TREATMENT OF HIGH-STRENGTH AND TOXIC ORGANIC INDUSTRIAL EFFLUENTS IN THE ANAEROBIC BAFFLED REACTOR

J Bell • P Dama • R Mudunge • CA Buckley

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TREATMENT OF HIGH-STRENGTH AND TOXIC ORGANIC INDUSTRIAL EFFLUENTS IN THE ANAEROBIC BAFFLED REACTOR

J BELL, P DAMA, R MUDUNGE and CA BUCKLEY

Pollution Research Group

School of Chemical Engineering

University of KwaZulu-Natal

Durban

Head of Pollution Research Group: Professor C A Buckley
Project Leader and Researcher: Dr J Bell

Report to the Water Research Commission on the project

"The Assessment of a Baffled (Compartmentalised) Anaerobic Digester for the Treatment of High-Strength and Toxic Organic Industrial Effluents"

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EXECUTIVE SUMMARY

The predicted high industrial growth rate in South Africa, coupled with the limited water resources, will necessitate the implementation of advanced effluent treatment systems. The results of previous research (Sacks, 1997) identified a number of industries, in KwaZulu-Natal, that produced effluent streams that would be amenable to treatment by anaerobic digestion. The organic content of these effluents (based on COD) was too high to permit conventional treatment at a wastewater treatment works. In the short term, these effluents could be treated in the under-utilised anaerobic digestion capacity in the region. However, with the projected increase in the load on wastewater treatment works, this available capacity will ultimately be needed for sewage treatment.

The challenge in advancing anaerobic digestion for high-strength or toxic organic waste streams lies largely in enhancing the bacterial activity taking place per unit of reactor volume and, in the case of xenobiotics (substances having structural features that are not normally encountered in nature), in the acclimation of the biomass to the compound. Taking into consideration the slow growth rate of many anaerobic micro organisms, particularly methanogens, the main objectives of efficient reactor design must be high retention time of bacterial cells within the reactor, together with good mixing to ensure contact between cells and their substrate (Grobicki, 1989). The anaerobic baffled reactor (ABR) achieves both objectives by means of a design that is both simple and inexpensive to construct, since there are no moving parts or mechanical mixing devices. High rates of hydraulic throughput are possible with very little loss of bacteria from the reactor.

The ABR has alternately hanging and standing baffles (Figure 1), which divide it into 8 compartments. The lower edges of the hanging baffles are slanted (45°) to route the flow of liquid and to reduce channelling. The liquid flow is alternately upward and downward between the partitions. The downflow chamber is narrower than the upflow chamber to prevent accumulation of biomass in the latter. On its upward passage, the waste flows through an anaerobic sludge blanket. Hence, the waste is in contact with the active biomass but, because of the design, most of the biomass is retained within the reactor. In principle, all phases of the anaerobic degradation process can proceed simultaneously. The sludge in each compartment will differ depending on the specific environmental conditions prevailing and the compounds or intermediates to be degraded (Nachaiyasit and Stuckey, 1997a). A staged reactor can provide higher treatment efficiency since non-labile substrates will be in an environment conducive to degradation. Process stability is good.

The concentrated, variable and intermittent nature of industrial effluents make them intrinsically unsuitable for treatment in a completely mixed system. The ABR provides resilience to intermittent organic and hydraulic loads as well as toxic components of an effluent. Cleaner production is defined as the continuous application of an integrated environmental strategy, applied to processes, products and services to increase eco-efficiency and to reduce risks for humans and the environment. Implementation of cleaner production and waste minimisation practices, at the effluent source, will lead to the production of more concentrated effluents. Agro-industries characteristically produce effluents of a xenobiotic nature and/or of high organic strength. Industries such as the food, leather and textile industries also utilise synthetic colorants in their processes, resulting in coloured effluents.

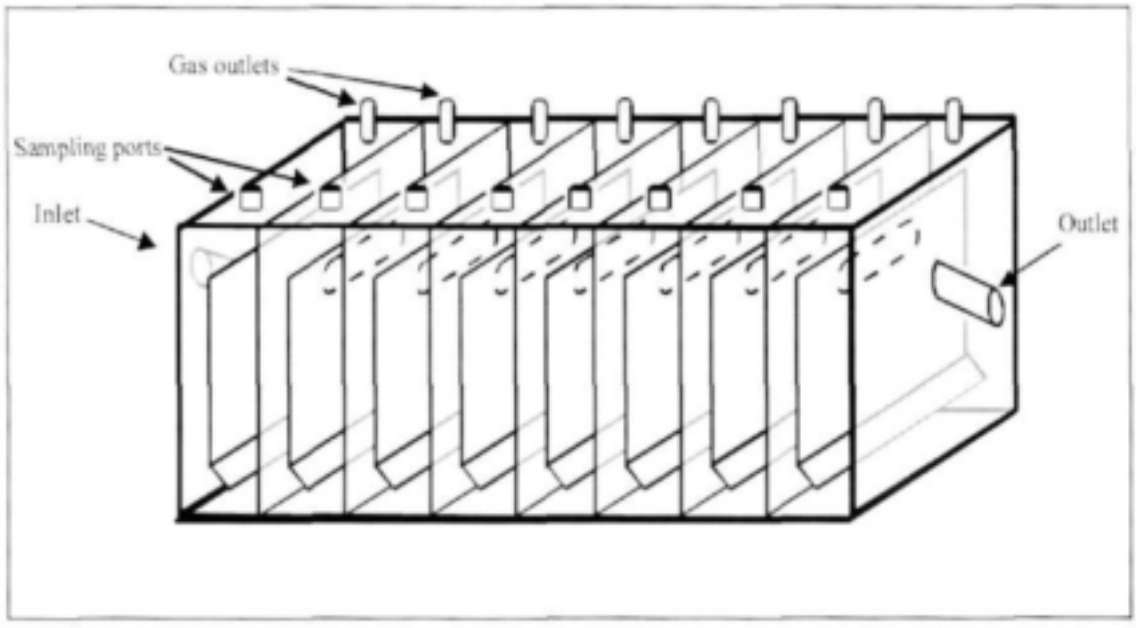

Figure 1: Schematic diagram of the anaerobic baffled reactor

Dyes and pigments are usually released into the environment in the form of a dispersion or a true solution in the industrial effluent (Seshadri et al., 1994). The presence of very small amounts of dyes in water (less than 1 mg/L) is highly visible and aesthetically unpleasant (Cooper, 1995). Public perception of water quality is influenced by colour; unnatural colour is associated with contamination. Strong colours could reduce light penetration, thus affecting the growth of plants and the aquatic ecosystem. The current environmental concern with azo dyes revolves around the potential carcinogenic health risk that they present or their intermediate biodegradation products when exposed to microflora in the human digestive tract (Seshadri et al., 1994). There is the potential for the degradation products of these dyes to build up in the environment since many of them pass through wastewater treatment plants virtually untreated. Azo dyes are intentionally designed to be recalcitrant under typical usage conditions, and it is this property, allied with their toxicity to micro organisms, that makes biological treatment difficult.

In South Africa, the ABR could be implemented on-site for pre-treatment of agro-industrial wastes, with high COD contents or those with xenobiotic components, which prevent conventional treatment at a wastewater treatment works. Current disposal options include marine discharge, co-disposal on municipal landfills or dilution with potable water. With implementation of the ABR, waste minimisation practices could be adopted to concentrate effluents on-site. Pre-treatment in the ABR, with a biomass acclimated to the particular effluent, should facilitate sufficient degradation such that the effluent could then be discharged to sewer for treatment.

1 Project Aims

The initial project aims were defined as follows:

1. Design and construct a laboratory-scale system for the assessment of the suitability of an organic industrial effluent for treatment in an anaerobic baffled reactor.
2. Develop experimental protocols for the assessment of the suitability of a high-strength or toxic organic industrial effluent for treatment in an anaerobic baffled reactor.

3. Assess the suitability of an anaerobic baffled reactor for the treatment of high-strength or toxic organic industrial effluents.
4. Design, construct and operate an efficient and cost-effective system for the treatment of a high-strength or toxic organic effluent.

Under the guidance of the Steering Committee, the aims were re-stated as:

1. Acquire knowledge on the design, operating procedures and functional aspects of the ABR.
2. Become familiar with current and previous investigations conducted on the ABR, to enable assessment of its potential applications.
3. Obtain sufficient understanding of the technology to facilitate identification and co-operation with an industrial partner.
4. Assess the biodegradability and inherent toxicity, of an industrial wastewater, to the anaerobic biomass.
5. Assess the efficiency of the ABR for treatment of an industrial wastewater, on a laboratory-scale.
6. Use Computational Fluid Dynamics and tracer tests to enhance understanding of the hydrodynamics, flow patterns and mixing patterns within the ABR.
7. Design, construct and commission a pilot-scale ABR.
8. Evaluate the pilot-scale ABR.

2 Summary of Results

Laboratory-scale screening tests were conducted on several food and textile dyes as well as industrial wastewaters. The objective of the *toxicity assays* was to determine the concentration at which each dye became inhibitory to the methanogenic biomass. The toxicity assays were specific to the methanogenic populations of the anaerobic digester sludge. A wide range of toxicity data were obtained with IC_{50} values ranging from > 20 g/L to 0.2 mg/L. The two most toxic dyes were Carmoisine Supra (IC_{50} of 0.25 g/L) and Erythrosine Supra (IC_{50} of 0.2 mg/L). The IC_{50} concentration of tartrazine was 14.3 g/L. The dye manufacturing effluent was relatively inhibitory to the methanogens with IC_{50} values of 22.5% and 15.9% (v/v), for the untreated and chemically treated effluents, respectively. The objective of the *biodegradability assays* was to evaluate whether the anaerobic biomass could utilise the dye as a sole substrate. Generally, the methanogenic activity was low, suggesting that these dyes were not readily utilised by the unacclimated methanogenic populations. Decolourisation due to adsorption or reduction of the azo bond was negligible for the majority of the dyes. These bioassays provided a more thorough understanding of the dye characteristics and degradation potential. This knowledge can be used to predict the optimal treatment option. Additional tests could be run with the supplementation of a carbon source, to investigate co-metabolism of the dyes. Adsorption bioassays could quantify the amount of adsorption of a particular dye to anaerobic biomass.

Several laboratory-scale ABRs were operated under varying conditions. One investigated the decolourisation of a pure food dye, tartrazine. Tartrazine, classified as Colour Index Food Yellow 4, is a monoazo synthetic organic colorant, with a maximum absorbance at 430 nm. Adsorption to anaerobic biomass played a role in the decolourisation of the dye. The COD of a tartrazine feed to the ABR resulted was reduced by 50 to 60%. Colour removal increased with time, suggesting acclimation of the biomass to degradation of the tartrazine. After approximately 2 months, the tartrazine colour removal was 90%. Most of the colour reduction was achieved in the

first compartment of the reactor. There was methanogenic activity in the first compartment of the ABR. Tartrazine was not readily degraded by anaerobic digestion, however, degradation may be improved with acclimation of the biomass. The use of molecular techniques (FISH) to identify the bacteria associated with the degradation of the tartrazine dye was unsuccessful as the dye interfered with probe hybridisation, resulting in the probes binding to the dye and not to the biomass.

The second laboratory-scale ABR investigated the degradation and decolourisation of the trade effluent from a food dye manufacturer. Anaerobic degradation of the wastewater was efficient. Methanogenic activity was high in the reactor, the organic content of the influent was reduced by ca. 70% and colour was reduced by almost 90%. Most of the colour reduction was achieved in compartment 1. Efficient degradation may be dependent on the composition of the wastewater, which was variable and could upset the degradation process. Whole cell oligonucleotide probe hybridisation showed definite shifts in the microbial populations due to the addition of the wastewater to the reactor. The general morphology of the Eubacteria changed as did their numbers, which decreased significantly in the last compartments of the reactor. Methanogens were observed throughout the reactor, with Methanosarcina clusters dominant in the first compartments of the reactor and the scavenging Methanosaeata species dominant in the last compartments.

A well-defined and previously researched reactive textile dye, CI Reactive Red 141, was added to the sugar/protein feed to a laboratory-scale reactor. Batch screening tests indicated that (i) the reactive dye was not inhibitory to the acidogenic populations; (ii) the biogas production increased with increasing dye concentration which suggests that the dyes were being actively metabolised by the acidogens; (iii) reactive dye compounds, present in textile dye wastewaters, did not adversely affect the anaerobic degradation process; (iv) Methanogenic activity was inhibited by the dye degradation products; and (v) there was no further reduction in COD or colour.

The results of the physical decolourisation tests suggested significant decolourisation due to adsorption to the biomass. However, it is possible that the dye chromophores were reduced due to the low redox potential environment within the test bottles. No dye break-through, due to adsorption saturation, was observed during operation of the reactor. The COD reduction was consistently > 90%, except for the period during the dye shock load. Colour reduction averaged 86%. The biomass showed acclimation to the dye, with increased methanogenic activity with each increase in dye concentration. The inaccurate biogas measurement resulted in the poor COD balance of 18.2%. The reactor operation was stable, even with increases in the dye concentration. The only observed response to the dye was when the concentration was increased to 1 g/L (shock load), resulting in temporary inhibition but recovery to stable operation within 5 HRTs.

This investigation has shown that successful treatment of a highly coloured wastewater is possible in the ABR. The design of the ABR facilitates efficient treatment of concentrated textile dye wastewaters by protecting the sensitive methanogens from the inhibitory dye molecules and promoting efficient colour and COD reduction, primarily by the acidogens in the first compartments. Metabolic activity was low in the final three compartments of the reactor. There was a definite shift in the microbial populations through the ABR, with a predominance of eubacteria in the first compartments (acidogenesis) and archaea (methanogenesis) in the later compartments.

