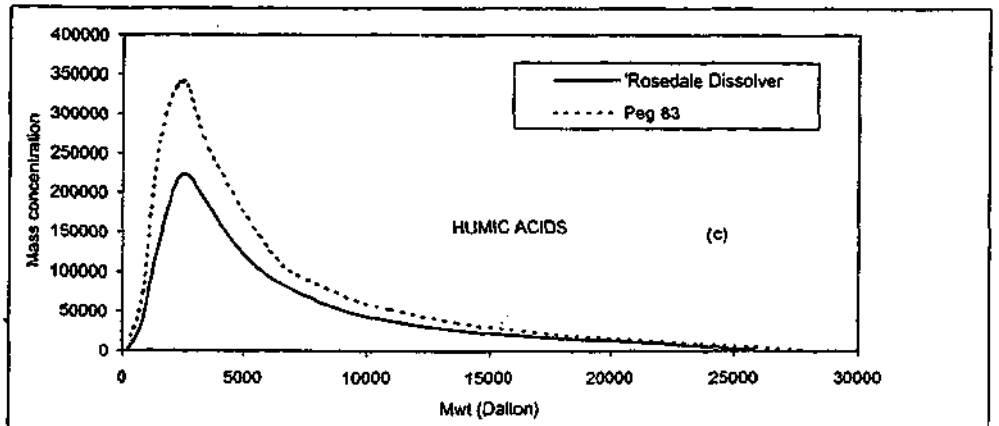
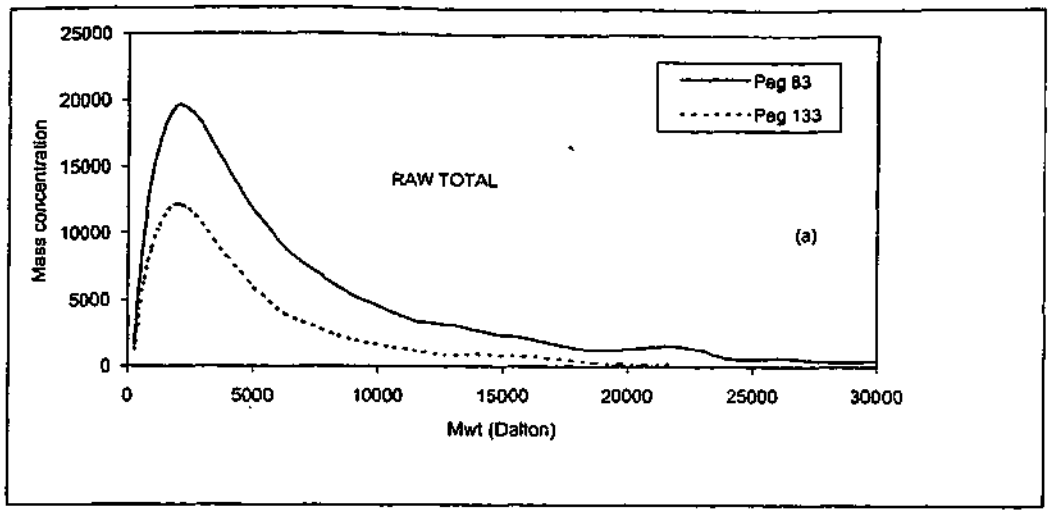
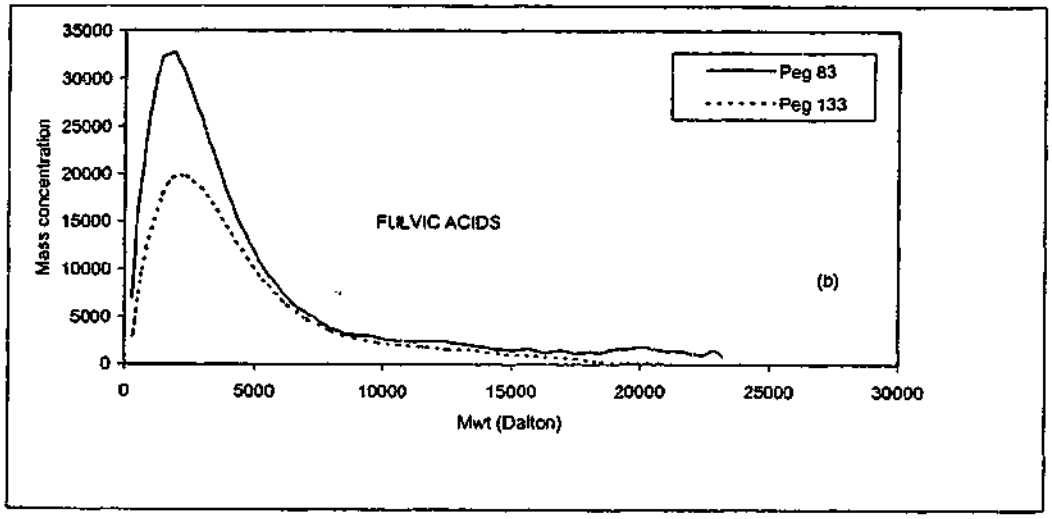


Fig. 5.5 Molecular weight distributions of wastewater collected from AMCOR mill downstream sites called Rosedale Dissolver and Peg 83





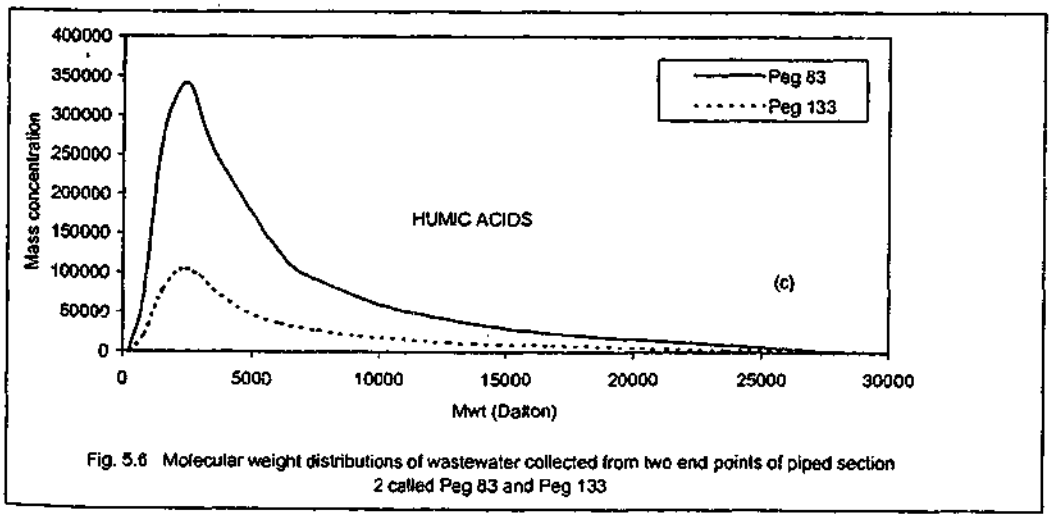
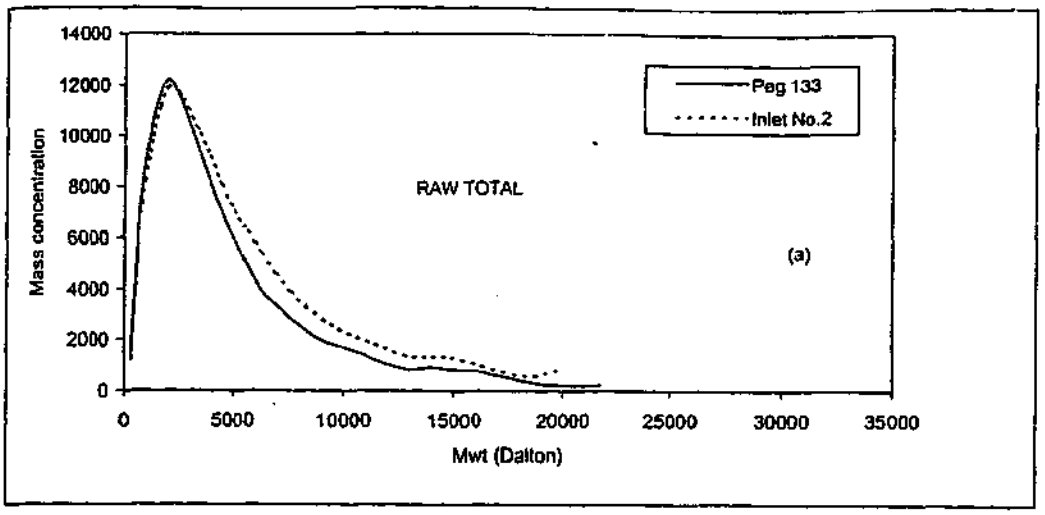
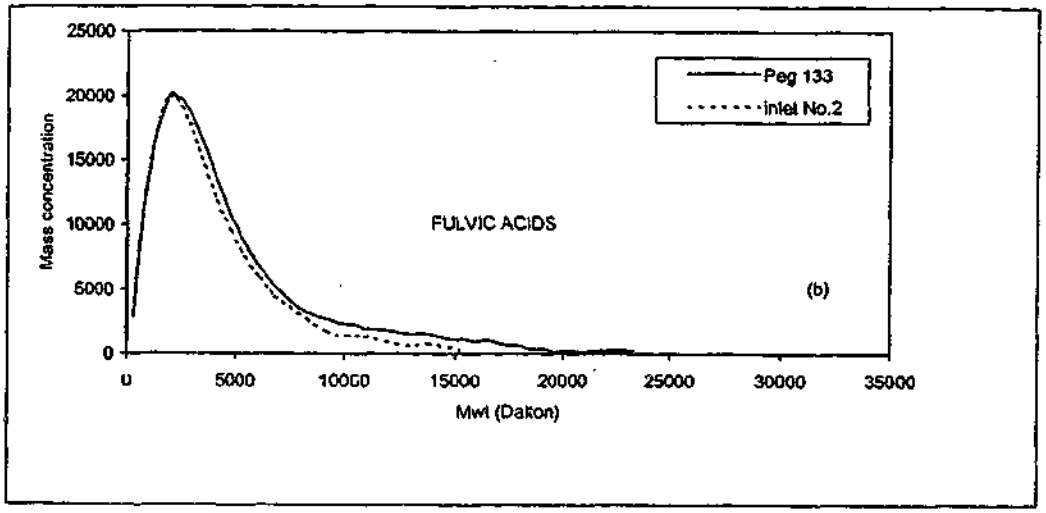


Fig. 5.6 Molecular weight distributions of wastewater collected from two end points of piped section 2 called Peg 83 and Peg 133





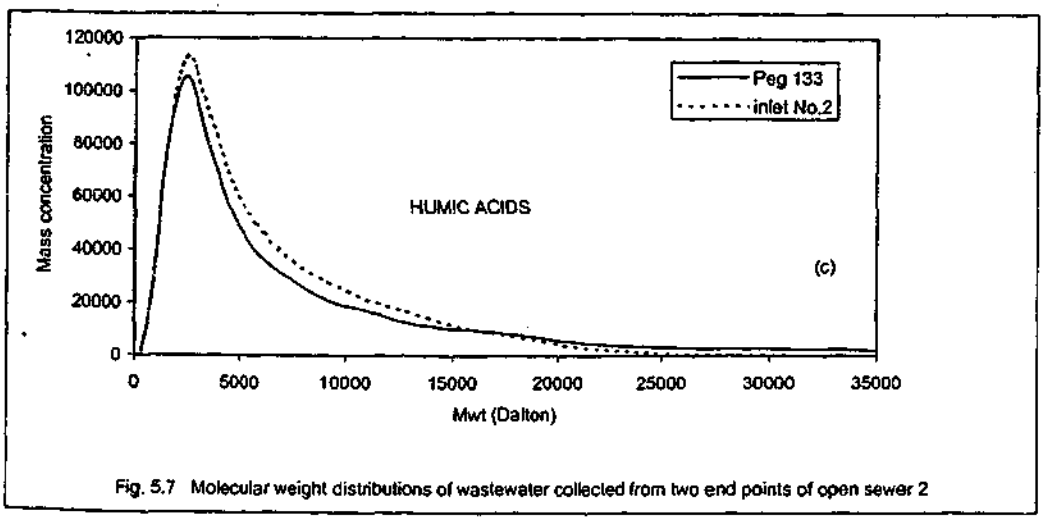
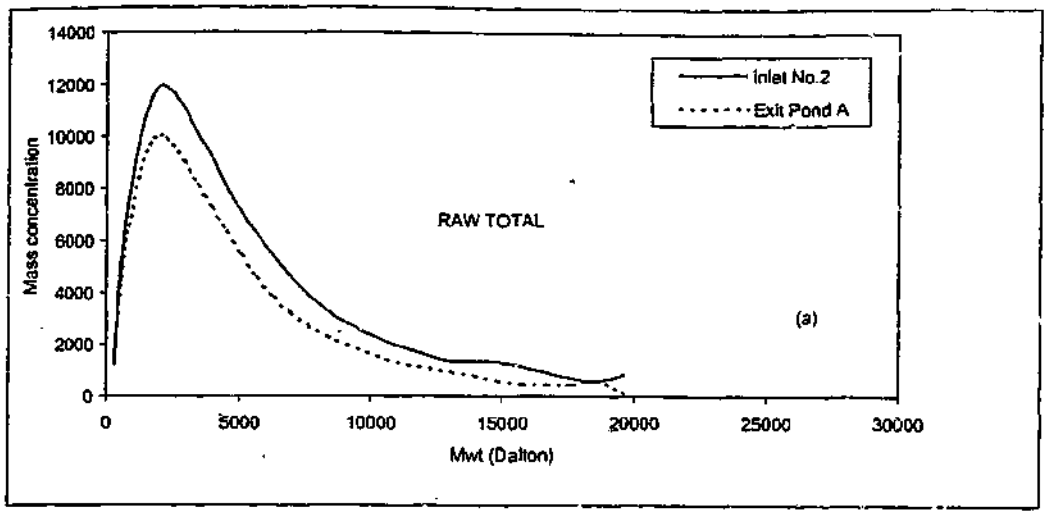
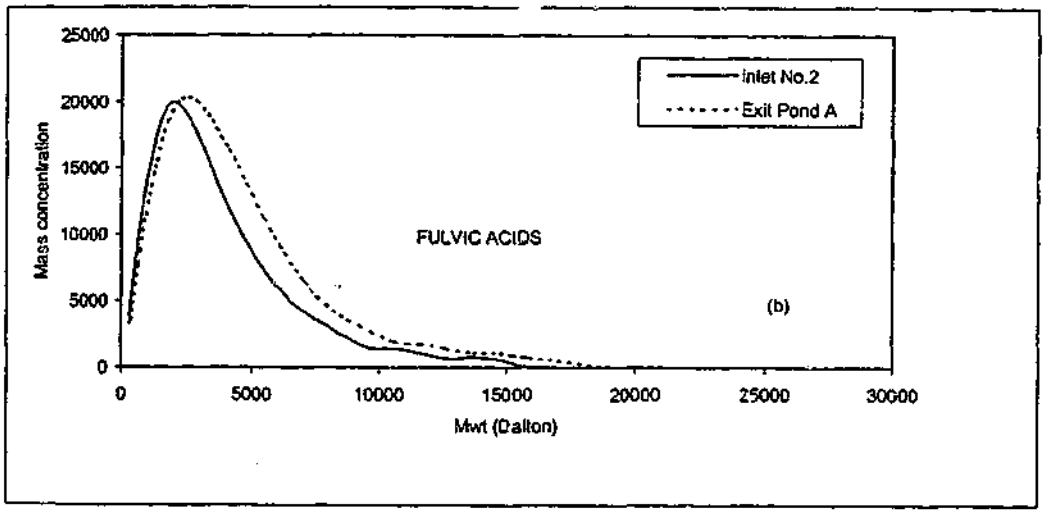


Fig. 5.7 Molecular weight distributions of wastewater collected from two end points of open sewer 2





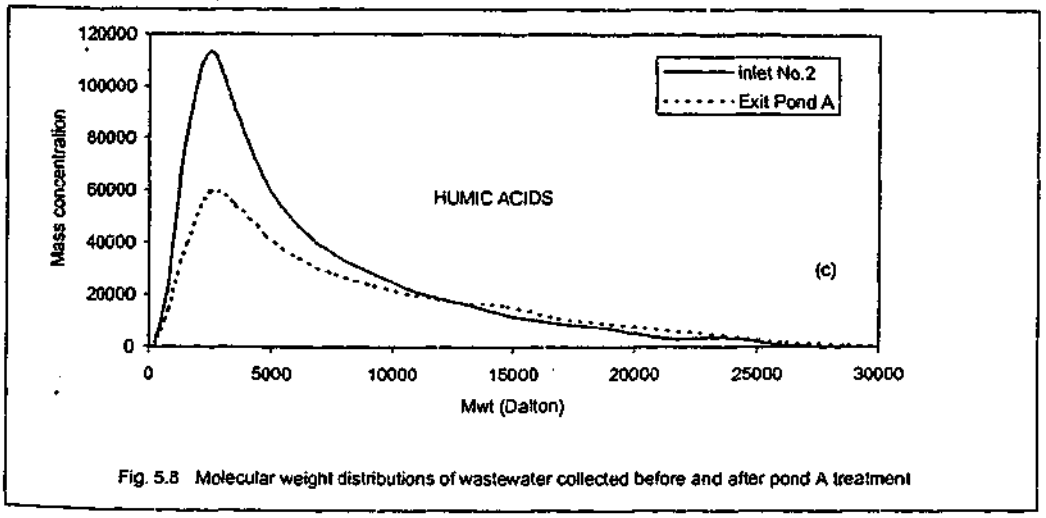
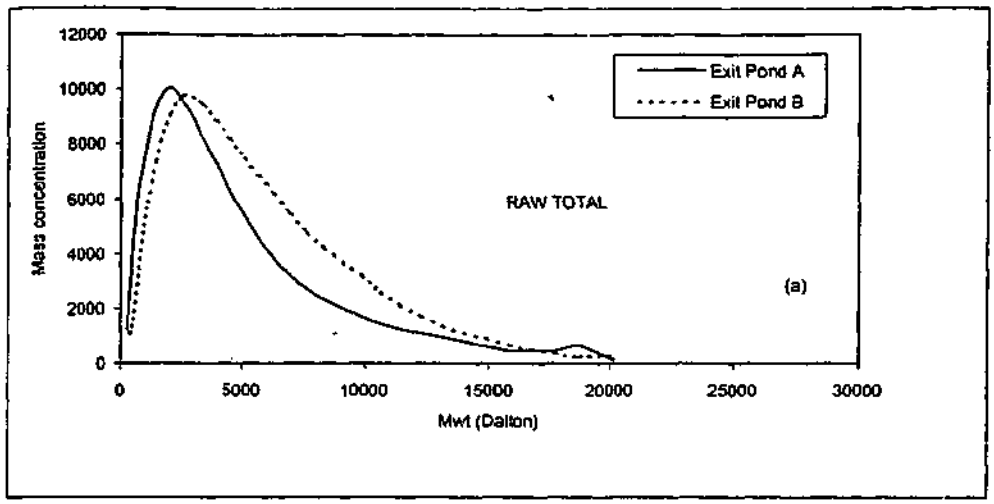
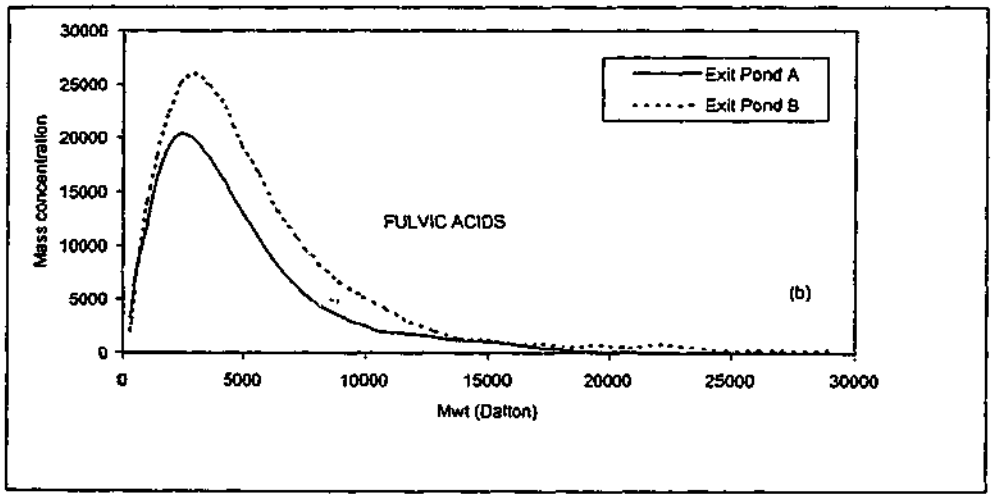


Fig. 5.8 Molecular weight distributions of wastewater collected before and after pond A treatment





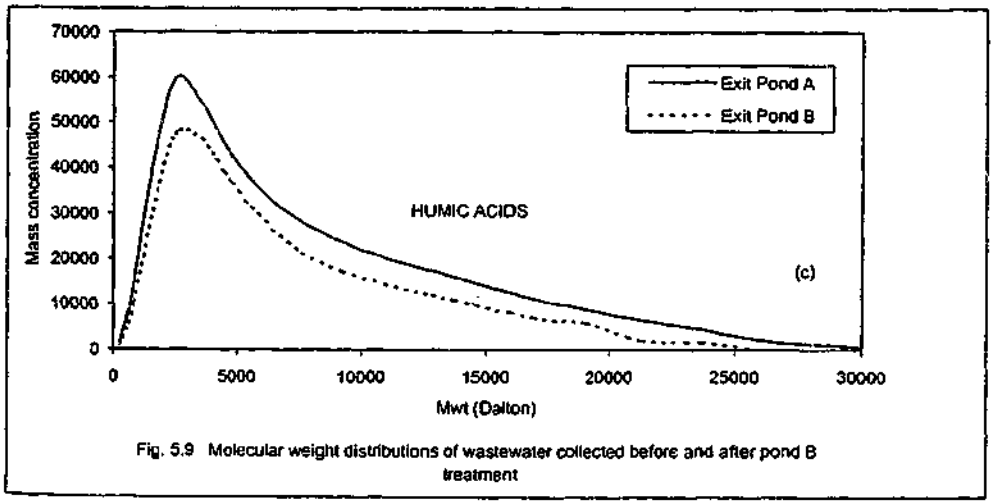
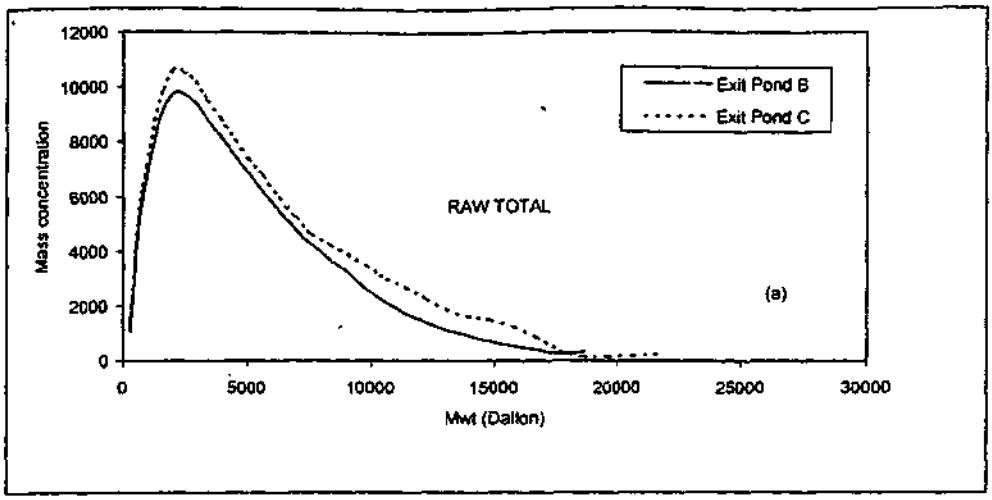
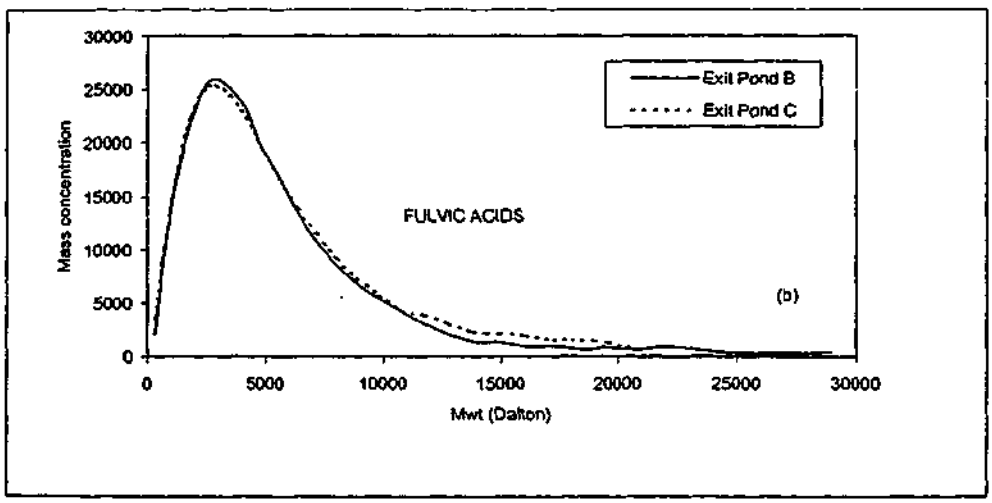


Fig. 5.9 Molecular weight distributions of wastewater collected before and after pond B treatment





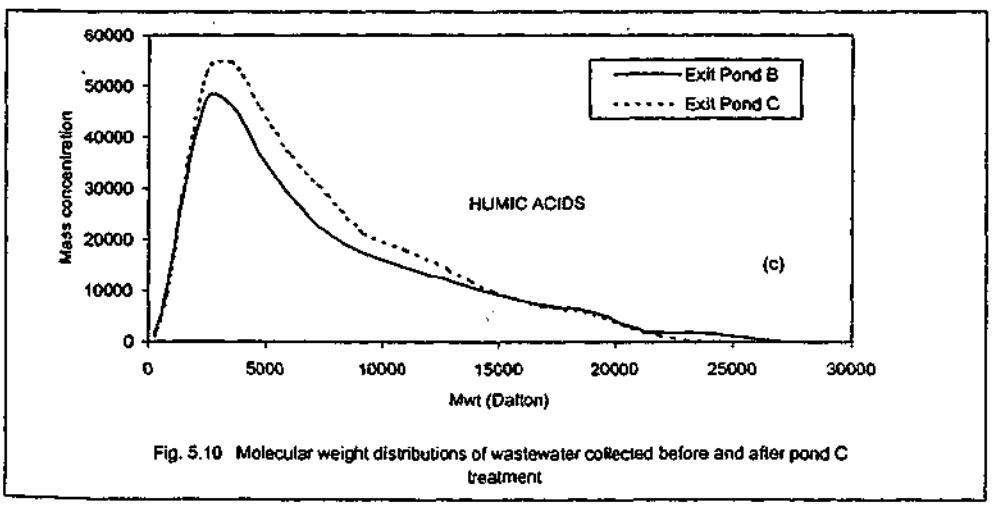
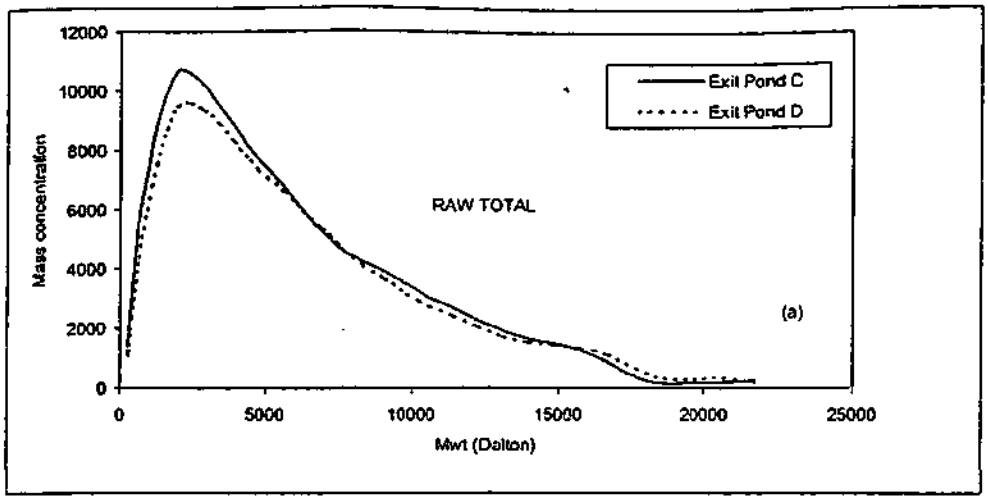
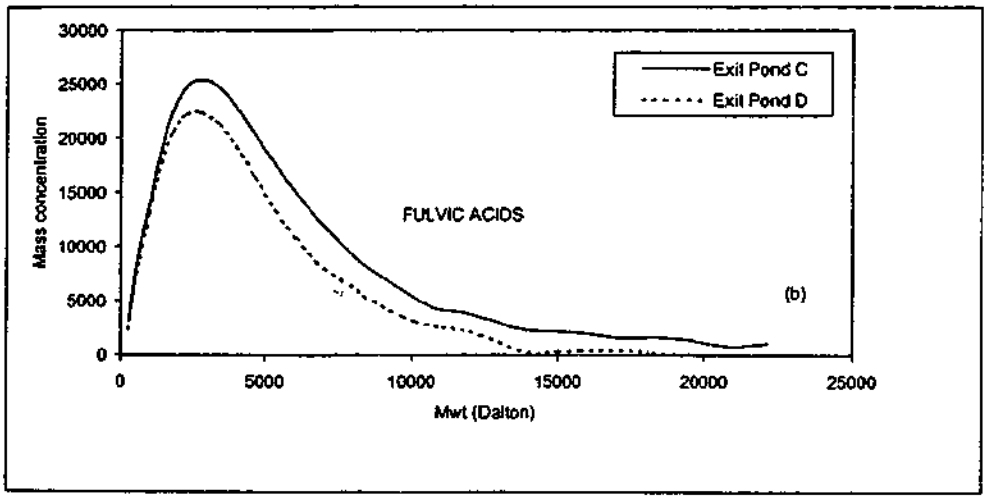


Fig. 5.10 Molecular weight distributions of wastewater collected before and after pond C treatment





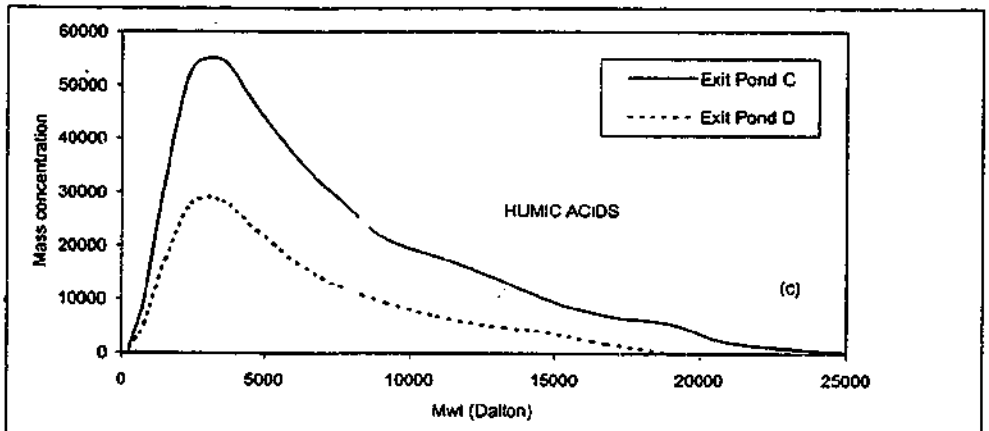
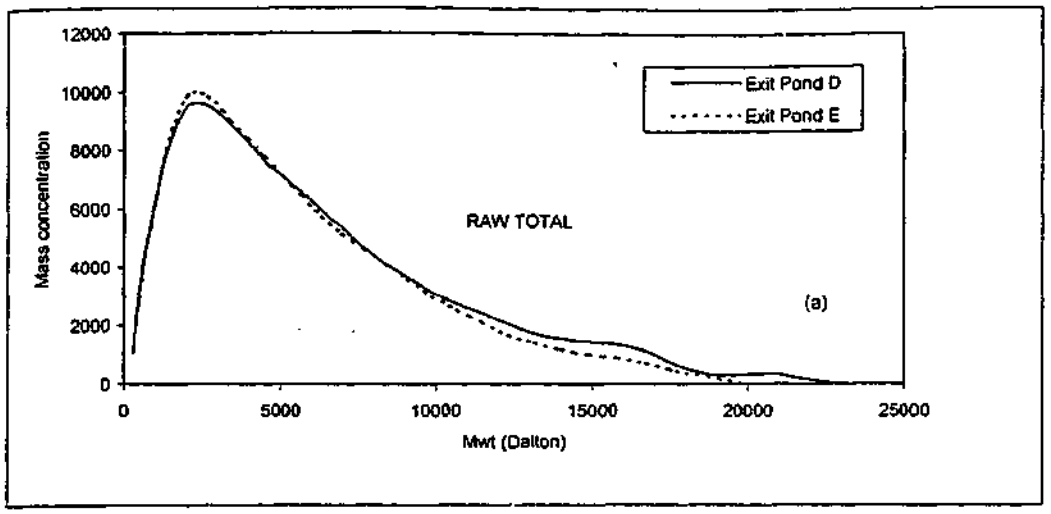
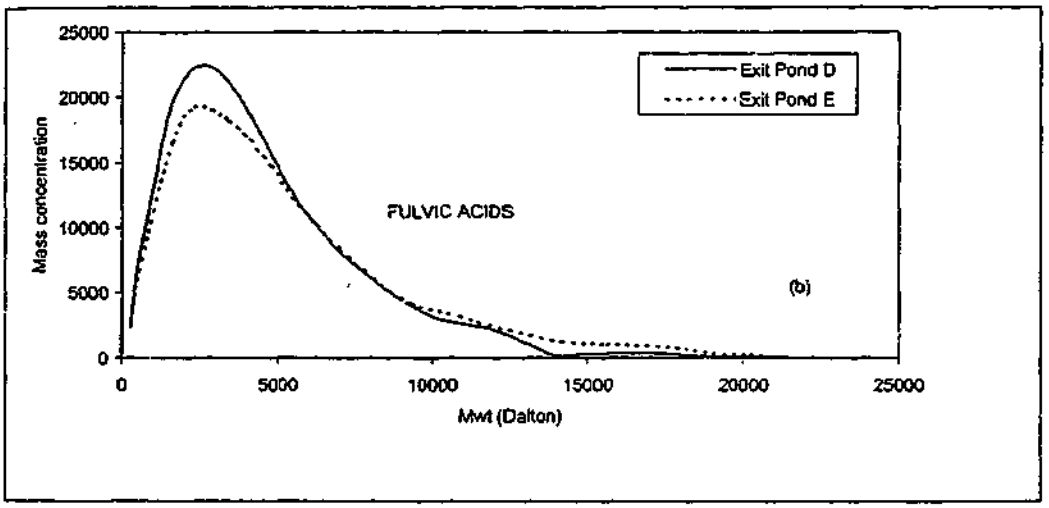


Fig. 5.11 Molecular weight distributions of wastewater collected before and after pond D treatment





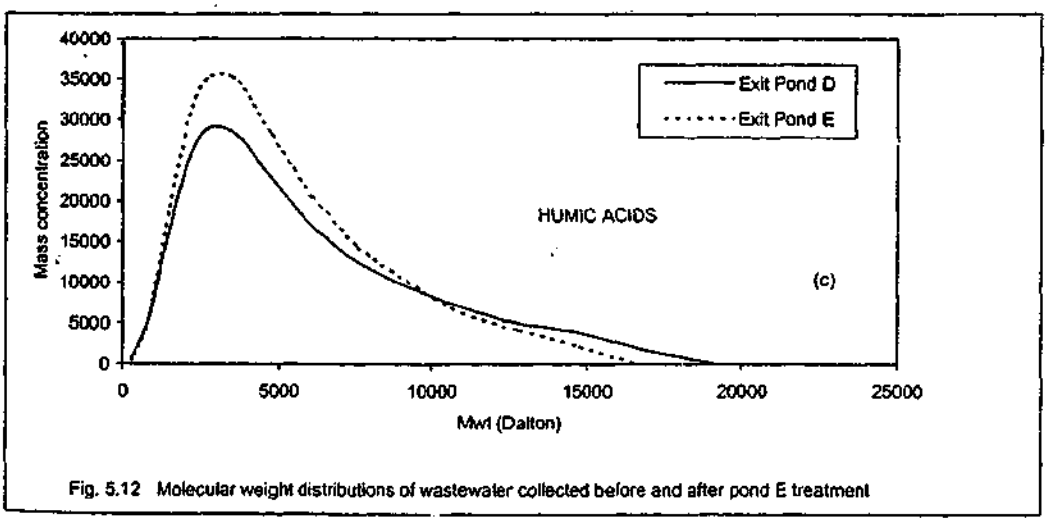


Fig. 5.12 Molecular weight distributions of wastewater collected before and after pond E treatment

Chapter 6

The Effect Of Biological Degradation On Doc And Molecular Weight Of An Australian Paper Mill Effluent And Recipient Water

Abstract

Preliminary screening experiments were done in order to establish the impact of biological processes on pulp and paper mill effluents and its receiving waters. AMCOR Maryvale pulp and paper mill effluent and the Latrobe Valley Outfall Sewer (LVOS) system were chosen for investigation. The extent of oxygenation of the laboratory experiments under natural aerobic condition was considered, and changes in dissolved organic carbon (DOC) were monitored to follow the rate and extent of biological oxidation of dissolved and particulate organic matter (POM). Aerobic and anaerobic conditions were established and molecular weights were analyzed at different stages by use of flow field-flow fractionation (FlFFF).

Comparatively low oxygen levels were observed with mill effluents and its receiving waters due to the high microbial activity. Depletion of oxygen was significant in mill effluents and wastewaters if proper stirring did not occur.

Studies were undertaken while both dissolved and particulate organic matter were present in the medium. DOC markedly decreased when proper agitation was provided. The results obtained imply that the aerobic bacteria preferentially remove DOC while anaerobic bacteria preferentially react on POM to release DOC.

Molecular weight studies were undertaken on the dissolved organic fraction after removal of suspended particulate matter. The results revealed that aerobes preferentially assimilate or microbially oxidize smaller solute molecules rather than larger solute molecules, while anaerobes preferentially react on larger solute molecules or release smaller molecules from POC. Therefore, after 24 to 48 hours higher average molecular weight was observed under oxic conditions while lower average molecular weight was observed under anoxic conditions.

6.1 INTRODUCTION

The Latrobe Valley Outfall Sewer (LVOS) system situated in Gippsland (Victoria, Australia) has been studied. The major input is the Maryvale Pulp and Paper mill effluent from the AMCOR plant. The study has focused on the LVOS and Dutson Downs (DD) treatment complex.

It was very difficult to confirm from field data (Chapter 3), which molecular weight fractions were affected by microorganisms under aerobic or anaerobic conditions in the system. Therefore, the effect of biological processes on the effluent-sewage pool has been studied in the laboratory. The objectives of the preliminary biological screening experiments were:

- to determine the most efficient degradation conditions, ie. aerobic degradation and/or anaerobic degradation
- to establish which molecular weight fractions, are selectively consumed by microbes under aerobic or anaerobic conditions.

The main hypotheses to be tested were: (1) that aerobes are more readily involved in DOC removal than anaerobes, and (2) that anaerobes are more readily involved in microbial conversion of the more refractory dissolved organic carbon (DOC) or suspended particulate organic matter (POM) into readily utilizable DOC that aerobes can readily assimilate.

6.2 EXPERIMENTAL

Three samples were chosen for this study. Two wastewater samples from the LVOS system (partially treated and fully treated) and one industrial effluent sample from the AMCOR pulp and paper mill plant were collected as described below.

- Industrial effluent sample:

  Pulp and paper mill effluent – the inlet stream to the 41 megalitre storage of the AMCOR pulp and paper mill plant.

- Partially treated wastewater sample:

  Rosedale Meter site – A point along the LVOS drain having continuous flow of the mixed effluent-sewage discharge.

- Fully treated wastewater sample:

  Exit Pond C – Residual wastewater from Dutson Downs treatment plant after considerable degradation in two prior ponds.

6.2.1 Procedure for the Aerobic/Anaerobic setup

The above three samples were collected and transported within 24 hours to the laboratory under refrigerated conditions. The sampling was undertaken by Gippsland Water. Upon arrival at the Monash laboratory, sample bottles were swirled several times to ensure thorough mixing. The aerobic experiment was set up by placing 250 mL samples in 1 L carbon sterilized (at 500°C) Erlenmeyer flasks and stoppered with cotton wool plugs. Carbon sterilized (500°C) 250mL glass bottles were filled up to the top and tightly capped with teflon lids for the anaerobic experiments. The experiments were conducted

at the original pH (~ 7.6) without pH adjustment. The dissolved oxygen was measured in each vessel at the start of the experiment.

6.2.2 Preliminary experiment for the screening of Aerobic/Anaerobic conditions

AMCOR pulp and paper mill effluent and wastewater collected from Exit Pond C at Dutson Downs treatment complex were chosen as samples for preliminary screening to establish the effect of aerobic and anaerobic conditions. They were filtered and prepared in separate vessels as described in the previous chapter. These reaction vessels were kept at a constant room temperature (23°C). The dissolved oxygen content was measured after 24 hours and 48 hours. The samples were withdrawn for determination of the molecular weight distributions by flow FFF.

6.2.3 Preliminary experiment for the screening of molecular weight changes due to Aerobic/Anaerobic conditions

AMCOR pulp and paper mill effluent and wastewater collected from Exit Pond C at Dutson Downs treatment complex were chosen for this study. Samples subjected to the preliminary screening of aerobic/anaerobic conditions were used for molecular weight analysis. Flow FFF fractionation was used to analyze molecular weights. The method of analysis and theory of the technique has been described in Chapters 2 and 3. These aerobic and anaerobic solutions were sterilized with sodium azide (final concentration 0.03%). The solids were separated by centrifugation at 3750 rpm and supernatant liquids were filtered through GF/C filter papers under vacuum. These filtrates were adjusted to pH 7.8 prior to injection in flow FFF analysis.

6.2.4 Flow FFF Analysis:

Flow FFF experimental conditions:

Channel flow rate = 5.0 mL / min

Cross flow rate = 5.0 mL / min

Carrier solution = 0.05 M tris + 0.00308 M NaN_3 + 0.0268 M HNO_3

Carrier solution pH = 7.8

Injection delay = 1 sec

Relaxation time = 15 sec

Dead time = 3 sec

Channel void volume = 1.24 mL

Pre-channel dead volume = 0.05 mL

Post-channel dead volume = 0.25 mL

Channel dimensions after new membrane installation:

Channel thickness = 0.0246 cm

Channel length = 22.4 cm

Channel breadth = 2.04 cm

Height from triangle base to tip = 2.43 cm

Triangle base = 2.0 cm

Calibration parameters with new membrane :

$\phi = 0.000263$

$n = 0.609$

Calibration Standards :

Poly(styrenesulphonate) having *Mw* = 4500, 6500 and 17500 Dalton

Concentration of standards = 1 mg mL^{-1}

6.2.5 Preliminary experiment for the screening of DOC changes due to Aerobic/Anaerobic exposure time

The wastewater sample collected from Rosedale Meter site at LVOS channel was chosen for this study. Aerobic and anaerobic experiments were set up with this sample as described in the previous chapter. The vessels were kept for microbial degradation at room temperature for 1 day, 2 days, 7 days and 14 days. At designated time intervals after the degradation began, the contents of the aerobic flasks were restored to the original volume with milliQ water and sterilized with sodium azide (final concentration 0.03%). The solids were separated by centrifugation at 3750 rpm and the supernatant liquid was filtered through GF/C filter papers under vacuum. The amount of dissolved organic carbon remaining in the liquid medium was measured.

A parallel set was prepared for aerobic to anaerobic cross over after 14 days. The contents in the aerobic flasks (i.e. flasks exposed to oxic condition) of the parallel set were transferred into glass bottles filled to the top and tightly capped. Also, the contents in the anaerobic bottles were transferred into erlenmeyer flasks and stoppered with cotton wool plugs. They were all kept at room temperature. Following aerobic to anaerobic cross over or vice versa, a further 14 days was allowed for degradation. After 14 days the aerobic flasks were restored to their original volume and sterilized with sodium azide

(final concentration 0.03%). Then solids were removed by centrifugation at 3750 rpm for 15 minutes and the supernatant liquids were filtered through GF/C filter papers before the DOC was measured.

6.2.6 Dissolved Organic Carbon analysis

Dissolved organic carbon (DOC) measurements were made on the centrifuged, filtered and sterilized solutions using a TOC-5000 Shimadzu total organic carbon analyzer. Freshly prepared potasium hydrogen phthalate (KHP) was used as total carbon (TC) standard and a $NaHCO_3$ / Na_2CO_3 mixture was used as inorganic carbon (IC) standard for instrument calibration. Three point calibration plots were made for TC and IC. The instrument automatically calibrated the TOC value by substracting the IC value from the TC value. The each sample was acidified to pH ~ 3.0 with 1M HCl acid before each measurement. The injections were usually performed in triplicate but went up to a maximum of five injections if the reproducibility was not satisfactory with the first three injections. The averaged value and standard deviation was automatically recorded.

6.2.7 Statistical Accuracy and Precision

Two sets of samples were collected from the LVOS and DD treatment complex. The experiments were conducted in duplicate with each set of samples. Then the whole set of experiments was repeated on a different occasion. For each sample withdrawn at a given time the TOC was analyzed in duplicate (by placing two subsamples in the TOC Autosampler). The TOC analysis procedure was repeated three times over three days. The instrument accuracy and reproducibility was checked by repeating the analyses three

times using a new instrument set up and new calibrations. The relative standard deviation was always less than 1%.

Flow FFF runs were undertaken in triplicate. The relative standard deviation for the average molecular weight analysis was 2-3%. To evaluate the channel reproducibility and recovery, a secondary reference humic substance sample isolated from Oakefenokee Swamp (GA, USA) was run and its relative standard deviation was within 2-3%.

6.3 RESULTS AND DISCUSSION

6.3.1 Preliminary Experiment:

Effect of Aerobic/Anaerobic conditions on untreated wastewater and treated wastewater

Two samples were subjected to this experiment. They were: (1) untreated mill effluent from AMCOR plant before undergoing storage (i.e. taken from the inlet to the 41 Megalitre storage); and (2) treated wastewater of the LVOS channel, taken from the exit point of pond C in the No.2 storage of the DD treatment complex.

In the laboratory these two samples were exposed to two different conditions with respect to the oxygen availability. The results are presented in Table 6.1. Both samples readily achieved oxic or anoxic conditions within 24 hours and the conditions persisted up to 48 hours. In the anoxic experiment the oxygen concentration was negligible (< 0.2 mg/L) in both samples. In the oxic experiment the DO was ~5.0 mg/L and this was the saturation

level for oxygen at 23°C. The oxygen level was always slightly less in the untreated mill effluent than in the treated wastewater. Since the raw mill effluent had not been treated, the reaction medium contains more readily available carbon sources so that the oxygen dissolved in the reaction medium would be rapidly removed from the solution as a result of bacterial oxidation. As the treated effluent had already exhausted much of the readily available carbon sources during the treatment, the rapid oxygen depletion observed in untreated mill effluent would not be expected.

6.3.2 Preliminary Screening for molecular weight changes:

Effect of Aerobic/Anaerobic conditions on molecular weight in (1)untreated wastewater (2)treated wastewater

Table 6.1 shows the weight average molecular weights of mill effluent (inlet 41 Megalitre storage) and treated wastewater (Exit Pond C) after 24 hours and 48 hours of aerobic or anaerobic treatment. An increase in the average molecular weights was evident in the aerobic set up and a decrease occurred in the anaerobic set up. It seems that the aerobes decompose lower molecular weight components, whereas the anaerobes preferentially decompose high molecular weight components, or POM, to release low molecular weight components.

Table 6.1 The conditions achieved in the OXIC / ANOXIC set up at room temperature under existing pH ~ 7.6 : PRELIMINARY SCREENING experiment with INLET 41 ML and EXIT POND C

	INLET 41 ML			EXIT POND C		
Contact time (hrs)	0	24	48	0	24	48
	Dissolved oxygen (mg O_2 / L)					
Aerobic set up	5.0	5.1	5.3	5.0	5.4	5.5
Anaerobic set up	5.0	0.2	0.2	5.0	0.2	0.2
	Weight average molecular weight (Dalton)					
Aerobic set up	9300	12350	14200	5200	6100	6550
Anaerobic set up	9300	7300	5700	5200	4600	4400

6.3.2.1 (a) Effect of aerobic conditions on molecular weight

It seems that low molecular weight fractions are being removed under aerobic conditions so that the molecular weight becomes biased towards high average molecular weight. The lower molecular weight fraction is considered to be the more bioactive fraction that bacteria actively uptake from the liquid phase. In fact this low molecular weight fraction may have the potential to diffuse into microbial cells (active uptake) rather than undergoing bacterial oxidation. Therefore, a higher weight average molecular weight in the medium would be a result from the active bacterial uptake of low molecular weight molecules.

If bacterial oxidation has occurred rather than bacterial uptake, the resultant medium would end up with a lower average molecular weight. This is because microbial oxidation is more favored with large molecules than with small molecules (Zo Bell 1946). Zo Bell C.E. (1946) has mentioned paraffin waxes are readily utilized by a large number of microorganisms, while short-chain molecules with less than five carbon atoms are oxidized by only a few organisms and very slowly. Strawinski and Stone (1940) found that hydrocarbons of high molecular weights are oxidized by soil bacteria more readily than smaller molecular weights. Their explanation was that the smaller the molecule the lower the ability to undergo microbial oxidization, because the smaller the molecule the less vulnerable are the sites for oxygen attack, and they are thermodynamically more stable. Higher homologs presumably undergo microbial oxidation readily, probably

because of the presence of more vulnerable sites for oxygen attack and they are thermodynamically less stable than small molecules.

The current studies in fact ended up with higher average molecular weights under the aerobic conditions. This is consistent with the removal of small solute molecules from the medium under aerobic conditions. That is, the resultant higher average molecular weight is most likely to be due to the removal of low molecular weight fractions. It appears from the discussion above that the removal of small molecules is due to active microbial uptake rather than microbial oxidation. The oxygen could have been effectively used for respiration and metabolism of so-called actively assimilated compounds. The active uptake of low molecular weight fractions by microbes seems to have occurred when oxygen is sufficiently available.

6.3.2.2 (b) Effect of anaerobic conditions on molecular weight

In contrast to the aerobic conditions, high molecular weight fractions are removed under anaerobic conditions so that the weight average molecular weight is biased towards a lower average molecular weight. The hypothesis for the anaerobic process is that large solute molecules are broken down into smaller molecules or reduced to CH_4 and CO_2.

The postulate is that anaerobic processes destroy the benzenoid skeleton of high molecular weight molecules. Biological destruction of benzenoid structures in strictly anaerobic conditions can occur through three different pathways (Evans 1977). They are: anaerobic photometabolism, anaerobic metabolism through nitrate respiration and

methanogenic fermentation. The methanogenic fermentation is the process that occurs in the absence of nitrate, sulphate and light. This phenomenon was described by Evans (1977) as the most widely occurring process in nature, especially in the processing of sewage and other waste effluents. The benzene nucleus was found to have been reduced and then cleaved to aliphatic acids by facultative anaerobic bacteria and converted to suitable substrates for methane bacteria.

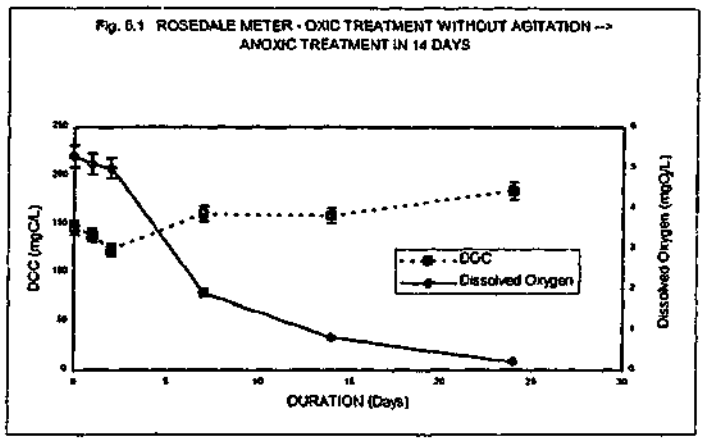
Previous workers have established that mixed microbial populations ferment aromatic compounds such as benzoate to methane and carbon dioxide. In this way lower molecular weights observed in anoxic media with mill effluent and its receiving water could possibly be attributed to the biological destruction of the benzenoid skeleton of larger molecules. This may give rise to both volatile compounds such as CH_4 and CO_2 and smaller fragments from the cleavage of large molecules.

6.3.3 Preliminary Screening for DOC changes:

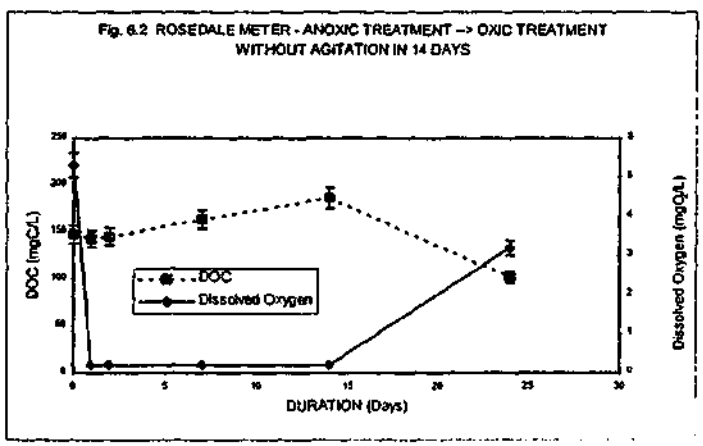
Effect of Aerobic/Anaerobic conditions on DOC in the mill effluent receiving wastewater, due to biological processes

Sample collected from the Rosedale Meter site was subjected to aerobic and anaerobic conditions. The biological effect on the DOC concentration was recorded with time and is presented in Figs.6.1, 6.2 and 6.3. The variation of DOC within the first 24 hours after aerobic exposure was insignificant. DOC in the aerobic medium after 48 hours (Fig.6.1) was significantly reduced to 124 mgC/L with respect to the original DOC (147 mgC/L). Further exposure to the same aerobic conditions for 14 days generated a higher DOC

concentration in the medium. Subsequent cross over of this aerobic condition to anaerobic conditions at day 14 increased the DOC concentration.



The population of aerobic bacteria may have simply grown in the effluent medium within 24 hours under the given oxygen saturation level and nutrient level. They seem to start removing DOC from the medium after 24 hours. The oxidative metabolism of bacteria is influenced by the presence of oxygen (Zo Bell, C.E. 1940).



There was no change in DOC within the first two days in the anaerobic experiment (Fig.6.2). Further exposure to the same anaerobic condition for up to 14 days produced a higher DOC concentration in the medium (Fig.6.2). However, after crossing over to aerobic conditions there was a significant reduction of DOC concentration after 14 days.

These findings suggest that the DOC is significantly removed by aerobic bacteria. In contrast, anaerobic bacteria either hardly removed any DOC or resulted in a release of DOC.

There is a lag period in both experiments (aerobic or anaerobic) as the particular bacteria population size is built up (Fig.6.2). The lag period is longer for the anaerobes than for the aerobes. There is a 48 hour-lag period was observed in the anaerobic medium, whereas 24 hour-lag period was observed in the aerobic medium. This is because the aerobic metabolism is more energetic than the anaerobic metabolism.

In fact the wastewater medium at the Rosedale Meter site is a pool of bleached kraft mill effluent carrying various organics and domestic sewage. Since the biomass in the domestic sewage is bacteria, DOC removal and POC leaching equilibrium time in this study was expected to be less than 30 minutes.

The other noticeable aspect of this experiment is that the reaction medium becomes richer in DOC as the anaerobic condition proceeds (Fig.6.2). Apparently there is no available DOC that anaerobic bacteria can readily utilize under anaerobic conditions. They seem to

preferentially react on suspended solids, probably in the form of cellulose fibers, which are liberated during the pulping process. They may release a substantial portion of organic solutes through stepwise enzymatic hydrolysis of insoluble lignocellulose fibers.

According to studies related to lake waters undertaken by previous researchers there are different microorganisms (bacteria, fungi and protozoa) that are known to be closely associated with particulate organic matter. Chrost and co-workers studied the stepwise enzymatic hydrolysis of insoluble polymeric material, and found that a substantial portion of dissolved organic solutes were liberated (Chrost 1989; Chrost and Overbeck 1987; Chrost et al. 1989 and Hoppe et al. 1988). These studies have shown that POM in lake water is an important source of DOM as a result of POM and leaching by microorganisms.

The increase in DOC under anaerobic conditions also suggests that anaerobic bacteria do not hydrolyse particulate suspended solids (fibrous matter) in a biochemically controlled process and do not assimilate most of the hydrolysis products, instead they diffuse into the environment. Previous researchers (Ammerman and Azam 1985) have revealed that microorganisms (including bacteria) preferentially assimilate (active uptake) orthophosphates originating from enzymatic hydrolysis rather than from orthophosphate in the bulk liquid medium. Also it has been shown that bacteria preferentially assimilate glucose originating from enzymatic hydrolysis of carbohydrates rather than from the bulk liquid phase (Chrost 1989), because glucose uptake by bacteria is tightly coupled to the rates of hydrolysis of carbohydrates which contain glucose. They postulated hydrolysis-

uptake coupling systems in aquatic bacteria. Such a hydrolysis-diffusion coupling by bacteria in pulp and paper mill wastewater medium could be occurring in the current project.

The release of DOC was observed only in the absence of oxygen. The results suggest the occurrence of a similar type microbial enzymatic hydrolysis of lignocellulose fibers in mill effluent wastewaters. The results also suggest that this process occurs preferentially under anaerobic conditions and hence it may be specific to anaerobes.

It is seen in Fig.6.1 that DOC in the medium tends to increase slightly in the aerobic experiment between days 2 and 7. It seems that the oxic media unintentionally achieved fairly anoxic conditions half way through the oxic experiment. That is the medium was not oxic any more. There are two reasons, which may account for this occurrence:

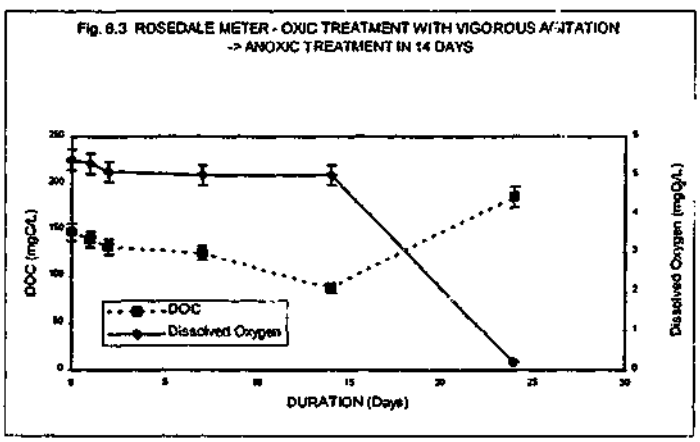
(1) the oxygen was respired by aerobic bacteria and cannot be compensated by diffusion from the atmosphere due to the lack of continuous agitation throughout the aerobic experiment, and/or

(2) the presence of reactive DOC that the aerobes can readily oxidize, thus using epoxy free oxygen.

Zo Bell (1946) also found that some readily oxidizable organic materials such as carbohydrates are preferentially attacked by microorganisms, and their oxidation results in depletion of oxygen and lowering of the reduction potential to a point where only anaerobes can function. Then facultative bacteria might have acclimatised to the oxygen

deficient environment and become established. They then seemed to start reacting on the suspended particles, such as cellulose fibers, possibly through stepwise enzymatic hydrolysis reactions. This may liberate organic solutes (as in the anaerobic experiment) resulting in the subsequent increase of DOC in the medium. This may be the reason for the subsequent increase in DOC in the aerobic experiment after a certain period of time elapsed.

The aerobic experiment, when repeated with proper agitation, does not show such a DOC increase (Fig.6.3). Instead continuous DOC depletion was observed throughout the aerobic time frame. Thus, the DOC concentration was considerably decreased over the 14 days, aerobic time frame provided with proper agitation (Fig.6.3).



Both Figs.6.1 and 6.3 clearly show that when the aerobic medium has crossed over to anaerobic conditions after 14 days and is subsequently held under anoxic conditions for an extended period (14 days), the medium achieves a higher DOC. This finding supports the hypothesis that when oxygen becomes a limiting factor bacteria can preferentially

react on suspended lignocellulose matter, rather than utilizing dissolved organic carbon in the bulk liquid phase.

When anaerobic conditions were crossed over to aerobic conditions after 14 days, the DOC in the medium decreased from 180 mgC/L to 102 mgC/L over 10 days (Fg.6.2). This could be explained as follows. The anaerobic bacteria preferentially feed on suspended lignocellulose fibers in the medium rather than on dissolved carbon when oxygen becomes deficient in the medium. However, facultative aerobic bacteria may regain their oxidative metabolism and start feeding on dissolved carbon when oxygen is available. The DOC concentration in the medium did not increase during the oxic phase due to the lack of agitation (Fig.6.2). Instead it decreased to 102 mgC/L. This clearly suggests that the original medium does contain aerobically utilizable DOC and the presence of oxygen favors the utilization of DOC already present in the medium. This also supports the hypothesis that degradable DOC is released to the medium after having been exposed to anoxic conditions and this is utilizable by facultative aerobes.

It seems both strict and facultative aerobic bacteria preferentially feed on dissolved organic carbon rather than on suspended lignocellulose fibers under aerobic conditions. In contrast, both strict and facultative anaerobic bacteria preferentially feed on suspended lignocellulose fibers under anaerobic conditions. This preliminary experiment suggests about 30% of DOC could be removed by exposing the medium to an oxygen deficient environment followed by supply of oxygen (Fig.6.2) without agitation. Only 16% DOC was removed when oxygen is available from the beginning (Fig.6.1) but without any

agitation. However, the highest DOC removal efficiency was observed with oxygen plus vigorous agitation from the beginning. In this current study it was 41%. The reason for lower DOC removal in the anoxic followed by the oxic without agitation experiment, than in the oxic with agitation followed by anoxic experiment, may be that there was less dissolved oxygen in the nonagitated medium than in the vigorously agitated medium, even though both flasks were open to the air.

6.4 CONCLUSIONS

The biological experiments conducted under oxic and anoxic conditions revealed that the aerobes seem to preferentially feed on DOC while anaerobes preferentially feed on suspended solids in the form of fibrous matter which have been liberated during the pulping process. In this way DOC would be leached to the surroundings under anoxic conditions. Anaerobes are not capable of removing these leached products, instead they are released to the surroundings. Therefore an increase in DOC was observed under anoxic conditions. Oxygen greatly favors the microbial utilization of DOC. The DOC, which are refractory to aerobes, may be converted to more susceptible forms of DOC under the anoxic conditions, which are then readily removed by aerobes. DOC removal seems more efficient with oxygenation.

The small molecules and low molecular weight fractions seem to be removed by active uptake (assimilation) by aerobes under the oxic conditions, which results in higher weight average molecular weight in the medium. In contrast, the larger molecules undergo only

microbial oxidation under oxic conditions, which could result in lower average molecular weight in the medium.

The high molecular weight fractions are removed under anoxic conditions so that the weight average molecular weight decreases. The anaerobic process will break down larger molecules into smaller molecules and the aerobic bacteria can utilize some of these small molecules. This probably occurs by the biological destruction of the benzenoid skeleton of the ill-defined large molecules.

DOC is removed under oxic conditions while DOC is released from POC under anoxic conditions.

Chapter 7

The Effect Of Biological Degradation On Chemical Fractions Of An Australian Paper Mill Effluent And Recipient Water

Abstract

Untreated mill effluent from the AMCOR pulp and paper mill plant was investigated in order to study the effect of biological processes on the major organic fractions present. The mill effluent is discharged to the Latrobe Valley Outfall Sewer (LVOS) channel. There are sewage influxes to the LVOS system. The study was extended to include two receiving wastewaters in the LVOS channel (see Chapter 3, Section 3.2, Figs.3.1-3.6):

- Piped channel with oxygen injectors
- Open channel with no oxygenation

Filtered samples were subjected to laboratory scale microbiological experiment as described in Chapter 6. The major organic groups were fractionated from over three samples by XAD-8 resin fractionation at various stages of the biological experiment (Chapter 4). The biological effect on the major organic groups was studied.

The aerobic degradation was efficient for dissolved organic carbon (DOC) removal. The anaerobic degradation also removed DOC, but to a lesser extent. The removed DOC was shown to be predominantly hydrophilic substances. The Fulvic acids were refractory in the presence of hydrophilic substances. Perhaps they were biologically degraded if hydrophilic substances were absent in the medium.

7.1 INTRODUCTION

The pulp and paper mill effluent from the AMCOR Maryvale plant situated in Gippsland Australia was investigated. The mill effluent is discharged to the Latrobe Valley Outfall Sewer (LVOS) channel. The study was also extended to two wastewater sites in the LVOS channel. The mill effluent is combined with domestic sewage influx while being transported down the channel (see Chapter 3, section 3.2).

The mill effluent and sewer was thought to consist of several major organic fractions with varying hydrophobicity. The effect of biological processes on the major organic fractions in the mill effluent and in the effluent sewage pool has been studied by carrying out controlled laboratory scale microbial digestion experiments. The major organic groups were fractionated by XAD-8 macroporous resin fractionation. The abundance of major organic groups was monitored during biological degradation.

The main objectives of these experiments were:

- to investigate the most efficient degradation process, i.e. aerobic degradation and/or anaerobic degradation, for dissolved organic fraction.
- to study which major organic group has been selectively consumed by bacteria under given degradation conditions.

7.2 EXPERIMENTAL

The LVOS effluents chosen for the laboratory microbiological study are as follows (see location maps in Chapter 3, section 3.1):

- pulp and paper mill effluent from the AMCOR plant, just before discharge into the LVOS channel – OUTLET 41 MEGALITRE STORAGE
- a piped channel which contains oxygen injectors – ROSEDALE DISSOLVER
- an open channel with no oxygenation – PEG 83

7.2.1 Biological experimental set up and pretreatment

The samples for the microbiological experiments were collected from the LVOS channel in Gippsland (Victoria) and transported within 24 hours to the Melbourne laboratory. The sampling was carried out by Gippsland Water, Gippsland. At the laboratory the samples were filtered through GF/C filter papers under the vacuum.

The aerobic experiment was set up by placing 100 mL samples in 250 mL carbon-sterilized (at 500°C) Erlenmeyer flasks, stoppered with cotton wool plugs. These flasks were agitated in a shaker bath at 30 strokes per minute at constant bath temperature (23°C). The pH and dissolved oxygen were measured in each flask. The anaerobic experiments were set up in carbon-sterilized (at 500°C) glass bottles (250 mL). They were filled up to the top and tightly capped with teflon lids and kept at constant room temperature (23°C). The experiments were conducted at their original pH, which was 7.6 and pH was measured after the designated experimental time intervals as well.

These vessels were shaken during microbial degradation at room temperature for 14 days. The contents of the aerobic flasks were restored to their original volume every day with milliQ water. The first set of vessels were withdrawn after 14 days, while the second set of vessels were crossed over (aerobic to anaerobic and anaerobic to aerobic) at that time. That is, the contents in aerobic flasks were transferred into glass bottles filled up to the top and tightly capped. The contents in the anaerobic bottles were transferred into Erlenmeyer flasks and stoppered with cotton wool plugs and placed in a shaker bath at room temperature. Having crossed over (aerobic to anaerobic and anaerobic to aerobic), 14 days were again allowed for degradation. The aerobic flasks were restored to their original volume with milliQ water every day as in the first 14 days. After 28 days the second set of vessels was withdrawn. The withdrawn vessels were sterilized with sodium azide (final concentration 0.03%). Then any solids were separated by centrifugation at 3750 rpm for ½ hour and the supernatant liquids were decanted into clean vessels.

7.2.2 Isolation of humic acids from LVOS samples

About 75 mL of the sterilized supernatants from the biologically treated and untreated solutions of the LVOS samples were adjusted to pH ~ 1.0 with concentrated HCl. They were kept for 72 hours in a refrigerator for humic acid precipitation, centrifuged at 3750 rpm for ½ hr and the humic acid precipitates were separated by decanting the supernatant fulvic acid liquid into clean glass vessels (burned overnight at 500° C in an oven). Humic acid precipitates were mixed with 10 mL 0.1 M HCl and centrifuged at 3750 rpm for 10 minutes. The supernatant HCl liquid was then decanted off, and added to the fulvic acid fraction. The precipitates of humic acid were transferred to glass vials with the aid of

milliQ water and the final volume was raised to 20 mL with milliQ water. The pH was immediately adjusted to 7.6 with 1 M NaOH to dissolve the precipitates.

7.2.3 Fractionation of dissolved organics by use of XAD-8 resin

The supernatant liquids left after the humic acid isolation is referred to as the non-humic acid fraction. Aliquots of 60 mL from these solutions were diluted ten times with milliQ water and the pH was adjusted to < 2.0 for XAD-8 resin fractionation. These solutions (pH<2) were pumped through the resin column at 2.5 mL/min with a Gilson peristaltic pump. The column void volume was ~ 13.0 mL, assuming the void volume within the XAD-8 resin is ~ 65% of its bulk column volume (Leenheer, 1981). One void volume of influent was displaced and discarded before collecting the effluent passing through the resin. The retained non-sorbed portion of the influent within the column was displaced from the resin by one void volume of 0.01 N HCl solution. The displaced portion of the column effluent was combined with the bulk column effluent (hydrophilic fraction) collected through XAD-8 resin.

The fraction, which passes directly through the XAD-8 column is known as hydrophilic substances although it includes hydrophobic bases as well (Leenheer, 1981). The XAD-8 sorbed fraction contains the hydrophobic acids plus hydrophobic neutrals.

The XAD-8 column was then eluted with 50 mL 0.1 M NaOH at half the effluent pump rate, i.e. 1.25 mL/min. One void volume of influent was first displaced and was discarded before collecting the hydrophobic acid fraction. The hydrophobic acid fraction collected

with 0.1 M NaOH elution is known as fulvic acids. At the end of the desorption one void volume of influent was displaced by 0.1 M NaOH and was combined with the hydrophobic acid fraction already collected. According to Leenheer, the hydrophobic neutral fraction is retained on the XAD-8 column under these experimental conditions (Leenheer, 1981).

It was possible to split the biologically treated samples into four major organic groups known as: humic acids; hydrophilic substances; hydrophobic acids (fulvic acid) and hydrophobic neutrals, depending on the nature of the hydrophobicity of the compound. Only three fractions were recovered: humic acids, hydrophobic acids (fulvic) and hydrophilic-substances. These fractions were analyzed for DOC.

7.2.4 Preparation of fulvic acid fraction for DOC analysis

The fulvic acid fractions were diluted with milliQ water and then adjusted to pH 7-8 with concentrated HCl and kept in the refrigerator until used for DOC analysis. The pH was reduced to 3-4 just prior to DOC analysis. Having adjusted the pH, the DOC analysis was done as soon as possible as there is a possibility of cloud formation or precipitation with longer storage at low pH. Further dilutions were performed where necessary for the DOC measurements. The measured values of DOC were used to calculate the DOC of the original sample concentrations by using their dilution factors.

7.2.5 Dissolved organic carbon analysis

The processed diluted samples were then used for DOC measurements using a Shimadzu total organic carbon analyzer (TOC-5000). Freshly prepared potassium hydrogen phthalate (KHP) solution was used as a total carbon (TC) standard. The instrument was calibrated with three KHP standards and DOC was measured as nonpergeable organic carbon (NPOC). The samples were acidified with 1 M HCl acid and sparged with zero grade air (synthetic air mixture of high purity O_2 and N_2 where $O_2\% : N_2\% = 21\pm1$) for 1 minute before each measurement.

7.2.6 Statistical accuracy and precision

The laboratory scale biological experiment was carried out in triplicate. Two subsamples from each sample vessel were measured for DOC at a time. The average of three sample injections was taken for each DOC measurement. This analysis of DOC was repeated a total of six times. The reproducibility and the accuracy of the instrument calibration line were checked on three occasions with freshly prepared standard solutions.