The use of molecular approaches provided useful descriptions of the methanogens actively involved within each compartment. These results showed a horizontal separation of a short filamentous archaeal micro organism and the long sheathed filamentous Methanosaeta spp. The short filamentous archaeal micro organism proliferated after

addition of the dye to the reactor and was metabolically active in the first four compartments of the reactor. Identification of the micro organism will require DNA extraction and sequencing. The application of molecular techniques to the ABR process improved the understanding of the metabolic processes occurring within each compartments and the micro organisms involved in these reactions.

A laboratory-scale investigation was conducted by R Mudunge to compare the performance of an ABR with a completely mixed anaerobic reactor. It was found that the ABR was more stable and could withstand a greater shock load than the completely mixed reactor (Mudunge, 2002).

During the course of the current project, the potential application of the ABR for the treatment of domestic wastewater from dense peri-urban settlements began to be appreciated. This resulted in the motivation of a new project, WRC Project No. 1248: *The Evaluation of the Anaerobic Baffled Reactor for Sanitation in Dense Peri-Urban Settlements*. Given the high developmental priority of this application in the region, in August 2000 the steering committee decided that the pilot plant phase of the current study should focus on the treatment of domestic wastewater as opposed to industrial effluent.

A pilot-scale ABR (3 200 L) was designed, constructed and commissioned at the Durban Metro Umbilo Wastewater Treatment Works and later moved to Kingsburgh WWTP close to Durban. The hydraulic design was based on a computational fluid dynamics analysis which was confirmed using tracer tests in the laboratory scale reactors. Partial funding for the pilot plant came from Durban Metro and Business Partners for Development (a World Bank initiative described in **Appendix B, Section B.2.1**). The results of the pilot plant study are presented in WRC Report 1248/1/06 (Foxon et al., 2006).

On a feed of 50% domestic wastewater and 50% industrial effluent and at hydraulic retention times ranging from 60 to 20 h, the ABR reduced the COD of the feed from a range of 600 to 1 000 mg/L to a range of 550 to 50 mg/L. There was some removal of pathogens (3 logs) but no removal of nutrients (nitrogen and phosphorus). The final effluent is not suitable for discharge to water courses without further treatment. Further evaluation is recommended.

3 Recommendations

In the South African context, the ABR could be implemented for on-site for pre-treatment of agro-industrial wastes, with high COD contents or those with xenobiotic components, which prevent conventional treatment at a wastewater treatment works. Further research should focus on the following:

1. Implementation of a pilot- or full-scale ABR for pre-treatment of a high-strength or toxic industrial effluent.
2. Extend the use of molecular techniques to further improve understanding of the microbial population dynamics within the various compartments.

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For the duration of this project, the following persons were nominated or served on the Steering Committee and their valuable contributions are gratefully acknowledged:

Mr Greg Steenveld	**WRC (Chairman)**
Mr J Bhagwan	**WRC**
Prof CA Buckley	**University of Natal**
Prof BK Loveday	**University of Natal**
Prof G Tivchev	**Natal Technikon**
Mr D Nozaic	**Umgeni Water**
Dr VL Pillay	**ML Sultan Technikon**
Prof KM Reineck	**University of Cape Town**
Prof E Senior	**University of Natal**
Mr P Gaydon	**Umgeni Water**
Ms M Pressend	**Environmental Monitoring Group**
Prof G Hansford	**University of Cape Town**
Ms JE Hewitt	**WRC (Committee Secretary)**

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LIST OF ABBREVIATIONS

ABR	Anaerobic Baffled Reactor
ADMI	American Dye Manufacturers Institute
APHA	American Public Health Association
BAP	Biomass-associated Products
BOD	Biochemical Oxygen Demand
ca	Approximately
CFD	Computational Fluid Dynamics
CI	Colour Index
COD	Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
DDGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetra-Acetic Acid
ETAD	Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry
FISH	Fluorescent in situ Hybridisation
GAC	Granular Activated Carbon
GC	Gas Chromatograph
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
IC	Inorganic Carbon
IC_{50}	Inhibition Concentration (for 50%)
MW	Molecular Weight
NMR	Proton Nuclear Magnetic Resonance Spectroscopy
OD	Optical Density
OLR	Organic Loading Rate
OTU	Operational Taxonomic Unit
PAC	Powdered Activated Carbon
PEC	Predicted Environmental Concentration
rDNA	Ribosomal Deoxyribonucleic Acid
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RTD	Residence Time Distribution
S	Svedburg Units
SMP	Soluble Microbial Products
SRB	Sulphate Reducing Bacteria
SRT	Solids Retention Time
SVI	Sludge Volume Index

TC	Total Carbon
TOC	Total Organic Carbon
TS	Total Solids
TSS	Total Suspended Solids
UAP	Utilisation-associated Products
UASB	Upflow Anaerobic Sludge Blanket Reactor
UV	Ultraviolet
UV-VIS	Ultraviolet-visible
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids
WWTW	Wastewater Treatment Works

GLOSSARY

Acclimation	The adaptation of a microbial community to degrade a previously recalcitrant compound, through prior exposure to that compound.
Acetogenesis	The reaction that degrades short chain fatty acids such as propionic acid, butyric acid, or longer chain fatty acids, as well as other intermediates such as ethanol, to acetic acid and hydrogen.
Acid dye	An anionic dye characterised by substantivity for protein fibres and often applied as an acidic dye solution.
Acidogenesis	The process in which long chain soluble monomers or dimers, such as carbohydrates and amino acids, are reduced to short chain volatile fatty acids, such as acetic acid, propionic acid, butyric acid, lactic acid and ethanol, or longer chain fatty acids.
Adsorption (dye)	Binding of dye compounds to surfaces such as microbial cells or activated carbon, usually through electrostatic interaction between the dye and the charged cell.
Aerobic	The condition of living or acting in the presence of molecular oxygen.
Anaerobic digestion	The microbial degradation of an organic compound in the absence of oxygen.
Anionic dye	A dye that dissociates in aqueous solution to give a negatively charged coloured ion.
Anthraquinone dye	Dye based on the structure of 9,10-anthraquinine, with powerful electron donor groups in one or more of the four alpha positions.
Auxiliary	A chemical or formulated product which enables a processing operation in preparation, dyeing, printing or finishing to be carried out more effectively or which is essential if a given effect is to be obtained.
Azo dye	Dye which contains at least one azo group (-N=N-) and can contain up to 4 azo bonds.
Basic dye	A cationic dye characterised by its substantivity for the acidic types of acrylic fibre and for tannin mordanted cotton.
Batchwise processing	Processing of materials as lots of batches in which the whole of each batch is subjected to one stage of the process at a time.
Biodegradation	The microbial degradation of organic compounds to inorganic molecules.
Carcinogenic	Cancer-causing
Chemical Oxygen Demand	A measure of the total amount of organic material in the waste stream.

Chromophore	A chemical group which, when present in a compound, is responsible for the appearance of colour.
Colour Index	An authoritative, descriptive catalogue of natural and synthetic colourants and intermediates in terms of generic name.
Colourant	Organic chemical used for colouring fabrics or food products and includes dyes and pigments.
Decolourisation	The removal of colour from solution by destruction of the chromophore.
Electron transport chain	A chain of carrier molecules with fixed orientation in the cell membrane, through which electrons are transported and ATP generated.
Enrichment	Selection of micro organisms with certain characteristics, from a mixed culture, through manipulation of culture conditions.
Exhaustion	The proportion of dye or other substance taken up by a substrate at any stage of a process, to the amount that was originally available.
Facultative anaerobe	An organism capable of either aerobic or anaerobic respiration.
Fermentation	Amino acids and sugars are degraded to propionic acid and other intermediary products, acetic acid and hydrogen by fermentative or acidogenic bacteria.
Headspace	The volume in a sealed vessel not occupied by the liquid phase.
Hydrolysis	Breakdown of complex long-chain macromolecules (carbohydrates, lipids and proteins), via the Embden-Meyerhof pathway, to short-chain compounds (sugars, fatty acids and glycerol, and amino acids, respectively). First phase of the anaerobic digestion process.
Inhibition	An impairment of bacterial function.
Intermediates (dye)	The compounds used to synthesise dyes.
Labile	Readily degradable.
Medium	Mixture of nutrient substances required by cells for growth and metabolism.
Metabolism	The physiochemical transformations through foodstuffs are synthesised into complex elements, complex substances are rendered into simple ones and energy is made available for use by the organism.
Metabolites	Intermediate compounds formed during dye catabolism.
Methanogenesis	The process by whereby low molecular weight substrates are degraded to form methane.
Mineralization	Microbial decomposition of an organic compound into inorganic constituents such as carbon dioxide, methane and water.
Mixed culture	Culture consisting of two or more types of micro organisms.
Pollution	An adverse alteration of the environment.

Reactive dyes	Coloured components capable of forming a covalent bond between the dye molecule and the fibre.
Recalcitrant	Resistant to microbial degradation.
Residence time distribution	The distribution of ages of liquid elements in a vessel.
Textile finishing	A collection of processes in which raw cloth/yarn is cleaned and prepared for dyeing and printing.
Volatile fatty acid	Short-chain organic acid formed by the anaerobic digestion process.
Xenobiotic	A compound not found in nature.

CHAPTER 1

INTRODUCTION

The predicted high industrial growth rate in South Africa, coupled with the limited water resources, will necessitate the implementation of advanced effluent treatment systems. The results of previous research (Sacks, 1997) identified a number of industries, in KwaZulu-Natal, that produced effluent streams that would be amenable to treatment by anaerobic digestion. The organic content of these effluents (based on COD) was too high to permit conventional treatment at a wastewater treatment works. In the short term, these effluents could be treated in the under-utilised anaerobic digestion capacity in the region. However, with the projected increase in the load on wastewater treatment works, this available capacity will ultimately be needed for sewage treatment.

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1.1 PROJECT AIMS

The initial project aims were defined as follows :

1. Design and construct a laboratory-scale system for the assessment of the suitability of an organic industrial effluent for treatment in an anaerobic baffled reactor.
2. Develop experimental protocols for the assessment of the suitability of a high-strength or toxic organic industrial effluent for treatment in an anaerobic baffled reactor.
3. Assess the suitability of an anaerobic baffled reactor for the treatment of high-strength or toxic organic industrial effluents.
4. Design, construct and operate an efficient and cost-effective system for the treatment of a high-strength or toxic organic effluent.

Under the guidance of the Steering Committee, the following additional project aims were agreed upon:

1. Acquire knowledge on the design, operating procedures and functional aspects of the ABR.
2. Become familiar with current and previous investigations conducted on the ABR, to enable assessment of its potential applications.
3. Obtain sufficient understanding of the technology to facilitate identification and co-operation with an industrial partner.
4. Assess the biodegradability and inherent toxicity, of an industrial wastewater, to the anaerobic biomass.
5. Assess the efficiency of the ABR for treatment of an industrial wastewater, on a laboratory-scale.
6. Use Computational Fluid Dynamics and tracer tests to enhance understanding of the hydrodynamics, flow patterns and mixing patterns within the ABR.
7. Design, construct and commission a pilot-scale ABR.
8. Evaluate the pilot-scale ABR.

1.2 POTENTIAL APPLICATION OF THE ABR TO PERI-URBAN SANITATION

The provision of water and sanitation services to previously unserved communities is a South African development priority. No single technological solution is universally applicable to solve this backlog and a solution for a particular community requires that a range of technologies to be available for consideration. eThekwini Municipality, where the current study took place, has adopted a policy of supplying dry sanitation options to low-income households in areas which still lack water-borne (Macleod, 2005). Many householders aspire to water-borne sanitation, however, there is a technology gap in water-borne sanitation options that are sustainable, affordable and practical for these conditions. It is estimated that it could take approximately 20 years for water-borne sewage to be provided to many of the peri-urban communities in the Municipality.

In this context, it was thought that the ABR could provide an immediate solution to the sanitation problem in dense peri-urban areas which have limited water available for consumption and household use and therefore produce concentrated wastewaters. The ABR meets several critical requirements, namely, it does not require energy for operation; requires low maintenance; is compact and could be mass-produced. Several ABRs could service small sub-groups within an area and eventually connect to a sewer system for further treatment at a WWTP.

During the course of the current project, the potential application of the ABR for the treatment of domestic wastewater from dense peri-urban settlements began to be appreciated. This resulted in the motivation of a new project, WRC Project No. 1248: *The Evaluation of the Anaerobic Baffled Reactor for Sanitation in Dense Peri-Urban Settlements*. Given the high developmental priority of this application in the region, in August 2000 the steering committee decided that the pilot plant phase of the current study should focus on the treatment of domestic wastewater as opposed to industrial effluent.

A pilot-scale ABR (3 200 L) was designed, constructed and commissioned at the Durban Metro Umbilo Wastewater Treatment Works and later moved to Kingsburgh WWTP close to Durban. The hydraulic design was based on a computational fluid mechanics analysis which was confirmed using tracer tests in the laboratory scale reactors. Partial funding for the pilot plant came from Durban Metro and Business Partners for Development (a World Bank initiative described in **Appendix B Section B.2.1**). The results of the pilot plant study are presented in WRC Report K5/1248 (Foxon et al., 2006). A brief summary of the main results and conclusions from the work at Umbilo is also presented in **Section 5.2** of the current report.