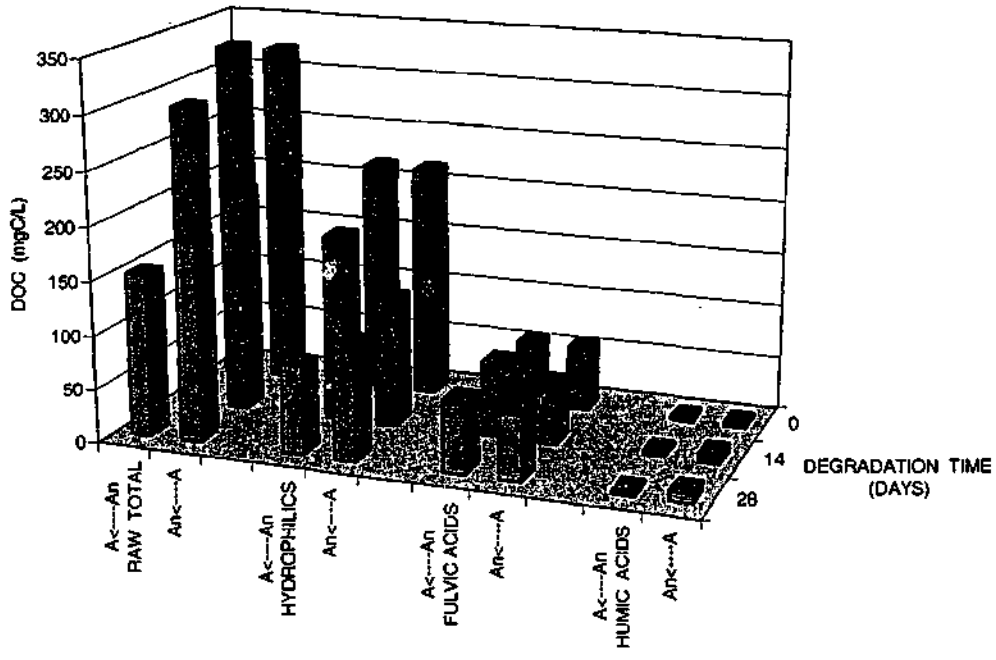
7.3 RESULTS AND DISCUSSION

7.3.1 AMCOR mill effluent (outlet stream from 41 megalitre storage)

The total DOC decreased markedly under aerobic conditions within 14 days (Fig.7.1). There was a much smaller decrease within the same duration under anaerobic conditions. The results reveal that DOC removal within 14 days was ~ 36% under aerobic conditions and ~ 12% under anaerobic conditions. Having crossed over from aerobic conditions to anaerobic conditions, only 11% of the total DOC was removed during the second 14 days

under anaerobic conditions. Similarly, having crossed over from anaerobic condition to aerobic conditions, 47% of the DOC was removed within 14 days under aerobic conditions.

Fig.7.1 Biological degradation of the Outlet 41 ML sample - DOC degradation of XAD-8 fractions under aerobic and anaerobic conditions (The conditions were reversed after 14 days, i.e.Aerobic-->Anaerobic etc.)



The effect of biological degradation on the major organic fractions is also shown in Fig.7.1. Under aerobic conditions, about 45% of the hydrophilic substances were removed within 14 days. After crossing over from aerobic conditions to anaerobic conditions, the additional removal of hydrophilic substances was only 10%. In the case of anaerobic experiment the additional removal of hydrophilic substances was only 21%.

However, after crossing over from anaerobic conditions to aerobic conditions, about 53% of hydrophilic substances were removed.

The results of XAD-8 fractionation have shown that the degradation trends observed for the raw solutions were due mainly to the degradation of the hydrophilic substances (Fig.7.1). Since hydrophilic acids are generally considered to be comparatively small molecules and with less carbon content (Painter et al 1959), bacteria would be expected to consume the hydrophilic fraction quite efficiently. However the results (Fig.7.1) reveal a certain fraction of hydrophilic substances are nondegradable by facultative anaerobes (i.e. in experiments starting with aerobic conditions then crossed over to anaerobic conditions). Fig.7.1 also reveals that the nondegradable hydrophilics fraction would remain after a lengthier period than a 28 day time frame.

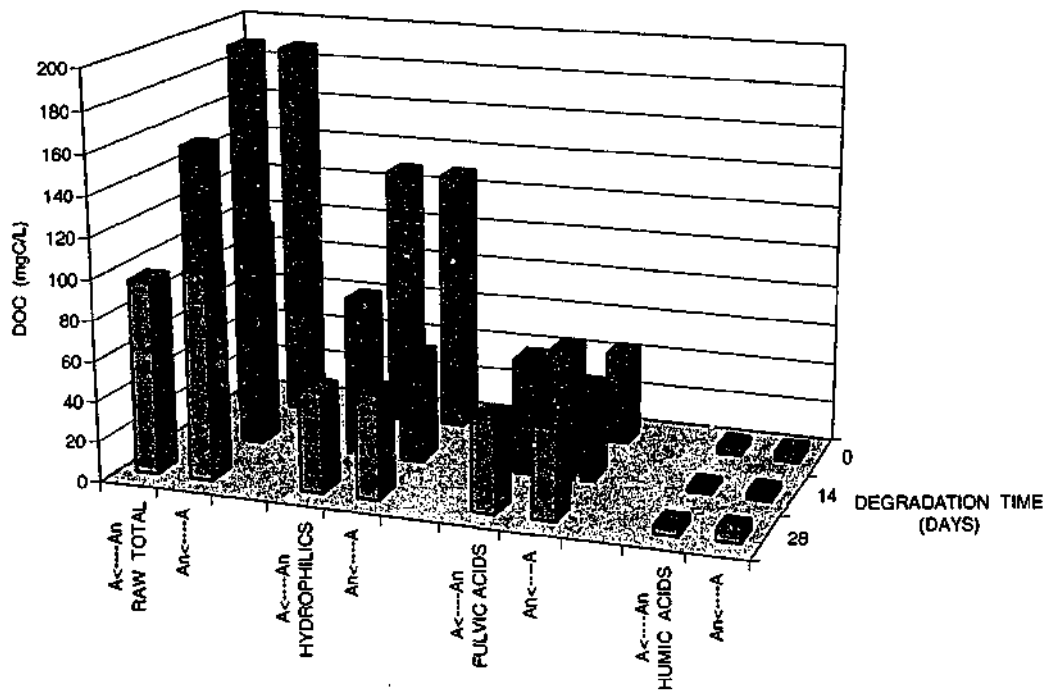
The fulvic acid fraction seemed to be fairly refractory under the conditions studied. Only 7% of the total fulvic acids were removed within 14 days under aerobic conditions. By crossing over from aerobic conditions to anaerobic conditions, the removal of fulvic acid did not change significantly and was only 9% by the end of 28 days treatment. With 14 days anaerobic treatment, the fulvic acid removal percentage was 18%. Only a further 7% fulvic acid was removed when the above anaerobic system was crossed over to aerobic conditions. These changes are not large in comparison to the removal occurring with the hydrophilic substances.

It is very difficult to interpret the DOC changes due to humic acids because of their very low concentration.

7.3.2 Rosedale Dissolver

Similar trends were observed to that of the mill effluent under the same experimental conditions. The total DOC has markedly decreased under aerobic condition within the 14 day experimental time frame (Fig.7.2). A smaller decrease was observed under anaerobic conditions within the same time. The DOC removal was ~ 42% within 14 days under aerobic conditions. It was 20% within the same time frame under anaerobic conditions.

Fig.7.2 Biological degradation of the Rosedale Dissolver sample - DOC degradation of XAD-8 fractions under aerobic and anaerobic conditions (The conditions were reversed after 14 days, i.e. Aerobic-->Anaerobic etc.)



However, having crossed over from aerobic condition to anaerobic conditions, the DOC removal declined a further 6% within 14 days. In the case where the anaerobic system crossed over to aerobic conditions, the DOC removal increased to a total of 36% within the same time frame. It seems (Fig.7.2) that almost all microbially removable DOC has been removed within the first 14 days in the experiment started under aerobic conditions. The facultative anaerobes do not seem capable of removing residual DOC after aerobic pretreatment for 14 days (Fig.7.2). The residual DOC left over after aerobic pretreatment is most probably refractory for facultative anaerobes so that only 6% more has been removed after crossing over from aerobic conditions to anaerobic conditions. A similar trend was also observed with the mill effluent. That is, the facultative aerobes seem to remove DOC efficiently after anaerobic pretreatment but not vice versa.

The concentration changes of hydrophobic and hydrophilic substances in untreated and biologically treated samples from Rosedale Dissolver is shown in Fig.7.2. Within the given experimental time frame (14 days), 60% of the hydrophilic substances were removed under aerobic conditions while only 40% were removed under anaerobic conditions.

Having crossed over from aerobic conditions to anaerobic conditions, the removal of hydrophilic substances was only a further 2% within 14 days. In the case where anaerobic conditions crossed over to aerobic conditions, the removal of hydrophilic substances was an additional 37%.

The XAD-8 fractionation has shown that the results observed were similar with that of the mill effluent under the same experimental conditions. That is, the degradation trends observed for the raw solutions were due mainly to the degradation of the hydrophilic fraction.

Again, the fulvic acids seemed to be more refractory under the conditions studied. DOC due to fulvic acids declined by only 4% under aerobic conditions, whereas it declined by 24% under anaerobic conditions. Having crossed over from aerobic condition to anaerobic conditions, only 4% more was removed. In the case where anaerobic conditions crossed over to aerobic conditions, a further 19% was observed. A similar outcome was observed with the mill effluent.

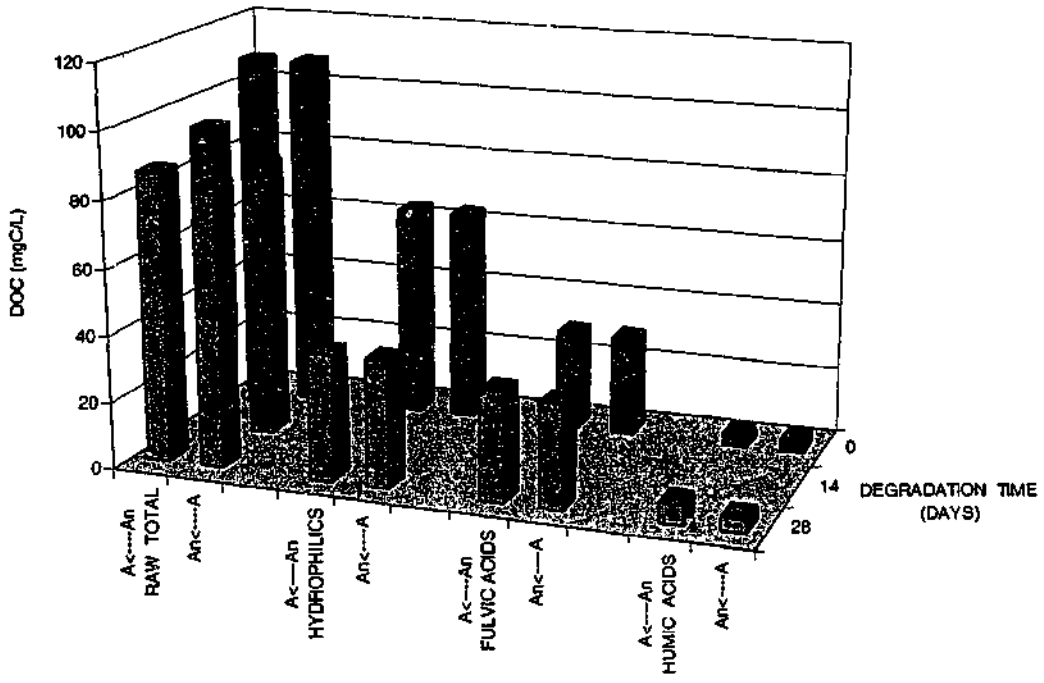
Again, it is very difficult to interpret changes in DOC due to humic acids because of the very low concentration of DOC.

7.3.3 Peg 83

The changes in DOC of the major organic groups due to biological degradation for the Peg 83 sample are shown in Fig.7.3. The XAD-8 fractionation was done with samples (1) prior to aerobic or anaerobic exposure; (2) after 28 days, having crossed over to the opposite condition after 14 days exposure to either aerobic or anaerobic condition. Again, the XAD-8 fractionation has shown that the degradation trends observed with the raw solutions were due mainly to the degradation of the hydrophilic substances.

the XAD-8 fractionation has shown that the degradation trends observed with the raw solutions were due mainly to the degradation of the hydrophilic substances.

Fig.7.3 Biological degradation of the Peg 83 sample - DOC degradation of XAD-8 fractions under aerobic and anaerobic conditions (The conditions were reversed after 14 days, i.e. Aerobic–>Anaerobic)



After the entire 28 days cycle, both cases were left with the same amount of hydrophilic substances. The hydrophilic substances left may be refractory to facultative microbes under the given time frame. A similar result was observed with the sample collected from Rosedale Dissolver, where a nondegradable hydrophilic fraction was left in the medium after 28 days. Thus both types of facultative bacteria do not assimilate all of the hydrophiiic substances. This particular fraction may be classified as 'refractory

The fulvic acid fraction was much more refractory than the hydrophilic fraction under the conditions studied. In fact there was not much change observed with fulvic acid as a result of microbial interaction under aerobic conditions. It seems anaerobic conditions removed them (~20%) significantly but not oxic. The humic acids seemed to increase slightly. However, it is very difficult to interpret the extent of the changes because of the very low DOC concentration.

7.4 CONCLUSIONS

For the AMCOR and LVOS samples, DOC in raw effluents is more effectively removed under aerobic conditions than under anaerobic conditions. There was no appreciable DOC removal observed under anaerobic conditions if the media were subjected to aerobic conditions initially. The DOC removal increased when the anaerobic medium was crossed over to aerobic conditions. The above trends were mainly due to the biological effect on hydrophilic substances.

After 28 days involving both aerobic and anaerobic treatment, both the mill effluent and its two receiving wastewater sites were left with a considerable amount of hydrophilic substances. The hydrophilic substances left are obviously refractory under the given conditions. Almost all of the fulvic acids seemed to be refractory under aerobic conditions. But significant amounts (~20%) can be removed by anaerobic treatment. Experiments with an extended time frame could be useful to determine whether DOC removal continues under both aerobic and anaerobic conditions.

The results revealed that the most efficient degradation condition for hydrophilic substances was aerobic degradation. The hydrophilic fraction is the major organic fraction that bacteria selectively and effectively removed under both types of degradation.

Chapter 8

Effect Of Biological Degradation On Colour And Molecular Weight Of Chemical Fractions Of An Australian Papermill Effluent And Recipient Water

Abstract

The current study focused on the Latrobe Valley outfall sewer (LVOS) piped channel in the vicinity of the AMCOR Maryvale plant. The AMCOR mill effluent was chosen since it is the major input to the LVOS channel. This study investigated rates of dissolved organic carbon (DOC) removal from the AMCOR mill effluent and the mill effluent receiving wastewater collected from a site within the piped channel, called Rosedale Dissolver. The major aim was to study the role of inverting the redox conditions (which is referred to as aerobic-anaerobic crossing over; i.e. oxic→anoxic or anoxic→oxic) on DOC, colour and molecular weight as a result of microbial mediated processes. The biological effects on the fulvic acid and humic acid fractions were also studied.

The rate of DOC removal is higher with aerobes than with anaerobes. The rate is higher in fresh mill effluent than in wastewater containing mill effluent. In the oxic→anoxic cycle, DOC was only removed during the oxic phase. The anoxic→oxic cycle also removed the same DOC content from the medium but over the total time frame involving both conditions.

The aerobes initially removed colour from the medium, but extended exposure to the same oxic conditions increased the colour in the medium. In the case of anaerobes, the colour increased initially but extended exposure to the same anoxic condition decreased the colour in the medium. The colour per DOC was increased by aerobes, but it did not change with anaerobes.

The higher the molecular weight of the raw effluent or wastewater, the higher the colour of the medium. The molecular weight of fulvic acid was decreased by aerobes, but did not change with anaerobes. In contrast, the molecular weight of humic acid was increased by aerobes and as well as by anaerobes. The colour of the humic acid increased due to the aerobes but decreased due to the anaerobes. The crossing over from oxic to anoxic or vice versa, reduced the molecular weights of humic acid further.

8.1 INTRODUCTION

Preliminary screening of biological effects on a wastewater sample collected from Rosedale Meter in the LVOS channel have established that the DOC removal was most efficient under aerobic conditions. This investigation was extended to the AMCOR mill effluent and the mill effluent receiving wastewater in the piped channel of the LVOS system.

The objectives of these experiments were to study the AMCOR effluent and its receiving wastewater to determine the:

- amount of DOC removal by aerobic and/or anaerobic reaction as a function of time
- oxic and/or anoxic effects on colour and molecular weight of the effluents
- oxic and/or anoxic effects on colour and molecular weight of fulvic acid and humic acid fractions in the effluents.

8.2 EXPERIMENTAL

The samples chosen for this investigation are given below:

- pulp and paper mill effluent from the AMCOR plant, which is about to discharge into the LVOS channel – OUTLET 41 MEGALITRE STORAGE
- piped channel with oxygen injectors – ROSEDALE DISSOLVER

The experimental set up was outlined in Chapter 6, section 6.2. The samples were treated aerobically and anaerobically in separate vessels as described in that section and were

withdrawn after time intervals of 1 day, 2 days and 14 days. The aerobic-anaerobic crossover was carried out after 14 days and further samples were withdrawn on day 15, day 16, day 21 and day 28. The withdrawn flasks of samples were restored with milliQ water up to the starting volume in order to correct the change in sample volumes due to water loss by evaporation. After sample processing (as described in Chapter 4), the filtrates were analyzed for DOC using a Shimadzu total organic carbon analyzer (TOC-5000).

8.2.1 Colour evaluation of biologically treated samples

The samples were collected for colour analysis on day 14 of each cycle. Filtrates of biologically treated solutions were refrigerated at 4°C if analysis was not completed on the day of withdrawal. Before colour analysis, filtrates were allowed to equilibrate to a constant room temperature. Dilutions were carried out when the filtrates appeared to be highly coloured (ie. when colour appeared to be greater than 500 Pt-Co units). The pH of the filtrates were adjusted to 7.6 ± 0.1 using 1 M HCl. The absorbances were measured at 465 nm against the Pt-Co standards, whose absorbances were measured prior to sample analysis in order to calibrate the instrument (HITACHI UV-Visible spectrophotometer). Subsequently, the colour values were read as Pt-Co units from the calibration line. All absorbances were measured in a 1 cm quartz cell and baseline adjustment was carried out with milliQ water. The milliQ water was also used as the reference with each measurement including the calibration solutions.

8.2.2 Preparation of calibration line for colour evaluation

The UV-Visible spectrophotometer (HITACHI) was calibrated by using a series of Pt-Co standard solutions. The calibration line was prepared by measuring absorbance values of these standard solutions at 465 nm using a 1 cm quartz cell. The instrument recorded the gradient (K), intercept (b) and Correlation co-efficient (R).

8.2.3 Pt-Co standard stock solution

The standard stock solution of 500 Pt-Co units was supplied by Gippsland Water, with the following preparation procedure. A measured quantity of 0.623 g potassium hexachloroplatinate (K_2PtCl_6) and 0.50 g crystallized cobaltous chloride ($CoCl_2.6H_2O$) was partially dissolved in approximately 200 mL deionised water in a 500 mL volumetric flask. A measured volume of 50 mL of concentrated hydrochloric acid was added to complete the dissolution of the solids. It was then diluted to 500 mL with deionised water. This stock solution had a colour value of 500 Pt-Co units and was stored at 4°C in the refrigerator.

8.2.4 Pt-Co standard solution series

A series of standard solutions (50, 100, 200, 300, 400 Pt-Co units) were prepared by diluting aliquots of 500 Pt-Co unit stock standard solution with milliQ water in milliQ washed 100 mL volumetric flasks.

8.2.5 Statistical Accuracy and Precision

The sample vessels were set up in triplicate with each effluent/wastewater. Each DOC measurement was carried out with three injections. The DOC analysis of each withdrawn sample at each designated time interval was repeated six times. The whole experiment was repeated two times. The instrument reproducibility and accuracy was checked with fresh calibrations on three occasions.

Several replicates of low colour value standards were analyzed to determine the reproducibility of absorbance. The coefficient of variation was determined and, if it was greater than 3%, samples were reanalyzed at a lesser dilution. All samples were analyzed in triplicate. Samples having a colour value less than 100 Pt-Co units were reanalyzed in duplicate with a lesser dilution.

8.3 RESULTS AND DISCUSSION

8.3.1 Biological effect on DOC

The relationship of the DOC in the mill effluent medium and exposure time in oxic conditions is graphically illustrated in Fig.8.1(a). It shows that the DOC remaining in the mill effluent medium decreased with exposure time. A similar trend also occurred under anoxic conditions (Fig.8.1(b)).

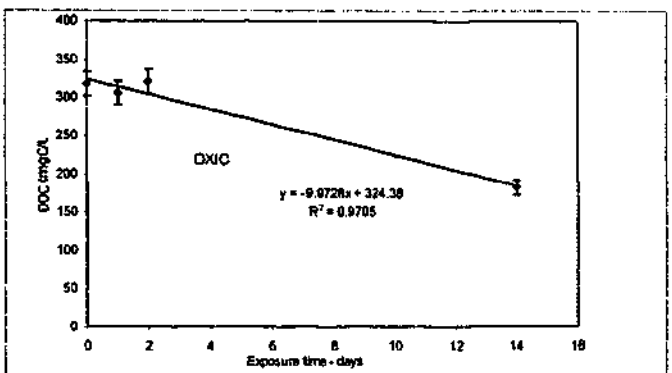


Fig. 8.1 (a) OUTLET 41 ML - Kinetics of DOC degradation under OXIC conditions

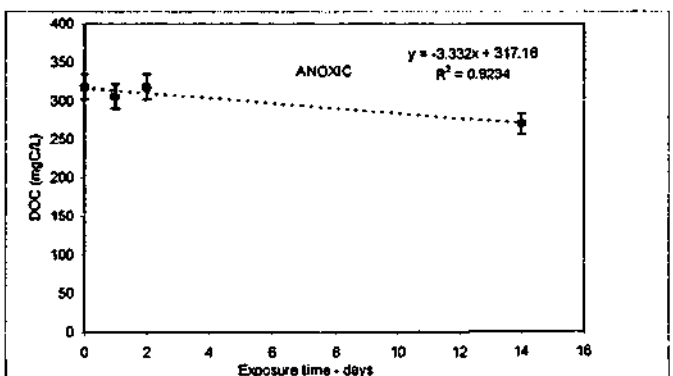


Fig. 8.1 (b) OUTLET 41 ML - Kinetics of DOC degradation under ANOXIC conditions

In the oxic medium up to 43% of the DOC has been removed after 14 days exposure; in the anoxic medium only 15% of the DOC has been removed after the same time. The rate of DOC removal in each case is given by the gradient of the curve of the DOC present in

the medium vs time. This revealed that the rate DOC removal in the mill effluent under the oxic condition is 3 times higher than that of the anoxic condition.

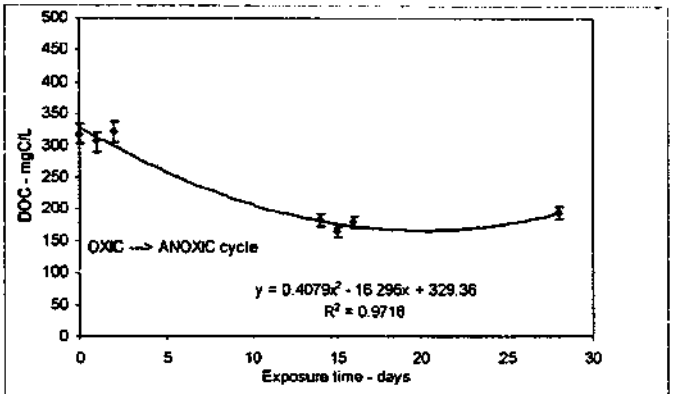


Fig. 8.2 (a) OUTLET 41 ML - Kinetics of DOC degradation when OXIC --> ANOXIC cross over occurs

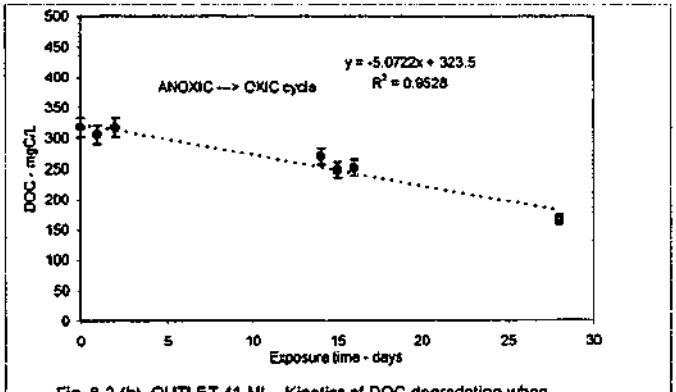


Fig. 8.2 (b) OUTLET 41 ML - Kinetics of DOC degradation when ANOXIC --> OXIC cross over occurs

The DOC degradation cycle for the mill effluent, comprising an oxic exposure period followed by an anoxic exposure period (oxic→anoxic cycle), was analyzed and graphically illustrated by Fig.8.2(a). For the oxic→anoxic experiment the DOC in the medium did not change after oxic samples crossed over to anoxic conditions. It shows that, even though the degradation rate was high when media were provided with sufficient oxygen at the beginning, it stops after crossover to anoxic conditions. In contrast, if the media had initially been exposed to an oxygen deficient environment, the

degradation rate did not vary even after cross over to oxic conditions (Fig.8.2(b)). In fact, the anoxic→oxic curve was approximately linear and the DOC in the medium continuously decreased with the exposure time.

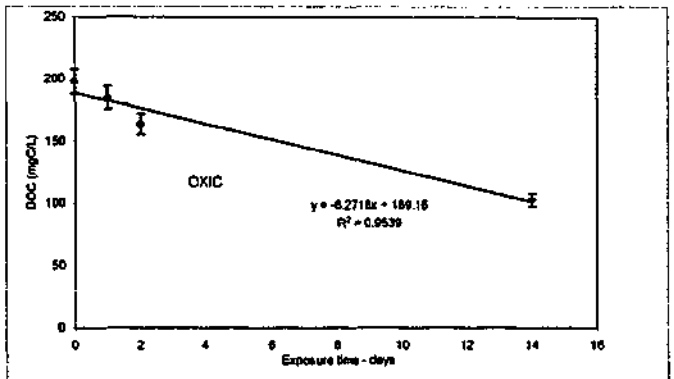


Fig. 8.3(a) ROSEDALE DISSOLVER - Kinetics of DOC degradation under OXIC conditions

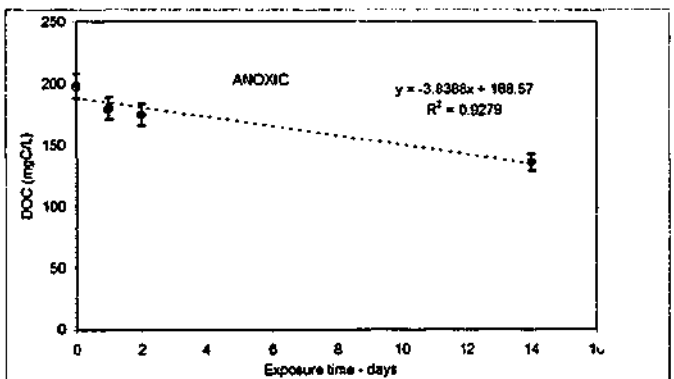


Fig. 8.3(b) ROSEDALE DISSOLVER - Kinetics of DOC degradation under ANOXIC conditions

Similar behaviour was observed with the wastewater collected from Rosedale Dissolver site. The gradients of the graphs of DOC remaining in the medium vs exposure time under oxic and anoxic conditions (Figs.8.3(a) and 8.3(b)) show that the rate of DOC removal under the anoxic condition (Fig.8.3(b)) was almost the same in both samples (mill effluent (Fig.8.1(b)) and mill effluent receiving wastewater at Rosedale Dissolver site (Fig.8.3(b)). However, the rate of DOC removal in the Rosedale sample under the

oxic condition (Fig.8.3(a)) was about 30% lower than for the mill effluent (Fig.8.1(a)) under the same conditions. In addition, the DOC removal rate under the oxic condition (Fig.8.3(a)) in the Rosedale wastewater was only 1.5 times than that in the anoxic medium (Fig.8.3(b)), compared to a 3 fold difference for the mill effluent sample. The reason may be that, for both samples under aerobic conditions, all the DOC that anaerobic bacteria can degrade is consumed by aerobes. The other possible reason may be that all anaerobes are killed in aerobic conditions but not vice versa.

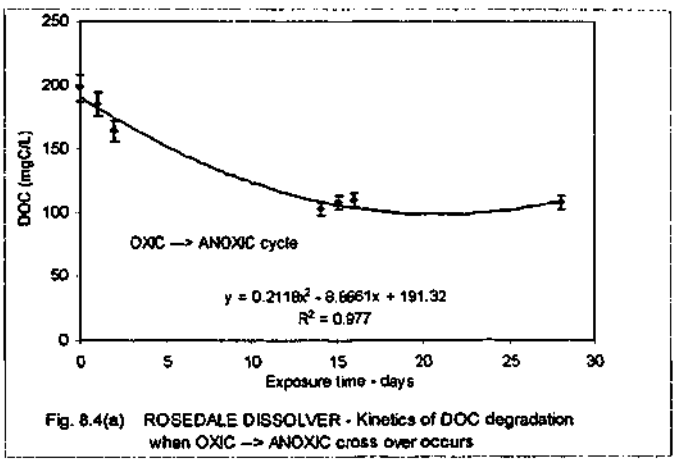


Fig. 8.4(a) ROSEDALE DISSOLVER - Kinetics of DOC degradation when OXIC —> ANOXIC cross over occurs

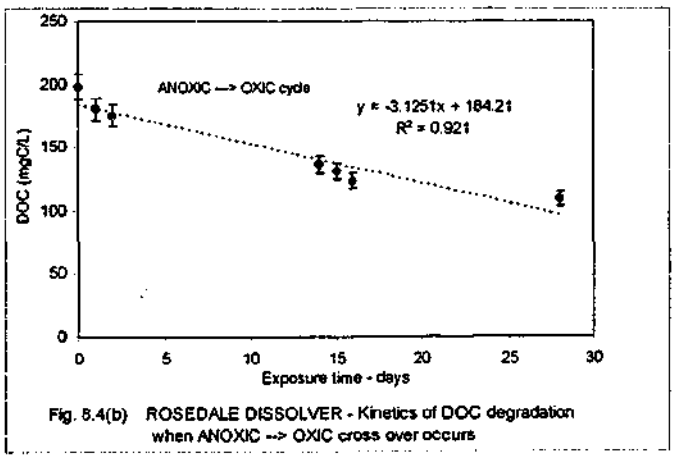


Fig. 8.4(b) ROSEDALE DISSOLVER - Kinetics of DOC degradation when ANOXIC —> OXIC cross over occurs

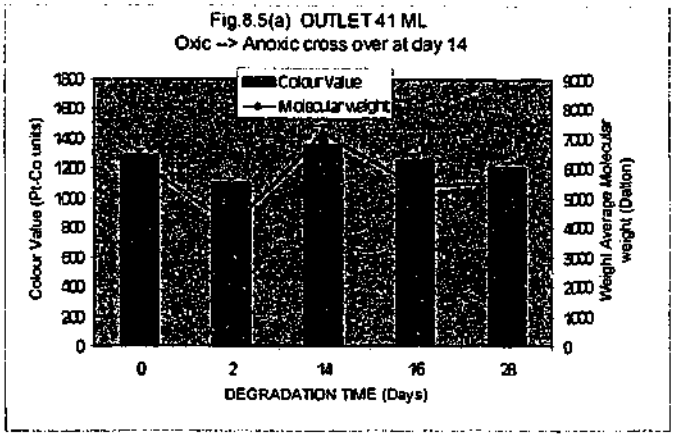
The oxic→anoxic cycle and the anoxic→oxic cycle for DOC in the medium with microbial exposure time studied with the wastewater at Rosedale site showed (Figs.8.4(a) and 8.4(b)) somewhat similar results as the fresh mill effluent under the same experimental conditions (Figs.8.1(a) and 8.1(b)).

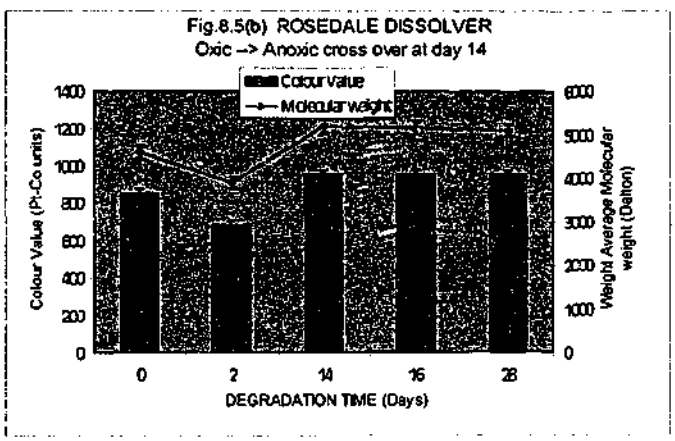
8.3.2 Biological effect on colour

The mill effluent and its receiving wastewater were tested for the effect of oxic or anoxic conditions on the colour of the medium. The results are illustrated in Figs.8.5(a,b) and 8.6(a,b). The colour value of the mill effluent was greater than the effluent receiving wastewater collected from the Rosedale site at the oxygen dissolver. This could be due to dilution of the mill effluent by ~1.5 times (refer to Chapter 3, Section 3.2) by the time it reaches the Rosedale Dissolver site.

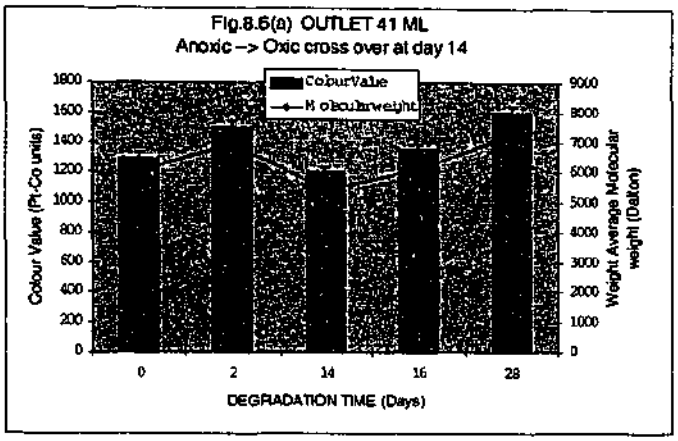
The colour of the mill effluent decreased within the first two days under the oxic condition, but further exposure to the same conditions up to day 14 seemed to increase the colour (Fig.8.5(a)) in the medium. It then decreased within the first two days after crossover to anoxic conditions, but further exposure to the anoxic condition did not change the colour further. The colour of the mill effluent receiving wastewater collected from the Rosedale site (Fig.8.5(b)) also demonstrated a similar behaviour except that after crossover from oxic to anoxic condition, the colour in the medium was stable. The results of both samples seemed to prove that the aerobes initially reacted on the coloured molecules, but eventually produced coloured biproducts. The stability of the colour during the anoxic phase in the oxic→anoxic cycle could be due to the refractory nature of

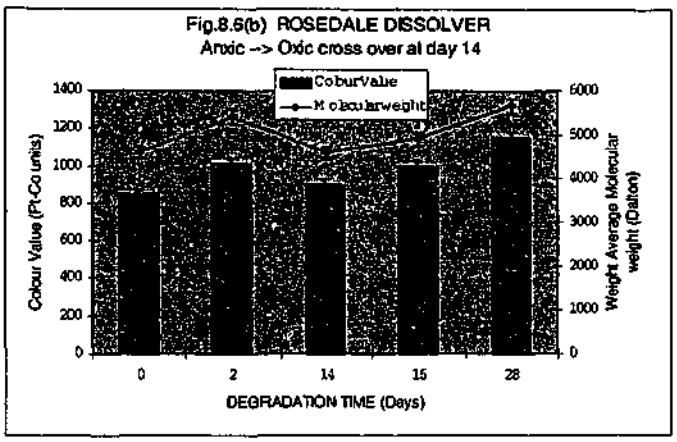
the DOC to anoxic reaction, or the fact that aerobic bacteria do not survive the initial 14 days of oxygenation.





The mill effluent subjected to the anoxic condition at the beginning (Fig.8.6(a)) showed an increase in colour within the first two days, but decreased to the same level at day 14. After crossing over to oxic conditions at day 14, the colour in the medium gradually increased until day 28. The wastewater at the Rosedale Dissolver site (Fig.8.6(b)) also behaved in a similar manner.

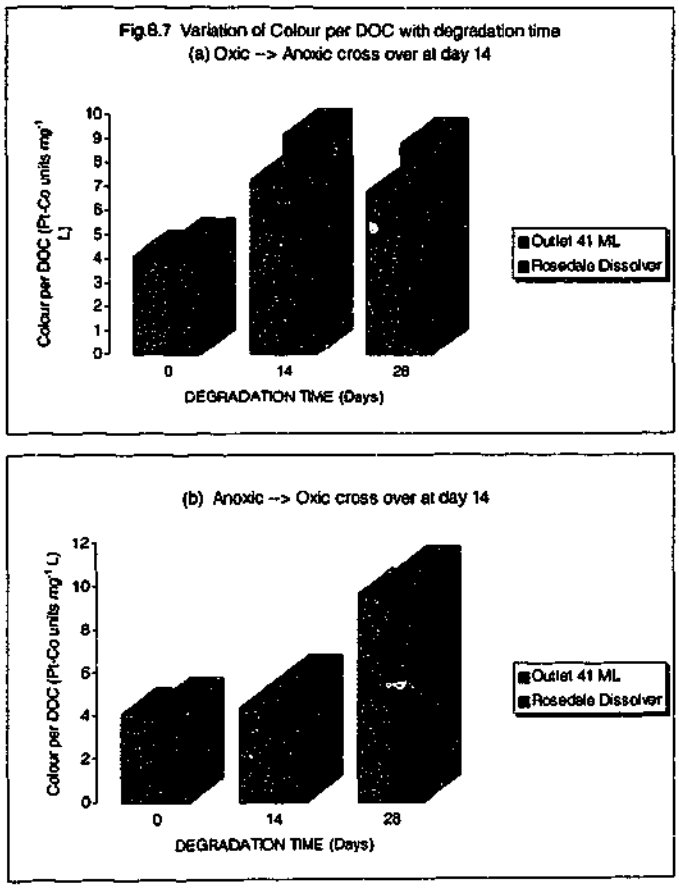




According to the results observed with both samples in the anoxic→oxic cycle (Fig.8.6(a) and 8.6(b)), it seems that the initial anoxic reaction (i.e. within the first two days) could lead to the formation of coloured biproducts while at the same time degrading some of the DOC (Figs.8.2(b) and 8.4(b)). Also, the oxic reaction in the anoxic→oxic cycle in both samples (Fig.8.6(a) and 8.6(b)) resulted in the formation of coloured biproducts while degrading the DOC (Figs.8.2(b) and 8.4(b)). The fact that the molecular weight trends are exactly the same as the colour suggests that colour is associated with high molecular weight fractions.