1.3 PROJECT APPROACH

The purpose of the investigation was to assess the suitability of the ABR for the pre-treatment of high-strength or toxic industrial effluents. A survey conducted during WRC project 762 (*Anaerobic Digestion of High-Strength or Toxic Organic Effluents in Available Digester Capacity* (Sacks and Buckley, 2004)) identified a number of industries producing effluents for potential pre-treatment in the ABR. The food and beverage industry was one of the main producers of high-strength organic effluents. Hence, the effluent produced by a company, using the spent grain from the local brewery to extract the solids as an animal feed additive, was investigated as the high-strength organic effluent. Since dyes and colorants are mostly xenobiotic and some have been shown to be inhibitory to biological processes, the biodegradability and toxicity of individual dyes and a dye wastewater were investigated.

The report begins with a review of current and retrospective literature (**Chapter 2**) on anaerobic digestion, the ABR, reactor hydrodynamics, microbial population characterisation, dyes and the treatment of dye containing effluents. **Chapter 3** describes the batch test experimental protocols used to assess the biodegradability and inherent toxicity of particular effluents, and the individual components.

Chapter 4 outlines the design, operation and results obtained from the laboratory-scale ABRs, operated on both the brewery effluent and the dye wastewaters, to assess the suitability of the ABR for treatment of high-strength or toxic organic effluents. **Chapter 5** provides information on the use of Computational Fluid Dynamics in the design of the pilot-scale ABR. A brief summary of the conclusions of the pilot plant work at Umbilo WWTP, which had a ~ 50% industrial wastewater feed is also presented.

The report is concluded with **Chapter 6**, summarising the main conclusions of the study and recommendations for future research are made. **Appendix A** describes the *capacity building* and **Appendix B** is a summary of technology transfer that was achieved during the duration of the project.

CHAPTER 2

LITERATURE REVIEW

Anaerobic digestion is a process by which a wide range of organic molecules can be converted into a methane-rich biogas. This literature review describes the mechanism and microbiology of anaerobic digestion; provides a description of the anaerobic baffled reactor (ABR) and reviews the literature on the ABR; reviews methods for elucidation of the hydrodynamics of the ABR; gives an introduction to molecular techniques for microbial population characterisation; and gives a brief review of the chemistry of dyes and colorants and the treatment of dye containing effluents. The literature on anaerobic digestion of domestic wastewater and peri-urban settlements is reviewed.

2.1 ANAEROBIC DIGESTION

There is a range of techniques available for the removal of organic molecules from wastewaters e.g. advanced oxidation, activated carbon and membranes. However, these processes are expensive and treat the symptoms and not the cause. Anaerobic digestion is a biological process in which organic matter is catabolised to methane and carbon dioxide. It has the potential to break down complex biorefractory organic compounds (Tracey et al., 1989). In aerobic respiration, molecular oxygen serves as an external electron acceptor, accepting electrons from electron carriers such as NADH by way of an electron transport chain (Brock and Madigan, 1991). In the absence of oxygen, carbon atoms associated with some incoming organic substrates will become electron acceptors and hence be reduced, while other compounds will be oxidised to carbon dioxide (Pohland, 1992).

The anaerobic degradation process has several advantages over aerobic treatment. In aerobic treatment the micro organisms use oxygen in the air to metabolise a portion of the organic waste to carbon dioxide and water. The micro organisms obtain energy from this oxidation, thus their growth is rapid and a large portion of the organic waste is converted to new cells (Speece, 1996). The portion converted to biomass is not stabilised but is simply biotransformed. Although these cells can be removed from the waste stream, the biological sludge they produce still presents a significant disposal problem. In contrast, the anaerobic conversion to methane gas provides relatively little energy to the micro organisms, resulting in a slow growth rate and only a small portion of the waste being converted to new biomass. Conversion to methane represents waste stabilisation since methane is poorly soluble and escapes from the waste stream where it can be collected. As much as 80 to 90% of the degradable organic portion of a waste can be stabilised in anaerobic treatment, even in highly loaded systems. This is in contrast to aerobic systems where only about 50% of the waste is actually stabilised, even with conventional loadings (McCarty, 1964).

Another advantage of anaerobic digestion is, since only a small portion of waste is converted to cells, the problem of disposal of excess sludge is greatly reduced. Since anaerobic treatment does not require oxygen, oxygen transfer does not limit the treatment rates and the power requirements are reduced. In contrast, the methane gas produced is a source of energy.

2.1.1 Anaerobic Microbiology

Anaerobic digestion is a complex microbial process involving a number of strongly interacting groups of micro organisms (Figure 2-1).

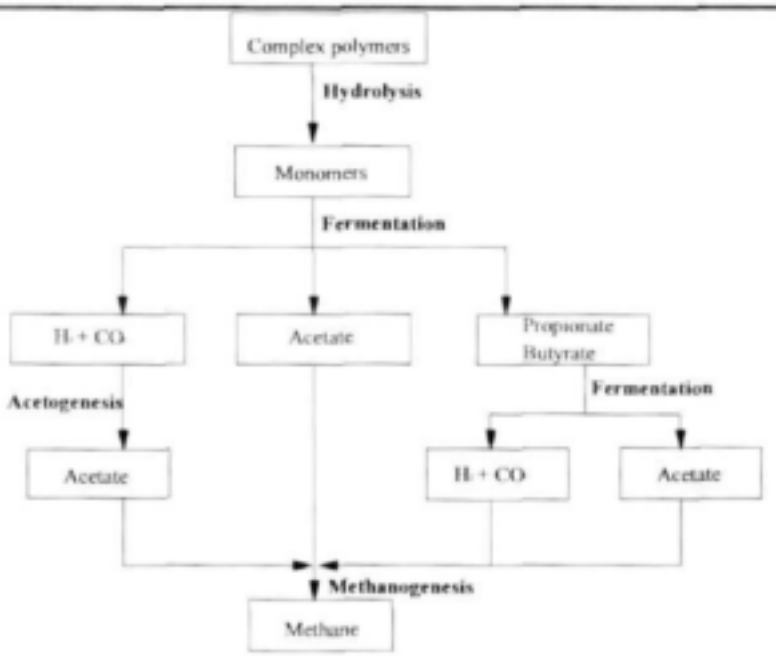


Figure 2-1 :Overall process of anaerobic decomposition. (Brock and Madigan, 1991)

During **hydrolysis**, complex long-chain macromolecules (carbohydrates, lipids and proteins) are hydrolysed extracellularly, via the Embden-Meyerhof pathway, to short-chain compounds (sugars, fatty acids and glycerol, and amino acids, respectively). Hydrolysis can be a slow process and can be the rate-limiting step in fermentation, particularly if the substrate contains particulate or large complex molecules in significant quantities. The resulting monomers are **fermented** to various intermediates, primarily acetate, propionate and butyrate, with the production of carbon dioxide and hydrogen. The biochemical pathways and end products for this phase depend upon the substrate and the hydrogen partial pressure (Figure 2-2). At a low hydrogen partial pressure, glucose is catabolised to acetate, carbon dioxide and hydrogen. At both low and high hydrogen partial pressures, glucose can be degraded to butyrate, carbon dioxide and hydrogen (McCarty and Smith, 1986); (Sam-Soon et al., 1991). When the hydrogen partial pressure is high, acetate , propionate, carbon dioxide and hydrogen will be formed from glucose. The propionate and butyrate cannot be used directly for methanogenesis and are converted to acetic acid, carbon dioxide and hydrogen in a second fermentation phase. This conversion can only occur under conditions of low hydrogen partial pressures. Additional acetic acid is produced by a second group of micro organisms called acetogenic bacteria (Pfeffer, 1979); (Sam-Soon et al., 1991). The acetic acid becomes the substrate for a group of strictly anaerobic methanogenic micro organisms. These micro organisms ferment acetic acid to methane and carbon dioxide. This methane, together with the methane formed by micro organisms which reduce carbon dioxide utilising hydrogen gas or formate produced by other species, accounts for the methane produced in this process (Pfeffer, 1979). The methane formed in this last stage, being poorly soluble in water, is lost to the gas phase. It can be collected and used for its energy value. The carbon dioxide that is evolved partially escapes to the gas phase

(Pfeffer, 1979); (Fang and Lau, 1996). Thus, two main substrate sources are used for methanogenesis, namely hydrogen and acetate. The methanogens are classified into three groups according to their energy source: hydrogenotrophs, which use hydrogen as the only energy source, acetoclastic methanogens, which use acetate as their sole energy source, and hydrogen/acetate utilisers, which can utilise both hydrogen and acetate.

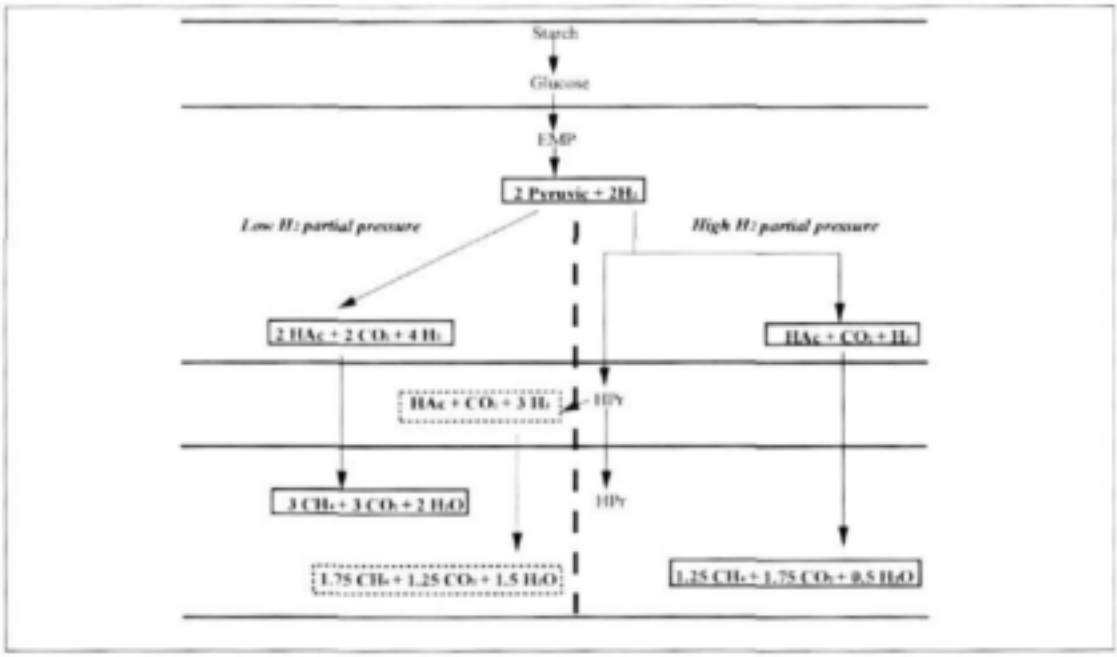


Figure 2-2 : Methane fermentation under high and low hydrogen partial pressure conditions (Sam-Soon et al., 1989)

The methanogens belong to a special group of micro organisms, the Archaea. They differ from other micro organisms in their type of metabolism and in the composition of cell constituents. Methanogens are obligate anaerobes with strict requirements for low redox potentials and the absence of dissolved oxygen. *Methanosaeta* species are sheathed rods, sometimes growing as long filaments, and are slow growing with minimum doubling rates of 4 d under mesophilic conditions (Zehnder, 1988). Their survival is due to their high affinity for acetate (K_s = 30 mg/L); these micro organisms are termed scavengers. *Methanosarcina* species (coccoid morphology) grow faster (minimum doubling time of 1.5 d), however, they have a poor affinity for acetate with K_s = 400 mg/L. Thus, in an anaerobic treatment system, the *Methanosaeta* would out compete the *Methanosarcina* at low substrate concentrations. However, with increasing substrate concentration, *Methanosarcina* species would dominate.

Many anaerobic micro organisms can perform electron-transport phosphorylation (regeneration of ATP) under anaerobic conditions, by transferring electrons derived from a substrate via a short electron-transport chain to an external electron acceptor supplied in the nutrient medium or an internal electron acceptor derived from substrate degradation (Senior, 1991); (Schlegel, 1992). In most cases, the energy sources used by organisms carrying out anaerobic respiration are organic compounds but several lithotrophic organisms are also able to carry out this process (Brock and Madigan, 1991). Nitrate, sulphate, carbonate and fumarate ions, as well as sulphur and carbon dioxide can function as electron acceptors (Schlegel, 1992). The presence of alternative electron acceptors may inhibit methanogenesis since sulphate-reducing bacteria (SRB) and nitrate-reducing bacteria can out-compete methanogens for available substrates (Pohland, 1992).

2.2 THE ANAEROBIC BAFFLED REACTOR

The successful application of anaerobic technology to the treatment of industrial wastewaters is dependent on the development of high rate anaerobic bioreactors. These reactors achieve a high reaction rate per unit reactor volume by retaining the biomass in the reactor, independently of the incoming wastewater (Barber and Stuckey, 1999). There is improved contact between the biomass and the wastewater; and biomass activity is enhanced due to adaptation (Iza et al., 1991).

2.2.1 Design of the ABR

The ABR is similar in design and application to the upflow anaerobic sludge blanket (UASB) but requires no special granule formation for its operation. Bachmann, Beard and McCarty (1985) (Bachmann et al., 1985) developed the ABR, however, baffled reactor units had previously been used to generate methane-rich biogas as an energy source (Chynoweth et al., 1980).