8.3.3 Biological effect on colour per DOC

Figure 8.7 illustrates the colour per DOC in the mill effluent and wastewater when both the oxic/anoxic and anoxic/oxic cross over occurs in experiments. Colour per DOC increased during the oxic phase in both cycles (Figs.8.7(a) and 8.7(b)), regardless of whether the medium was oxygenated at the beginning or after a deoxygenation phase. This is due to both an increase in colour and a decrease in DOC. Thus the formation of more chromophores is indicated and perhaps more noncoloured molecules are removed than coloured molecules.



The anoxic reaction did not seem to increase the colour per DOC significantly in either sample (Figs.8.7(a) and 8.7(b)). In the oxic→anoxic cycle the stability of the colour per

DOC during the anoxic phase is mainly due to the stability of the DOC (Figs.8.2(a) and 8.4(a)). Probably, the microbe susceptible DOC has already been exhausted from the medium during the oxic phase, so that the DOC left in the medium at the time of crossing over to the anoxic phase could be refractory for anaerobes. Alternatively the anaerobes were already killed off by the oxic phase.

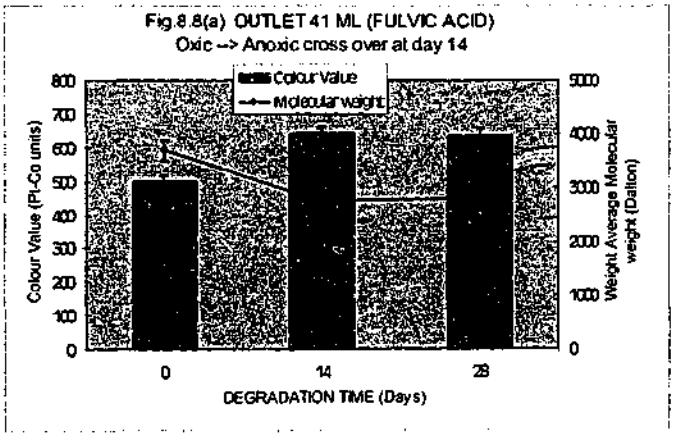
In the anoxic→oxic cycle, the slight increase in colour per DOC during the anoxic phase is due mainly to the removal of DOC while the colour is almost constant (Figs.8.6(a) and 8.6(b)) (Figs.8.2(b) and 8.4(b)). Probably the DOC removed by anaerobes in the fresh anoxic media (before being subjected to oxic conditions) is noncoloured DOC.

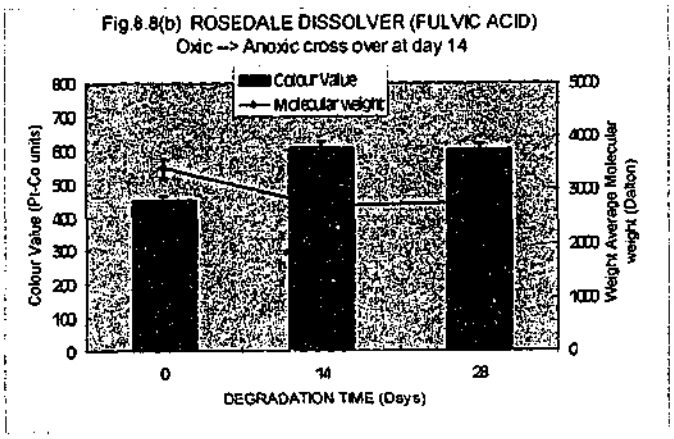
8.3.4 Biological effect on molecular weight

The behaviour of the weight average molecular weight with the exposure time for the mill effluent, under oxic or anoxic conditions with subsequent crossing over, is illustrated in Figs.8.5(a) and 8.6(a). In the oxic→anoxic cycle (Fig.8.5(a)), the weight average molecular weight after 14 days oxic exposure was higher than the molecular weight before the oxic exposure (day 0). In the case of the anoxic→oxic cycle (Fig.8.6(a)), the weight average molecular weight after 14 days anoxic exposure was similar to the molecular weight at day 0. These characteristics were observed with the Rosedale Dissolver sample as well (Figs.8.5(b) and 8.6(b)). The above molecular weight trends followed the behaviour of the colour of the medium. It seems that the colour of the medium is mainly associated with the high molecular weight organic matter.

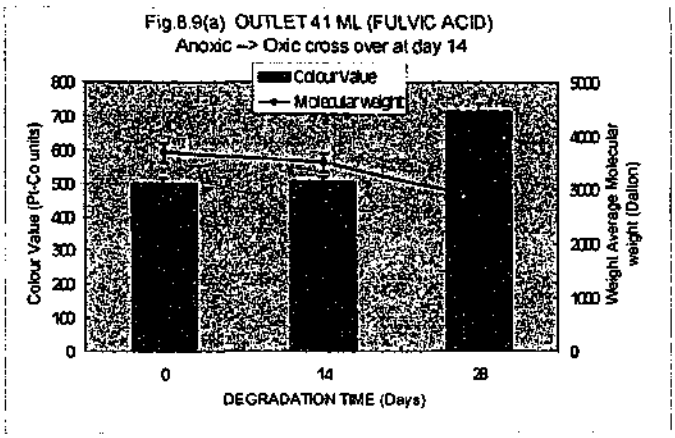
In the oxic→anoxic cycle with the mill effluent (Fig.8.5(a)), the decreased molecular weight after 14 days anoxic exposure is probably due to the simple fragmentation of large molecules, since the DOC was stable during the above interval (Fig.8.2(a)). With the Rosedale Dissolver sample (Fig.8.5(b)) in the same cycle, the weight average molecular weight did not change after 14 days anoxic exposure and this is probably due to the stability of the DOC (Fig.8.4(a)) to anoxic reaction. The DOC removed during the oxic phase in the anoxic→oxic cycle for both samples (Figs.8.2(b) and 8.4(b)) was apparently due to low molecular weight fraction (Figs.8.6(a) and 8.6(b)).

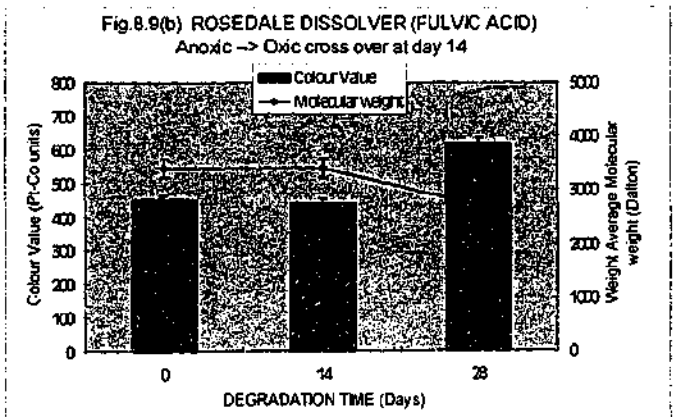
8.3.5 Biological effect on fulvic acid





For both samples, the colour of the fulvic acid fraction increased during the oxic phase in the oxic→anoxic cycle (Figs.8.8(a) and 8.8(b)). The colour did not change as a result of crossing over to anoxic condition. In the anoxic→oxic cycle also (Figs.8.9(a) and 8.9(b)), the same trend in colour was obtained, suggesting that the aerobes are capable of producing coloured fulvic acid whereas anaerobes are not capable of changing the colour of fulvic acid.

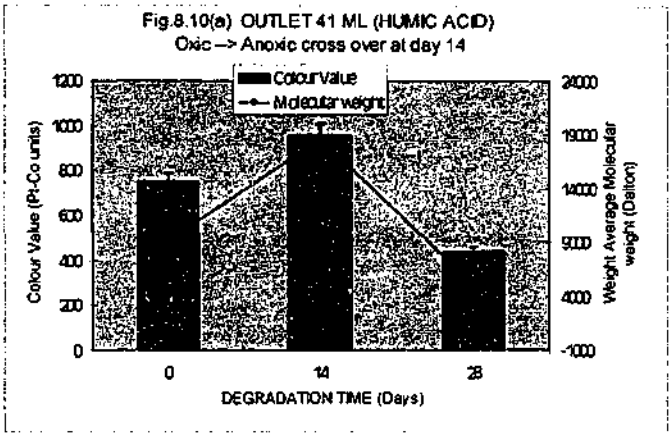


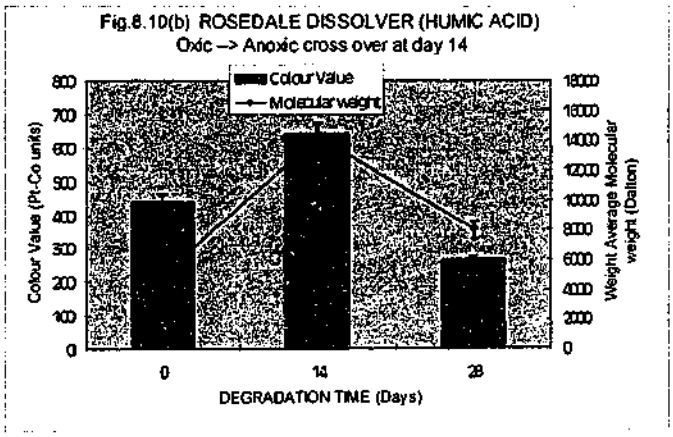


The weight average molecular weight of the fulvic acid extracted from the mill effluent after oxic/anoxic treatments showed that it decreased during the oxic phases but did not change during the anoxic phases (Figs.8.8(a) and 8.9(a)). The mill effluent receiving wastewater also demonstrated the same trend (Figs.8.8(b) and 8.9(b)) under the same

conditions. The oxic reaction seemed to remove the larger molecular weight fractions, but the anoxic reaction did not seem to affect the molecular weight. It seems that for the fulvic acid, the colour is associated more with the lower molecular weight fractions, or only the noncoloured molecules in the higher molecular weight fractions are removed.

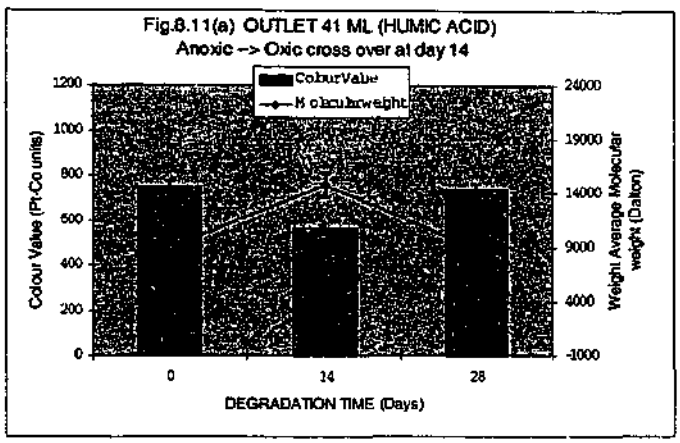
8.3.6 Biological effect on humic acid

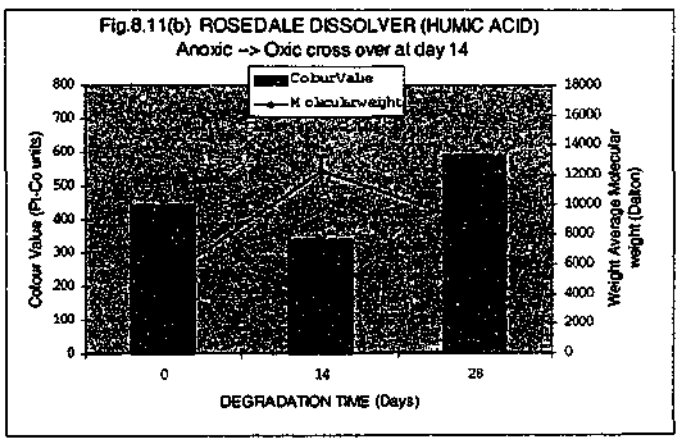




The colour value of humic acid extracted from both the mill effluent and wastewater increased after 14 days of oxic exposure (Figs.8.10(a,b) and 8.11(a,b)). However, on crossing over to anoxic conditions for 14 days, the colour of the medium was decreased.

If they were exposed to the anoxic→oxic cycle, the colour of the medium was decreased in the anoxic medium and increased in the oxic medium. This data suggests that the aerobes are capable of producing coloured organic byproducts while anaerobes are capable of removing coloured organic matter.





The humic acids of both samples after exposure to oxic↔anoxic cycles were analyzed for molecular weight changes. The results are illustrated in Figs.8.10(a,b) and 8.11(a,b). The weight average molecular weight of humic acid initially increased under both conditions (Figs.8.10(a,b) and 8.11(a,b)). The molecular weight then decreased after crossing over the redox condition in the medium. The microbes still active after the

crossover (i.e. facultative microbes) probably reacted on high molecular weight humic acid fractions irrespective of whether the second stage of the cycle was oxic or anoxic. The molecular weight follows the same trend as colour in the oxic→anoxic cycle (Figs.8.10(a) and 8.10(b)), suggesting that the colour is associated with the high molecular weight humic acid fractions. However, in the anoxic→oxic cycle (Figs.8.11(a) and 8.11(b)), the opposite trend in colour occurs but not for the molecular weight. The data suggests that the facultative microbes preferentially remove higher molecular weight molecules but produce coloured byproducts.

8.4 CONCLUSIONS

The rate of DOC removal is higher with aerobes than with anaerobes. The DOC removal rate is higher with the mill effluent than with the mill effluent receiving wastewater at the Rosedale Dissolver site. Some fractions of the DOC were refractory to anaerobic reaction. The DOC removed in the first two days of the oxic phase is coloured but coloured material is generated later. However, if the medium was anoxic from the beginning, the colour was generated initially but removed later. The colour of the raw effluent media was due mainly to the high molecular weight organic fractions.

The colour of the fulvic acid is mainly associated with the low molecular weight fulvic acid fractions. Noncoloured high molecular weight fulvic acid fractions also could be removed. The aerobes preferentially remove higher molecular weight fulvic acid fractions. The anaerobes did not affect the colour or molecular weight of the fulvic acid.

The aerobes produced coloured humic acid material. Perhaps the low and high molecular weight humic acid fractions removed by aerobes in both cycles were noncoloured. The anaerobes removed coloured humic acid material. The high and low molecular weight humic acids removed by anaerobes in both cycles were coloured. Generally speaking, the aerobes are involved in producing coloured humic acid fractions, whereas the anaerobes are involved in removing coloured humic acid fractions. Aerobes as well as anaerobes preferentially remove low molecular weight humic acid fractions. Subsequently they remove high molecular weight humic acid fractions as well.

Chapter 9

Studies On Effluents From A New Zealand Paper Mill And Recipient Water

Abstract

A series of sites along the lower reaches of the Tarawera river in New Zealand were selected for analysis where the major inputs were effluents from the Tasman pulp and paper mill. The study has focussed on five different sewers discharged at five different processing stages of the mill as well as the clarifier (primary treatment plant), the aerated lagoons (secondary treatment plant) and a few selected sites along the Tarawera River (recipient waters) lower reaches.

The initial phase of the primary treatment process (clarification) was shown to break down larger molecules into smaller molecules. The resultant smaller molecules have more colour than the larger molecules. This process did not seem to affect the DOC level in the medium. The latter phase of the primary treatment process (sludge lagoon) preferentially removes larger molecules and they were more coloured than smaller molecules. In other words, the colour/DOC ratio has been affected by sludge lagoon treatment.

The settling basin (pond 1) of the secondary treatment process did not affect the DOC level or molecular weights of the dissolved organic matter. The aeration of the secondary treatment process (pond 2) has removed small dissolved organic molecules, which were coloured. The aerated ponds seemed to maintain a constant low DOC level throughout the aeration process. Perhaps molecular dissociation and association could be occurring during the pond treatment while the colour increases. Biological studies have shown that the hydrophilics were preferentially removed when the concentration of fulvic and humic acids were low. Also, they may have been preferentially removed if fulvic and humic acids were refractory. However, some hydrophilics are nonbiodegradable while some humic and fulvic acids are degradable. Hydrophilics were formed while humic acids were degraded. In the absence of degradable hydrophilics the fulvic acids were removed.

The average molecular weight of the raw effluent could be decreased or increased depending on which organic fraction is being removed. The colour of the raw effluent is mainly dependent of the concentration of humic acid or fulvic acid present. In the Caustic Sewer it is due to fulvic acids and in the Pulp mill sewer it is due to humic acids. The colour of the raw effluent varies, mainly due to which acid (i.e. fulvic or humic acid) in the medium is degraded.

9.1 INTRODUCTION

The lower reaches of the Tarawera River and the Tasman's aerated lagoons situated in Kawerau in New Zealand were selected for analysis. The major inputs were effluents from the Tasman pulp and paper mill plant. The Tasman pulp and paper mill plant discharges five effluents produced at five different processing stages into the lower reaches of the Tarawera River (Fig.9.1). The study also extended to the primary and secondary treatment complexes of Tasman pulp and paper mill plant. The primary treatment complex consists of a clarifier and a sludge lagoon. The secondary treatment complex consists of the Tasman's aeration lagoon.

The sewers generated from the different processes of the Tasman pulp and paper mill plant are discharged into the primary treatment plant in excess of 206 million litres per day. All sewers except the acid sewer are combined and discharged to the clarifier in the primary treatment plant where clarification of the mill output is carried out before discharge to the aerated lagoons. The resultant clarified mill output main sewer is then combined with the acid sewer effluent and discharged to the ponds in the Tasman's aeration lago ns.

The filtered samples from each site were analyzed for DOC, colour and molecular weight. The water quality at downstream sites along the Tarawera River may reflect either biological plus physicochemical changes or may simply be a result of dilution by influxes from canals and tributary streams. Laboratory experiments were carried out with the caustic sewer and pulp mill sewer discharges to investigate biological

effects on these two mill effluents. Isolation and prefractionation of humic acids and XAD-8 resin fractionation was carried out for chemical characterization. Flow FFF analyses were carried out for physical characterization (molecular weight distributions).

9.2 STUDY SITE

This study has focussed on the Tarawera River and Tasman's aerated lagoons situated in Kawerau in New Zealand. A series of sites along the lower reaches of the Tarawera River were selected for analysis where the major inputs were effluents from the Tasman pulp and paper mill plant (Fig.9.1). There are five effluents being discharged to the Tarawera River lower reaches system from the Tasman pulp and paper mill plant (Fig.9.1). These effluents are in fact sewers discharged at five different processing stages of the Tasman pulp and paper mill plant. These five different sewer discharges were as follows :

- Paper machines → Paper Mill Sewer
- Bleach Plant → Caustic Sewer
- Bleach Plant → Acid Sewer
- Wood storage → Groundwood Sewer
- Pulping Plant → Pulp Mill Sewer

The study was extended to the primary and secondary treatment complexes of the Tasman pulp and paper mill plant. The primary treatment complex consists of the Dorr-Oliver clarifier (initial phase of the primary treatment plant) and a sludge lagoon (latter phase of the primary treatment plant). The inlet and outlet streams of the Dorr-

Oliver clarifier were the effluents collected from the primary treatment plant, and they were labelled as follows:

- Clarifier in
- Clarifier out

The Tasman's secondary treatment complex consists of some aeration lagoons. The effluents collected from the secondary treatment plant were as follows:

- Pond in
- Pond 1-2
- Pond 2-3
- Pond 3-4
- Pond out

The sewers generated from the different processes of the Tasman pulp and paper mill plant are discharged into the primary treatment plant. The total discharge is in excess of 206 million litres per day. All sewers except the acid sewer are combined and discharged to the clarifier where clarification of the mill output is carried out before discharge to the aerated lagoon (Fig.9.1). The combined sewer enters the clarifier in 20 million litre batches and its retention time in the clarifier is 2 hours.

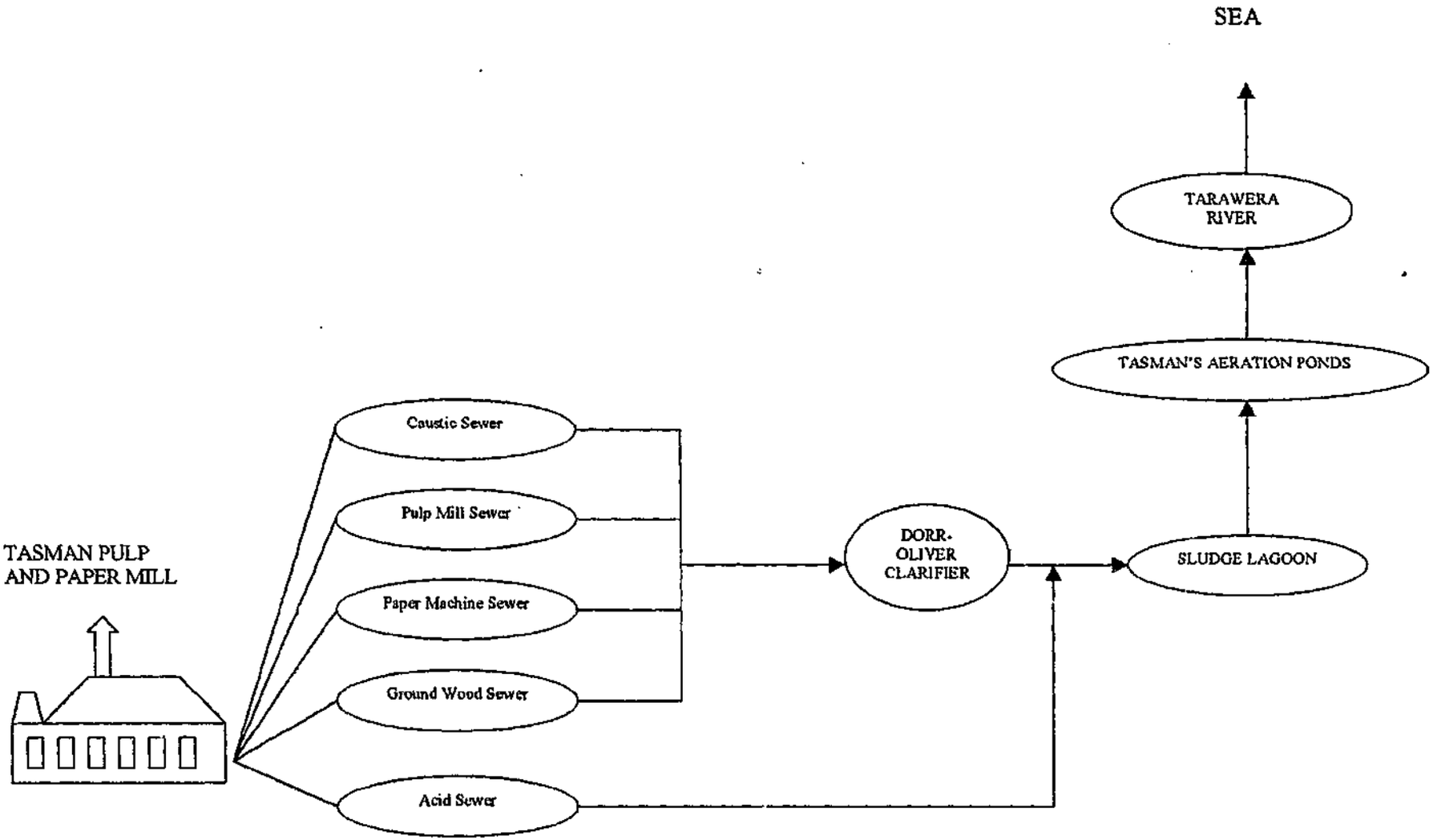


Fig.9.1 Schematic diagram of Tasman Pulp and Paper Mill effluent treatment system

The resultant clarified mill output main sewer is then combined with the acid sewer. The average volume of acid sewer that combines with the main sewer and exits from the clarifier is 20 million litres per day. There is no need to adjust the pH of the combined mill output because after the acid sewer is combined with the main sewer effluent after exiting the clarifier, the pH is close to neutral. This resultant neutral combined effluent is then discharged to the sludge lagoon and then to the ponds in the Tasman's aeration lagoons, which are called the secondary treatment plant. The characteristics of the water in the main sewer that enters the treatment ponds and exits from treatment ponds when samples were collected are given in the table below:

Table 9.1. Characteristics of combined main sewer before and after the treatment ponds (Data supplied by Tasman's pulp and paper mill in New Zealand)

	pH	Suspended Solids (mg/L)	Colour (Pt-Co units)	Dissolved Oxygen (mg/L)	BOD (mg/L)
Pond IN (from clarifier)	8.4	88	590	1.5	161
Pond OUT (to the Tarawera River)	7.6	21	556	4.3	24

The retention time in the aerated lagoons is 5-6 days and then it is discharged to the Tarawera River. The sites from where receiving waters (wastewaters) were collected in the lower reaches of the Tarawera River were as follows:

- Kawerau Bridge
- Tasman Bridge
- Seed Orchard
- O'Sullivans Farm

- Above Braemar outlet
- Below Braemar outlet
- State Highway No.2 Road Bridge
- Matata Main Road

9.3 EXPERIMENTAL

9.3.1 Sample Pretreatment

9.3.1.1 Effluents and their receiving wastewaters for characterization

The samples were received as azide treated (0.075 g of sodium azide in 250 mL sample) solutions. Upon receipt at the Melbourne laboratory, samples were filtered through 0.2 μm nylon membrane (50 mL per membrane) on a nylon mesh membrane holder under vacuum. The nylon membranes were pre-washed with milliQ water before use for filtering. The filtered samples were analyzed for DOC, colour and molecular weight as described in Chapters 3-8.

9.3.1.2 Effluents for laboratory scale biological treatment experiments

The samples were received as fresh raw samples (without adding azide) and they were transported to the Monash laboratory under refrigerated conditions (< 4°C). The samples were immediately stored at < 4°C until experiments were commenced. They were swirled several times and laboratory scale microbial experiments were arranged without doing the filtering process.

9.3.2 Laboratory tests for the effect of biological processes

Two effluents were considered for this experiment and they were:

- Caustic Sewer
- Pulp Mill Sewer

The experimental set up was outlined in Chapter 7, Section 7.2. The samples were treated aerobically and anaerobically in separate vessels as described previously (Chapter 7, Section 7.2). Samples were withdrawn after designated time intervals of 7, 14, 21 and 28 days. The withdrawn samples were restored to the original volume to compensate the water loss due to evaporation. They were sterilized, centrifuged and filtered as described in Chapter 7, Section 7.2. Then filtrates were analyzed for DOC as described in Chapter 4, Section 4.2, using a Shimadzu total organic carbon analyzer (TOC-5000).

The following experimental conditions were chosen in this study.

(1) The samples were swirled several times and the microbial experiment was carried out without any filtering process.

(2) The effluent from the bleaching plant, which had pH 13.7, was neutralized with conc. HCl before commencing the experiment.

(3) The effluent media were innoculated with sewage using a 10:1 (effluent:sewage) volume ratio and the experment was performed without any dilution.

(4) The sewage treated effluent media were diluted 2 times and the experiment was performed in duplicate.

(5) Subsamples were withdrawn after the designated time intervals and filtered through a 0.2 μm nylon membrane under vacuum before commencing analysis.

The isolation and prefractionation of humic acids and XAD-8 resin fractionation was carried out as described in Chapter 4, Section 4.2. The procedures for DOC analysis, flow FFF analysis and colour analysis, also have been described in Chapters 3, 4 and 5 respectively.

9.4 RESULTS AND DISCUSSION

Since the pulp mill sewer and caustic sewer have the highest colour values, these two sewers and the combined sewer that enters and exits the aerated treatment ponds were tested for DOC, molecular weight and colour with freshly collected samples at two different occasions (batch 1 and batch 2). The results are given in Tables 9.2(a) and 9.2(b). Since all sewers are discharged into the clarifier, the high concentrations originally in any of them are reduced to a moderate level within the clarifier as a result of rapid dilution caused by less concentrated paper machine sewer.

Table 9.2(a) Characteristics of Tasman Pulp and Paper Mill effluents and treated wastewaters from secondary treatment plant

Sample Batch 1

Effluent	DOC (mgC/L)	Colour Value (Pt-Co Units)	Weight Average Molecular Weight (Dalton)
Pulp Mill Sewer	190	1140	11200
Caustic Sewer	468	2190	3400
Acid Sewer	334	830	3050
Ground Wood Sewer	190	83	No FFF Peak
Paper Machine Sewer	32	10	No FFF Peak
Clarifier IN	192	236	5000
Clarifier OUT	197	280	4700
Pond IN	140	210	4500
Pond OUT	78	260	5200

Table 9.2(b) Characteristics of Tasman Pulp and Paper Mill effluents and treated wastewaters from secondary treatment plant

Sample Batch 2

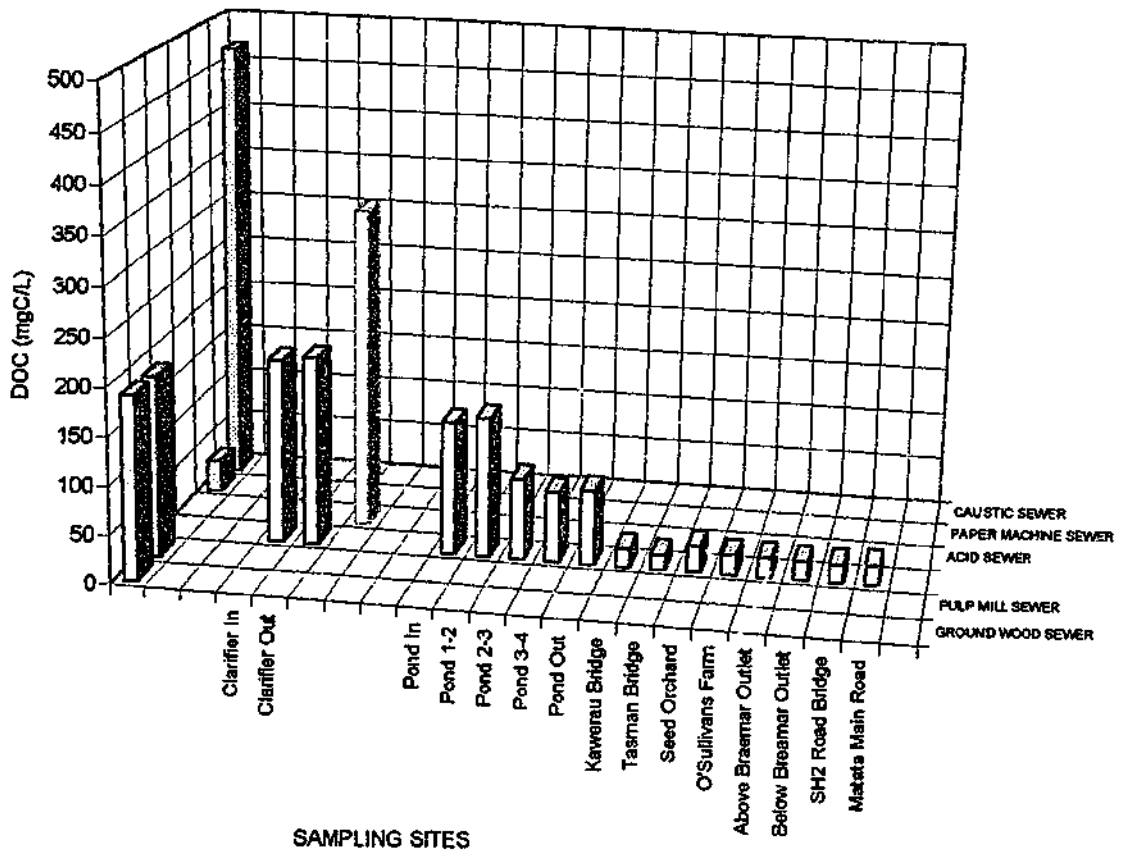
Effluent	DOC (mgC/L)	Colour Value (Pt-Co Units)	Weight Average Molecular Weight (Dalton)
Pulp Mill Sewer	388	1100	18750
Caustic Sewer	479	2050	4250
Pond IN	170	260	4600
Pond OUT	110	310	5600

9.4.1 Characterization of mill effluents

The caustic sewer has the highest DOC level while the paper machine sewer (PM1) has the lowest DOC level (Fig.9.2). Among the five sewer discharges, four of them are combined prior to discharge into the clarifier. The exception is the acid sewer. The caustic sewer is rapidly diluted to a moderate level as a result of combining it with the paper machine sewer before discharge into the clarifier. The DOC level in the combined main sewer that enters the clarifier ($clarifier_{in}$) was almost equivalent to the DOC level of the pulp mill sewer and the groundwood sewer (Fig,9.2).

The paper machine sewer is a colourless clear solution and the groundwood sewer is a foamy opaque white suspension even after filtration through a 0.2 μm nylon membrane. Among the five sewer discharges, the paper machine sewer has the lowest colour value. The groundwood sewer also has a very low colour value. The flow FFF analyses were unsatisfactory with these two sewers because of their low UV absorbance values.

Fig.9.2 TASMAN PULP AND PAPER MILL EFFLUENTS AND SAMPLING SITES OVER TREATMENT PLANTS AND TATAWERA RIVER LOWER REACHES



The caustic sewer and pulp mill sewer have higher colour values than the other sewers while the acid sewer has a medium colour value (Table 9.2(a)). Therefore, flow FFF analyses were possible using UV detection with sewers from the bleach plant (caustic and acid sewers) and the pulp mill plant (pulp mill sewer). The caustic sewer and acid sewer have low weight average molecular weights while pulp mill sewer has a high weight average molecular weight (Tables 9.2(a) and 9.2(b)).

9.4.2 Characterization of effluents from the primary treatment plant

9.4.2.1 Effluents before and after clarification process

The DOC level in the combined main sewer that enters the clarifier ($clarifier_{in}$) was lower than in the caustic sewer due to dilution caused by merging sewers (Fig.9.2). The DOC level of the main sewer exit from the clarifier ($clarifier_{out}$) was almost equivalent to the DOC level of the combined effluent entering the clarifier ($clarifier_{in}$). This shows that the clarification process in the Dorr-Oliver Clarifier has not affected the DOC level in the medium (Fig.9.2).

The weight average molecular weight of the main sewer (5000 Dalton) that enters the clarifier ($clarifier_{in}$) is little higher than the caustic sewer and lower than the pulp mill sewer (Tables 9.2(a) and 2(b)). The reason for this is, the high molecular weight pulp mill sewer and low molecular weight caustic sewer are combined and discharged to the clarifier. The sewer exit from the clarifier ($clarifier_{out}$) has a lower molecular weight than that of $clarifier_{in}$. Since DOC remains unchanged (Fig.9.2), while the molecular weight is reduced during the clarification process, it suggests that

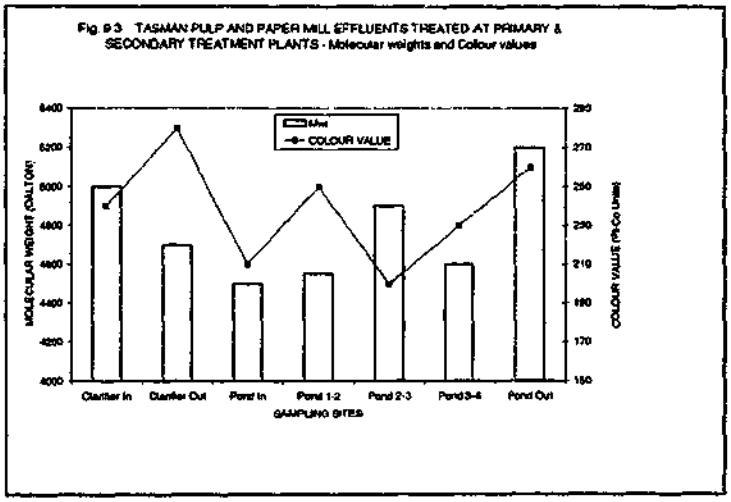
dissociation of high molecular weight dissolved organics or cleavage of large molecules into small molecules occurs during the clarification process.

The colour value in the $clarifier_{in}$ is much lower than that of caustic sewer and pulp mill sewer due to the dilution caused by the combination with other sewers. However, the colour value in the $clarifier_{out}$ is higher than that of the $clarifier_{in}$. That is, the colour value has increased during the clarification process (Table 9.2(a)). The dissociation of larger molecules into smaller molecules (according to the molecular weight studies) would support the idea that the smaller molecules impart more colour than the larger molecules.