The ABR has alternately hanging and standing baffles (Figure 2-3), which divide it into 8 compartments. The lower edges of the hanging baffles are slanted (45 °) to route the flow of liquid and to reduce channelling. The liquid flow is alternately upward and downward between the partitions. The downflow chamber is narrower than the upflow chamber to prevent accumulation of biomass in the latter. On its upward passage, the waste flows through an anaerobic sludge blanket. Hence, the waste is in contact with the active biomass but, because of the design, most of the biomass is retained within the reactor. In principle, all phases of the anaerobic degradation process can proceed simultaneously. The sludge in each compartment will differ depending on the specific environmental conditions prevailing and the compounds or intermediates to be degraded (Nachaiyasit and Stuckey, 1997a). A staged reactor can provide higher treatment efficiency since non-labile substrates will be in an environment conducive to degradation. Process stability is good.

The laboratory-scale reactors were made of Perspex, with a 10 L volume (7.68 L working volume). The reactor dimensions were: length of 500 mm (450 mm working) x 150 mm; width (130 mm working) x 330 mm height (305 mm working). The internal baffles were 10 mm thick. The reactor lid and sides were removable. Sample ports on the lid allowed for the removal of gas and liquid samples.

The hydrodynamics and degree of mixing that occur within a reactor of this design strongly influence the extent of contact between substrate and bacteria, thus controlling mass transfer and potential reactor performance. Micro organisms within the reactor gently rise and settle due to the flow characteristics and gas production, however, their rate of movement down the reactor is slow. The main driving force behind reactor design has been to enhance the solids retention capacity.

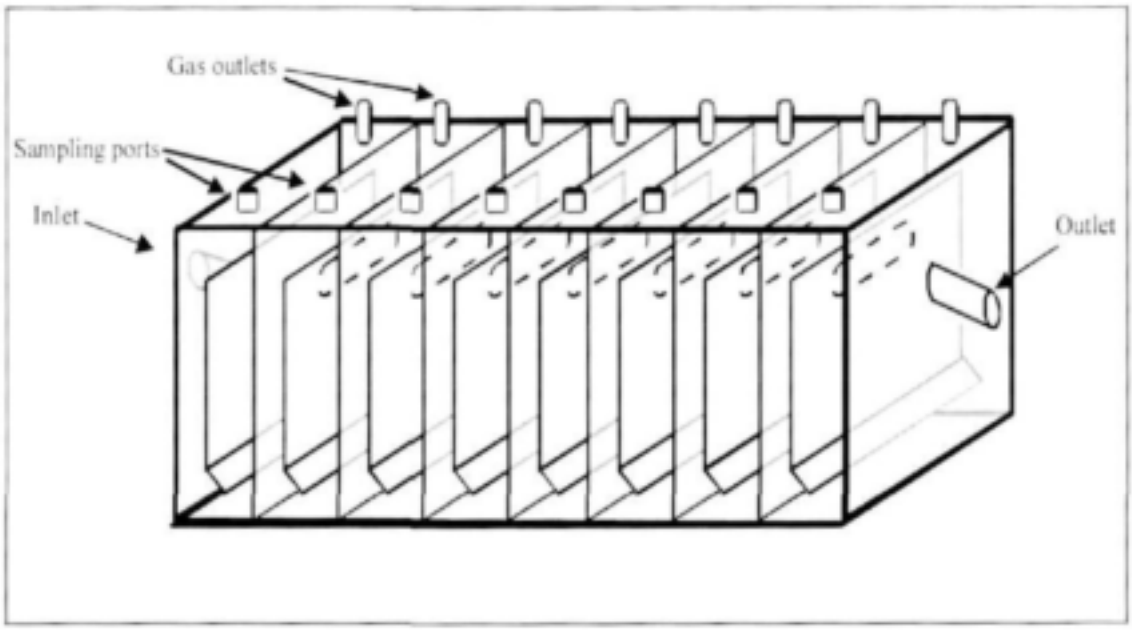


Figure 2-3 : Schematic diagram of the anaerobic baffled reactor

2.2.2 Advantages of the ABR

The ABR has several advantages over other high-rate reactor systems. Probably the most significant advantage is its ability to separate acidogenesis and methanogenesis longitudinally, allowing the reactor to behave as a two-phase system without the associated control problems and high costs.

The reactor design is simple, with no moving parts or mechanical mixing, making it relatively inexpensive to construct. There is no requirement for biomass with unusual settling properties. Sludge generation is low and solids retention time (SRT) is high; this is achieved without the need for biomass to be fixed to media particles or a solid-settling chamber. Gas separation is not required.

Since the hydraulic retention time (HRT) and SRT are separate, increased volumes of wastewater can be treated, relative to a CSTR where HRT = SRT. Intermittent operation is possible, which would facilitate treatment of seasonal wastewaters. The ABR has been found to be stable to hydraulic and organic shock loads and the reactor configuration provides protection of the biomass to toxic compounds in the influent.

The concentrated, variable and intermittent nature of industrial effluents make them intrinsically unsuitable for treatment in a completely mixed system. The ABR is well suited to intermittent high organic or hydraulic loads; due mainly to the high efficiency with which the active biomass is retained within the reactor. The high rates and efficiency with which the substrates are degraded are due to the compartmentalisation of different microbial associations that have been acclimated to the range of effluent constituents. The series of microbial associations allow acclimated bacteria to degrade the effluent stepwise producing degradation products that may be toxic or inhibitory to a mixed culture

2.2.3 Literature Review of the ABR

One of the major problems associated with anaerobic treatment systems is the start-up procedure. The overall objective of start-up is the development of the most appropriate microbial culture for the waste stream to be treated.

Initial loading rates should be low so that the slow growing micro organisms are not over-loaded and both gas and liquid upflow velocities should be low to facilitate flocculent and granular sludge growth. The recommended initial loading rate is ca. 1.2 kg COD/m³.d (Speece, 1996). Barber and Stuckey (1997) (Barber and Stuckey, 1997) showed that by starting with a long HRT (80 h) and gradually reducing it, in a stepwise fashion, whilst keeping the substrate concentration constant, greater reactor stability is maintained, with superior performance in comparison to a reactor started up with a constant and low HRT coupled to a stepwise increase in substrate concentration. This assessment was based on improved solids accumulation, promotion of methanogenic populations and faster recovery to hydraulic shocks.

In 1992, Grobicki and Stuckey (Grobicki and Stuckey, 1992) conducted a series of residence time distribution studies in the ABR. They found that the ABR could be characterised as a series of completely stirred tank reactors (CSTRs) and that there were low levels of dead space (8 to 18% hydraulic dead space) in comparison with other anaerobic reactor designs. Investigations of the hydrodynamics to date have not taken into account various other factors which may be influential, such as: biogas mixing effects, viscosity changes due to extracellular polymer production and biomass particle size. The rate of movement of biomass down the reactor has not yet been defined.

Recycling the ABR effluent stream tends to reduce removal efficiency because the reactor approaches a completely mixed system and therefore, the mass transfer driving force for substrate removal decreases despite a small increase in the loading rate. Mixing caused by recycle has also been found to cause a return to single phase digestion, therefore, the benefits arising from the separation of the acidogenic and methanogenic phases are partially lost (Barber and Stuckey, 1999). However, the addition of a recycle stream can alleviate the problems of low pH caused by high levels of volatile acids at the front of the reactor (Chynoweth et al., 1980) and has the benefit of dilution of toxicants and reduction of substrate inhibition in the influent.

The ABR has been shown to tolerate hydraulic and organic shock loads. To a steady-state reactor, with an HRT of 20 h and an organic loading rate of 4.8 kg COD/m³ (synthetic carbohydrate/sucrose protein feed), Grobicki (1989) (Grobicki, 1989) introduced a hydraulic shock by decreasing the HRT to 1 h, for a period of 3 h. The reactor returned to its previous COD removal efficiency of > 95% within 24 h of resuming normal operating conditions. Less than 15% of the active biomass was lost. In a similar experiment, the organic loading rate was increased to 20 kg COD/m³ and, under these conditions, a COD removal efficiency of 72% was still achieved.

Several authors have treated low-strength wastewaters effectively in the ABR (Barber and Stuckey, 1999). Dilute wastewaters inherently provide a low mass transfer driving force between the biomass and substrate, subsequently reducing biomass activities according to Monod kinetics. As a result, treatment of low-strength wastewaters has been found to encourage the dominance of scavenging micro organisms, such as *Methanosaeta* species (Polpraset et al., 1992). Biomass retention is enhanced significantly due to lower gas production rates, suggesting that low hydraulic retention times are feasible during low-strength treatment. Witthauer and Stuckey (1982) (cited in (Barber and Stuckey, 1999) observed that biogas mixing was greatly reduced and this resulted in minimal biomass/substrate mass transfer. The authors suggested that when treating dilute wastewaters, baffled reactors should be started-up with relatively high biomass concentrations in order to obtain a sufficiently high sludge blanket and better gas mixing.

Whereas low retention times are possible for dilute wastewaters, the opposite applies when treating concentrated wastes. This is due to the high gas mixing caused by improved mass transfer between the biomass and substrate. The

reactor configuration and the improved settling ability of the biomass have reduced solids loss caused by the increased gas production (Boopathy and Tilche, 1991). According to kinetic considerations, high substrate concentrations will encourage both fast growing bacteria and organisms with high K_s values and methane production will be derived mainly from acetate decarboxylation.

Nachaiyasit and Stuckey (1997) (Nachaiyasit and Stuckey, 1997b) did an extensive study on operation of the ABR at low temperatures. Generally, biochemical reactions double in relative activity for every 10 C° increase in temperature, however, these authors found no significant reduction in overall COD removal efficiency when the temperature of an ABR was dropped from 35 °C to 25 °C. Further reduction in temperature, to 15 °C, resulted in a 20% decrease in COD removal. Changes in performance were gradual which is advantageous since this slow response would inherently provide improved protection to shocks, in comparison to other reactor systems. After 12 weeks of operation, at the low temperature, the performance had not improved. This was attributed to the fact that K_s increases substantially as temperature falls, leaving volatile fatty acids (VFAs) that cannot be degraded. It was found that the production of refractory material (soluble microbial products - SMPs) increased substantially at the lower temperatures.

One of the main constituents of the effluent from a wastewater treatment plant is SMPs, which are produced in the reactor during metabolism and endogenous decay (Schiener et al., 1998). In their investigation of SMP production in the ABR, Schiener *et al.* found that a decrease in HRT and an increase in the organic loading rate (OLR) both resulted in increased effluent SMPs. Quantitatively, between 26 and 48% of the incoming feed COD was converted to SMPs in the first compartment of the ABR. SMPs are categorised as (i) those that are produced at a rate proportional to the rate of substrate utilisation (utilisation-associated products, UAP); and (ii) those formed at a rate proportional to the concentration of active biomass (biomass-associated products, BAP) (Rittman et al., 1987).

Fox and Venkatasubbiah (Fox and Venkatasubbiah, 1996), investigated the efficiency of sulphate reduction in the ABR. Reactor profiles showed that sulphate was almost completely reduced to sulphide within the first compartment and a concomitant increase in sulphide levels down the reactor indicated that sulphate redirected electron equivalents to hydrogen sulphide in preference to methane. Increasing sulphate concentrations with glucose and isopropanol (increase the COD:SO_4 ratio) showed inhibition of sulphate reduction caused by elevated sulphide concentrations. Volatile fatty acids concentrations as high as 4 500 mg/L were observed during inhibition.

Barber (1999) showed that an aerobic polishing step can be inserted within an ABR with no detrimental effect on reactor performance. This is due to the fact that the methanogenic Archeae are well shielded from oxygen by the outer layers of facultative anaerobes, in immobilised aggregates. Therefore, processes which inherently require both anaerobic and aerobic treatment, e.g. textile dye wastewaters, could be treated within a single reactor unit.

The ABR shows promise for industrial wastewater treatment, on a full scale, since it can withstand severe hydraulic and organic shocks, intermittent feeding, temperature changes, and tolerate certain toxic materials due to its inherent two-phase behaviour. Despite comparable performance with other well-established technologies, its future use will depend on exploiting its structure in order to treat wastewaters, which cannot be readily treated.

Boopathy and Tilche (1991) investigated the treatment of a concentrated raw molasses wastewater in a hybrid ABR (Boopathy and Tilche, 1991). A 77% reduction in soluble COD was attained at an OLR of 20 kg COD/m^3.d. A

change of feed affected the performance of the reactor for a short period and the biomass adapted to the new substrate after a short acclimation period.

2.3 DYE WASTEWATERS

Dye wastewaters enter the environment from dye manufacturers and dye consumers e.g. textile, leather and food industries (Cooper, 1995). The majority of dyes are xenobiotic chemicals, substances having structural features that are not normally encountered in nature. With modern methods of manufacture and use, dyestuffs do not enter the environment in major quantities, but losses are inevitable and it is important to consider to what extent such products may persist (Brown and Laboureur, 1983).

Dyes and pigments are usually released into the environment in the form of a dispersion or a true solution in the industrial effluent (Seshadri et al., 1994). The presence of very small amounts of dyes in water (less than 1 mg/L) is highly visible and aesthetically unpleasant. The predicted environmental dye concentration (PEC) averages ca. 1 mg/L, but can be higher since batch dyeing is common practice. The PEC is calculated from the daily usage of the dye; the degree of fixation; the degree of removal in the effluent treatment plant; and the dilution factor in the receiving water (Cooper, 1995).

2.3.1 Dye Chemistry

The vast number of dyes available commercially required a form of classification, based on the dye chemistry. The *Colour Index* (Society of Dyers and Colourists) is the internationally accepted classification system for dyes. Almost two-thirds of the listed organic colorants are azo dyes.