9.4.2.2 Effluents before and after sludge lagoon treatment

The high DOC level in the acid sewer was thought to increase the DOC level in the main sewer exit from the clarifier since the acid sewer is connected to the main sewer after the clarifier. Thus the high DOC originally in the acid sewer is reduced to a moderate level as a result of rapid dilution caused by the main sewer that exits from the clarifier (Fig.9.2). However, the main sewer stream that enters the aerated treatment ponds ($pond_{in}$) in fact has a lower DOC level than in the sewer exits from the clarifier ($clarifier_{out}$) even after merging with the high strength acid sewer. That is, as the combined effluent is transported across the sludge lagoon, DOC seems have been removed from the medium. This may reflect either of the two hypotheses; i.e. biological degradation or adsorption/physical settling in the sludge lagoon.

The resultant sewer that enters the treatment ponds ($pond_{in}$) has even lower average molecular weight (Tables 9.2(a) and 9.2(b)) than that of the exit stream ($clarifier_{out}$) because the sewer exit from the clarifier ($clarifier_{out}$) is combined with the low molecular weight acid sewer before it enters the treatment ponds. However, the decrease in DOC (Fig.9.2) between the $clarifier_{out}$ and the aerated lagoon inlet ($pond_{in}$), suggests the removal of high molecular weight substances across the sludge lagoon by means of adsorption and/or settling or biodegradation. The colour value has further decreased in $pond_{in}$, which is probably due to the removal of high molecular weight molecules across the sludge lagoon by means of adsorption/settling or biodegradation (Fig.9.3).



9.4.3 Characterization of effluents from secondary treatment plant

Pond 1 is a settling basin so that no aerators are encountered in pond 1. The DOC and molecular weight at exit pond 1 (i.e. pond 1-2) was equivalent to that of $pond_{in}$. The results show that the DOC and molecular weight has not been affected due to the settling basin. We can infer that changes occuring within the pond 1 treatment are

negligible or simultaneous processes of microbial reaction on dissolved organics and suspended or settled solid matter occur. However, DOC has been considerably decreased and molecular weight has increased during the pond 2 treatment. Pond 2 is equipped with aerators. Probably aeration has promoted the removal of low molecular weight DOC by means of microbial assimilation or microbial oxidation.

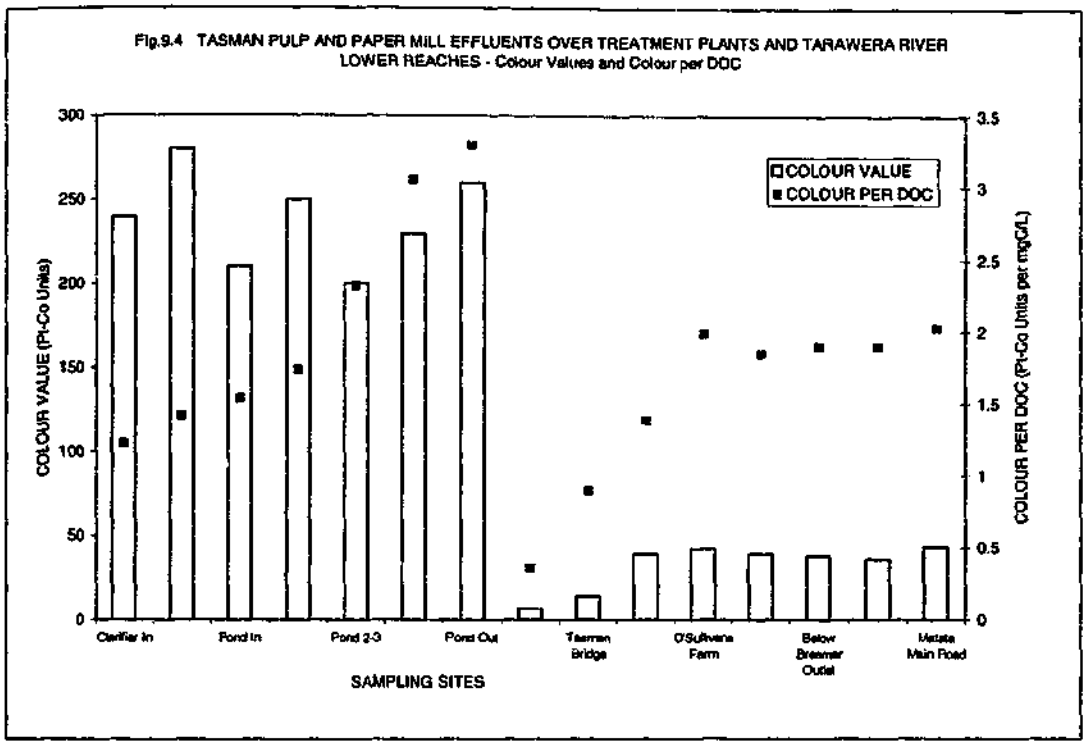
Pond 3 treatment has no significant effect on DOC but the molecular weight has decreased. We can attribute this observation to dissociation of high molecular weight dissolved organics or cleavage of large molecules into small molecules during the pond 3 treatment. The degradation of high molecular weight organic material and release of DOC from bottom sludge also may be possible.

Treatment in pond 4 has no significant effect on DOC but the molecular weight has increased (Fig.9.3). If this observation is due to the microbial reaction on dissolved organics, that it would suggest molecular association or aggregation of low molecular weight dissolved organics into larger molecules during the treatment in pond 4 has occured. However, whether these observations are due to the microbial reaction on dissolved organics or on suspended solid matter is uncertain.

9.4.4 Characterization of receiving wastewaters from Tarawera River

The DOC levels in the downstream sites along the Tarawera River were very low compared to the discharged mill sewer concentrations (Fig.9.2). The elevated DOC and colour value at Seed Orchard (Figs.9.2 and 9.4) show that the merging inputs

and/or in situ production between Kawerau Bridge and Seed Orchard have introduced some DOC and colour to the Tarawera River lower reaches.



The changes in DOC along the Tarawera River beyond O'Sullivan's Farm may reflect either of the two hypotheses: (1) biological degradation and physical settling/aggregation, or (2) they may simply be the result of dilution by low DOC influxes from canals and tributary streams.

9.4.5 Biological Experiment

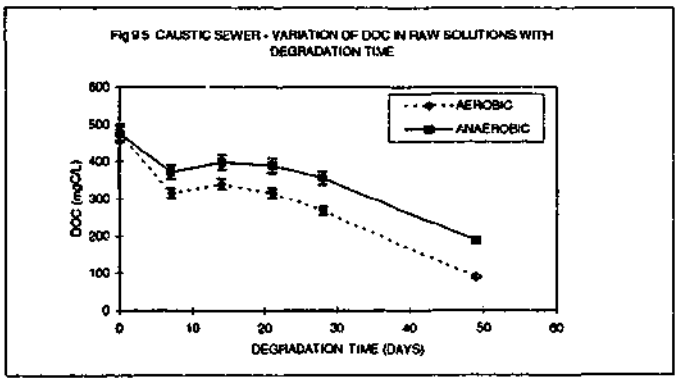
Two sewer samples were subjected to this experiment. They are caustic sewer (pH = 13.7) and pulp mill sewer (pH = 10.8). The pHs of the media were adjusted to 6.5-7.0 with conc. HCl and domestic sewage was added to each flask before leaving for degradation. A test experiment carried out with one drop of domestic sewage in the

caustic sewer produced no change in DOC. Use of the sewer with domestic sewage in a 10:1 volume ratio (sewer:sewage) was found to yield significant changes in DOC. The reason may be lack of enough bacteria in the drop of innoculum.

9.4.5.1 Caustic Sewer

9.4.5.1.1 Chracterization and trends in DOC

The variation of total DOC with time is illustrated in Fig.9.5. The DOC has significantly decreased within seven days under both conditions (aerobic or anaerobic). The most efficient degradation type seems to be the aerobic reaction. This is consistent with the laboratory experiments undertaken within 14 days with the AMCOR mill effluent and LVOS samples, where aerobic degradation seemed to be the most efficient.

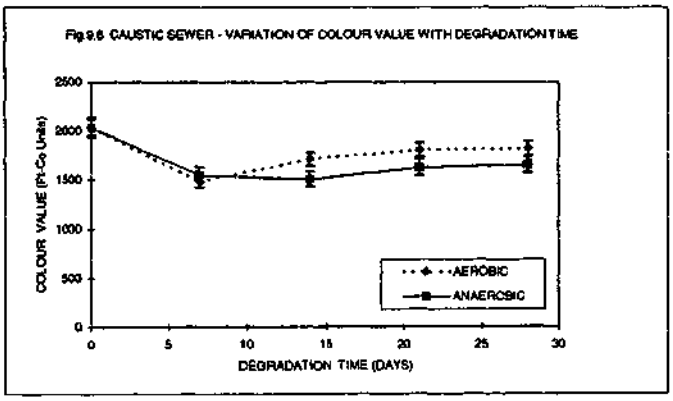


Further exposure to the same conditions for more than 14 days yielded insignificant changes in the DOC. This observation is consistent with the LVOS wastewater sample, which contained some refractory dissolved organic substances (e.g. Peg 83). Only some of the DOC in the caustic sewer has degraded within 28 days and most of the DOC was refractory during that period. However, the continuation of the

experiment under the given conditions proved that microbes were forced to use the available substrate within the rest of the period. This caused DOC to be progressively removed over the extended three week period from t=28 to 49 days (Fig.9.5).

9.4.5.1.2 Characterization and trends in colour value

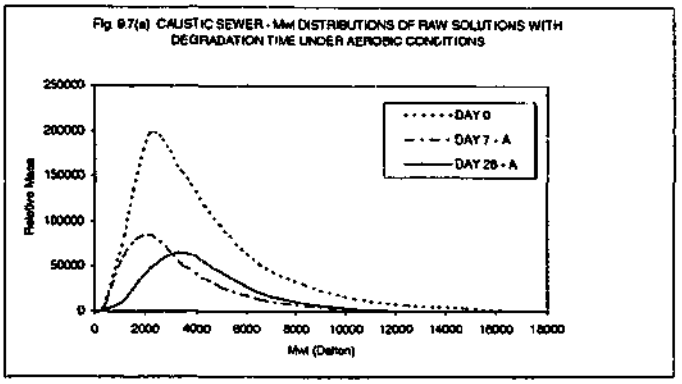
The colour decreased within first seven days under both conditions (Fig.9.6). There was no significant variation after 7 days under the anaerobic conditions. However, there was a tendency to increase under aerobic conditions. Coloured DOC could possibly be leached from particulate organic matter under aerobic conditions after 7 days (Fig.9.6). The DOC removed subsequently (till t= 49 days) under both conditions were both coloured and non-coloured. This indicates the presence of non-biodegradable coloured DOC or possibly the creation of a small amount of coloured DOC material due to microbial reactions.

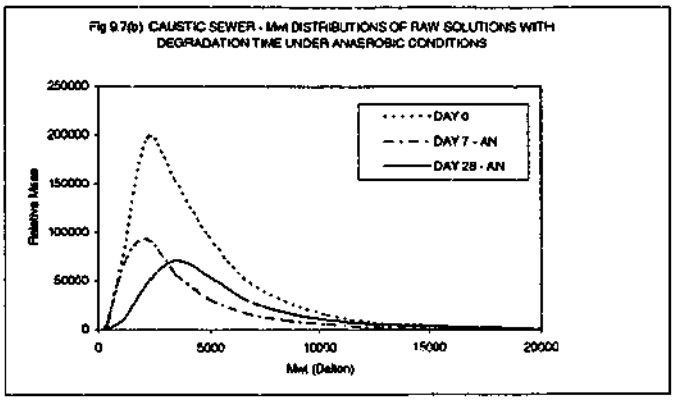


9.4.5.1.3 Characterization by molecular weight

The weight average molecular weight of the raw caustic sewer sample and the humic acids has decreased within seven days under both conditions (Table 9.3). The molecular weight distributions show that almost all molecular weight fractions have

been reduced within seven days under both conditions (Figs.9.7(a,b)). In the humic acid fraction the low molecular weight (<7000 Dalton) organic material has increased within seven days under both conditions (Figs. 9.8(a,b)).





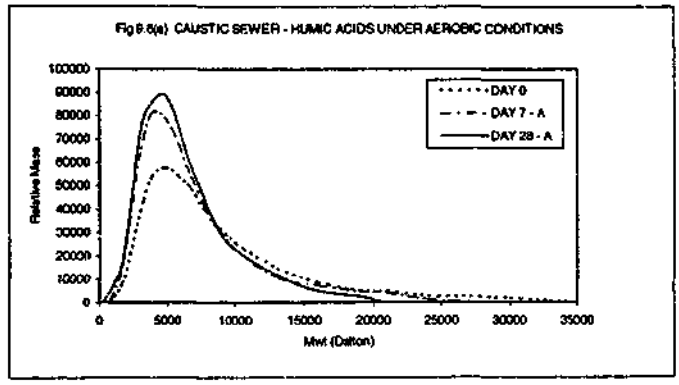
In 28 days, the weight average molecular weight has increased in the raw solutions under both conditions (Table 9.3). However, the average molecular weight of humic acids did not vary from day 7 to day 28. The molecular weight distributions of the raw solutions show that the high molecular weights (>3000 Dalton) have increased from day 7 to day 28 under both conditions. However, the molecular weight distributions of humic acids remain unchanged from day 7 to day 28. Therefore we can conclude that the changes of the weight average molecular weights in the raw solutions from day 7

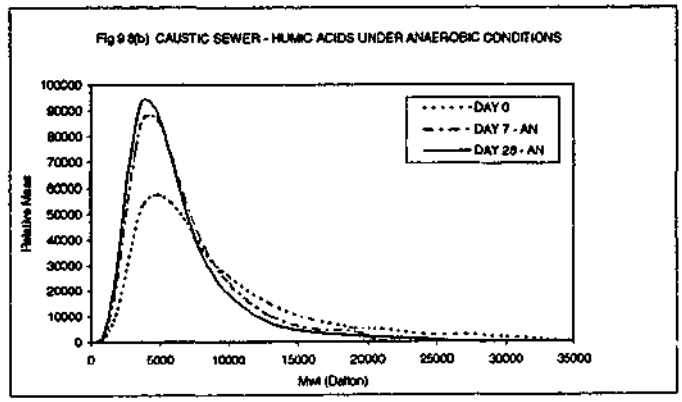
to day 28 are due to substances other than humic acids. They may be fulvic acids or hydrophobic bases.

Table 9.3 CAUSTIC SEWER : Weight and Number average molecular weights of raw solutions and Humic Acids extracted from them

BEFORE DEGRADATION	
Raw Solution	Humic Acids
Mw=4300 Mn=2950 ρ=1.46	Mw=8750 Mn=6000 ρ=1.46

	AEROBIC DEGRADATION		ANAEROBIC DEGRADATION	
Duration (Days)	Raw Solution	Humic Acids	Raw Solution	Humic Acids
7	Mw=3200 Mn=2250 ρ=1.42	Mw=7050 Mn=5050 ρ=1.40	Mw=3700 Mn=2400 ρ=1.54	Mw=6550 Mn=4900 ρ=1.34
28	Mw=4250 Mn=3350 ρ=1.27	Mw=7000 Mn=4950 ρ=1.41	Mw=5400 Mn=3900 ρ=1.39	Mw=6300 Mn=4650 ρ=1.36

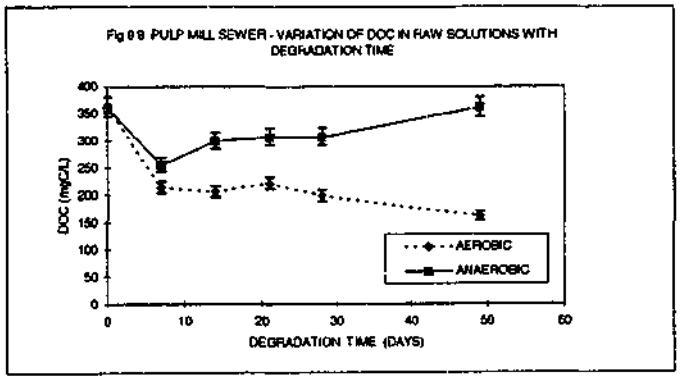




9.4.5.2 Pulp mill sewer

9.4.5.2.1 Characterization and trends in DOC

The total DOC changes are with time illustrated in Fig.9.9. The DOC has decreased under both conditions within seven days. Further exposure showed an increase in DOC under anaerobic conditions, while under aerobic conditions there appears to be a constant in DOC for a certain period and then a decrease. It seems that a fraction of the DOC (~250 mgC/L) in the pulp mill sewer is somewhat refractory to anaerobes and that the anaerobes may be reacting on particulate matter causing some release of DOC. Some of the DOC seemed to be relatively refractory to aerobes as well, causing the DOC removal to be stopped for a certain period (after seven days) before proceeding. This observation is consistent with the DOC results of the caustic sewer under the same conditions, where some of the dissolved organic substances in the medium were more refractory. Microbes eventually utilized them after a lengthier period.

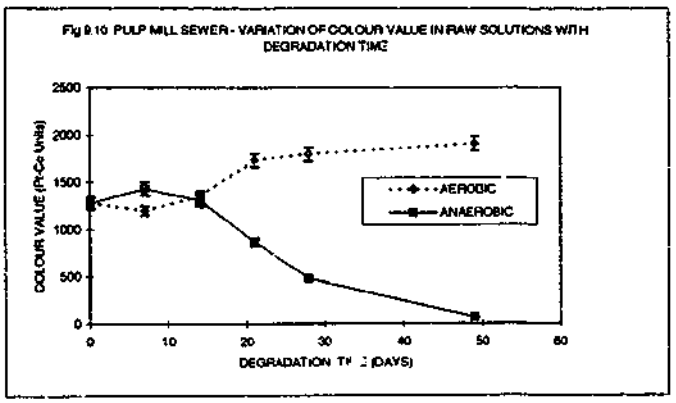


The most efficient degradation type conditions seem to be the aerobic reaction medium. This is consistent with laboratory experiments undertaken with the caustic

sewer as well as the AMCOR effluent and the receiving wastewaters at LVOS channel.

9.4.5.2.2 Characterization by colour

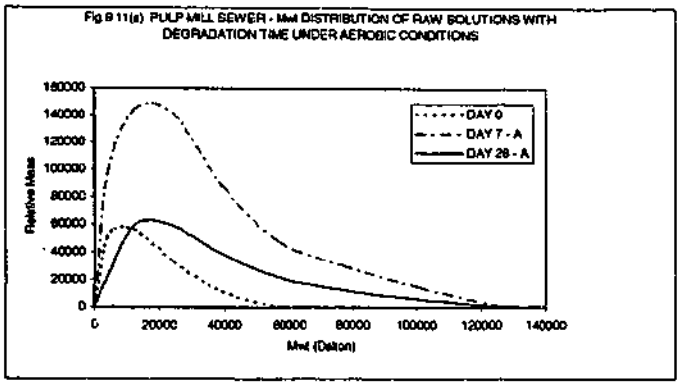
The colour did not change much within the first 14 days under both conditions (Fig.9.10). Perhaps only noncoloured compounds are degraded or the rate of decomposition of noncoloured compounds is the same as the rate of production of coloured compounds. Colour increased gradually over the rest of the period under the aerobic conditions, whereas it decreased after 14 days under anaerobic conditions. Perhaps the leached DOC under the anaerobic conditions is primarily noncoloured (Fig.9.10). The subsequent removal of DOC (i.e. after 7 days) under aerobic conditions would be noncoloured material.

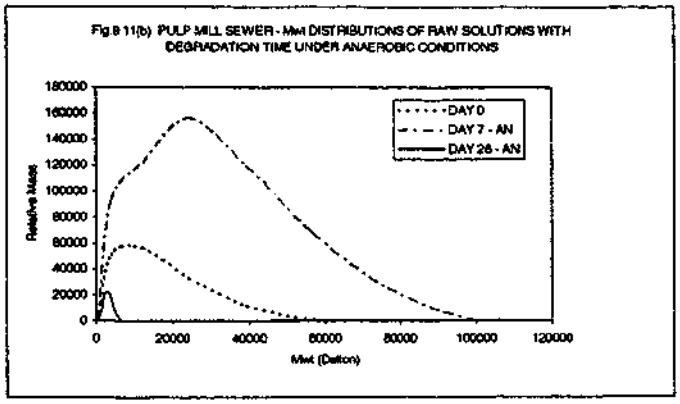


9.4.5.2.3 Characterization of molecular weight

The average molecular weight of the nonfiltered raw sample increased within seven days under both conditions (Table 9.4). The average molecular weight of the humic acids also increased. The molecular weight distributions of the raw solutions show

that the higher molecular weight fractions (MW>2000 Dalton) have increased after seven days under both conditions (Figs.9.11(a,b)).

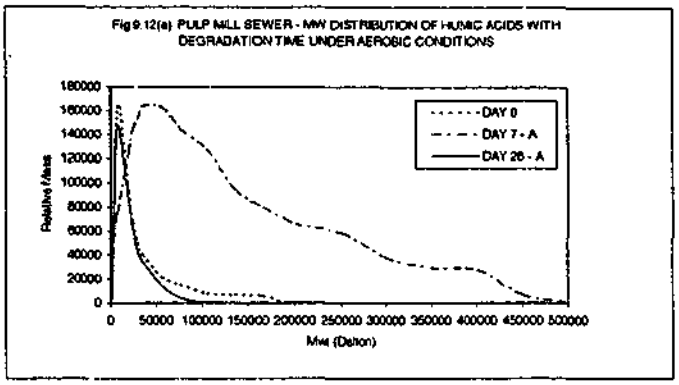


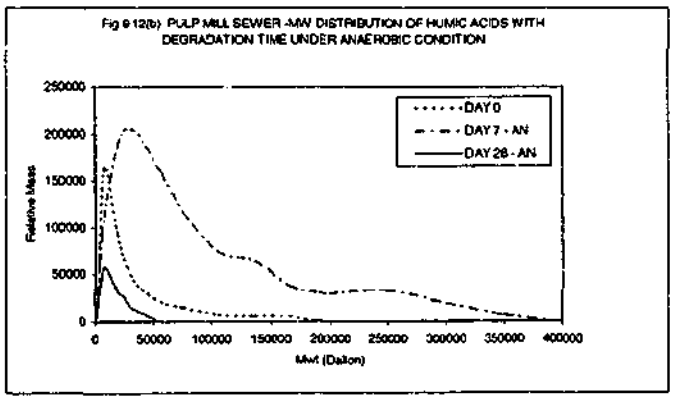


The molecular weight distributions of humic acids show that the very high molecular weight fractions (MW>20,000 Dalton) have increased within seven days under both conditions (Figs.9.12(a,b)).

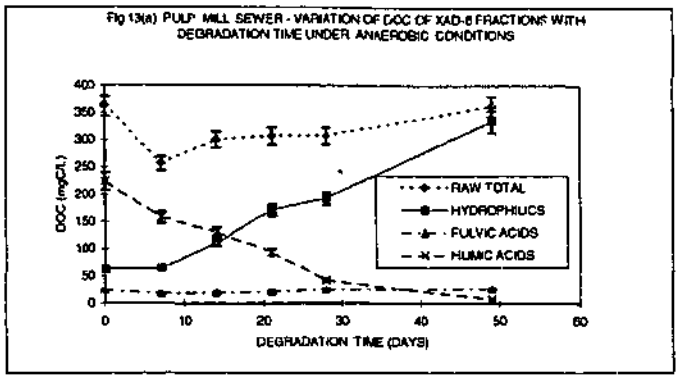
After 28 days under aerobic conditions, the average molecular weight (Table 9.4) of the raw solution remains same. The molecular weight distributions of raw solutions under aerobic conditions (Fig.9.11(a)) show that all molecular weight fractions have decreased under aerobic conditions from day 7 to day 28, which leads to the average molecular weight remaining unchanged.

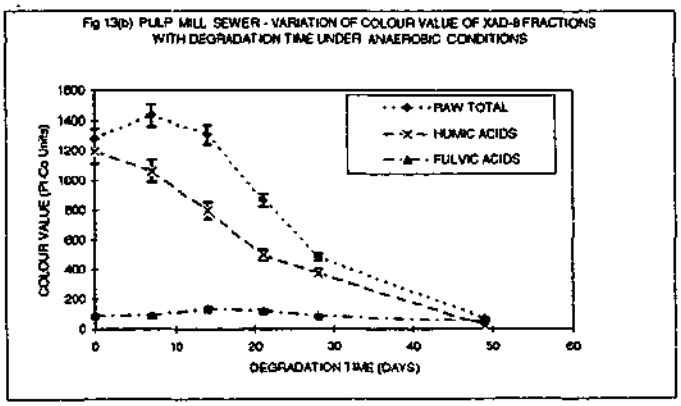
In contrast, in humic acids only the high molecular weight fractions have been reduced from day 7 to day 28 (Fig.9.12(a)) under aerobic conditions, which leads to a lower weight average molecular weight (Table 9.4). Therefore we can conclude that the reason for the constant weight average molecular weight in the raw solution from day 7 to day 28 under aerobic conditions would be due to substances other than humic acids.





The raw solution after 48 days under anaerobic conditions was an almost noncoloured clear solution (Figs.9.13(a,b)) and only a very minute FFF peak was observed (Fig.9.12(b)) even after 28 days.





However, the average molecular weight of the isolated humic acids at day 28 under anaerobic conditions was lower than that at day 7 (Table 9.4). The molecular weight distributions of humic acids have shown that almost all of the high molecular weight fractions have disappeared from day 7 to day 28 under anaerobic conditions (Fig.9.12(b)). The weight average molecular weight of the humic acids fraction in that case has decreased accordingly. However, the raw solution after 28 days under anaerobic conditions was a clear solution due to the low concentration level of humic acids present. Therefore, the average molecular weight of the raw solution after 28 days under anaerobic conditions is probably rather inaccurate and not representative of the total organic matter present (Table 9.4).

Table 9.4 PULP MILL SEWER : Weight and Number average molecular weights of raw solutions and Humic Acids extracted from them

BEFORE DEGRADATION	
Raw Solution	Humic Acids
Mw=18800 Mn=10900 ρ=1.73	Mw=38100 Mn=15300 ρ=2.49

	AEROBIC DEGRADATION		ANAEROBIC DEGRADATION	
Duration (Days)	Raw Solution	Humic Acids	Raw Solution	Humic Acids
7	Mw=35800 Mn=16900 ρ=2.12	Mw=154000 Mn=64900 ρ=2.37	Mw=34000 Mn=18200 ρ=1.87	Mw=97400 Mn=40100 ρ=2.43
28	Mw=35900 Mn=20450 ρ=1.76	Mw=29400 Mn=13200 ρ=2.23	No peak Clear soloution	Mw=20100 Mn=11900 ρ=1.69

9.4.6 Characterization by major organic groups

9.4.6.1 Caustic Sewer

The changes in DOC occurring in the total sample and the hydrophilic, fulvic and humic acid fractions as a result of the biological degradation, is shown in Fig.9.14. The total DOC decreased more under aerobic conditions than under anaerobic conditions. Under aerobic conditions 34% of the total DOC was removed after 7 days, but only 22% of the total DOC was removed under anaerobic conditions after the same duration. Further exposure to the same conditions to day 28 reveals that 15% of the total DOC left in the medium was removed under aerobic conditions, but only 4% of the total DOC left in the medium was removed under anaerobic conditions.

XAD-8 fractionation has shown that the degradation trend observed for the raw solution is due mainly to degradation of the hydrophilic substances fraction, although

fulvic acids were also degraded to some extent within 7 days. Between days 7-28, there was no further change in the fulvic acid concentration. However, humic acids seem to be produced to some extent due to microbiological action.

Fig.9.14 Fractionation of CAUSTIC SEWER - DOC in XAD-8 fractions with degradation time under aerobic or anaerobic conditions

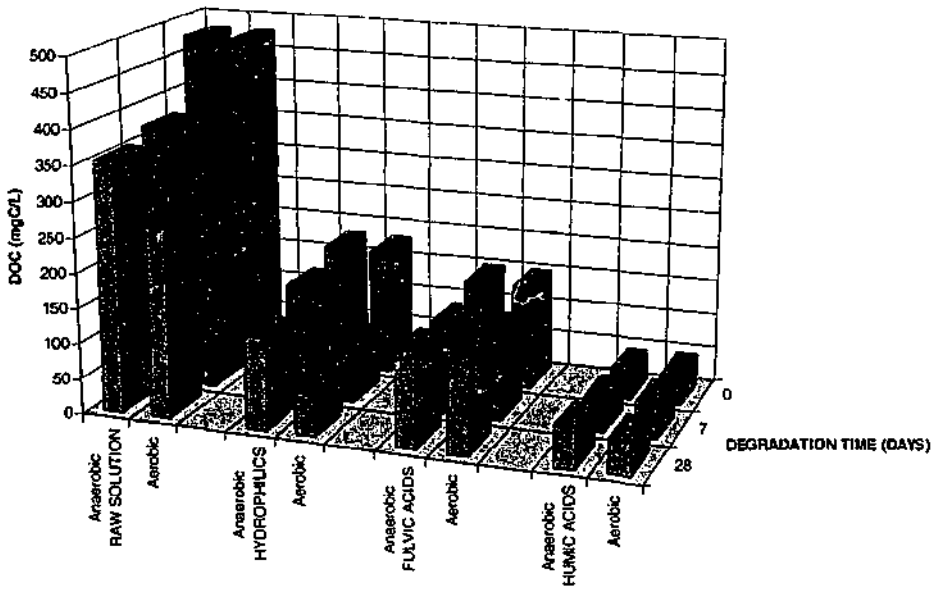
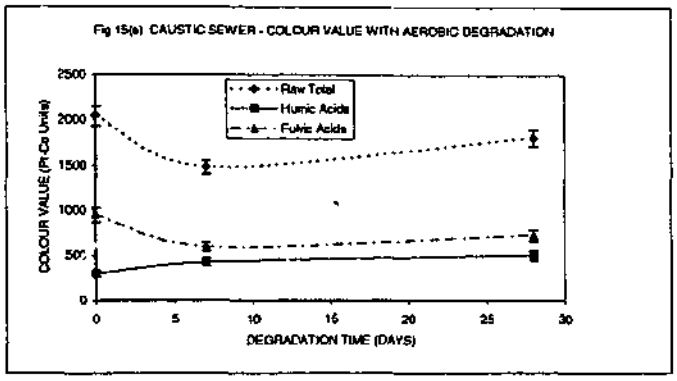
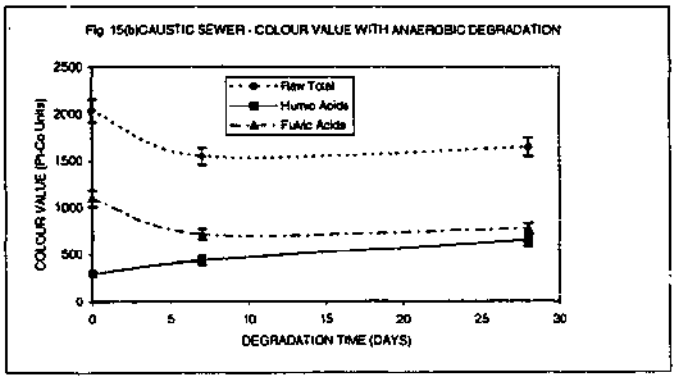


Figure 9.14 also indicates that the hydrophilic fraction may be further degraded in an extended aerobic degradation period. The colour values of the humic and fulvic acids suggest that the colour changes observed with raw solutions were due mainly to the fulvic acids (Figs.9.15(a) and 9.15(b)).

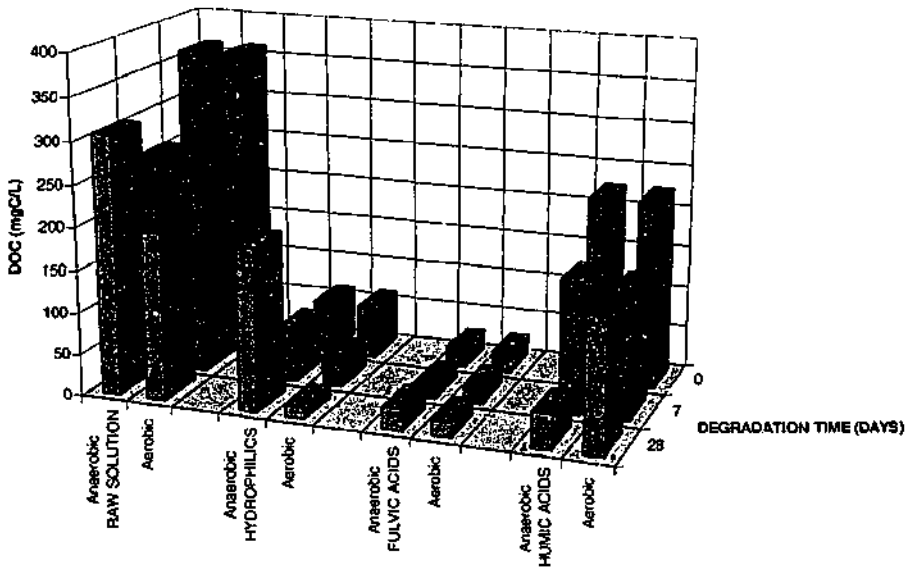




9.4.6.2 Pulp Mill Effluent

The total DOC has decreased under both conditions within seven days (Fig.9.16). The trend observed in the DOC with raw solution within seven days under aerobic conditions was due mainly to degradation of both the humic acids and the hydrophilic substances. Under anaerobic conditions it was mainly due to degradation of humic acids alone. Under both conditions the fulvic acids also appear to be involved in the loss of DOC within seven days (Fig.9.16), but to a lesser extent.

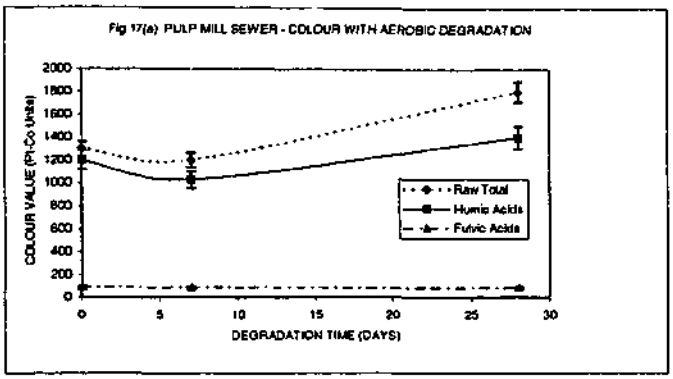
Fig. 9.16 Fractionation of PULP MILL SEWER - DOC in XAD-8 fractions with degredation time under aerobic or anaerobic conditions

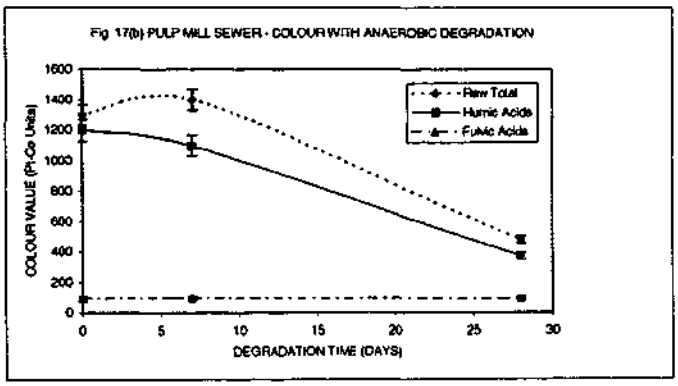


The DOC increased in the raw solution from day 7 to day 28 under anaerobic conditions. This is mainly due to the production of hydrophilic substances. Since a dramatic decrease in humic substances occurs, perhaps the humics are being converted into hydrophilic substances. Furthermore, the results suggest that the conversion of humic acids into hydrophilics under anaerobic conditions is extensive and may also point to a minor conversion into fulvic acids.

In contrast, under aerobic conditions, the slight decrease of the total DOC from day 7 to day 28 in the raw solution was due mainly to the degradation of the hydrophilic substances only, and not due to the humic acid degradation (Fig.9.16). The colour

values of humic and fulvic acids suggests that the colour changes observed with raw solutions were due mainly to the humic acids (Figs.9.17(a) and 9.17(b)).





9.5 CONCLUSIONS

Bleaching effluents released from bleach plants as a result of bleaching processes are the most highly concentrated dissolved organic effluents. The paper machine sewer release from paper machine plant as a result of paper manufacturing processes is the least concentrated in that respect. The wood milling and pulp milling processes occurring in the wood storage and in the pulp plant generate moderately concentrated dissolved organic effluents.

The caustic bleaching process is the one that imparts the most colour to the combined effluent, although the pulp milling process also imparts much colour to the effluent. The acidification process in bleach plants, also imparts some colour to the effluent medium but less than due to caustic bleaching and pulp milling processes. The colour imparted to the effluent medium as a consequence of wood milling process is negligible compared to the bleaching and pulping processes. The paper maufacturing process does not impart colour to the effluent at all with respect to the other processes.

The pulping process in pulping plant generates higher molecular weight dissolved organic compounds than the bleaching processes in bleach plant. The wood milling and paper manufacturing processes do not produce organic wastes with molecular weights high enough to even analyze.