Azo dyes are water-soluble, synthetic organic colorants possessing the characteristic –N=N- (azo) bond and showing great structural diversity. Generally azo dyes contain between one and three azo linkages, linking phenyl and/or naphthyl rings that are usually substituted with some combination of functional groups including: amino, chloro, hydroxy, methyl, nitro and sulphonate groups (Razo-Flores et al., 1997). Azo dyes can be used to colour many different substrates, such as synthetic and natural textile fibres, plastics, leather, paper, mineral oils, waxes, foodstuffs and cosmetics.

The attachment of an **acid dye** to a substrate is dependent on the presence of one or more acidic groups. **Basic dyes** form ionic bonds with acid or anionic groups on the substrate. They have a basic amino group which becomes protonated under acidic conditions. **Direct dyes** have sulphuric acid groups but are not acid dyes because these groups are not used for attachment. The dye molecules are large, flat, linear molecules and NaCl is used to enhance the dyeing process by the sodium ions promoting equilibrium between the dye and the substrate. The dyeing process is reversible and used because it is economical and easy to apply. **Sulphur dyes** are insoluble dyes, which need to be reduced with sodium sulphide, to transform them to a soluble form, which adsorbs to the substrate. Upon exposure to air, the dyes become oxidised to form the original insoluble form, which is now present inside the substrate and, therefore, resistant to removal by washing (Maynard, 1983). **Reactive dyes** form covalent bonds with hydroxyl or amino groups on the substrate. They have excellent fastness because the dye becomes a part of the substrate. **Disperse dyes** are non-ionic dyes with a low water solubility. Colouring is due to the formation of a solid solution in the substrate.

2.3.2 Discharge Standards and Treatment Options

Public perception of water quality is influenced by colour. Unnatural colour is associated with contamination. Strong colours could reduce light penetration, thus affecting the growth of plants and the aquatic ecosystem. The current environmental concern with azo dyes revolves around the potential carcinogenic health risk that they present or their intermediate biodegradation products when exposed to microflora in the human digestive tract (Seshadri et al., 1994). There is the potential for these dyes to build up in the environment since many of them pass through wastewater treatment plants virtually untreated. Azo dyes are intentionally designed to be recalcitrant under typical usage conditions, and it is this property, allied with their toxicity to micro organisms, that makes biological treatment difficult.

In the setting of discharge standards, dye concentration is not used as a measuring parameter since different dyes have different intensities and hues. Colour standards are, therefore, based on absorbance. Samples are filtered (0.45 μm) and the absorbance read between 400 and 700 nm (Cooper, 1995). No such standards are set in South Africa; instead the colour discharge standard to receiving waters is that *no colour should be visible*.

Several chemical and physical decolourisation methods are available, such as coagulation/flocculation and precipitation; oxidation treatment, with chlorine (sodium hypochlorite), ClO_2, hydrogen peroxide (Fenton's oxidation) or ozone; adsorption of the dye could be achieved with activated carbon (GAC or PAC), clays or bioadsorbants; electrolysis; and membrane extraction (Cooper, 1995). However, these methods are costly, therefore, the move has been toward biological decolourisation of wastewaters.

Azo dyes are resistant to aerobic degradation by bacteria. The strong electron withdrawing character of the azo group stabilises these aromatic pollutants against conversions by oxygenases (Razo-Flores et al., 1997). Seshadri *et al.* describe an experiment in which the partitioning of 18 azo dyes in an activated sludge process showed that most of the dyes passed through the system largely unchanged, four dyes were significantly adsorbed onto the mixed liquor solids (bioelimination), and three dyes were apparently degraded.

The initial step in the degradation of azo dyes involves the cleavage of the azo bond. This has been achieved under anaerobic conditions (Brown and Hamburger, 1987):

$$(R_1 - N = N - R_2) + 4e + 4H^+ \rightarrow R_1 - NH_2 + R_2 - NH_2$$

where R_1 and R_2 are various phenyl and naphthol residues. The aromatic amines are generally more basic than the azo compounds, thus the pH of the reactor may increase after cleavage of the azo linkages (Knapp and Newby, 1995). Further degradation of the intermediates, which are recalcitrant under anaerobic conditions, is readily achieved under aerobic conditions; thus suggesting sequenced anaerobic/aerobic treatment system for the total degradation of azo dyes.

Investigations have shown that the addition of an additional carbon source, such as glucose or a VFA mixture enhances decolourisation. The carbon source functions as a donor of reduction equivalents, and thus enhances the cleavage of the azo linkage. The addition also results in more actively respiring cells which deplete any oxygen present and thereby enhance the azo reductase activity (Haug et al., 1991). However, Razo-Flores *et al.* (1997) found that a pharmaceutical azo dye, azodisalicylate, was completely decolourised and mineralised to CH_4 without an additional carbon source, at dye loading rates of up to 225 mg/L. These results indicated that some azo dyes

could be degraded in anaerobic environments in contrast to the common assumption that they are only biotransformed to mutagenic and carcinogenic aromatic amines.

The primary rate-limiting step in the degradation of azo dyes is the rate of permeation of the dye through the cell membrane (Haug et al., 1991). Dye permeability has been shown to be a function of the adsorption-desorption equilibrium of the dye at the cell membrane and the food : micro organism ratio. Older cells with a reduced supplemental nutrient supply have been shown to have better degradative capabilities. There is increased azo reductase efficiency with damaged cells.

2.3.3 Food Dyes

Freedom from toxicity is the first consideration in the choice of colorants for foods (*Colour Index*). After that, the properties commonly required are high solubility in water, alcoholic solvent, edible oils etc.; freedom from reaction with other components of the foodstuff e.g. flavourings and preservatives; freedom from attack by bacteria; solubility to light and heat; and an aesthetically acceptable hue.

Azo dyes are widely used as colorants in foods such as soft drinks, candy, hot dogs, ice cream and cereals (Chung et al., 1978). The extent of use is related to the degree of industrialisation of the society. Several carcinogenic aromatic amines have been identified in food dyes (Prival et al., 1993). There is increasing legislative control of the dyes used in foodstuffs. Use is restricted to colorants which have not shown any harmful effects when subjected to rigorous examination.

2.3.4 Textile Dyes

An environmental problem facing the textile industry is the coloured effluent from the dyeing of cellulosic fibres with reactive dyes. Reactive dye loss during dyeing operations is about 10 to 40% indicating the need to learn more about the fate of these dyes. Increasing environmental regulations are driving technical innovation to manage this problem (Hansa, 1999).

2.4 HYDRODYNAMICS

The flow patterns found in real processes usually lie between the two extremes of perfect mixing (complete mixing of the fluid) and plug flow (no mixing in the direction of flow). Three interrelated factors constitute the contacting or flow patterns within a reactor: the residence time distribution (RTD) of material which is flowing through the vessel; the state of aggregation of the flowing material, its tendency to clump and for a group of molecules to move about together and; the earliness and lateness of mixing of material in the vessel (Levenspiel, 1999). Deviation from ideal plug flow or ideal mixed flow can be caused by channelling of fluid, by recycling of fluid, or by the creation of stagnant areas (Barnett, 1995). In bypassing, some elements of the fluid bypass the entire process whereas in channelling some elements of fluid move through the process significantly faster than others. Dead space refers to a region in the process with no flow. This does not often occur in real processes as there is usually some contact between the dead space and the bulk fluid. As this contact is extremely slow, it is usually assumed that there is no flow. Recycling occurs when fluid is re-circulated to the process inlet or to another region of the process (Rabbitts, 1982).

Biomass growth and retention, together with gas production and turbulence provided by rising gas bubbles or effluent re-circulation, strongly affect the retention time distributions in reactors (Harper and Suidan, 1991). The influences of biomass growth and gas production are directly linked to substrate loading and other operational strategies such as flushing and wasting of accumulated biomass. The importance of mixing in achieving efficient substrate conversion has been noted by several researchers (Monteith and Stephenson, 1981); (Smith et al., 1996) although the optimum mixing pattern is a subject of debate. Under plug flow conditions, incoming substrate remains in the reactor for one retention time, allowing maximum time for conversion. However, high substrate concentrations resulting from lack of dispersion may inhibit bacterial activity. On the other hand, excessive dispersion may result in short-circuiting of the substrate. An intermediate degree of mixing appears to be optimal for substrate conversion (Smith et al., 1996).

2.4.1 Tracers

Wastewater treatment processes are aqueous flow processes in which dissolved or suspended solids are removed. The most common electrolytes for tracing water processes are lithium chloride (LiCl) and sodium chloride (NaCl). Sodium is generally present in higher concentrations in water treatment processes than lithium. The background concentration is often variable (Barnett, 1995). Methanogens have the ability to adapt to high concentrations of NaCl (Jackson-Moss et al., 1989). Lithium chloride is relatively inexpensive and lithium is detectable in µg/L. concentrations by flame photometric methods. Lithium is stable in solutions and is not lost by deposition.

Anderson, Campos, Chernicharo and Smith (1991) (Anderson et al., 1991) investigated the potential inhibitory effects of the lithium ion on anaerobic sludge. It was found that with Li concentrations > 2.0 g/L, there was a degree of inhibition, with a sharp drop in gas production and methane content, followed by a long period of reactor upset with very poor performance.

The two most used methods of tracer injection are pulse input and step input. In a pulse input, an amount of tracer *No* is suddenly injected into the feed stream in as short a time as possible. A material balance, for a pulse addition of tracer, compares the actual amount of tracer added to the amount that leaves the process:

$$N_0 = A \cdot q \qquad \textbf{Equation 2.1}$$

where: A = area under the response curve

q = the volumetric flow rate of the effluent stream

The area under the response, A, is found from:

$$A = \int_0^\infty C(t)\,dt \qquad \textbf{Equation 2.2}$$

In a step input, a constant rate of tracer addition to the feed is initiated at time $t=0$. Before this time, no tracer is added to the feed. The concentration of the tracer in the feed is kept at this level until the concentration in the effluent in indistinguishable from that in the feed. The test is then discontinued.

2.4.2 Residence Time Distribution Test

A technique for determining the flow model of processes is the residence time distribution method. Danckwerts (1953) (Danckwerts, 1953) developed the residence time distribution concept to characterise the overall flow behaviour in a process. The effluent stream from a continuous flow process is a mixture of fluid elements, which have resided in the process for different lengths of time. The distribution of these residence times is an indicator of flow patterns within a process. Analysis of these data allows calculation of the actual hydraulic retention time in the digester, a parameter which is controlled by the extent of mixing (Tenney and Budzin, 1972).

The residence time distribution (RTD) is determined experimentally by injecting an inert chemical, (tracer), into the reactor at some time $t=0$ and then measuring the tracer concentration, C, in the effluent stream as a function of time (Nachaiyasit, 1995). If the tracer has the same flow attributes as the fluid, this residence time distribution can be said to approximate to the residence time distribution of the fluid. In general, tracer tests cannot be used to determine the residence time distributions of processes with open boundaries, that is, systems that allow material that has left the system to re-enter.

In an ideal plug-flow reactor, all the material leaving the reactor has been inside it for exactly the same time (Fogler, 1992). Similarly, in an ideal batch reactor, all the material within the reactor has been inside it for an identical length of time. The time the material has spent in the reactor is called the *residence time*. In all other reactor types, the various atoms in the feed spend different times inside the reactor, that is, there is a distribution of residence times of the material within the reactor.

2.4.3 Residence Time Distribution Modelling

For first order reactions, the residence time distribution (RTD) curve can be used directly to predict conversion in the reactor. For more complex reaction kinetics, it is necessary to first set up a model for the flow patterns in the reactor before an estimate of conversion can be made. The next step is, therefore, to fit simple non-ideal flow models to the RTD curves. Selection of a flow model is based on the physical configuration of the reactor, visual observations of the flow patterns where possible, and the shape of the RTD curve (Rabbitts, 1982). The model is fitted to the RTD curve by comparing the theoretical model with the experimental RTD curve. The parameters of the theoretical flow model are varied until the closest fit between the theoretical and experimental curves is achieved.

The two simplest models which describe deviation from ideal flow are the dispersion model and the tanks-in-series model (Levenspiel, 1961). In the **dispersion model** the dispersion number ($D/\mu L$) is the inverse of the dimensionless Peclet number, Pe:

$$Pe = \frac{\mu L}{D} \qquad \textbf{Equation 2.3}$$

where D = longitudinal dispersion coefficient (m^2/s)

μ = fluid velocity (m/s)

L = fluid path length (m)

Pe is used to characterise the spread of the concentration-time response around the mean residence time caused by longitudinal mixing. When the dispersion number is small (< 0.002) the flow within the reactor approximates to plug flow (Nachaiyasit, 1995).

The **tanks-in-series model** assumes that the flow system can be characterised by a number, *n*, of perfectly-mixed tanks in series. Ideal flow occurs when $n=1$. The greater the number of tanks in series needed to approximate the flow pattern, the closer the system is to plug flow.

A computer program, IMPULSE, was written by Baddock, Barnett, Brouckaert and Buckley (1993) (Baddock et al., 1992) which allows easy modelling of systems using curves obtained from tracer response tests. The user assumes a flow model for the system. The program determines the theoretical response for the model and optimises a chosen set of parameters of the model to fit the experimental curve.

2.4.4 Hydrodynamics of the ABR

In 1992, Grobicki and Stuckey (Grobicki and Stuckey, 1992) conducted a series of residence time distribution studies in the ABR. They found that the ABR could be characterised as a series of completely stirred tank reactors (CSTRs) and that there were low levels of dead space (8 to 18% hydraulic dead space) in comparison with other anaerobic reactor designs.

Dead space can be divided into the categories of biological and hydraulic dead space. Hydraulic dead space is a function of flow rate and the number of baffles. Nachaiyasit (1995) investigated the effect of recycle on hydraulic dead space. It was found that the percentage of dead space increased initially and then levelled off at a recycle ratio of 0.25. The hydraulic dead space increases with increasing Reynolds number (flow rate) (Nachaiyasit, 1995). The amount of dead space decreased with decreasing HRT (increasing Reynolds number). This indicates that an increase in liquid velocity resulted in increased dispersion and enhanced mixing. The biological dead space is a function of biomass concentration, gas production and flow rate. Intrachandra (1998) (Intrachandra, 1998) showed that an increase in the biomass concentration did not effect the volume of dead space in the ABR.

Investigations of the hydrodynamics to date have not taken into account various other factors which may be influential, such as: biogas mixing effects, viscosity changes due to extracellular polymer production and biomass particle size. The rate of movement of biomass down the reactor has not yet been defined.

2.5 MICROBIAL POPULATION CHARACTERISATION

The fundamental aspects of the anaerobic digestion process have been investigated, yet there is still the need for more basic information on the biological aspects of the anaerobic digestion ecosystem (Godon et al., 1997). The ABR promotes the separation of various trophic groups into different compartments of the reactor. In principle, this facilitates a fundamental analysis of the effects of various components in the inlet stream on the population dynamics and microbial interactions.

Until recently, it has been difficult to characterise and quantify microbial species within a population. Viable plate count and most-probable number techniques have been used for quantification of active cells in environmental samples (Amann et al., 1995). For oligotrophic to mesotrophic aquatic habitats, it has been frequently reported that direct microscopic counts exceed viable cell counts by several orders of magnitude. By now there is little doubt in

most cases, the majority of microscopically visualised cells are viable but do not form visible colonies on plates (Amann et al., 1995). Two different types of cells contribute to this silent but active majority: (i) known species for which the applied cultivation conditions are not suitable or which have entered a non-culturable state and (ii) unknown species that have never been cultured before for lack of suitable methods (Amann et al., 1995). With the availability of innovative techniques, many more micro organisms will become culturable.

Molecular based methods, such as ribosomal RNA (rRNA) probe hybridisation, allow the direct identification and enumeration of microbial populations in complex environments (Griffin et al., 1998). These techniques will provide a clearer insight into the interactions, concentrations and growth rates of the various trophic groups involved in anaerobic digestion.

The ability to focus on the population dynamics of particular groups within a consortium of organisms will allow for a more interventionist approach. For instance, upon evidence of toxicity, specific organisms could be mutated and selected to provide resistant organisms, which could then be used to inoculate the reactor. This *forced evolution* would prevent the characteristic lag period caused by inhibition of the biomass.

2.5.1 16S rRNA Probes

Ribosomal RNA-based methods can be used to detect phylogenetically defined groups of organisms and quantify metabolic activity, because activity is directly related to ribosome production (Griffin *et al.*, 1998). Standard protocols for sampling and extraction should facilitate the use of labelled species and group specific oligonucleotide probes to follow dynamic changes in populations, in response to toxic stresses such as the introduction of refractory organic molecules. Thus, coupled with standard chemical and biochemical analyses, this strategy should enable the identification of the most sensitive members of the communities following initial responses and subsequent acclimation.

The ribosomes are the sites of protein synthesis (Schlegel, 1992). An actively growing bacterial cell contains up to 10^4 ribosomes. The number varies with the growth rate and is greatest in the fastest growing cells. Bacterial ribosomes are comprised of a mixture of nucleic acids (ribosomal ribonucleic acids – rRNA)) and proteins and have an average size of 70S (Svedburg units). Ribosomes are functionally very conservative. Ribosomal RNA is present in all cellular organisms and has an essential function in protein synthesis. Consequently it has remained relatively constant in structure because its essential function dictates that it must be relatively resistant to evolutionary change. It also comprises a mosaic of regions of highly conserved and highly variable regions of sequence. These properties make rRNA molecules useful for inferring phylogenetic relationships (Schlegel, 1992). Because rRNA homologues are found in every organism, analysis of rRNA sequences recovered from environmental samples should not show any bias towards particular groups of organisms. In general, essential rRNA sequence domains are conserved across all phylogenetic lineages thus *universal* tracts of sequence can be identified. Species and subspecies specific sequences have also been identified. The number of ribosomes within a cell and hence the number of rRNA molecules is proportional to the growth rate of the cell. It is therefore possible to identify and characterise the active fraction of the microbial population by targeting the rRNA itself rather than the genes that encode it. In whole cell hybridisation, individual bacteria can be identified and enumerated using fluorescent probes targeted at specific signature sequences in ribosomal RNA.

Within the bacteria, the small subunit rRNA is the 16S rRNA and the genes that code for this molecule are 16S rDNAs. In most cases, the 16S rDNA is exactly transcribed to form the 16S rRNA. Additionally, ribosomes of the bacteria contain the larger 23S rRNA. An average bacterial 16S rRNA molecule has a length of 1 500 nucleotides, and 23S rRNA molecules are around 3 000 nucleotides (Amann et al., 1995). Nucleotide sequence analysis of rRNA led to the discovery that one group of micro organisms is so different from all other groups that a very clear division of the prokaryotes into two branches was assumed. This group is the Archeae (formerly Archaebacteria) and all other groups are collectively designated as Eubacteria. The Archaea cell wall does not contain a peptidoglycan skeleton; only proteins and polysaccharides are present. The RNA polymerases and some of the co-enzymes of the Archaea differ from those of the Eubacteria. Methanogenic bacteria fix carbon dioxide via the acetyl-CoA pathway, and not via the ribulose bisphosphate cycle. Even though great advances in our understanding of the role of methanogens in anaerobic processes have been made since the discovery of the Archaea and the placement of the methanogens in this domain, much needs to be learnt about microbial interactions in anaerobic systems. From a practical point, given the importance of methanogens in anaerobic treatment processes, an understanding of their ecology is essential to make effective control of the start-up and operation of anaerobic bioreactors possible (Raskin et al., 1994).

2.5.2 Fluorescent In Situ Hybridisation (FISH)

Oligonucleotides (short strands of nucleic acids; usually 15-30 nucleotides in length), complementary to the 16S rRNA sequence regions with an intermediate degree of conservation and characteristic for phylogenetic entities like genera, families and subclasses, have been used successfully for rapid identification of bacteria. The oligonucleotides, or probes, are able to enter fixed bacterial cells and once inside the cells, they may form stable associations (hybrids via hydrogen bonding between complementary nucleotides) with the 16S rRNA in the ribosomes (Bell, 2002). If the complementary sequence for the probe is not present in the 16S rRNA in the ribosome, stable hybridisation does not occur and the probe is washed from the bacterial cell. In order to observe when hybridisation occurs, the probes are labelled with fluoroscein or rhodamine. Cells in which the fluorescently labelled oligonucleotide has hybridised with the 16S rRNA in the ribosome can be directly visualised by epifluorescent microscopy. The use of whole cell hybridisation provides a basis to estimate the *in situ* growth rates of species in natural populations, since the cellular ribosome content and, consequently, the rRNA concentration vary with the growth rate (this would be detected by changes in the strength of the fluorescent signal).

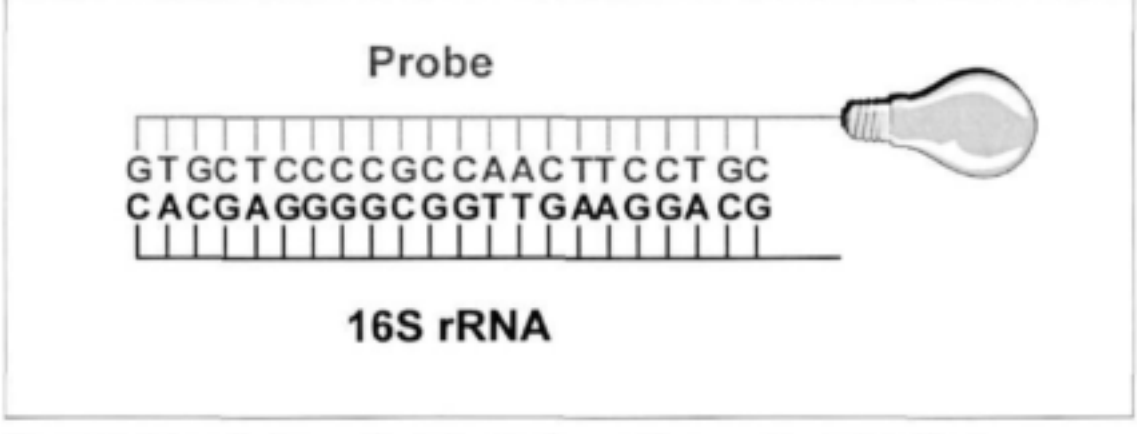


Figure 2-4 : Schematic diagram illustrating the theory of fluorescent *in situ* hybridisation

Epifluorescence microscopy: Fluorochromes, such as fluoroscein and rhodamine, are excited by particular wavelengths of light and generate secondary emitted wavelengths, which are detected as an image of a fluorescing object. The excitation process generally requires light of short wavelengths in the near-UV or blue range. The light source and the arrangement of specific filters in the light path are important. The filters vary depending on the type of fluorochrome being detected. In epifluorescence, the objective acts as both the objective and the condenser. There is no direct light beam from the source to the eye of the operator. Instead, the excitor beam is reflected to the objective from a rear port by a beam-splitting mirror that reflects the exciting wavelength but transmits visible light back from the objective, through the eyepieces. The filters are between the lamp and the specimen. The filters pass wavelengths of light required for excitation and adsorb most other wavelengths. The confocal laser microscope can be used to evaluate the spatial configuration of microbial granules or flocs.

2.5.3 Other Molecular Techniques

An alternative way to compare the rDNA sequences (genes for rRNA) is by separating them into discrete bands on the basis of their sequence using denaturing gradient gel electrophoresis (DGGE). This method exploits the difference in the strength of bonding between thymidine and adenine base pairs and guanine and cytosine base pairs. In DGGE the double-stranded DNA products undergo electrophoresis through a polyacrylamide gel containing an increasing linear gradient of denaturants. Strand separation occurs when sufficiently high denaturant concentration is reached. The electrophoresis gels can be probed with diagnostic oligonucleotides to identify particular sequences or bands can be excised, re-amplified and sequenced.

One great advantage of FISH over other methods in molecular microbial ecology is that it is relatively rapid and information on the numbers and distribution of specific organisms can be obtained within a single working day. One important conclusion is that close morphological similarity between bacteria does not necessarily reflect true relatedness at a genomic level. Knowing the 16S rDNA sequence of a particular organism then means that a unique segment of that gene can be recognised and a specific probe synthesised. The probe can be tagged and used to specifically identify only that organism in complex natural communities of bacteria.

2.5.4 Population Dynamics in the ABR

With the unique construction of the ABR, various profiles of microbial communities may develop within each compartment. The microbial ecology within each reactor compartment will depend on the type and amount of substrate present as well as external parameters such as pH and temperature.

CHAPTER 3
Laboratory Screening Tests

The objective of this phase of the study was to assess the toxicity of a range of food and textile dyes to the methanogens in anaerobic digester sludge. The anaerobic toxicity of the brewery effluent was also investigated. Only representative results are presented in this report, the full set of results and experimental techniques can be found in Bell, 2002.

Bioassay techniques for measuring the presence or absence of inhibitory substances are effective since they are simple to set-up, several substances can be tested simultaneously, they are inexpensive, and do not require knowledge of specific inhibitory substances (Owen et al., 1979). In these batch bioassays, anaerobic toxicity was determined as the adverse effect of a dye or the brewery effluent on the predominant methanogens. Methanogenic activity was stimulated, at the start of the test, by the addition of the methanogenic precursors, acetate and propionate. The methanogenic metabolism of the acetate-propionate solution was monitored by total gas production, in the control. Inhibition due to dye or wastewater addition was determined as a decreased rate of gas production, relative to the control. The first week of incubation is critical as these data reflect the true, unadapted response of the micro organisms to the substrate.

The objective of a toxicity assay is to determine the concentration at which a substance becomes inhibitory to the biomass. Thus, it is important that assay concentrations are selected to provide a range from non-inhibitory to severely toxic.

Batch biodegradability assays can function as preliminary screening tests to assess the anaerobic degradability of a particular substrate. It is critical that these tests are conducted prior to operation of a continuous reactor in order to evaluate the efficiency of the degradation process and to assess volumes and concentrations of the substrate that can be treated effectively, i.e. without causing reactor failure. A detailed methane balance should be kept to assess the methanogenic utilisation of the substrate and the efficiency of the anaerobic degradation process.

In these screening tests, the results of the anaerobic toxicity assays were used to guide the set-up of the biodegradability assays; to prevent inhibition of the biomass, dye or wastewater concentrations lower than the measured IC50 concentrations were added to the assay bottles. Biodegradability of the dyes was determined by monitoring the cumulative methane production during anaerobic incubation, according to the method of Owen et al., 1979. The aim of this phase of the investigation was to assess the anaerobic biodegradability of several food and textile dyes and the brewery effluent, by the microbial populations present in the anaerobic digester sludge.

3.1 FOOD DYES

Food dyes have similar chromophores to textile dyes, but are frequently simpler in chemical structure as they not have to attach to substrates such as textile fibres or leather. Food dyes are discharged to the environment from food dye manufacturing plants, food processes or as kitchen and human waste. Food dyes were used as model substrates prior to investigating textile dyes.