The clarification process increases the colour of the effluent but does not seem to affect the DOC concentration. However, dissociation or cleavage of large molecules into small molecules may occur during this process. The small molecules may impart more colour to the effluent than large molecules. The effluent transportation across the sludge lagoon seemed to remove some DOC and colour while reducing the average molecular weight. This may reflect either of the two hypotheses; ie. biological degradation or adsorption/physical settling of coloured large molecules.

Treatment in pond 1 (settling basin) has a negligible effect on DOC and molecular weight, but introduced more colour substances into the medium. The removal of low

molecular weight dissolved organics was observed in pond 2. Probably the aeration has promoted some DOC removal in pond 2 due to microbial assimilation or microbial oxidation. However, pond 3 and pond 4 treatments did not affect DOC concentration, but a decrease and increase in average molecular weight was observed. The molecular dissociation in pond 3 and molecular association in pond 4 is most likely to occur due to microbial processes. The resulting smaller molecules in pond 3 have more colour than the larger molecules.

The DOC at Kawerau bridge reflects dilution due to merging with the Tarawera River. The merging inputs at Seed Orchard also have introduced colour to the Tarawera River lower reaches. Simple dilution at downstream sites occurred after O'Sullivans Farm.

Laboratory scale biological experiments with the caustic sewer have shown that the most efficient degradation process seems to be the aerobic reactions. The DOC in the caustic sewer is somewhat refractory. In the raw caustic sewer medium under both conditions, the decrease in average molecular weight within seven days is due mainly to the removal of high molecular weight fractions of humic acids. The increase in molecular weight after 7 days is due to the removal of low molecular weight fractions of substances other than humic acids.

The biological experiments with the pulp mill sewer also have shown that the more efficient degradation type seems to be the aerobic reaction. The DOC left in the medium after 7 days were somewhat refractory to anaerobes. The anaerobes have

presumably reacted on particulate matter resulting in the DOC of the medium increasing after 7 days under anaerobic conditions. The DOC was also resistant to removal by aerobes after seven days.

The increase in average molecular weight of the raw pulp mill sewer within seven days under both conditions is due mainly to the increase of high molecular weight fractions of humic acids. The DOC removed within seven days seemed to be low molecular weight fractions of both humic acids and fulvic acids. The removal of low molecular weight fractions in the raw solutions after 7 days seemed to be mostly hydrophilic substances under aerobic conditions but mainly humic acids under anaerobic conditions.

XAD-8 fractionation of the caustic sewer has shown that the degradation trend observed with the raw solution is due mainly to the degradation of the hydrophilic substances fraction, although fulvic acids were also degraded to a lesser extent. Humic acids seem to be produced due to biological action at least to a certain extent. The hydrophilic fraction may be further degraded with longer degradation periods. The colour changes observed in the raw solutions were due mainly to the fulvic acids.

XAD-8 fractionation has shown that the degradation trend observed with raw pulp mill sewer solution under aerobic conditions was due mainly to the degradation of the humic acids and the hydrophilic substances. Under anaerobic conditions it appeared to be due mainly to the degradation of the humic acids and conversion into hydrophilic substances. The fulvic acids appear to be degraded to a lesser extent within seven

days under both conditions. The conversion of humic acids into hydrophilics under anaerobic conditions is significant whilst generation of fulvic acids is minor. The changes of colour observed with the raw solutions followed the trends in changes of colour observed with humic acids. When humic acids were degraded under anaerobic conditions, the colour of the humic acid medium was also reduced. However, in aerobic media the humic acids may undergo structural changes to increase the chromophore sites in humic acid molecules.

Chapter 10

Comparison Of The Effect Of Biological And Physico-Chemical Treatments On A Coal Washing Effluent

Abstract

Coal washings from a hydrothermal drying (HTD) process of brown coal at the Latrobe Valley power plant of the State Electricity Commission of Victoria (SECV) was investigated to compare the effect of biological and physico-chemical treatment. Brown coal is the main source of energy for power generation in Victoria, Australia. These HTD effluents obtained after anaerobic digestion at two temperatures and filtered HTD effluent after alum coagulation were subjected to investigation. Thus they represent biologically treated and physico-chemically treated samples respectively.

The anaerobic digestion and alum coagulation were equally effective in removing the hydrophobic fraction from the HTD wastewater. Further characterization of the hydrophobic fraction revealed that different hydrophobic components are removed to different extents. Biological treatment effectively removes fulvic acids and to a certain extent hydrophobic neutrals as well. It is inefficient for humic acids.

Physico-chemical treatment removes humic acids effectively and also fulvic acids to a lesser extent. It is inefficient for hydrophobic neutrals.

Biological treatment removes high molecular weight fulvic acids while physico-chemical treatment removes low molecular weight fulvic acids. The high molecular weight humic acids are generated in the biological system but they are removed in the physico-chemical system. Biological treatment preferentially removes the hydrophilic fraction, the physico-chemical treatment preferentially removes the low molecular weight component from the hydrophobic fraction.

10.1 INTRODUCTION

The washings left after hydro-thermal drying (HTD) of Latrobe Valley brown coal in Victoria, Australia were chosen for this study. The heat treatment process used for drying brown coal is called hydrothermal drying, during which coal undergoes both physical and chemical changes. The HTD is a newly developed process for drying brown coal efficiently and for minimizing the salt content of the dried coal. The process reduces the amount of water, which is being held in microscopic pores. The released water can then be easily removed as coal wastewater.

Latrobe Valley brown coal wastewater from the SECV plant, which has been subjected to anaerobic digestion at two temperatures, were considered as examples of biologically treated coal washings, while filtered effluent after alum coagulation was considered as a physico-chemically treated coal washing. The efficiency of these two types of treatments was studied with respect to the removal of specific organic fractions under biological and physico-chemical conditions.

The main objective of this study was to compare:

- which organic fractions are effectively removed by each treatment
- which molecular weight fractions are affected by each treatment.

10.2 EXPERIMENTAL

Samples from coal washings of the State Electricity Commission of Victoria (SECV), situated in the Latrobe Valley, about 150 km east of Melbourne, were transported under refrigerated conditions to the Water Studies Centre at Monash University. This is one of the largest electricity utilities in Australia and the largest individual coal producer. Samples were refrigerated at 4°C until needed for the experimentation at Monash laboratory. Four samples were selected for this study:

- raw coal washings from the hydro-thermal drying process
- HTD effluent from the Anaerobic digester at 37°C degrees
- HTD effluent from the Anaerobic digester at 60°C degrees
- filtered HTD wastewater after flocculation with alum.

The hydro-thermal drying process of brown coal generates HTD wastewater (coal washings) is described below.

A slurry of coal is formed by milling crushed raw coal with added water. A series of heat exchangers are provided to heat and cool while the slurry is pumped under high pressure. The slurry temperature is raised to 300C degrees for few minutes and then is cooled down. Some molecules of the coal containing oxygen decompose at these high temperatures and produce carbon dioxide. During this process the coal particles shrink and eject water held in internal pores within them. The excess water can be removed by a filter press or centrifuge after cooling down and is called HTD coal washings.

10.2.1 Sample pretreatment and preparation for DOC and FFF analysis

About 10 mL aliquots from each sample were withdrawn for FFF and DOC analysis. These samples were checked for pH and half of the volumes were adjusted to pH 7.8 with 1 M NaOH where necessary to prepare them for FFF analysis. The remaining half volumes were adjusted to pH 3-4 with 1 M HCl where necessary for DOC analysis. Then above pH adjusted samples were diluted ten times and filtered through 1.2 μm GF/C filter paper under vacuum.

10.2.2 Sample pretreatment and preparation for XAD-8 Resin Fractionation

About 30 mL from each raw sample were made into 300 mL with milliQ water. The pH in the medium was adjusted to pH 1 with conc. HCl acid and kept overnight in the refrigerator for humic acid precipitation. They were then filtered through 1.2 μm GF/C filter paper under gravity and 0.2 μm millipore (GS 0.22 μm) filter paper under vacuum to separate the precipitate from the supernatant. The filtrate was brought to pH 2 with 5 M NaOH before passing through an XAD-8 column.

10.2.3 XAD-8 Resin Fractionation

The filtrate adjusted to pH 2 was passed through an XAD-8 column and the hydrophobic fraction, which adsorbed to the XAD-8 column was eluted with 30 mL of 0.1 M NaOH. The Humic acid precipitate was dissolved in 0.1 M NaOH and the pH was brought to 7.8 with conc. HCl acid. The final volume of the humic acid solution was brought to 30 mL with milliQ water. Further experimental details are outlined in Chapter 4, Section 4.2.

10.2.4 Flow FFF analysis

The effluents and two isolated hydrophobic acid fractions were analyzed by flow FFF to study molecular weight changes. The experimental procedure and experimental conditions were outlined in Chapter 3, Section 3.2.

10.3 RESULTS AND DISCUSSION

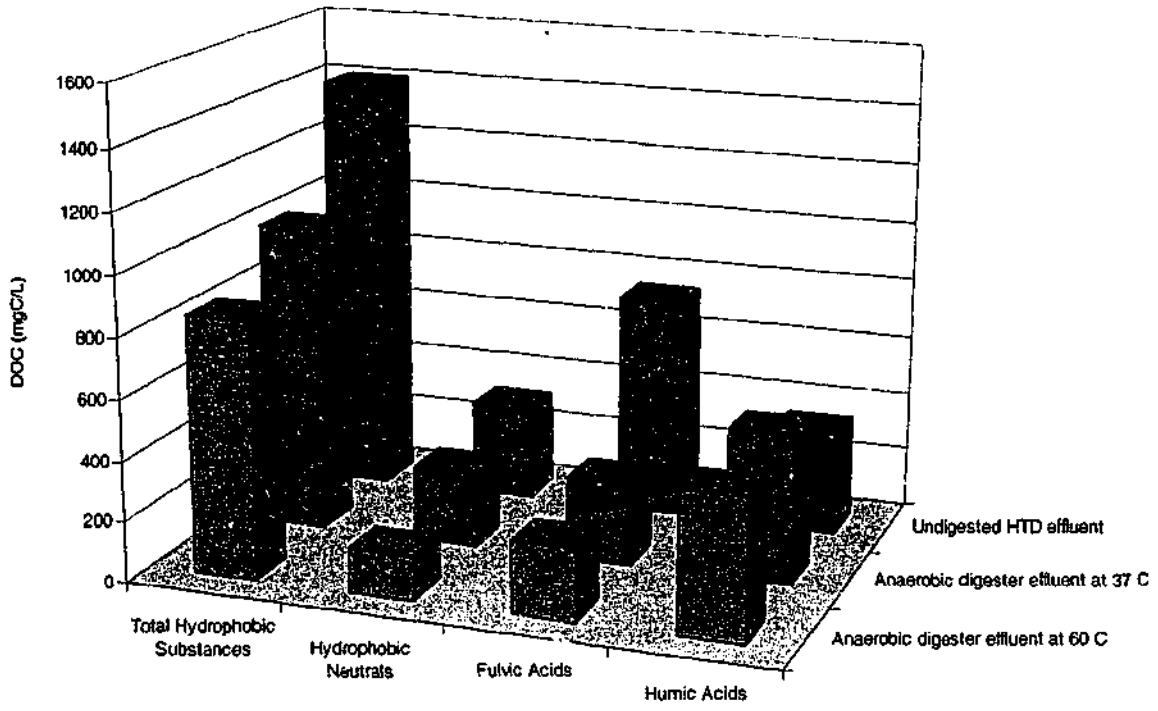
10.3.1 Effect of biological treatment on hydrophobic substances

10.3.1.1 Hydrophobic Fraction

The effect of biological treatment on the hydrophobic fraction of coal washings left after the hydro thermal drying (HTD) process of brown coal was studied. The effluent from the HTD process was treated in an anaerobic digester at 37°C and 60°C, which removes only some of the hydrophobic organic matter. The amount of total hydrophobic substances appears to have decreased in the anaerobic digester effluents (Fig.10.1). This was due to the removal of some of the fulvic acid and hydrophobic neutrals. In fact, fulvic acid reduced dramatically compared to the hydrophobic neutrals. Humic acid was not removed by anaerobic digestion and instead increased.

It seems that anaerobic digestion at 37°C preferentially removes most of the fulvic acid while some of the other hydrophobic components remain. A fraction of the hydrophobic neutrals also seemed to be removed by anaerobic digestion, but to a lesser extent. Perhaps anaerobic digestion may eventually remove hydrophobic neutrals when the remaining fulvic acids are refractory for anaerobic digestion.

Fig. 10.1 Fractionation of HTD COAL Wastewater - DOC in XAD-8 fractions from the Anaerobic digester effluents operated at two different temperatures



In the 60°C digestion, the fulvic acid fraction was stable but hydrophobic neutrals were removed. The anaerobic digestion at 60°C was inefficient in removing humic acid. Removal of hydrophobic neutrals appeared to be responsible for the decrease in hydrophobic mater in the 60°C anaerobic digester.

10.3.1.2 Molecular weight distributions

The flow FFF fractograms and molecular weight distributions for the total effluents of undigested coal washings and digested effluents from anaerobic digester are illustrated in Figs.10.2 and 10.5.

Fig.10.2 Fractograms of HTD effluents before and after biological treatment - After void peak removal

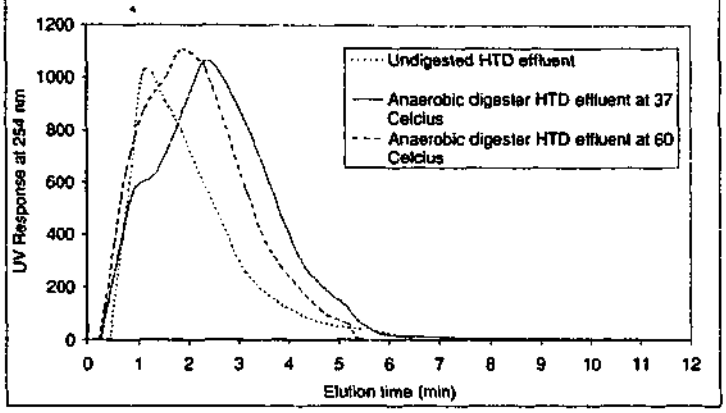


Fig.10.3 Fractograms of FULVIC ACIDS in HTD effluents before and after biological treatment - After void peak removal

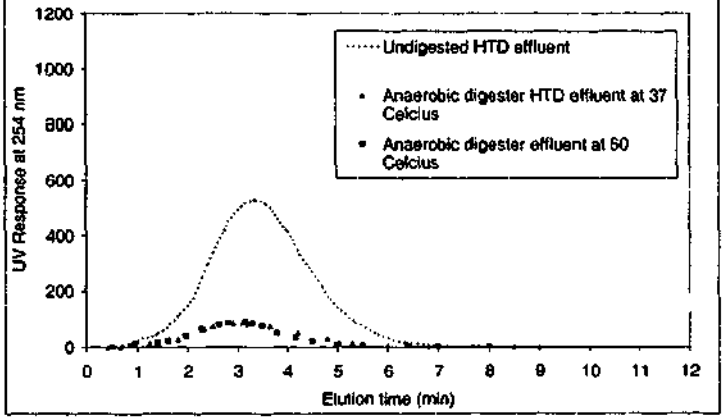
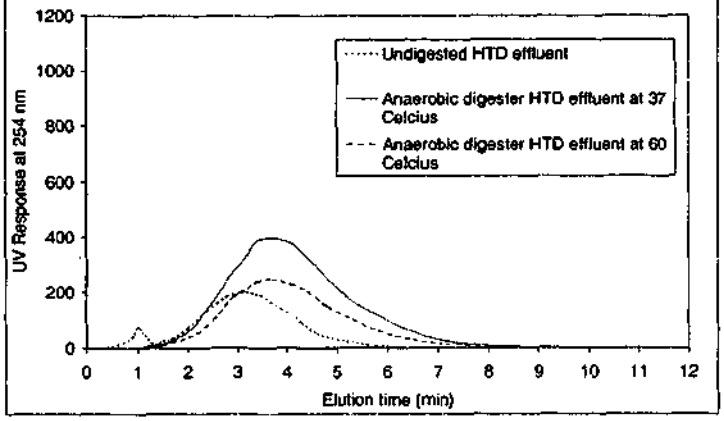


Fig.10.4 Fractograms of HUMIC ACIDS in HTD effluents before and after biological treatment



The elution profile for the total coal washings was shifted to higher retention times as a consequence of anaerobic digestion. This is due to the generation of high molecular weight organic material in the anaerobic digester effluents. Despite the generation of some high molecular weight organic material, Fig. 10.1 shows some removal of total hydrophobic substances in this study. These removed hydrophobic organic substances are mainly the low molecular weight components.

The two isolated hydrophobic acid fractions (fulvic and humic) were also analyzed for fractograms and molecular weight distributions. These plots are given in Figs.10.3, 10.4, 10.6 and 10.7. The isolated fulvic acid fractograms (Fig.10.3) showed that they do not follow the behavior of the total systems (Fig.10.2) for undigested and digested effluents. Molecular weight distributions of fulvic acids in the digester effluents at the above two temperatures were almost identical and both of them were slightly shifted to lower retention times (Fig.10.3).

The fractograms were converted to molecular weight distributions using calibration constants as outlined in chapters 2 and 3, and are illustrated in Figs.10.5, 10.6 and 10.7. The frequency function plotted against the molecular weight presented in Fig.10.5 shows the changes that occurred to the total effluent due to anaerobic digesters. The results revealed that generation of high molecular weight fractions and removal of low molecular weight fractions occur in these digesters.

Fig.10.5 Molecular weight distributions of HTD effluents before and after biological treatment

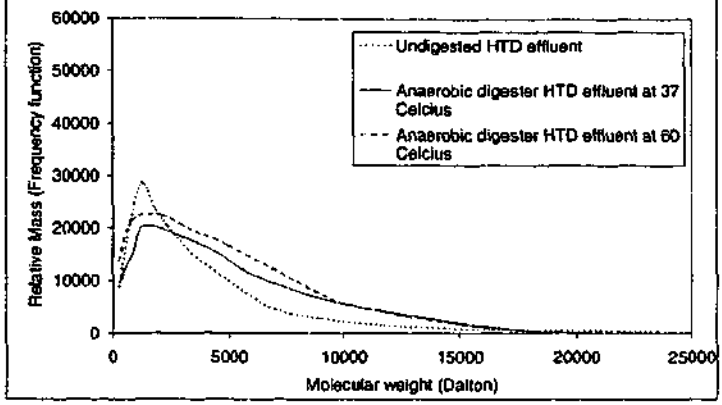


Fig.10.6 Molecular weight distributions of FULVIC ACID in HTD effluents before and after biological treatment

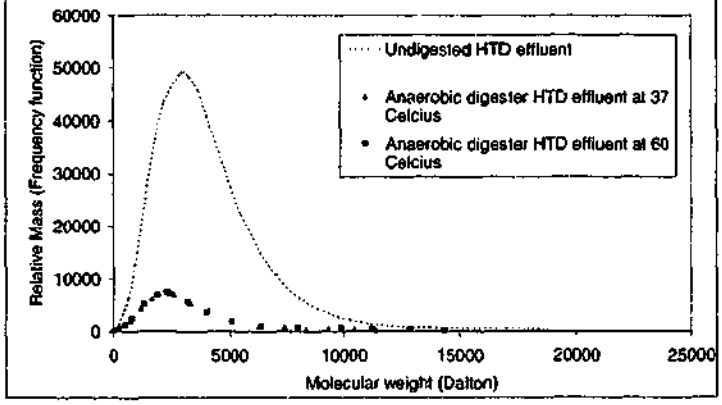
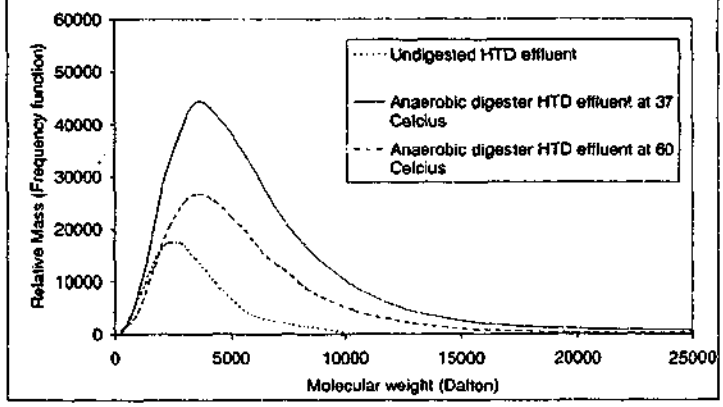


Fig.10.7 Molecular weight distributions of HUMIC ACID in HTD effluents before and after biological treatment



It seems that the anaerobic digester has predominantly reduced the amount of the higher molecular weight fulvic acid fractions, hence the molecular weight distributions have shifted to lower molecular weight (Fig.10.6). In fact it shows some removal of fulvic acids over the entire molecular weight range as a consequence of anaerobic digestion, but more of them were high molecular weight fulvic acids. In contrast, the higher molecular weight of humic acids range have increased in the anaerobic digesters, thus the humic acid molecular weight distributions have shifted to higher molecular weight (Fig.10.7).

The two anaerobic digester effluents studied in this work demonstrated similar molecular weight distribution patterns for the fulvic acid component (Fig.10.6), but have a significantly different molecular weight distribution pattern for the humic acid component (Fig.10.7). The fulvic acids remaining after the anaerobic digestion would be fairly stable even at 60°C. Some how in the total system (Fig.10.5) removed hydrophobic neutrals (Fig.10.1) may be lower molecular weight matter.

10.3.1.3 Molecular weight characteristics

The corresponding number average and weight average molecular weights were calculated for the total system and two isolated hydrophobic acid fractions before and after anaerobic digester treatment of the HTD effluent. The molecular weight distribution parameters (characteristics) are tabulated in Tables 10.1, 10.2 and 10.3.

Table 10.1 Molecular weight characteristics of HTD effluents before and after anaerobic digestion

HTD Effluent	Mw	Mn	Mw/Mn	Skew(sk)
Undigested effluent	4500 ± 200	2100 ± 50	2.14	2.66
Anaerobically treated at 37°C	5550 ± 250	2800 ± 60	1.98	1.72
Anaerobically treated at 60°C	5250 ± 250	2550 ± 50	2.06	1.85

Table 10.2 Molecular weight characteristics of FULVIC ACID in HTD effluents before and after biological treatment

HTD Effluent	Mw	Mn	Mw/Mn	Skew(sk)
Undigested effluent	4150 ± 200	2950 ± 60	1.41	2.81
Anaerobically treated at 37°C	3400 ± 150	2300 ± 50	1.48	1.81
Anaerobically treated at 60°C	3650 ± 180	2350 ± 50	1.55	2.01

Table 10.3 Molecular weight characteristics of HUMIC ACID in HTD effluents before and after biological treatment

HTD Effluent	Mw	Mn	Mw/Mn	Skew(sk)
Undigested effluent	3500 ± 200	2550 ± 50	1.37	1.15
Anaerobically treated at 37°C	6550 ± 300	4250 ± 80	1.54	2.61
Anaerobically treated at 60°C	5800 ± 250	4000 ± 80	1.45	2.49

As a result of anaerobic digestion, the weight average and number average molecular weights of the anaerobic digester effluents were increased compared to the undigested HTD coal washings (Table 10.1). The hydrophobic fractions isolated by XAD-8 resin fractionation shown that humic acid (Table 10.3) has followed the same trend as the total HTD effluent, while fulvic acid (Table 10.2) has followed the opposite trend. Anaerobic digester effluents were found to contain a much lower concentration of fulvic acid

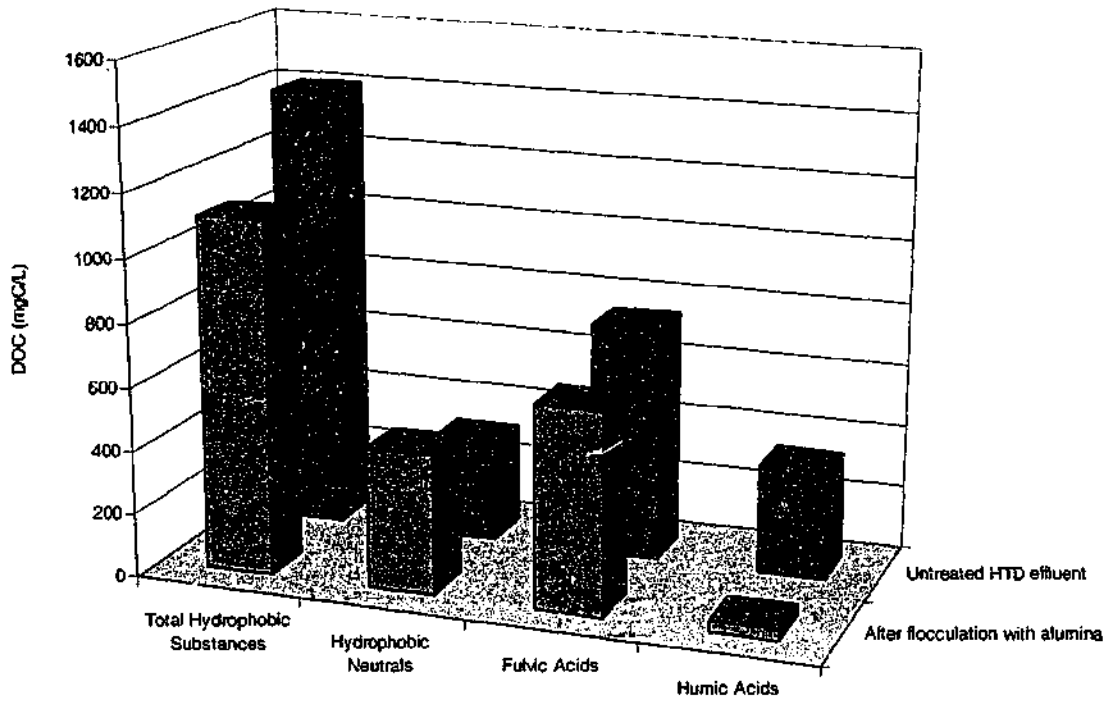
(Fig.10.1) than in the undigested effluent, and the residual fulvic acid seemed to contain lower molecular weight material (Table 10.2). Most of the fulvic acid removed during the anaerobic digestion (Fig.10.1) was high molecular weight fulvic acids (Fig.10.6), resulted in a lower average molecular weight for the fulvic acid fraction in the digester effluents. Even though humic acid follows the same trend as in the total effluent with respect to the average molecular weights (Tables 10.1 and 10.3), humic acid does not appear to be removed by anaerobic digestion (Fig.10.1). Even though the total hydrophobic substances (Fig.10.1) removed as a consequence of anaerobic digestion seem to consist of lower molecular weight material (which results in a higher average molecular weight with the digested effluents), these low molecular weight materials could be due to hydrophobic neutrals. The higher average molecular weights observed with the total digester effluents are most likely due to the appearance of higher molecular weight humic acids.

However, the polydispersity values for the total samples did not vary much after anaerobic digestion (Table 10.1). In fact they decreased marginally. Polydispersity appears to increase in the fulvic and humic acid fractions (Tables 10.2 and 10.3), which interestingly may suggest that high molecular weight hydrophobic neutrals present in the HTD effluent have been removed to a certain extent during the anaerobic digestion. Further proof comes from the skew parameter of the molecular weight distribution curves, which decreased after anaerobic digestion for the total sample (Table 10.1) and for the fulvic acid fraction (Table 10.2), but strikingly increased for the humic acid fraction (Table 10.3). The behavior of the total sample must be certainly linked with the changes in the hydrophobic neutrals fraction as well.

10.3.2 Effect of physico-chemical treatment on hydrophobic substances

10.3.2.1 Hydrophobic Fraction

Fig. 10.8 Fractionation of HTD wastewater - DOC in XAD-8 fractions for both the untreated and physico-chemically treated samples



The washings left, after HTD effluent was flocculated with alum and filtered, were characterized in this work to study the effect of this physico-chemical treatment on the hydrophobic components in the effluent. The XAD-8 resin fractionation results are illustrated in Fig.10.8. Some of the total hydrophobic substances were removed by this treatment. The isolated fractions have revealed that almost all of the humic acids were removed as well as some of the fulvic acid, however the hydrophobic neutrals appeared to have increased.

10.3.2.2 Molecular weight distribution

Fractograms were recorded for the untreated coal washings and the filtered coal washings left after treating with alum. The elution profile of alum-treated coal washings was shifted to lower retention times (Fig.10.9). The corresponding fractograms of isolated fulvic acid and humic acid fractions are illustrated in Figs.10.10 and 10.11. These fractograms did not seem to follow the trends for the total system.

The computed molecular weight distributions are presented in Figs.10.12, 10.13 and 10.14, and they clearly illustrate what changes occurred under physico-chemical treatment. Figure 10.13 shows that the frequency function of low molecular weight fulvic acid has been reduced, while that of high molecular weight fulvic acid increased slightly. An increase in high molecular weight fulvic acid material could be simply due to intermolecular associations or condensation type reactions. The intermolecular association of lignin has been discussed by Sarkenan et al. (1981). Lindberg (1955), Gross et al. (1958) and Lindstrom (1979) have also suggested that hydrogen bonding, hydrophobic interactions and long-range van der Waals forces, etc., give rise to molecular associations, which in turn give rise to high molecular weights.

In the case of humic acid (Fig.10.14) also, where materials in all molecular weight ranges have been removed, low molecular weight range has remarkably decreased. In this study, the physico-chemical treatment with alum was more likely to remove low molecular weight fractions from fulvic (Fig.10.13) and humic acid (Fig.10.14).

Fig.10.9 Fractograms of HTD effluents before and after physical treatment - After void peak removal

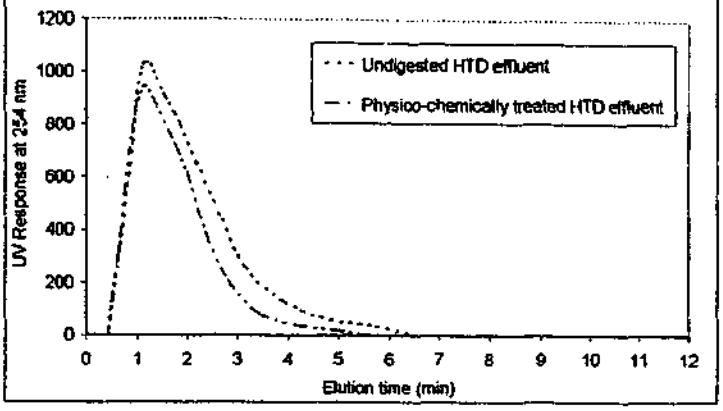


Fig.10.10 Fractograms of FULVIC ACIDS in HTD effluents before and after physical treatment - After void peak removal

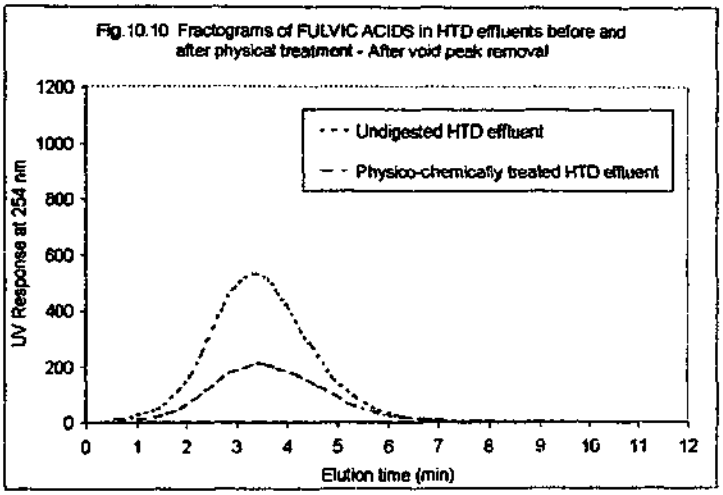


Fig.10.11 Fractograms of HUMIC ACIDS in HTD effluents before and after physical treatment - After void peak removal

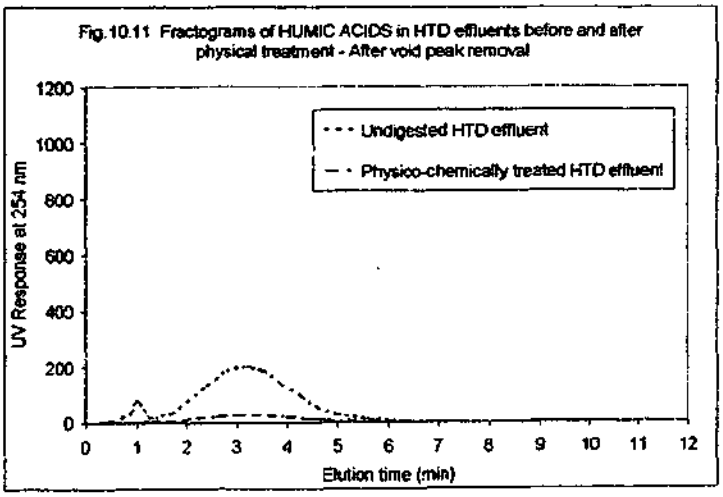


Fig.10.12 Molecular weight distributions of HTD effluents before and after physical treatment

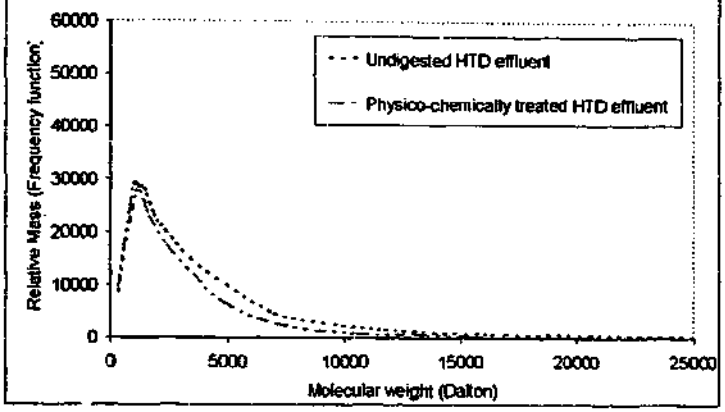


Fig.10.13 Molecular weight distributions of FULVIC ACID in HTD effluents before and after physical treatment

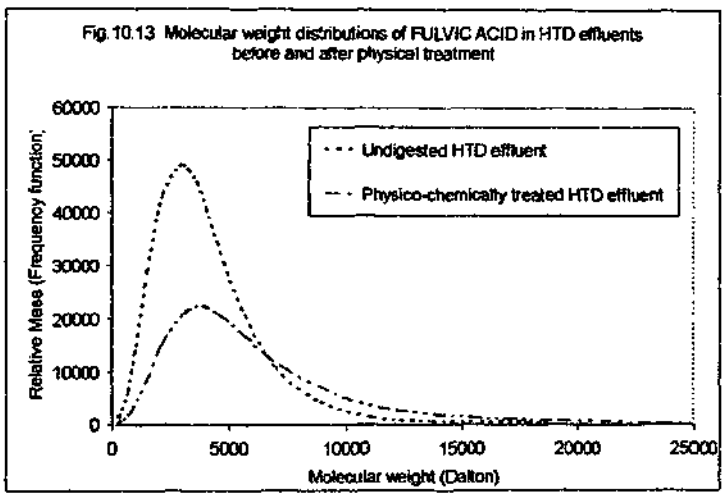
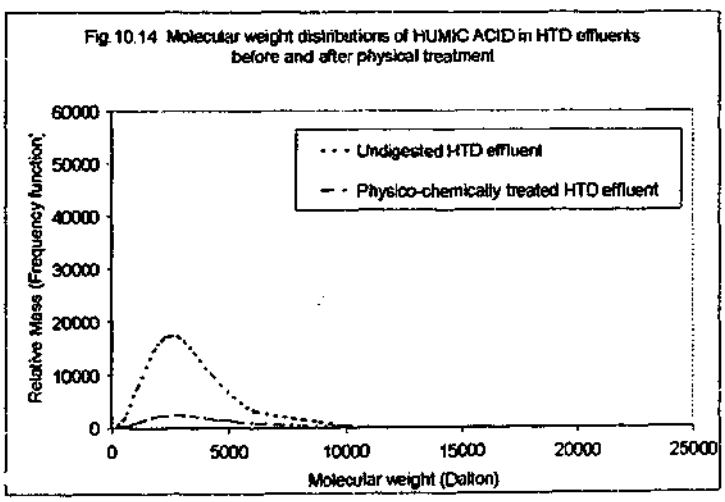


Fig.10.14 Molecular weight distributions of HUMIC ACID in HTD effluents before and after physical treatment



The work carried out by Beckett et.al. (1989), found the removal of hydrophobic substances by various physico-chemical treatments, including alum coagulation, affected the molecular weight distribution of the total effluent. Their data indicated the preferential removal of hydrophobic substances. However, in the current study, fractionation of the total hydrophobic fraction revealed that physico-chemical treatment has affected different hydrophobic components in different ways. According to the current study, humic acids (Figs.10.8 and 10.14) were removed over the entire molecular weight range. However, the removed the low molecular weight range fulvic acids (Figs.10.8 and 10.13) were preferentially removed. Consequently the molecular weight distribution of the total system shifted slightly to lower molecular weight. The hydrophobic neutrals could be produced with lower molecular weight material, which have compensated for the removal of low molecular weight fractions of fulvic and humic acid in the total sample.

10.3.2.3 Molecular weight characteristics

Tables 10.4, 10.5 and 10.6 summarize the parameters calculated from the molecular weight distributions of the untreated and alum treated coal washings. As a consequence of physico-chemical treatment, the changes in weight average and number average molecular weights of the fulvic and humic acids fractions are given in Tables 10.5 and 10.6. For the isolated fulvic acid fraction, these two parameters were increased (refer toTable 10.5) by alum coagulation. As far as humic acids are concerned, the above two parameters were only slightly increased by alum treatment.

Table 10.4 Molecular weight characteristics of HTD effluents before and after physical treatment

HTD Effluent	Mw	Mn	Mw/Mn	Skew(sk)
Undigested effluent	4500 ± 200	2100 ± 50	2.14	2.66
Physico-chemically treated	3070 ± 150	1670 ± 30	1.84	2.67

Table 10.5 Molecular weight characteristics of FULVIC ACID in HTD effluents before and after physical treatment

HTD Effluent	Mw	Mn	Mw/Mn	Skew(sk)
Undigested effluent	4150 ± 200	2950 ± 60	1.41	2.81
Physico-chemically treated	6400 ± 300	4150 ± 100	1.54	2.61

Table 10.6 Molecular weight characteristics of HUMIC ACID in HTD effluents before and after physical treatment

HTD Effluent	Mw	Mn	Mw/Mn	Skew(sk)
Undigested effluent	3500 ± 200	2550 ± 50	1.37	1.15
Physico-chemically treated	4350 ± 200	2950 ± 60	1.48	1.32

The explanation for this difference could be the removal of low molecular weight fulvic acid fractions (Fig.10.13), as well as condensation type reactions to form high molecular weight fulvic acids. However, as most of the removed humic acids were from over the entire molecular weight range including both low and high molecular weight fractions, the average molecular weights before and after treatment (Table 10.6) were close.

The weight average and number average molecular weights of the total samples were reduced (Table 10.4) as a consequence of physico-chemical treatment. However, for the

fulvic acids and humic acids fractions (Tables 10.5 and 10.6) these two parameters did not follow the trend of the total system (Table 10.4). The reason may be the behavior of the hydrophobic neutrals for the above treatment in the total system. The resultant hydrophobic neutrals due to physico-chemical treatment (Fig.10.8) probably contain low molecular weight material, which in turn attributes to the lower average molecular weights in the total system.

The polydispersity value for the total system was lower after the physico-chemical treatment than for the untreated sample (Table 10.4). Fulvic acid and humic acid isolated from coal washings after this treatment shows a higher sample polydispersity (Tables 10.5 and 10.6). The behavior of the total system must be linked with some hydrophobic component, other than the fulvic and humic acids. Perhaps the other component involved was hydrophobic neutrals, which are not isolated in the XAD-8 fractionation scheme used as they stay on the column.

Further proof comes from the skew value, which shows a constant value for the total system before and after physico-chemical treatment. Both fulvic and humic acids almost followed the same trend. This may lead to the conclusion that high molecular weight fractions of fulvic and humic acids did not affect much as a consequence of physico-chemical treatment. This shows that the decrease in average molecular weights for the total system is certainly not due to the removal of high molecular weight material from fulvic or humic acid, instead it could be due to formation of low molecular weight

hydrophobic neutral material. On the other hand, the removed hydrophobic material (Fig.10.8) from fulvic and humic acids fractions must certainly be low molecular weight matter (<6000 Dalton), which results in high polydispersity with these two acids since removal of low molecular weight matter affects (reduces) Mn value rather than Mw value.

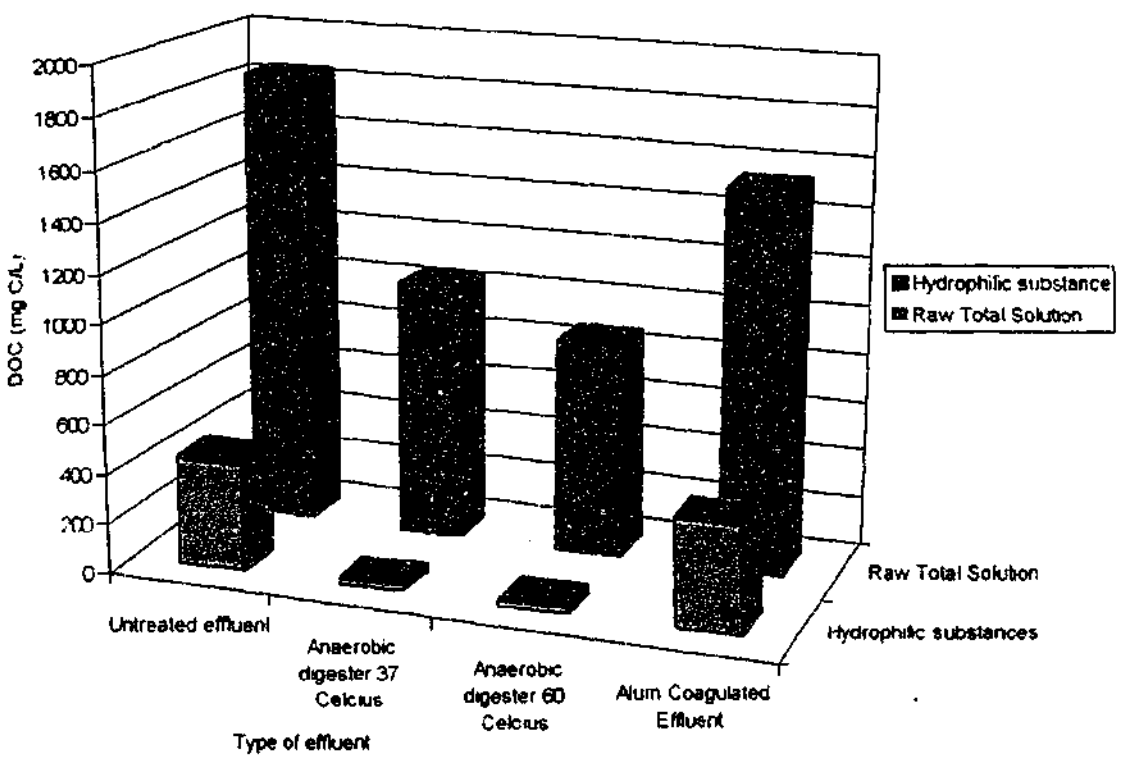
The other noticeable aspect of this study is that the molecular weight distribution for the total sample is highly skewed (Table 10.4) which was due to the fulvic acid fraction (Table 10.5) and not due to the humic acid fraction (Table 10.6). This further suggests that the high molecular weight fractions in the molecular weight distribution of the total system mainly consists of the fulvic acids, as it can be seen in Figs.10.13 and 10.14. The similarity in skew value for the total system and for the fulvic acid fraction further suggests that the removed hydrophobic material was not high molecular weight fulvic acids. In other words, the removed fulvic acids, if any, were low molecular weight fulvic acids and high molecular weight fulvic acids still remain in the medium even after physico-chemical treatment. Similarly, skew value of humic acid supports the idea that most of the removed humic acids were not high molecular weight humic acids and they were more likely to remain in the medium.

10.3.3 Effect of biological or physico-chemical treatment on hydrophilic substances

The effect of anaerobic digestion and physico-chemical treatment on the hydrophilic substances before and after these treatments are shown in the Fig.10.15. It shows that the hydrophilic fraction was totally exhausted from the medium by the anaerobic digestion, but the hydrophobic fraction was only partially degraded. Probably, the anaerobic digestion has preferentially removed almost all of the hydrophilic substances from the HTD effluent than has the hydrophobic fraction. Due to lack of enough hydrophilic substances for microbial digestion after they were exhausted from the medium, the microbes may eventually have reacted on hydrophobic substances. Thus the hydrophobic fraction may also be eventually removed to a significant extent. Probably, the hydrophobic fraction may not be affected by microbes since a significant amount of susceptible hydrophilic fraction is remaining in the medium. It seems the anaerobic digestion is capable of removing some hydrophobic substances as well, depending on the availability and susceptibility of the hydrophilic fraction.

The results of laboratory biological experiments carried out with pulp and paper mill effluent and recipient water at LVOS channel (Chapter 7, Section 7.3), clearly show that the bacteria preferentially react on hydrophilic fraction rather than on the hydrophobic fraction.

Fig 10 15 HTD Wastewater Effect of biological and physical treatments on hydrophilics fraction



In contrast, the physico-chemical treatment significantly removed the hydrophobic fraction from the HTD effluent, but almost all of the hydrophilic substances remained in that medium

10.4 CONCLUSIONS

Hydrophobic substances in coal washings left after hydro thermal drying of brown coal were removed to a certain extent (27%) by anaerobic digestion at 37°C degrees

As far as the total hydrophobic substances are concerned, the anaerobic digestion preferentially removed fulvic acids whereas physico-chemical treatment preferentially removed humic acids, even though fulvic acids are also removed to a lesser extent. In fact, anaerobic digestion was inefficient in removing humic acid while physico-chemical treatment was inefficient in removing fulvic acid and hydrophobic neutrals. The anaerobic digestion also removed hydrophobic neutrals but appears to generate in the physico-chemical process. Humic acids appear to be generated during the biological process, perhaps by anaerobic reaction. Anaerobic digestion at high temperature did not much affect the concentration or the molecular weight distribution of the fulvic and fraction. The hydrophobic neutrals seem to be susceptible to anaerobic digestion at high temperature.

As far as fulvic acids are concerned, the anaerobic digestion efficiently removed high molecular weight fulvic acids whilst physico-chemical treatment removed low molecular weight fulvic acids more efficiently. In relation to this occurrence the average molecular weights of fulvic acids fraction in effluents after anaerobic digestion were lower, whereas in physico-chemically treated effluents they were higher than that found in the untreated effluent.

As far as humic acids are concerned, the anaerobic digestion generated high molecular weight humic acids, perhaps by anaerobic reactions on particulate matter, whilst physico-chemical treatment removed humic acids over the entire molecular weight range. As a consequence, the average molecular weights of humic acids fraction in effluents after

anaerobic digestion were higher, whereas in physico-chemically treated effluents they were more or less similar to that of untreated effluent.

The high molecular weight component in the molecular weight distribution of the raw effluent was mainly due to the fulvic acid fraction. In biologically treated effluents, the high molecular weight component in the molecular weight distribution of the total sample was due to humic acid fraction. Whilst in physico-chemically treated effluent the high molecular weight component in the molecular weight distribution of the total sample was due to fulvic acid fraction.

Biological treatment preferentially removed hydrophilic material whilst physico-chemical treatment preferentially removed hydrophobic material. These materials seem to be low molecular weight and high molecular weight materials respectively. Therefore, higher average molecular weights are observed with biologically treated effluents. In contrast, lower average molecular weights are observed with physico-chemicaly treated effluents.

Chapter 11

Studies On Biological Degradation Of Wastewater From A Sugar Mill

Abstract

An effluent called dunder from the CSR Pty Ltd Sugar Mills at Plane Creek in Queensland, Australia, was chosen for this study. Dunder is a concentrated disposal wastewater removed from the wash column, where the yeast free phase of the fermenter wash, or broth, after molasses fermentation is distilled. This effluent was fractionated into three major organic fractions by means of XAD-8 resin adsorption and these three major fractions were subjected to microbial degradation under laboratory conditions. The above three XAD-8 coloured fractions after humic acid separation were hydrophilic substances, fulvic acid and weak hydrophobic bases (HCl acid eluate). They were sewage treated and periodically analyzed for DOC, colour and molecular weight. Refractionation of the sewage treated coloured samples revealed some chemical transformations into other organic fractions during the microbial treatment. Flow FFF was used to study the molecular weight changes.

The following outcomes were observed with the three microbially treated coloured fractions mentioned above. The DOC effectively degraded within 14 days under the aerobic conditions. Anaerobic conditions also degraded the same amount but within a lengthier period of time. The nondegradable DOC residual was left under aerobic conditions. Chemical transformations were observed due to microbial degradation under both aerobic and anaerobic conditions. Fulvic acids formed or left in the media did not degrade under aerobic condition, but did under anaerobic condition. The noncoloured low molecular weight fulvic acid degraded initially, while the coloured high molecular weight fulvic acid degraded eventually. The noncoloured hydrophilics were degradable under both conditions. The coloured hydrophilics degraded under anaerobic conditions. Hydrophobic neutrals seem to be formed under anaerobic conditions. Under the aerobic conditions, hydrophobic neutrals were observed only in the hydrophilic medium.

11.1 INTRODUCTION

Since the microbial degradation experiments carried out with raw effluents from the AMCOR paper mill (Australia), SECV power plants (Australia) and TASMAN paper mill (New Zealand) have shown that the degradation of DOC is due mainly to the hydrophilic fraction, it was decided to study the microbial effect on major organic groups present in an effluent.

The major organic groups were isolated, depending on the hydrophobicity of the molecules, by XAD-8 resin fractionation from an effluent obtained from the CSR Sugar Mills at Plane Creek, Sarina, Queensland, Australia. These fractions were subjected to microbial degradation under laboratory conditions. After being biologically treated, samples were again XAD-8 fractionated into major organic fractions depending on the hydrophobicity of the molecules present in the medium. Both aerobic and anaerobic conditions were used in separate experiments. The main objectives of these experiments were to study:

- the effect of microbial degradation on coloured organic fractions in wastewaters with respect to the DOC, colour and molecular weight
- whether chemical transformations occur in XAD-8 fractionated sugar mill effluents during microbial treatment
- which process (i.e. aerobic or anaerobic), is more responsible for removing and/or transforming coloured organic fractions in the sugar mill effluents.

Dunder is the concentrated residue left after molasses is fermented. It is a valuable byproduct used as a fertilizer. As only small amounts of process chemicals are present, such as nutrients for yeast growth and defoaming oils, its composition largely reflects that of molasses without the original sugar content. Its value as a fertilizer stems mostly from its potassium content.

11.2 EXPERIMENTAL

The following process describes the generation of dunder effluent during the fermentation:

A concentrated substrate of molasses and water called heavy mash is continuously metered into the fermenter at a preset rate to ensure a low residual glucose concentration of 1-3 g/L. The fermenter wash, or broth, is at the same time being pumped to centrifuges, where the yeast is separated and recycled to the fermenter. The yeast free phase is pumped to the wash column where ethanol is removed as 30-40% WW ethanol/water vapour from the top. As a result, a concentrated effluent is removed from the base and is called Dunder. Chemical constituents of dunder effluent are moisture (60%), fat (0.7%), protein (5.5%), carbohydrates (23%) and ash (9%).

A 10 L plastic drum of dunder effluent was transported to the Monash WSC laboratories in Victoria from the CSR Sugar Mills in Sareena, Queensland. It was stored at room temperature in the laboratory. About 150 mL of the dunder effluent was taken and made up to 300 mL with milliQ water. This stock solution was used for subsequent dilutions. 5 L of solution was prepared for filtration by diluting 250 mL of the above stock solution

with milliQ water. This 5 L solution was filtered through GF/C filter papers sitting on a glass-sintered frit under vacuum. The filtered 5 L solution was made up to 10 L with milliQ water.

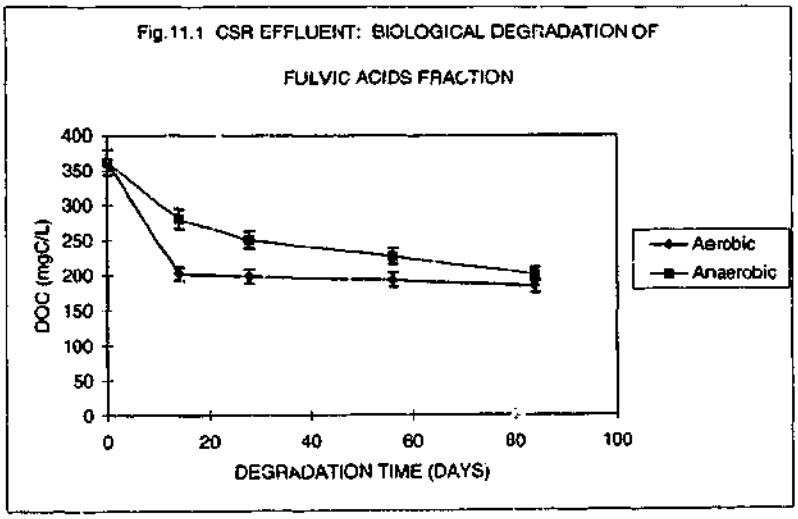
About 2L of the diluted effluent was adjusted to pH 2 and kept 24 hours for any humic acid precipitation. It was centrifuged at 3750 rpm for ½ hr and the decanted solution was XAD-8 fractionated as described in Chapter 4, Section 4.2 using a large scale resin fractionation scheme with a 200 mL XAD-8 resin column. After passing through the resin column with the above diluted dunder effluent at pH 2, the 0.1 M NaOH solution and 0.1 M HCl acid solution were passed through.

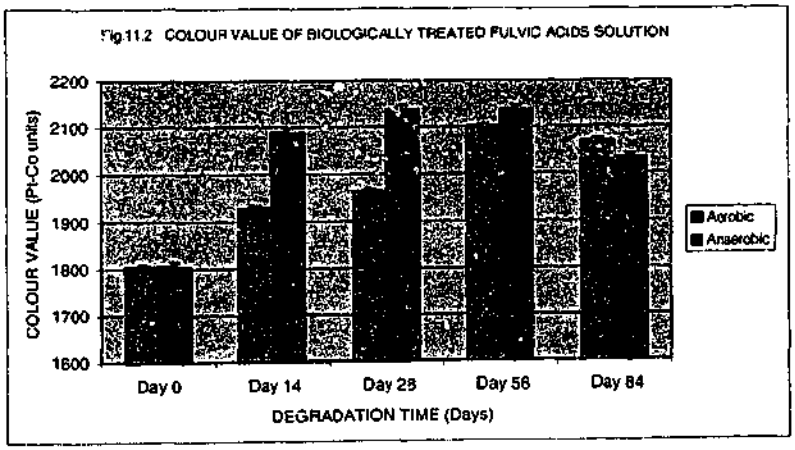
Three coloured fractions were collected, which are designated as the hydrophilics, weak hydrophobic bases (0.1 M HCl acid eluate) and fulvic acids respectively. These fractions were subjected to microbial degradation. The hydrophilic and fulvic acid fractions were diluted 4 times, while the weak hydrophobic bases (0.1 M HCl acid eluate) fraction was diluted two times, before microbial degradation experiments were performed.

11.3 RESULTS AND DISCUSSION

11.3.1 Fulvic Acid Fraction

The microbiological experiments carried out with XAD-8 fractionated fulvic acid solution (Fig.11.1) show that some of the fulvic acid were degraded by microbes. Most of them were degraded, within 14 days under aerobic conditions. As there was about 180 mgC/L of fulvic acid remaining in the medium after 14 days, there was a considerable amount of refractory fulvic acid in the sample under aerobic conditions. In contrast, under anaerobic conditions the DOC degraded more gradually but reached about the same DOC level after 84 days.





Under aerobic conditions, the colour value (Fig.11.2) in the medium increased gradually till day 56. However, it seems that the colour value in the medium tends to level off after 56 days in the aerobic experiment. Probably microbes produce chromophore structures in some fulvic acid molecules under the given conditions.

In the case of the anaerobic experiment, the colour in the medium increased within 14 days and then tended to level off. Apparently the molecules which undergo formation of chromophoric structures react more quickly under anaerobic conditions.

Both experiments attained an overall increase in colour of about 15%. The fact that the rate of colour change and rate of DOC degradation did not follow the same trends suggests that these are quite distinct processes, which are not directly related.

Table 11.1 Molecular weights of raw fulvic acid as a function of degradation time
Raw Fulvic Acid - Molecular Weight (Dalton)

Aerobic Degradation			
Duration (Days)	Mw	Mn	Polydispersity
0	5700	4100	1.39
14	6500	4200	1.55
28	5100	3800	1.34
56	4900	3700	1.32
84	4600	3500	1.31

Anaerobic Degradation			
Duration (Days)	Mw	Mn	Polydispersity
0	5700	4100	1.39
14	5000	3500	1.43
28	5100	3500	1.46
56	4500	3400	1.32
84	4300	3400	1.26

The molecular weight studies (Table 11.1) show that some of the low molecular weight fractions are removed within 14 days under aerobic conditions. As a result, the weight average molecular weight in the medium has increased after the first 14 days in the aerobic experiment. Further exposure to aerobic condition has removed some of the large molecular weight fractions, so that the weight average molecular weight of the medium has decreased after 14 days to day 84. The weight average molecular weight has decreased over the entire experiment under anaerobic conditions, suggesting that some of the high molecular weight fractions were removed under anaerobic conditions.

Fig. 11.3(a) XAD-8 FRACTIONS ISOLATED AFTER BIOLOGICAL DEGRADATION OF FULVIC ACID FRACTION

AEROBIC EXPERIMENT

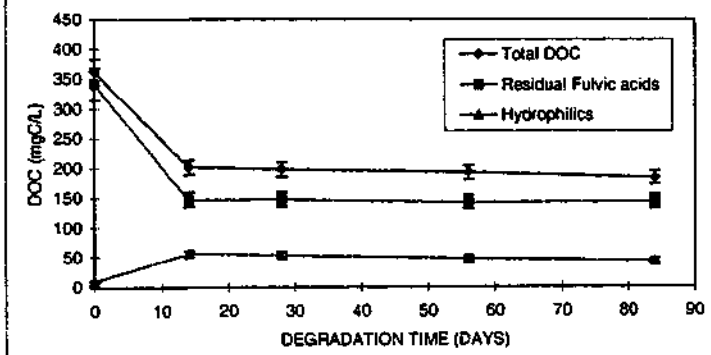
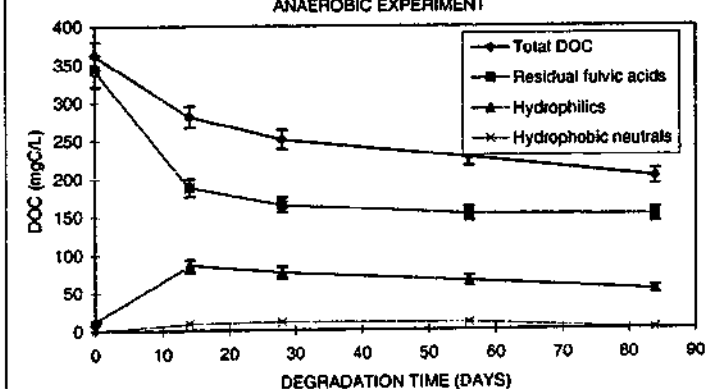
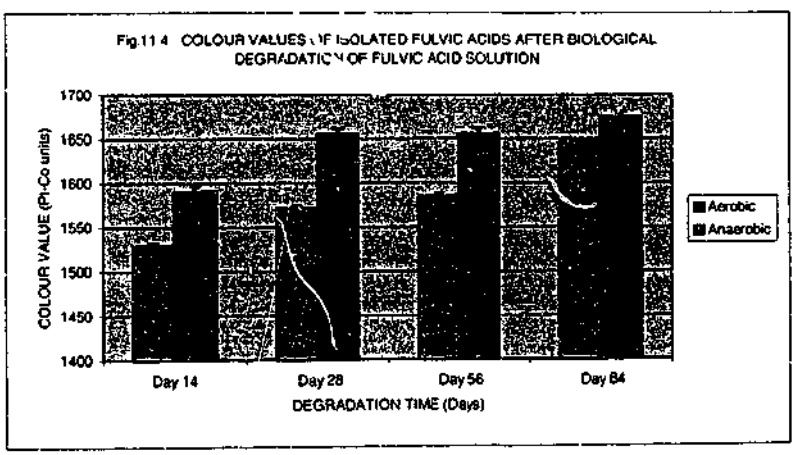


Fig.11.3(b) XAD-8 FRACTIONS ISOLATED AFTER BIOLOGICAL DEGRADATION OF FULVIC ACIDS FRACTION

ANAEROBIC EXPERIMENT

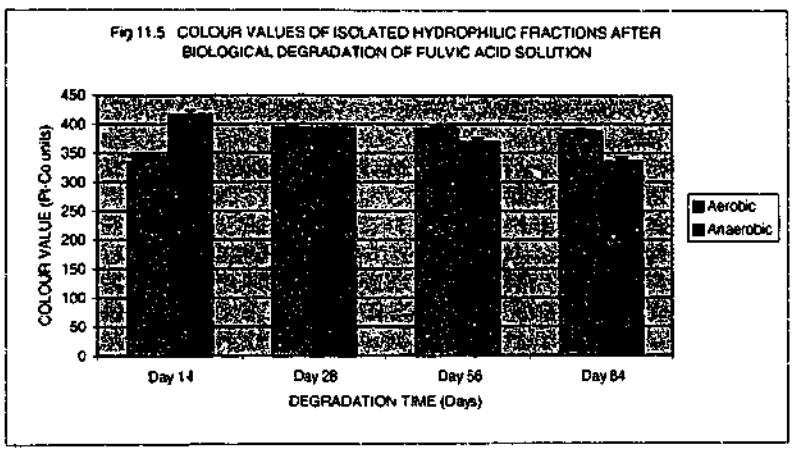


The DOC results obtained by XAD-8 resin fractionation of laboratory scale biologically degraded raw fulvic acid solutions are shown in Figs.11.3(a,b). Within the first 14 days some of the fulvic acids have been converted to hydrophilic substances under both aerobic and anaerobic conditions. Subsequently, these biologically produced hydrophilic substances appear to have been slightly degraded as a function of time under both conditions. The fulvic acid left after the microbial degradation could be described as the residual fulvic acid and its content under aerobic conditions did not change over the entire time frame. In the anaerobic experiment, the fulvic acid continued to degrade after 14 days. The hydrophilic substances were generated from fulvic acids under the aerobic condition due to chemical transformation caused by microbes. In the anaerobic degradation media, some hydrophobic neutrals also formed in addition to hydrophilic substances.



The colour values of the biologically degraded raw fulvic acid solutions and the organic fractions isolated from XAD-8 resin fractionation after the laboratory microbial treatment are given in Figs.11.2, 11.4 and 11.5. The colour value of the residual fulvic acid in the

aerobic medium has increased over the entire experimental time frame (Fig.11.4). The residual fulvic acid appears to produce chromophore structures under aerobic conditions. The colour formation in the residual fulvic acids is most likely the reason for colour formation in the raw fulvic acid solutions. In the case of the anaerobic experiment, the colour of the isolated residual fulvic acid increased over only 28 days. Further exposure to anaerobic conditions to day 84 did not affect the colour of the residual fulvic acid significantly.



The hydrophilic fractions, biologically produced from the original fulvic acid source under both conditions, were coloured (Fig.11.5). The colour of these hydrophilic fractions in the aerobic experiment did not change significantly after 14 days. Thus it appears that the coloured hydrophilic substances which were aerobically produced were not removed by microbes under the aerobic conditions. In contrast, the colour of hydrophilic substances in the anaerobic experiment degraded slightly with time. That is, during degradation of fulvic acids the anaerobically produced coloured hydrophilic substances seemed to be removed by microbes under prolonged anaerobic conditions.

Table 11.2 Molecular weights of residual fulvic acid in the fulvic acid solution as a function of degradation time (Dalton)

Aerobic Degradation			
Duration (Days)	Mw	Mn	Polydispersity
0	5600	3400	1.65
14	5200	3700	1.41
28	5000	3600	1.39
56	4800	3500	1.37
84	4700	3400	1.38

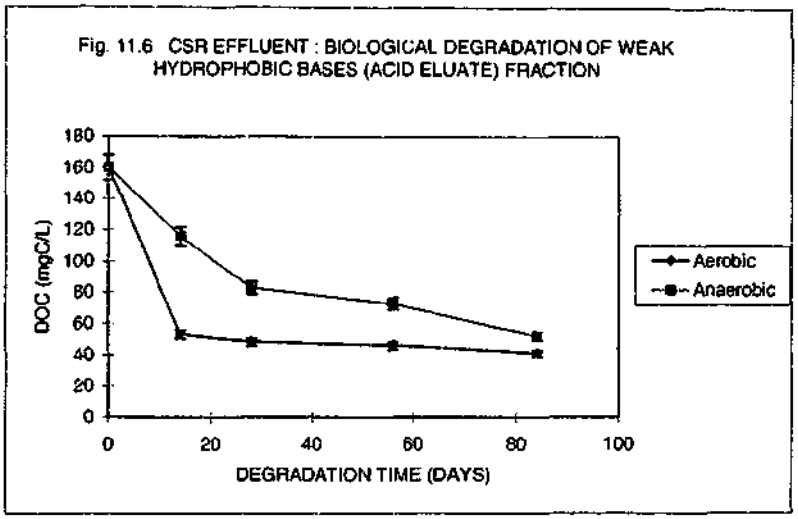
Anaerobic Degradation			
Duration (Days)	Mw	Mn	Polydispersity
0	5600	3400	1.65
14	4700	3500	1.34
28	4800	3500	1.37
56	5100	3600	1.42
84	5000	3600	1.39

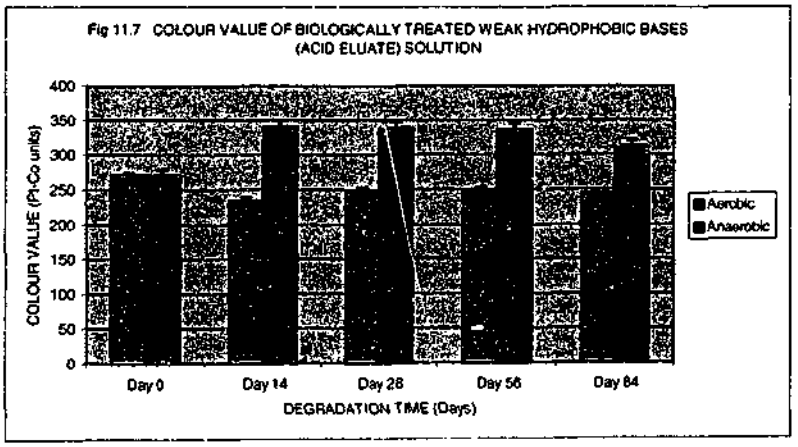
The weight average molecular weights of the residual fulvic acid fractions obtained by XAD-8 resin fractionation of samples collected at various times in the fulvic acid degradation experiment (Table 11.2) have shown a decreasing trend over the period of 84 days under the aerobic condition. In the anaerobic experiment, it decreased over the first 14 days and then kept constant over the rest of the period. Since the fulvic acids did not degrade any further after 14 days under aerobic conditions (Fig.11.3(a)), the decreasing trend in weight average molecular weight could be attributed to molecular cleavage of large fulvic acid molecules or some transformation during the microbial reaction. During the degradation of the fulvic acids under anaerobic conditions (Fig.11.3(b)), the weight average molecular weight decreased, suggesting that more of the high molecular weight

fractions were removed over the 28 days (Table 11.2). Subsequently it seems that more of the low molecular weight fractions were removed over the rest of the period.

11.3.2 Weak Hydrophobic Bases (0.1 M HCl Acid Eluate) Fraction

The 0.1 M HCl acid eluate fraction (Fig.11.6) of the dunder sample was degradable by microbial treatment under both aerobic and anaerobic conditions, but to different extents. The aerobic reaction seemed to be more rapid in removing DOC than the anaerobic system.





The colour value of the above acid eluate fraction (Fig.11.7) decreased slightly within the first 14 days under the aerobic conditions, and kept at a constant level over the rest of the period. In contrast, under the anaerobic conditions, the colour value in the acid eluate medium increased within first 14 days and then kept at a constant level over the rest of the time frame. It appeared to decrease marginally between days 56 and 84.

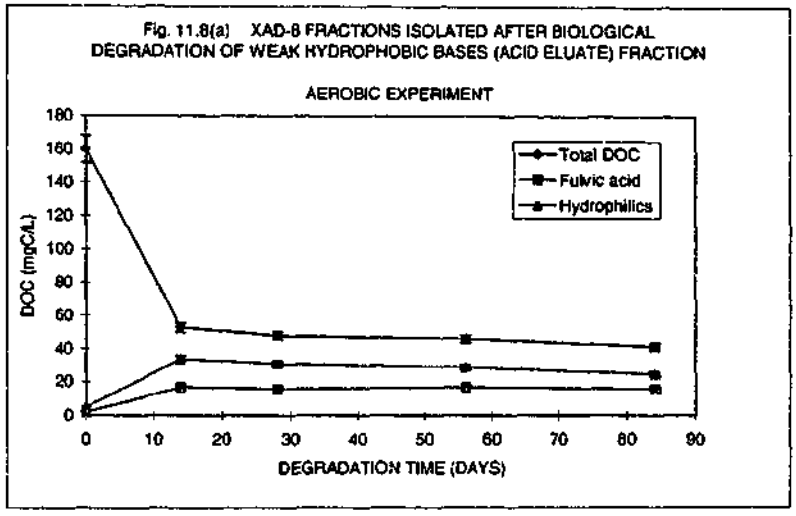


Fig. 11.8(a) XAD-8 FRACTIONS ISOLATED AFTER BIOLOGICAL DEGRADATION OF WEAK HYDROPHOBIC BASES (ACID ELUATE) FRACTION

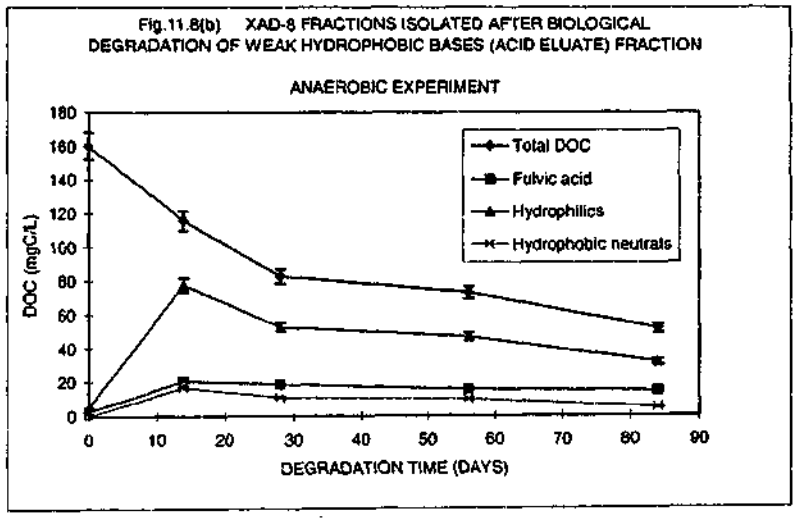
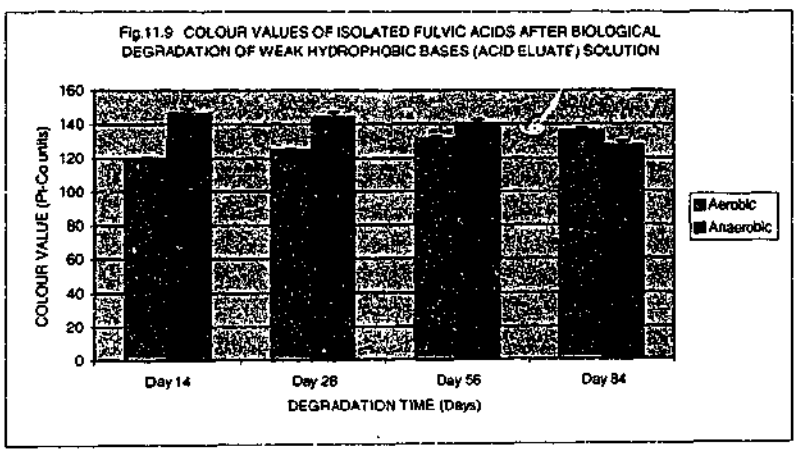


Fig.11.8(b) XAD-8 FRACTIONS ISOLATED AFTER BIOLOGICAL DEGRADATION OF WEAK HYDROPHOBIC BASES (ACID ELUATE) FRACTION

The XAD-8 fractionation of biologically treated acid eluate samples (Figs.11.8(a,b)) has shown that some of the material in the raw acid eluate solution has been transformed to

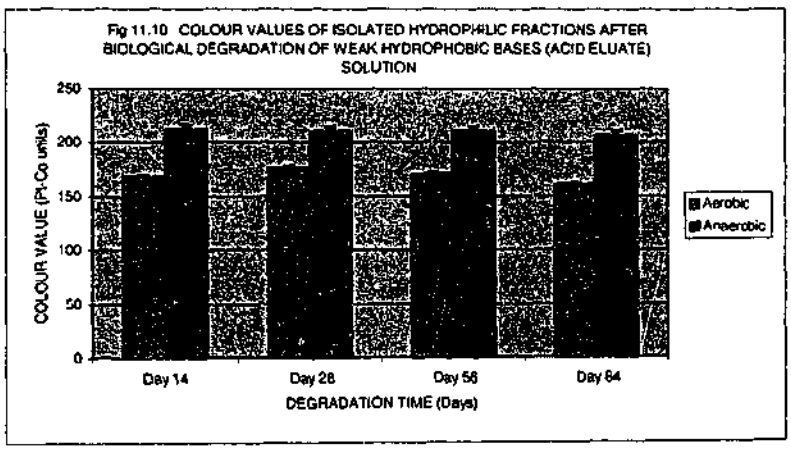
fulvic acid and hydrophilic substances within the first 14 days under both aerobic and anaerobic conditions. These biologically produced fulvic acids were biologically nondegradable under further aerobic conditions. In fact, about 60 mgC/L of the hydrophobic base is decomposed and 50 mgC/L is transformed to fulvic acid and hydrophilic substances.