3.1.1 Materials and Methods

Anaerobic Toxicity Assays : a food dye manufacturer, provided samples of 15 food dyes, of varying chemical classes. The dyes are listed in Table 3-1, with both the commercial and *Colour Index* names. The classification into chemical class is dependent on the structure of the dye molecule and, most importantly, the chromophore type.

Dye stock solutions (10% w/v) were made up by dissolving 10 g of each dye powder, listed in Table 3-1, in deionised water, to make up 100 mL. The experiments were performed in 160 mL glass serum bottles, which were sealed with butyl rubber septa and aluminium crimp seals. A defined nutrient medium containing trace elements, minerals and vitamins was prepared according to Owen et al., 1979, with some modifications. The method for the preparation of the stock solutions and the nutrient medium are presented in Bell, 2002.

Table 3-1 : List of food dyes investigated for inhibition of methanogenic activity

Commercial Dye	Colour Index Classification	Dye Class
Sunset Yellow Supra	CI Food Yellow 3	Monoazo
Amaranth Supra	CI Food Red 9	Monoazo
Carmoisine Supra	CI Food Red 3	Monoazo
Brown FK Standard	CI Food Brown 1	Monoazo
Allura Red AC Supra	CI Food Red 17	Monoazo
Red 2G Supra	CI Food Red 10	Monoazo
Tartrazine Supra	CI Food Yellow 4	Monoazo
Ponceau 4R Supra	CI Food Red 7	Monoazo
Black PN Extra	CI Food Black 1	Disazo
Green S Supra	CI Food Green 4	Triarylmethane
Patent Blue V Supra	CI Food Blue 5	Triarylmethane
Brilliant Blue Supra	CI Food Blue 2	Triarylmethane
Quinoline Yellow Extra	CI Food Yellow 13	Quinoline
Erythrosine Supra	CI Food Red 14	Xanthene
Indigo Carmine Supra	CI Food Blue 1	Indigoid

The serum bottles were dosed with a range of dye concentrations, to provide a range from non-inhibitory to toxic. The dye concentrations investigated, for each dye were: 50 mg/L; 250 mg/L; 1 g/L; 5 g/L; 10 g/L and 20 g/L.

The assay bottles were overgassed with a gas mixture containing 70% N_2, 30% CO_2 at a flow rate of 0.5 mL/min for 15 min. A 20% (v/v) inoculum was used in each serum bottle, which was equivalent to 20 mL of anaerobic digester sludge (suspended) in a total working volume of 100 mL. The biomass was mixed with 30 mL of the nutrient medium. The dye stock solution was diluted to the required concentration, with deionised water, to make up a volume of 48 mL. The bottles were overgassed and sealed. After equilibration for 1 h, at the incubation temperature of 37 °C, the gas volumes were zeroed to ambient pressure with a glass syringe. The acetate-propionate solution (75 mg acetate and 26.5 mg propionate in 100 mL working volume) was added to each bottle.

The anaerobic toxicity assays were also run with four industrial effluents. Two of these were sampled at the factory of the food dye manufacturer, where a chemical treatment of the final effluent (sodium dithionite) was employed in an attempt to remove some of the colour. Effluent samples before and after chemical treatment were taken. Two leather tannery effluents were tested. A range of effluent concentrations were investigated: 20; 40; 60; 80 and 100% (v/v) of the effluent, diluted in deionised water. The serum bottles were set up in the same manner as for the dye tests.

The controls, or blanks, contained only the inoculum sludge, the anaerobic nutrient medium and the acetate-propionate spike. The methanogenic metabolism of the acetate-propionate solution was monitored by total gas production, in the controls. Inhibition due to the addition of a dye was determined as a decreased rate of gas production, relative to the control. Gas volume measurement during incubation was performed with a graduated glass syringe.

The dye concentration at which 50% of the methanogenic population was inhibited (IC_{50}) was calculated for each dye, according to Figure 3-1. Total biogas was measured during the incubation period. The methane composition of the biogas was determined, thus the methane fraction of the biogas was known. At the commencement of the tests, the total suspended solids (TSS) and volatile suspended solids (VSS) of the inoculum sludge was measured. The methanogenic activity could thus be calculated (mL CH_4/g VSS) for each dye concentration, and calculated as a fraction of the methanogenic activity in the controls. The activity at each concentration was plotted and a best-fit line was plotted through the data points. The IC_{50} was then calculated. These results are given in the following sections.

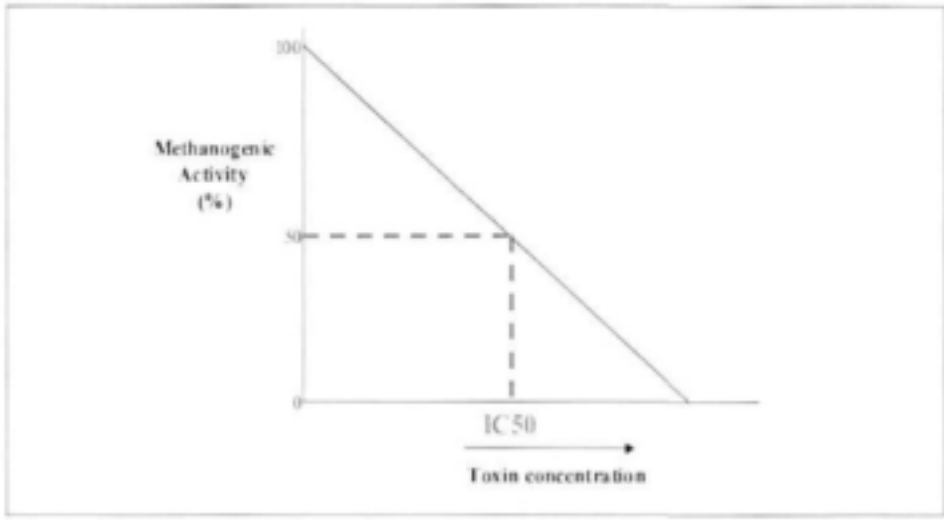


Figure 3-1 : Schematic diagram showing the calculation of the IC_{50} value of each dye

Biodegradability assays : the same dyes were investigated as in the anaerobic toxicity assay (Table 3-1). The chemical structures of the investigated dyes have not been included in this report. The anaerobic degradability of the four industrial effluents was also evaluated. The nutrient medium was prepared as described by Bell, 2002. The inoculum sludge was obtained from an operating anaerobic digester at the Mogden Sewage Works. The TSS and VSS were measured on the sludge.

The concentration of dye added to each assay bottle was calculated according to the theoretical COD of the dye; the theoretical gas production (to produce ca. 100 mL biogas), according to the Tarvin and Buswell (1934) (Tarvin and

Buswell, 1934) equation; and the measured IC_{50} concentrations determined in the anaerobic toxicity assays. The investigated concentration of each dye is given in Table 3-2.

The investigated concentrations for the industrial effluents were 20% (v/v) and 100% (v/v) for the dye manufacturer treated and untreated effluents, and 50% (v/v) and 100% (v/v) for the two leather tannery effluents.

The bottles were prepared in the same manner as for the anaerobic toxicity assays. Each sample was run in triplicate. A 10% (v/v) inoculum was added to each bottle. The dye stock solutions were diluted, to the required concentration, in the anaerobic medium. No additional carbon source and no acetate-propionate solution was added The serum bottles were equilibrated and then incubated in a water bath, at a constant temperature of 37 °C. The bottles were shaken manually to facilitate contact between the micro organisms and the substrate.

Table 3-2 : Bioassay conditions to assess anaerobic biodegradability

Dye	Chemical Formula	Methanogenic IC_{50}	Theoretical COD (g COD/g dye)	Assay Dye Conc. (g/L)
Sunset Yellow	$C_{16}H_{10}N_2O_7Na_2$	19.6	0.96	1.25
Carmoisine Supra	$C_{20}H_{12}N_2O_7Na_2$	0.25	1.15	0.1
Brown FK Standard	$C_{31}H_{27}N_{10}O_9Na_3$	2.48	0.97	0.24
Allura Red AC	$C_{18}H_{14}N_2O_8Na_2$	> 20	1.48	0.7
Red 2G Supra	$C_{18}H_{11}N_3O_8Na_2$	> 20	1.46	1.26
Tartrazine Supra	$C_{16}H_9N_4O_9Na_3S$	14.3	0.644	1.5
Ponceau 4R Supra	$C_{20}H_{11}N_2O_{10}Na_3$	> 20	0.86	1.0
Black PN Extra	$C_{28}H_{21}N_5O_{14}Na_2$	> 20	0.826	0.28
Green S Supra	$C_{23}H_{27}N_2O_7Na_3$	19.5	1.5	1.0
Patent Blue V	$C_{27}H_{31}N_2O_6NaS$	2.15	1.14	0.6
Brilliant Blue Supra	$C_{37}H_{36}N_2O_9Na_2$	5.55	1.61	1.85
Quinoline Yellow	$C_{18}H_{11}NO_6S_2$	8.38	1.35	1.0
Erythrosine Supra	$C_{20}H_6O_5Na_2I_4$	0.0002	0.69	0.01
Indigo Carmine	$C_{16}H_{10}N_2O_8Na_2$	14.03 g/L	0.89	0.52

Three sets of controls were set up, each in triplicate. The first set contained only the inoculum sludge and the nutrient medium, to account for gas production due to degradation of residual organic molecules in the inoculum sludge and any gas production associated with the nutrient medium. The second set of controls contained the nutrient medium and the assay concentration of each dye, to identify any decolourisation caused by reducing agents in the defined medium. In order to assess whether the dyes were adsorbed to the butyl rubber septa, the third set of controls contained only the dye solutions in sealed serum bottles.

Biogas production and composition were measured according to the methods described for the anaerobic toxicity assays. Biogas composition was determined whenever gas was wasted.

Samples were withdrawn from each bottle, on the first day of incubation. The samples were centrifuged (4 000 rpm) and the supernatants filtered. The COD and colour of each sample was measured, according to the methods outlined Bell, 2003. These are referred to as the *initial*, or starting measurements. The same parameters were measured after 60 d of incubation, to assess the reduction in both COD and colour.

3.1.2 Results and Discussion

Anaerobic Toxicity Assays : The full gas production results, for each dye and wastewater investigated, are contained in Bell, 2002. The methanogenic activity for each dye concentration was calculated from the measured biogas volume and the analysed methane fraction. This activity was calculated as a percentage of the activity in the controls; no inhibition would have an activity of 100%. From these data, the dye concentration at which 50% of the methanogenic population was inhibited (IC_{50}) was calculated for each dye. These values are given in Table 3.3.

The literature has indicated that dye compounds and their degradation products can be toxic. An investigation by ETAD studied the effect of dyes on wastewater bacteria to determine whether dyes reaching a wastewater treatment plant could adversely affect the operation of the biological process. Eighteen out of 200 tested dyes showed significant inhibition of biomass respiration (Cooper, 1995).

The aim of these tests was to determine the potential toxicity of a range of food dyes to methanogenic populations in anaerobic digester sludge. Amaranth Supra was not included in the study as it was insoluble in both water and ethanol. The defined nutrient medium ((Bell, 2002)) contained nutrients and vitamins required by anaerobic cultures. Resazurin was added to detect oxygen contamination. Sodium sulphide was included to provide a reducing environment and sodium bicarbonate to provide buffering, for alkalinity control.

Table 3.3 gives the molecular mass of each dye molecule, to provide an indication of the variability in dyes that are available commercially. The molecular weights ranged between 400 and 900 g/mole. According to the literature, an average dye concentration, in an effluent, is ca. 1 mg/L. Dye concentrations usually investigated in laboratory studies range between 1 and 250 mg/L. Thus, it was believed that the wide concentration range used in these toxicity assays (50 mg/L to 20 g/L) should incorporate concentrations at which each dye was both non-inhibitory and inhibitory to the methanogens.

Table 3-3 : Calculated methanogenic IC_{50} values for the investigated food dyes

Commercial Dye	Molec. mass (g/mol)	Methanogenic IC_{50}
Sunset Yellow Supra	452.2	19.6 g/L
Carmoisine Supra	502.4	0.25 g/L
Brown FK Standard	848.8	2.48 g/L
Allura Red AC Supra	496.0	> 20 g/L
Red 2G Supra	509.0	> 20 g/L
Tartrazine Supra	534.4	14.3 g/L
Ponceau 4R Supra	604.3	> 20 g/L
Black PN Extra	871.7	> 20 g/L
Green S Supra	530.0	19.5 g/L
Patent Blue V Supra	663.0	2.15 g/L
Brilliant Blue Supra	794.9	5.55 g/L
Quinoline Yellow Extra	401.2	8.38 g/L
Erythrosine Supra	879.9	0.2 mg/L
Indigo Carmine Supra	468.3	14.03 g/L
Untreated Effluent	-	22.5% (v/v)
Treated Effluent	-	15.9% (v/v)
Leather Tannery (1)	-	> 100% (v/v)
Leather Tannery (2)	-	> 100% (v/v)

A wide range of toxicity data were obtained from these tests (Table 3.3) with IC_{50} values ranging from > 20 g/L (highest dye concentration investigated) to as low as 0.2 mg/L. It was surprising to find that some of the dyes, currently used to colour foodstuffs, were toxic at concentrations lower than 1 mg/L. These dyes could be problematic in the anaerobic treatment of dye wastewaters since they could cause inhibition of the methanogens present in the treatment system, resulting in reactor failure and inefficient treatment of the wastewater.