In the case of the anaerobic experiment, biologically produced fulvic acids in the acid eluate medium were not degraded by microbes from day 14 (Fig.11.8(b)). At the same time the hydrophilic substances in the anaerobic acid eluate media were degraded significantly (Fig.11.8(b)). Most of the hydrophilic substances produced from the acid eluate medium within the first 14 days under the anaerobic conditions are not degradable within that period (Fig.11.8(b)). Also, it can be seen that some of the material in the acid eluate medium has been converted to hydrophobic neutrals under anaerobic conditions (Fig.11.8(b)).



The colour value of the XAD-8 fractions obtained after microbial degradation of the acid eluate fraction are given in Figs.11.9 and 11.10. The results show that the colour value of

the biologically produced fulvic acid increased slightly with time under aerobic conditions, whereas it decreased slightly under anaerobic conditions. It seems that even though this fulvic acid, which was biologically produced under aerobic conditions (Figs.11.8(a,b)), were nondegradable and they form more chromophore structures if oxygen is provided. On the other hand, the colour value of the biologically produced fulvic acid seemed to degrade gradually under the anaerobic conditions (Fig.11.9). That is, the fulvic acids, which were biologically produced under anaerobic conditions, subsequently degrade to some extent and it appears that the coloured fulvic acids degrade more readily under these conditions than under aerobic conditions.



The colour value of the biologically produced hydrophilics in the weak hydrophobic bases solution (Fig.11.10) did not change under anaerobic conditions, but a slight decrease was observed under aerobic conditions.

The weight average molecular weights of biologically produced fulvic acid in the microbially treated acid eluate media are given in Table 11.3. Since those fulvic acids

were biologically nondegradable under the aerobic condition, the changes in weight average molecular weight under the aerobic condition could be due to molecular association or molecular dissociation. However, the degradation of fulvic acid in the acid eluate medium (Fig.11.8(b)) under the anaerobic condition seems to preferentially target the low molecular weight fractions to day 56. After 56 days high molecular weight fractions seem to be removed.

Table 11.3 Molecular weights of fulvic acid in acid eluate solution after the microbial degradation (Dalton)

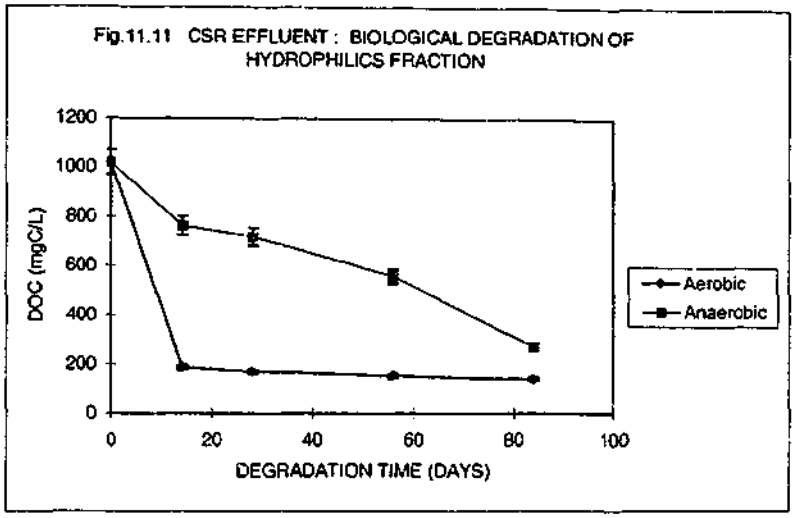
Aerobic Degradation			
Duration (Days)	Mw	Mn	Polydispersity
14	3700	2500	1.48
28	5200	3000	1.73
56	3200	2300	1.39
84	3200	2400	1.33

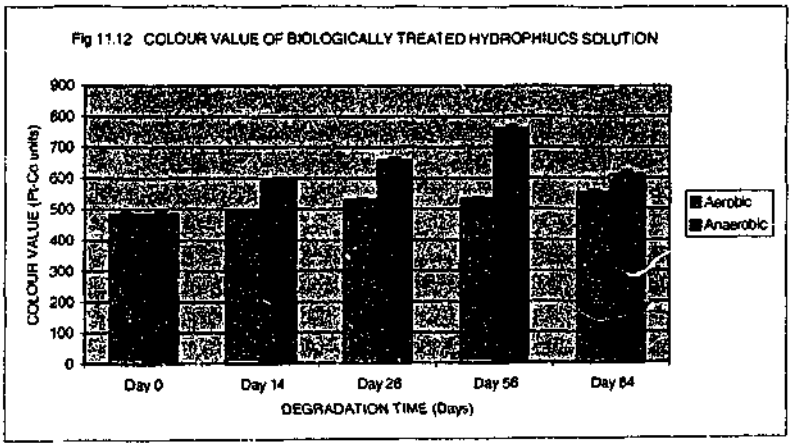
Anaerobic Degradation			
Duration (Days)	Mw	Mn	Polydispersity
14	3400	2400	1.42
28	4300	2800	1.54
56	5600	3500	1.6
84	3400	2500	1.36

11.3.3 Hydrophilic Fraction

The microbial degradation of the hydrophilic fraction of the dunder solution (Fig.11.11) shows that the DOC degradation trend was very similar to that of the fulvic acid fraction (Fig.11.1) and the acid eluate fraction (Fig.11.6). That is, most of the organic matter degraded within 14 days under aerobic conditions, while almost the same amount was

degraded over the entire experimental time frame (till day 84) under anaerobic conditions. As in the other two microbial experiments (fulvic acid and 0.1 M HCl acid eluate), a nondegradable hydrophilic fraction was left in the medium after 14 days under aerobic conditions. In contrast, under the anaerobic conditions, the hydrophilic substances continuously degraded over the 84 day period. It is not clear whether degradation will continue even further after 84 days.





The colour values of the microbially degraded hydrophilic fraction of the dunder solution increased with time under both aerobic and anaerobic conditions (Fig.11.12). The colour

in the aerobic experiment did not change in the first 14 days even though about 80% of the hydrophilic DOC was removed (Fig.11.11). Thus the degraded hydrophilic substances must be noncoloured. In addition, most of the hydrophilics, which have been degraded over the entire experimental phase under anaerobic conditions, are also noncoloured.

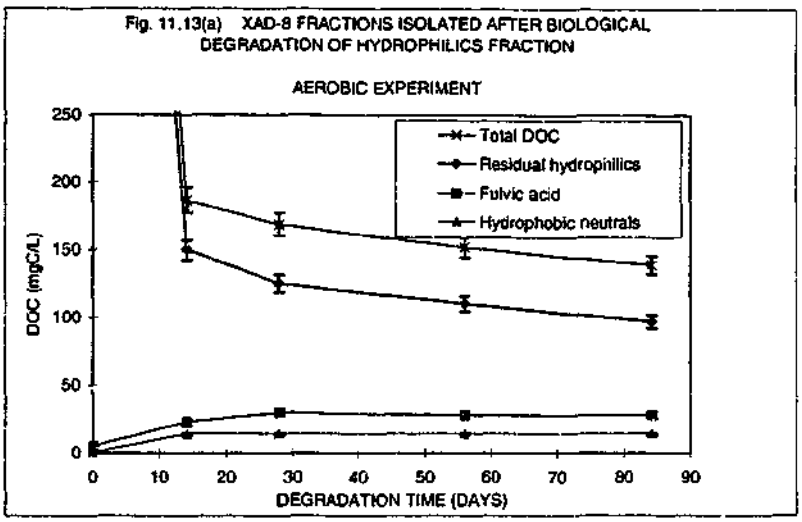


Fig. 11.13(a) XAD-8 FRACTIONS ISOLATED AFTER BIOLOGICAL DEGRADATION OF HYDROPHILICS FRACTION

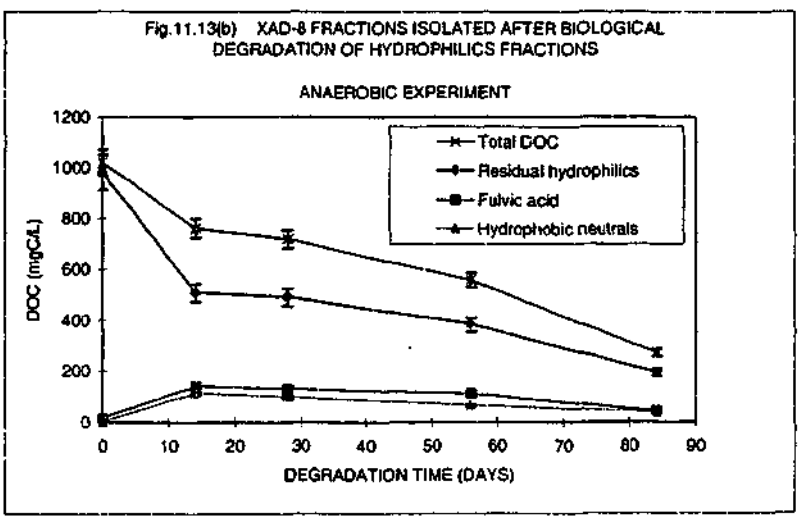


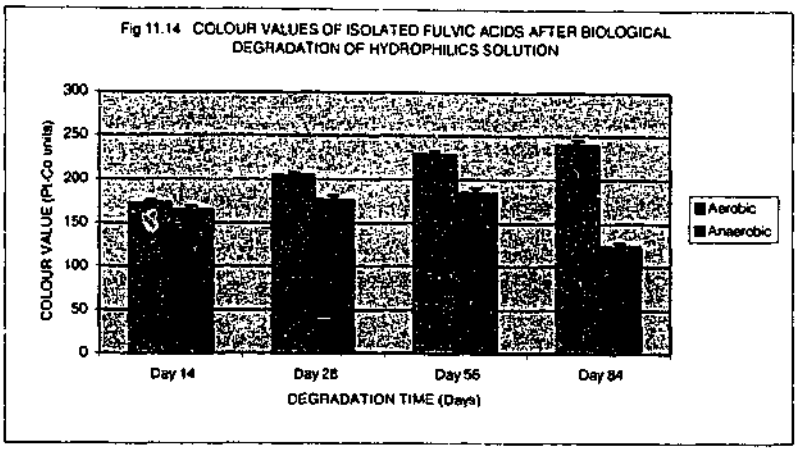
Fig.11.13(b) XAD-8 FRACTIONS ISOLATED AFTER BIOLOGICAL DEGRADATION OF HYDROPHILICS FRACTIONS

The DOC values of the XAD-8 fractions obtained from the biologically treated solution of hydrophilic substances are given in Figs.11.13(a,b). It seems that some of the

hydrophilic substances have been transformed into fulvic acid and hydrophobic neutrals during microbial treatment.

A high proportion of the total DOC in the hydrophilic solution was hydrophilic substances in both experiments (aerobic and anaerobic) even after the microbial treatment. About 250 mgC/L of hydrophilic substances were transformed to fulvic acid and hydrophobic neutrals in the anaerobic media, while only about 35 mgC/L of hydrophilic substances were transformed into fulvic acid and hydrophobic neutrals in the aerobic media.

The biologically produced fulvic acids were nondegradable under the aerobic condition, whereas 70% of them (about 100 mgC/L of fulvic acid) were degradable under the anaerobic condition within 84 days. The hydrophilic substances continued to degrade to some extent under the both conditions over the entire experiment. Not much hydrophobic neutrals (~14 mgC/L) were formed under aerobic conditions and they did not seem to degrade under aerobic conditions. In contrast, more hydrophobic neutrals (~113 mgC/L) were formed under anaerobic conditions than aerobic conditions, and also they tended to degrade somewhat (70%) with time.



The colour values of two XAD-8 fractions (fulvic acid and hydrophilic substances) from the biologically treated hydrophilic solution (Figs.11.14 and 11.15) show that these materials behave differently depending on the oxygen availability in the medium. Noncoloured hydrophilics are removed under aerobic conditions, whereas those removed under anaerobic conditions were coloured. The fulvic acids that degraded under these two conditions were noncoloured. The exception to this general observation is that some of the coloured fulvic acids were degraded under anaerobic conditions after 56 days.

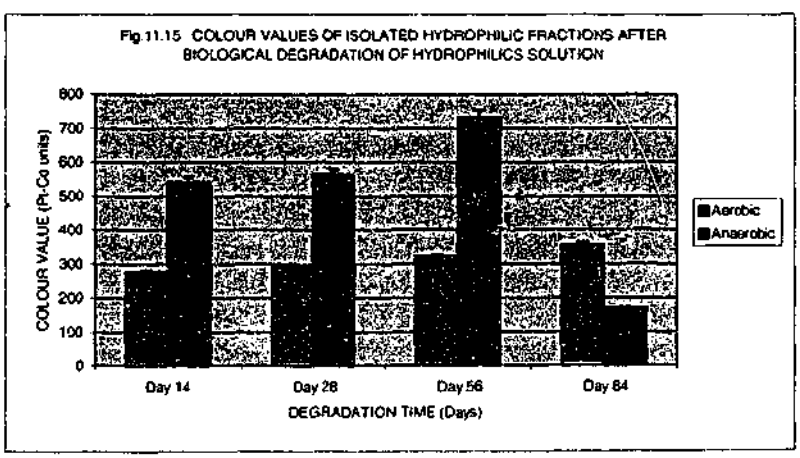


Table 11.4 Molecular weights of fulvic acid in hydrophilic solution after the microbial degradation (Dalton)

Aerobic Degradation			
Duration (Days)	Mw	Mn	Polydispersity
14	4000	2700	1.48
28	4400	2600	1.69
56	6700	3100	2.16
84	4900	2800	1.75

Anaerobic Degradation			
Duration (Days)	Mw	Mn	Polydispersity
14	2700	2100	1.29
28	3300	2400	1.38
56	4900	3000	1.63
84	2400	1900	1.26

The weight and number average molecular weights of fulvic acid biologically produced from the raw hydrophilic medium (isolated by XAD-8 fractionation) under either aerobic or anaerobic condition are given in Table 11.4. Since microbially formed fulvic acid was nondegradable under aerobic conditions, an increase in the weight average molecular weight (to day 56) could be due to molecular association of fulvic acid molecules and likewise, the decrease after day 56 could be due to dissociation. However, since fulvic acid in the microbially treated hydrophilic medium degraded under anaerobic conditions, an increase in the weight average molecular weight to day 56 and subsequent decrease to day 84 could be due to removal of low molecular weight fractions and high molecular weight fractions respectively. Under both conditions, the molecular weight distributions of microbially produced fulvic acid became more highly polydispersed as the degradation time proceeded. However, this broadening of the molecular weight distribution only

occurred up to 14 days. Then microbes seemed to start react on large molecules, which could give rise to a less polydispersed distribution. It seems aerobes were involved only in dissociation, whereas anaerobes were able to degrade them.

11.4 CONCLUSIONS

The initial rate of DOC degradation for three major fractions isolated by XAD-8 resin fractionation (fulvic acid, weak hydrophobic bases (0.1 M HCl acid eluate) and hydrophilics) of the dunder effluent at the CSR sugar mill, were more rapid under aerobic conditions than under anaerobic conditions. The biodegradable portion of all three fractions degraded within 14 days under the aerobic condition. The degradation rate of anaerobic digestion is appreciable over the entire 84 days. The percentage degraded in each fraction under both conditions, reaches about the same level after 84 days. However, the percentage of degradation after 84 days varies for each fraction; being 50% for fulvic acid, 75% for acid eluate and 85% for hydrophilic substances. That is, more of the fulvic acid is refractory.

Some of the XAD-8 fractionated organic materials have undergone chemical transformations into other forms under both aerobic and anaerobic reactions. In most cases the transformation occurs within 14 days and these biologically formed new fractions are refractory. However, there is evidence of subsequent degradation of some biologically formed fractions during the days 14 to 84. For example, the hydrophilics biologically produced from acid eluate solution under anaerobic conditions were

degraded up to 60%. The residual hydrophilics in the hydrophilic media under aerobic and anaerobic conditions were degraded up to 35% and 60% respectively.

Under anaerobic conditions, hydrophobic neutrals were found in the fulvic acid and acid eluate media. Hydrophobic neutrals were also found in the hydrophilic solution under both conditions, but higher amounts were found in the anaerobic experiment than in the aerobic experiment. These biologically formed hydrophobic neutrals under anaerobic conditions were subsequently degraded.

A significant increase in colour occurred after treatment of fulvic acid in both aerobic and anaerobic conditions. This was mainly due to an increase in colour of the residual fulvic acid fraction. Anaerobic degradation of the acid eluate and hydrophilic fractions resulted in an increase in colour, but aerobic degradation resulted in little change. The increase in colour of the hydrophilic fraction under anaerobic conditions was mainly due to increased colour in the nondegraded residual hydrophilic material. The increase in colour in the acid eluate fraction under anaerobic conditions was not caused by the fulvic acid or hydrophilics. Perhaps it was due to an increase in colour of the residual acid eluate fraction.

The changes that occurred in the molecular weight in most degradation experiments were presumably due to preferential removal of either low or high molecular weight fractions. In general there was a small decrease in molecular weight for the fulvic acid degradation in both aerobic and anaerobic conditions. The changes in weight average molecular

weight of this biologically produced fulvic acid under aerobic conditions may be due to molecular association and dissociation processes. In the anaerobic degradation of the above three fractions, the low molecular weight fulvic acid fractions degraded initially and they were noncoloured. But coloured high molecular weight fulvic acid fractions were subsequently degraded (after 56 days).

The hydrophilics degraded from hydrophilic media under both aerobic and anaerobic conditions were noncoloured. In the case of fulvic acid and acid eluate media, the degraded hydrophilics were noncoloured under aerobic conditions, but coloured under anaerobic conditions.

Chapter 12

Conclusions

This work has demonstrated that flow FFF can be successfully applied in molecular weight studies on industrial effluents and receiving wastewaters. Raw effluents as well as treated effluents were effectively characterized for their molecular weight distributions. Since interactions between the sample and the solid stationary phase are minimal in flow FFF, the sample elution times should be more precise than in conventional chromatography. Flow FFF is capable of providing accurate analysis of industrial effluents and recipient waters. In this work it was used to study the effect of treatment on the polydispersed and ill-defined group of molecules found in various wastewaters.

An XAD-8 resin fractionation method was used to separate several broad chemical groups present in the industrial effluents and recipient waters. The humic acid fraction was isolated from the total effluent sample to separate fulvic acid fraction for XAD-8 resin fractionation. This technique was then used to separate hydrophilic substances from the fulvic acid component in the above left solution. This separation principle depends on the hydrophobic nature of the solute molecules.

In most industrial effluents, the hydrophilic fraction is the organic group that is most susceptible to removal by microbes. It is removed by both aerobes and anaerobes, but is most effectively removed by aerobes. In some effluents (e.g. caustic sewer from the bleach plant of the Tasman mill) hydrophilics were hardly removed by anaerobes, but in

others they may be refractory to anaerobes (pulp mill sewer from the pulp plant of the Tasman mill).

Laboratory experiments, carried out with the major organic groups isolated from the effluent released by molasses fermentation at the CSR sugar mills, also showed that hydrophilic fraction is the major organic group effectively removed under both conditions (aerobic or anaerobic). Two other chemical groups (fulvic acid and weak hydrophobic bases) isolated from the same effluent were also removed under both conditions but to a lesser extent.

In some effluents (e.g. AMCOR mill effluent and its recipient water of the LVOS) the only degradable organic group was the hydrophilics due to the refractory nature of the other organic groups present, such as fulvic acid and humic acid. In some instances a nondegradable fraction of the hydrophilics were left in the medium (e.g. the laboratory studies with pulp mill effluents (AMCOR and Tasman mills) and recipient water of the LVOS). In other effluents almost all of the hydrophilics are degradable by microbes (e.g. coal washings from SECV).

The fulvic acids were also removed to a certain extent by both aerobes and anaerobes, but to a lesser extent than hydrophilics. It is more effectively removed by anaerobes than aerobes. In some effluents (e.g. AMCOR mill effluent and recipient water of the LVOS channel) fulvic acids were refractory to aerobes, but were removed by anaerobes to a

certain extent. Fulvic acids are significantly removed by anaerobes when hydrophilics are not in the medium (e.g. coal washings from SECV).

The humic acids were not susceptible to removal by microbes when hydrophilics or fulvic acids or both were present in the medium (e.g. caustic sewer from the bleach plant of the Tasman mill, coal washings from SECV). It was removed by both aerobes and anaerobes when hydrophilics were refractory and fulvic acids were negligible in the medium, but was most effectively removed by anaerobes (e.g. pulp mill sewer at the pulp plant of the Tasman mill).

Perhaps anaerobes may react on humic acids if concentration of fulvic acids in the medium is negligible and when the hydrophilics, if present, are refractory to anaerobes. Perhaps microbes preferentially react on fulvic acids rather than on humic acids if hydrophilics are not present in the medium (e.g. coal washings from SECV). The order of preference of DOC removal by microbes from industrial effluents and recipient waters seem to be hydrophilics, fulvic acids and humic acids respectively.

Effluents from pulp mills possess relatively high average molecular weights with high colour and high DOC values. However, bleaching of them in acid or caustic plants will result in lower average molecular weights. For example, the combined effluent from the pulp plant and paper plant of the AMCOR mill situated in Gippsland, Victoria, had a high average molecular weight and the molecular weights were distributed from 1000 Dalton to 25000 Dalton. Also, the average molecular weight of an effluent from the pulp plant

(pulp mill sewer) of the Tasman mill situated in Tarawera, New Zealand, were high (11000-18000 Dalton); whereas effluents from the caustic and acid bleach plants of the same mill were low (3000-3500 Dalton).

As far as effluent treatments in aerated and nonaerated ponds are concerned, the average molecular weight was increased by aerobic pond treatment whereas it was decreased by anaerobic pond treatment. In the DD treatment ponds and as well as in the Tasman aeration ponds, the average molecular weights in treated effluents exiting from aerobic ponds were higher than those which enter them. Treatment in aerobic ponds removed low molecular weight-dissolved organic matter and generated high molecular weight matter. These two processes may have occurred simultaneously. Anaerobic pond treatment resulted in the removal of high molecular weight-dissolved organic matter and an increase in low molecular weight-dissolved organic matter. An effluent from the HTD brown coal washing process was treated biologically in an anaerobic digester, which removed high molecular weight fulvic acids. Surprisingly, high molecular weight humic acids were generated at the same time. It seems that generation of humic acids can be achieved by both aerobes as well as anaerobes The data appears to indicate that low molecular weight solutes are preferentially degraded by aerobes and high molecular weight solutes are preferentially degraded by anaerobes.

In the open sewer sections of the LVOS channel, high average molecular weights were observed and this could be due to the natural macromolecules derived from allochthonous sources, leached from particulate organic matter after partial degradation or simply due to

generation of humic acids. It seems that microbes preferentially react on lower molecular weight compounds within the open sewers. In that respect it would be similar to the microbial processes occurring in aerobic ponds.

When effluents are subjected to storage in a pond or vessel, microbial reaction on suspended particulate organic matter would perhaps release DOC into the medium. The leached DOC material would most probably be high molecular weight organic matter with high colour.

The DOC at sites within the LVOS channel downstream of the AMCOR mill (Victoria, Australia) is biologically degraded within the two piped sections and open sewer no.2 but not within open sewer no.1. However, the behavior of the molecular weight did not show such a regular trend. The molecular weights of recipient water at downstream sites in the LVOS channel varied depending on whether the channel consisted of oxygenated piped sections or non-oxygenated open sewers. Within the piped sections, an increase of low molecular weight material and depletion of high molecular weight material was evident. Probably microbial oxidation of high molecular weight-dissolved material, rather than microbial degradation, could have occurred within the oxygenated piped sections which are subjected to vigorous oxidation. Low average molecular weights were observed in oxygenated piped sections while high average molecular weights observed in non-oxygenated open sewers. Consequently, the average molecular weights at downstream sites could be high or low, depending on whether the degrading material is hydrophilic or hydrophobic, and whether site is in an oxygenated piped channel or in an open sewer.

With respect to the behavior of fulvic acids in the LVOS channel, the low molecular weight fulvic acids were degraded by aerobes within the piped sections. The open sewers (AMCOR upstream before reach SCC; AMCOR downstream at Peg 83, etc.) introduced low molecular weight fulvic acids to the LVOS system (probably by in situ production), whilst merging inputs introduced high molecular weight fulvic acids at the same time. Whether the low molecular weight fulvic acids have undergone condensation type reactions under vigorous oxidation in the piped sections is uncertain.

Both open sewers plus piped sections in the LVOS channel introduced humic acids to the LVOS flow. The low molecular weight humic acids were predominantly introduced from merging inputs within piped sections in the LVOS channel, whilst high molecular weight humic acids seem to be from in situ productions in open sewers. However, humic acids were degraded within the piped section 2 and most of them were from the low molecular weight range.

During the aerobic phase of the DD treatment ponds (pond A to pond C), the low molecular weight humic acids were removed first and the high molecular weight humic acids were removed later. At the same time formation of high molecular weight fulvic acids have occurred. During the anaerobic phase of the DD treatment ponds (pond C to pond E) the high molecular weight fulvic and humic acids were removed. Pond E seems to be a steady state.

Most of the removed organic matter along the LVOS channel was hyrophilics. About 30% of the hydrophilics were removed within the initial phase of the LVOS channel. The hydrophobics were removed within the latter phase of the same and also during the treatment at DD ponds. Hydrophilics proved to be exhausted from the medium first under well aerated conditions, whereas hydrophobics were removed eventually but preferred anaerobic conditions. Within the treatment ponds no remarkable removal of hydrophilic substances was observed, instead much of the hydrophobics were removed. The residual hydrophilic substances found in the treatment ponds must be refractory.

The laboratory biological experiments conducted under oxic and anoxic conditions revealed that aerobes preferentially degrade DOC while anaerobes preferentially degrade suspended solids. The solids present in these samples may be in the form of lignocellulose fibrous matter and have been liberated during the pulping process. In this way DOC could be leached to the surroundings under anoxic conditions. Anaerobes are not capable of removing hydrolysis products, instead they diffuse into the water. Probably oxygen has greatly favored the utilization of DOC by microbes.

The biological experiments conducted with the same effluents after filtration (AMCOR mill effluent and the LVOS recipient water) under oxic and anoxic conditions revealed that aerobes preferentially react on low molecular weight dissolved organics, while anaerobes preferentially react on high molecular weight dissolved organics. However, DOC was removed under both conditions, with degradation of DOC being more efficient under oxic conditions.

In the laboratory experiments, the behavior of colour and molecular weight of fulvic acids and humic acids were studied. The colour of fulvic acid is associated with low molecular weight fulvic acids. Also, noncoloured high molecular weight fulvic acids could be removed by aerobes. The aerobes generated coloured humic acids, but the humic acids removed by aerobes were noncoloured, whereas the anaerobes removed coloured humic acids. Both aerobes and anaerobes initially remove low molecular weight humic acids but later removed high molecular weight humic acids. In fact, the colour of the raw total effluents varies depending on whether fulvic acids or humic acids are involved, and which molecular weight fraction of them is involved with the treatment. Caustic sewer experiments under both conditions shows that the colour of the effluent is mainly due to fulvic acids, whilst in pulp mill sewer it is mainly due to humic acids.

The biological experiments conducted with isolated organic fractions from CSR sugar mill effluent revealed that fulvic acids and weak hydrophobic bases are also degradable. But in the presence of hydrophilics, microbes selectively remove the hydrophilics first. The other noticeable aspect of these experiments was the occurrence of some chemical transformations between each major organic fraction. This could have also occurred in the combined medium composed of all major organic fractions.

Degradation of small molecules by aerobes seems to be due to active uptake into the bacterial cell. However, degradation of larger molecules by aerobes seems to be due to microbial oxidation. The low molecular weight fractions being removed under oxic

conditions will result in a higher average molecular weight. Conversely the high molecular weight fractions being removed under anoxic conditions will result in a lower average molecular weight. The anaerobic process breaks down larger molecules into smaller molecules, which in turn aerobic bacteria can readily utilize. This would most probably be caused by the biological destruction of the benzenoid skeleton.

12.2 GENERAL CONCLUSIONS

Flow FFF can be successfully applied to study the effect of treatment on the ill-defined group of organic molecules found in various industrial effluents and receiving wastewaters. Separation by XAD-8 resin fractionation can also be successfully used to separate the hydrophilic component from the hydrophobic substances contained in these effluents.

Parameters such as DOC and colour, together with the amount of the chemical fractions isolated by using XAD-8 resin fractionation (depending on the hydrophobicity nature of the molecules), can be used as a tool for chemical characterization of industrial effluents and receiving wastewaters. Parameters such as weight and number average molecular weights of the dissolved organic component in raw effluents and in the major chemical fractions isolated, together with their molecular weight distribution patterns (obtained by flow FFF) can be effectively used as a tool for physical characterization.

The hydrophilic component found in most industrial effluents and receiving wastewaters is the most susceptible organic group among the dissolved organic components. This fraction is effectively removed by biodegradation. Microbes significantly remove hydrophilic substances from the medium even if other chemical groups, such as fulvic and humic acids, are present in the same medium. Microbes preferentially react on the hydrophilic component in the effluent/wastewater organic pool, and it is thus removed first from the reaction medium.

Depending on the refractory nature of the other chemical groups present, the hydrophilic component could be the only biodegradable organic group in some effluents. Often, almost all of the hydrophilic substances may be degradable by microbes. However, in some effluents, a fraction of nondegradable (refractory) hydrophilic substances may be left in the medium even after microbial degradation.

The other chemical groups present, such as fulvic acid and humic acid may also be biologically degradable, but to a lesser extent. Microbes preferentially react on fulvic acids if the hydrophilics are either refractory or not found in the medium. In the presence of degradable hydrophilics, microbes selectively remove the hydrophilics first.

Humic acids are generally more refractory to biological degradation than other chemical fractions. However, humic acids may also be biologically degradable, either in the absence of hydrophilics and fulvic acids or if these fractions are refractory. The order of preference in removal of chemical groups by biodegradation is; hydrophilics > fulvic acids > humic acids.

Both aerobes and anaerobes are capable of removing dissolved organic matter in industrial effluents and wastewaters. Aerobes preferentially react on solutes (DOC) in wastewater media, even in the presence of solids. In contrast, anaerobes preferentially react on solids (POC) in the medium, even in the presence of dissolved organics. In fact, DOC may increase in an anaerobic medium as a result of DOC leaching from POC into the surrounding solution due to anaerobic reaction.

In the absence of solids (POC) in an effluent medium, DOC removal by anaerobes is substantial, but degradation of DOC is more efficient under oxic conditions than under anoxic conditions. As far as the dissolved organic component is concerned, it appears that aerobes preferentially remove lower molecular weight solutes, whereas anaerobes preferentially react on higher molecular weight solutes.

Concerning the issue of which chemical group is most efficiently removed from a raw effluent/wastewater medium (non-fractionated total organic medium) under which conditions, it seems that aerobes and anaerobes selectively react on different organic groups in the medium. However this selectivity also depends on the availability and susceptibility of other chemical groups present.

For example, aerobes are generally more efficient in removing hydrophilics than anaerobes and anaerobes are more efficient in removing hydrophobic substances than aerobes. However, if hydrophilics are absent, aerobes more effectively react on hydrophobic components than anaerobes. The hydrophilics are hardly removed by anaerobes in the presence of hydrophobics. On the other hand, hydrophobics are hardly removed by aerobes in the presence of hydrophilics.

The presence of POC in the medium affects the net outcome of anaerobic processing of the DOC component, since anaerobes preferentially react on POC rather than on DOC in the medium. This is because, anaerobic reaction on POC introduces high molecular weight solutes to the medium, whilst anaerobic reaction on DOC introduces low molecular weight solutes.

As a result of microbial reactions, some forms of chemical transformation (from one organic group to another organic group or to several organic groups) occur between the dissolved organic groups present. This can occur in the combined media containing all organic groups; for example raw effluent medium or raw wastewater medium, as well as in isolated media fractionated using XAD-8 resin fractionation.

Both aerobes and anaerobes are involved in this chemical transformation process. Aerobes are more efficient in removing biologically produced (transformed) material from the medium than anaerobes. However, most of the biologically produced matter remains in the medium under both conditions. Perhaps, biologically produced organic material may refractory to microbes to a certain extent.

The colour of an effluent is mainly associated with fulvic and humic acids but may be due to hydrophilics as well, depending on the type of effluent. However, the colour of a treated effluent varies with the type of treatment process, such as whether it is an aerobic or anaerobic process. An aerobic process increases the colour of an effluent, whereas the anaerobic process usually decreases the colour.

This is because aerobes preferentially remove noncoloured DOC from an effluent, whereas anaerobes preferentially remove coloured DOC. Also, aerobes may selectively remove noncoloured fractions from organic compounds that result in from biological transformations, which occur during a biological treatment process of an effluent. Likewise, anaerobes may selectively remove coloured organic fractions from such organic matter result in from biological transformations.

According to field studies, it seems that vigorous oxidation in wastewater channels results in microbial oxidation of high molecular weight dissolved organic material. Perhaps, aerobes may selectively oxidize high molecular weight fractions under vigorous oxidation conditions.

In the absence of external addition of oxygen, the degradation occurring in open sewers would be an anaerobic process, due to insufficient oxygen in the medium. These anaerobic processes on both DOC and POC can account for the total degradation in open sewers.

The generation of fulvic and humic acids can occur in open sewers due to in situ production. It seems that these fulvic acids are in the lower molecular weight range whilst humic acids are in the higher molecular weight range. The average molecular weight of a wastewater sample collected from downstream sites in a channel carrying an industrial effluent could be low or high, depending on whether the site is in an oxygenated region or in an unoxygenated open sewer.

Effluent treatment in aerobic and anaerobic ponds can result in diverse trends from plant to plant depending on the conditions prevailing as well as the type of effluent. Due to the refractory nature of some chemical groups in some effluents, effluent treatment in aerobic or anaerobic ponds may greatly favor a particular degradation trend of a particular susceptible organic group.

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APPENDIX

Molecular weight of a macromolecular solution

The molecular weight of a macromolecular substance may be determined, by utilizing the colligative properties of solutions, such as lowering of vapor pressure, boiling point elevation, freezing point depression or osmotic pressure. Such methods are particularly useful for low molecular weight solutions.

However, the variations in colligative effects due to high molecular weight macromolecules are smaller than for small molecules. This is because, for a given mass concentration of a polydispersed macromolecular substance in a solvent, the number of solute molecules of high molecular weight is very much less than for solute molecules of low molecular weight. Since the most macromolecular systems are mixtures of different molecules of different molecular weights, the molecular weight of such a solution is then an average. The diffusion or sedimentation coefficients of macromolecules in a solution can be used to determine the molecular weight of such high molecular weight materials.

Molecular weight averages

(a) Number average molecular weight

The colligative properties are defined as the properties that primarily depend on the number of molecules and not on their nature. For example, since the osmotic pressure primarily depends on the number of solute molecules in a macromolecular solution and not on their macromolecular size, the average molecular weight obtained by this method is called the number average molecular weight (*Mn*). The number average molecular weight is the total mass of dispersed material divided by the total number of molecules present.

(b) Weight average molecular weight

The macromolecules in a polydisperse solution are distributed according to their molecular size. Since the sedimentation of macromolecules in such a polydisperse system under the influence of centrifugal forces is dependent on their molecular size, the average molecular weight obtained by this method is called weight average molecular weight (*Mw*). The weight average molecular weight is the summation of the molecular weights of each molecular size of the dispersed material multiplied by the fraction of the total weight.

Monodisperse and Polydisperse systems

In some macromolecular samples, almost all of the dispersed molecules have the same molecular weight. Such solutions are said to be monodisperse systems. Generally, the low molecular weight macromolecular samples are monodispersed systems. However, some macromolecular samples are often mixtures of molecules of different molecular weights. Such samples are said to be polydisperse systems. Generally, the high molecular weight macromolecular samples are polydispersed systems.

Polydispersity

If a material consists of a mixture of different molecular weights, then the two average values obtained for the molecular weight based on number and weight, may be quite different and this difference may be a useful guide to the homogeneity of the macromolecular sample. For a monodisperse system, these two values of *Mw* and *Mn* are equal. For a polydisperse system, *Mw* is always greater than *Mn*, since the effect of the small molecules on the *Mw* value is less than that of larger molecules. The large molecules have a greater effect on *Mw* than on *Mn*. The ratio of *Mw/Mn* is called the Polydispersity of the system and it is an indication of the sample heterogeneity.