Since food dyes are ingested it is not generally expected that they would be toxic, however, these tests showed a range of toxicity values to the methanogenic populations. No inhibition was observed in several of the dyes, including Sunset Yellow, Allura Red AC Supra, Red 2G Supra, Ponceau 4R Supra, Black PN Extra and Green S Supra. Addition of these dyes did not cause 50% inhibition at concentrations as high 20 g/L, and since it is unlikely that they would be present in higher concentrations in a wastewater, it can be concluded that they would not have an inhibitory effect on an anaerobic treatment system. However, some of the investigated dyes did show toxicity to the methanogens. The two most toxic dyes were Carmoisine Supra (IC_{50} of 0.25 g/L) and Erythrosine Supra (IC_{50} of 0.2 mg/L). These dyes could be problematic in anaerobic treatment if they are present in concentrations greater than the calculated IC_{50} concentrations. This could easily occur with wastage or washing procedures at the factory resulting in a large volume of the dye being present in the final effluent. Further tests

would have to be conducted to determine whether the methanogenic biomass could acclimate to these inhibitory dyes.

The monoazo dye, tartrazine was of interest due to the large production volume by the dye manufacturer. The anaerobic toxicity assays showed the dye to be relatively non-inhibitory with an IC_{50} concentration of 14.3 g/L. These results were promising as they indicated that anaerobic treatment was a possibility for tartrazine waste streams. Results from the tests with the real industrial wastewaters showed that after chemical treatment, the effluent became more inhibitory to the methanogenic biomass. Overall, the tests showed the effluent to be inhibitory to the methanogens with IC_{50} values of 22.5% and 15.9% (v/v), for the untreated and chemically treated effluents, respectively. The increased inhibition after chemical treatment could be due to the presence of toxic aromatic amines which have been found to be released, from the combined form, by reduction with dithionite (Prival et al., 1993).

The toxicity tests on the leather tannery effluents showed them to be non-inhibitory to the methanogens, with the calculated IC_{50} values greater than 100% (v/v) wastewater, for both effluent samples. These results indicate the potential for anaerobic treatment of leather tannery wastes.

In similar batch toxicity assays, Flores et al. (1997) also found selected azo dye compounds to be toxic towards methanogenic activity in anaerobic granular sludge. Considering the ability of anaerobic micro organisms to reduce and decolourise azo compounds, acclimation of the methanogens to the azo dyes is likely during anaerobic treatment. The objective of these experiments was to provide an initial indication of the characteristics of the food dyes, and to provide toxicity data on which further biodegradability tests could be based. Biodegradability tests would provide information on microbial metabolism of the dyes and acclimation of the micro organisms to the inhibitory dyes.

Biodegradability Assays : Measurements were taken and results calculated after 60 d of incubation at 37 °C. The full results of the biodegradability assays are presented in Bell, 2002.

The initial biogas production rate (mL/d) was the rate measured on day 2 of incubation. This provided an indication of degradability of the dye by the micro organisms; the lower the gas production rate, the greater the inhibition. An extended lag period was observed with some of the assays, during which time the micro organisms acclimated to the dye, resulting in biogas production. The biogas production rate of the acclimated biomass is given for these bioassays.

The volume of biogas produced was measured throughout the test period. Biogas composition was determined whenever gas was wasted from a serum bottle and after 60 d of incubation. The total volume of methane gas produced during the 60 d incubation period was determined. This was corrected for the amount of methane produced in the controls, to give the net methane production due to degradation of the added substrate (dye or industrial effluent). The COD equivalent of the methane produced was calculated from the known conversion of 1 g COD being equal to 0.350 L CH_4 at 35 °C (Speece, 1996).

The amount of methanogenic activity in each serum bottle was estimated by calculating the fraction of dye COD converted to methane COD. The *theoretical* utilisation was based on the theoretical COD of the dye. The *actual* utilisation used the measured COD at the start of incubation. These values provide an indication of the extent of methanogenic utilisation of the dyes as substrates.

The COD balance was calculated from the measured COD values. *CODin* represents the initial COD measurement; *CODout* is the total of the final soluble COD measurement and the COD transformed into methane. The measured reduction in COD is given as a percentage.

The maximum wavelength for each dye and industrial wastewater was determined by a spectrum scan on the UV-VIS spectrophotometer. Colour reduction (%) was determined by the change in absorbance (at the maximum wavelength) from the initial starting colour, to the colour after 60 d of incubation. Decolourisation was corrected for by the controls to assess the amount of decolourisation due to reduction by reducing agents in the nutrient medium and adsorption of the dye to the butyl rubber stoppers. These values were negligible for most of the dyes. The results of the biodegradability bioassays are summarised in Table 3.4.

In these biodegradability assays the added substrate, i.e. the food dye or the industrial effluent was added as the sole substrate. The objective of these tests was to evaluate whether the anaerobic microbial populations would be able to utilise the added dye as a sole substrate. Experimental work has shown that an additional carbon source, such as glucose or VFA mixture enhances decolourisation (Razo-Flores et al., 1997). However, Razo-Flores et al. (1997) also found that a pharmaceutical azo dye, azodisalicylate, was completely decolourised and mineralised to CH_4 without the supplementation of an additional carbon source, at dye loading rates up to 225 mg/L.d. These results indicated that some azo dyes could be degraded in anaerobic environments in contrast to the common assumption that they are only biotransformed to mutagenic and carcinogenic aromatic amines.

Three sets of controls were set up for these bioassays. The function of the controls containing only the inoculum sludge and the anaerobic nutrient medium was to determine the amount of gas produced due to the microbial degradation of residual organic molecules in the inoculum or gas production associated with the nutrient medium. The measured gas volumes, for the experimental bottles, were corrected by subtracting the volume of gas produced in the controls to quantify the gas produced as a result of the degradation of the dye alone.

The controls containing the anaerobic medium and the assay concentration of each dye functioned to evaluate decolourisation due to the reducing agent, sodium sulphide, in the medium. Decolourisation may be attributed to adsorption and not necessarily degradation of the dye. To determine whether dyes were adsorbed to the butyl rubber septa, controls were set up containing only the dye solution in sealed serum bottles. Decolourisation due to adsorption or reduction of the azo bond was found to be negligible for the majority of the dyes.

Table 3-4 : Results of the anaerobic biodegradability assays (60 d)

Dye	Methanogenic Utilisation	COD Reduction	Colour Reduction
Sunset Yellow Supra	0	64.3	78.5
Carmoisine Supra	0	68.6	69.4
Brown FK Standard	0	66	72
Allura Red AC Supra	0.9	55.5	90
Red 2G Supra	1.3	52.5	89.8
Tartrazine Supra	0.84	48.2	94.4
Ponceau 4R Supra	1.64	48.5	86.9
Black PN Extra	1.9	55	74.2
Green S Supra	0	28.6	94.9
Patent Blue V Supra	0	61.8	33.6
Brilliant Blue Supra	2.08	54	0
Quinoline Yellow Extra	0	57.4	0
Erythrosine Supra	0	41.3	0.8
Indigo Carmine Supra	2.6	34	16.6
Untreated (20%)	2.2	37	65.8
Untreated (100%)	0.43	64	79.8
Treated (20%)	1.96	54	31
Treated (100%)	0	61	36.6
Leather (1) (50%)	5.0	69.8	-
Leather (1) (100%)	7.17	63	-
Leather (2) (50%)	2.65	13.9	-
Leather (2) (100%)	0.97	62.7	-

The dye structure or chemical formula of each dye was known, thus the theoretical COD could be calculated per g of dye. The theoretical gas production, assuming complete mineralisation to methane and carbon dioxide, was calculated, according to the Tarvin and Buswell (1934) equation:

$$CnHaOb + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \qquad \textbf{Equation 3.1}$$

The amount of dye required to produce ca. 100 mL of biogas was calculated. The theoretical COD of the calculated mass of dye was calculated, according to the equation:

$$CnHaObNc + d\,Cr_2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + \frac{a + 8d - 3c}{2}H_2O + cNH_4^+ + 2dCr_3^+ \qquad \textbf{Equation 3.2}$$

where $d = 2n/3 + a/6 - b/3 - c/2$

The theoretical COD ($3/2\ d$) is calculated based on the amount of oxygen required to oxidise it (Speece, 1996). The amount of dye added to each serum bottle was corrected if the theoretical COD was greater than 2 g and if the calculated dye concentration (based on the production of 100 mL biogas) was greater than the IC_{50} concentration, calculated during the anaerobic toxicity assays. The dye concentration added to each assay bottle is shown in Table 3-2.

Gas production is indicative of metabolic activity, thus the shape of the gas production curve indicates the degree of degradability of a substrate. Biogas production was monitored throughout the incubation period. Determination of the biogas composition gave the fraction of methane in the total biogas. The volume of methane could then be calculated to give an indication of the extent of methanogenic activity within the serum bottles. It is known that in an anaerobic environment, COD is not destroyed, it is only transformed. Thus, a methane balance can be used to evaluate the methanogenic activity within a batch culture by calculating the amount of COD converted to methane. These values were calculated for each assay to assess the extent of methanogenic activity. The amount of methane produced was corrected for the volume of methane produced in the controls, such that the equivalent methane COD was attributed to degradation, or utilisation, of only the dye. The methanogenic activity was low, suggesting that these dyes were not readily utilised by methanogenic populations.

There is a degree of inaccuracy associated with the data presented for the COD balances, resulting in the poor balances attained. The discrepancy lies with the measured COD values, relative to the theoretical values. For each assay, the theoretical COD of the dye added was calculated, from the mass of dye added. The COD of the nutrient medium was assumed to be negligible (measured at 36.7 mg/L). However, the COD values, measured at the start of the incubation period, do not correlate with the theoretical values; they are generally larger. The final soluble COD was measured and the COD equivalent of the methane produced was calculated to give the final *CODout*.

Soluble COD was measured since it was assumed that biomass production would be negligible. Also, COD measurement of solids is inaccurate unless the samples are properly homogenised. The presence of a larger floc in a sample would greater influence the measured COD. The total COD (soluble and insoluble) of each bottle was measured to assess the influence on the COD balances (data not shown). The samples were homogenised by passing them through a 0.6 mm syringe needle. The measured values were much greater than the measured initial COD values and, therefore, did not provide a solution to the poor COD balances

The *loss* of COD may be attributed to adsorption of the dye (and its associated COD) to the biomass. An accurate measure of insoluble COD should, therefore, be used to assess this assumption. Tests could also be run to evaluate the extent of adsorption of each dye to the biomass.

In terms of effluent discharge, decolourisation is critical. However, a delicate balance exists because, as investigations have shown, the degradation products of dyes can be toxic, or carcinogenic. Thus, before implementation of a particular treatment system, for decolourisation, it is critical that the components of the effluent are analysed. The Microtox test could be used to identify inhibitory compounds and HPLC could be used to analyse the compounds present in an effluent. Colour removal can be achieved by physical, chemical or biological means. Colour reduction in these bioassays could have been due to the reducing environment within the serum bottles. If the redox potential is low enough it may result in the cleavage of the azo bond. Decolourisation could also be achieved

by adsorption, to the biomass or the butyl rubber stoppers. The aim was to achieve biological decolourisation, i.e. utilisation of the dye by the micro organisms resulting in breakdown of the dye molecules and removal of colour. Decolourisation was of particular interest for samples with low IC_{50} concentrations because biodegradation was not expected but reduction, or breakage, of the dye molecules, could occur resulting in decolourisation. Since bioadsorption tests were not conducted, decolourisation in these assays could have been caused by either adsorption or degradation, or both.

Very little gas production was observed in the Sunset Yellow bioassays (Table A2.2). The gas production approximated that of the controls, suggesting that gas production was due to degradation of residual organic molecules in the inoculum or associated with the nutrient medium. The plot of the corrected gas production was negative at points, showing that gas production was lower than that in the controls, indicating inhibition due to addition of the dye. This was not expected since the IC_{50} concentration was calculated at 19.6 g/L. This inhibition value was specific for the methanogens, therefore, the dye could be inhibitory to the other microbial populations present in the digested sludge. However, there was no methanogenic activity either. These results suggest that the dye would be unsuitable for anaerobic degradation. Reduction in COD and colour were relatively high at 65% and 78.5%, respectively. These reductions were obviously not due to microbial activity and are, therefore, attributed to adsorption to the biomass.

Gas production in the Carmoisine Supra bottles was lower than in the controls, which suggested that the dye was inhibitory to the anaerobic micro organisms. This correlated with the results of the anaerobic toxicity assays, where the IC_{50} concentration was low at 0.25 g/L. No methanogenic activity was present in the bottles. This dye would, therefore, also not be suited to anaerobic treatment. Again, the COD and colour reductions were relatively high at 68.4% and 68.6%, respectively, which could have been due to adsorption to the biomass. Similar results were obtained for Brown FK Standard, which also had a low IC_{50} concentration at 2.48 g/L. This resulted in inhibition of the microbial populations, including the methanogens which showed no activity. COD reduction was 66% and there was a 72% reduction in colour.

Biogas production was greater than in the controls, for Allura Red AC Supra. The methanogenic IC_{50} concentration was calculated at > 20 g/L, however, in these assays, methanogenic activity only attributed to 0.9% of the utilisation of the dye. From this it can be deduced that, although the dye is not inhibitory to the methanogens, it is not readily utilised. This could be overcome by addition of a carbon source, which may result in co-metabolism of the dye. The bioassay COD was reduced by 55.5% and the colour by 90%. This could have been achieved by other bacterial populations, which is suggested by the volumes of biogas produced. Similar results were obtained for Red 2G Supra, which also had an IC_{50} concentration of > 20 g/L. Methanogenic utilisation accounted for 1.3% of the dye. COD reduction was 52.5% and colour was reduced by 89.8%. Tartrazine Supra had an IC_{50} concentration of 14.3 g/L, and was, therefore, assumed not to be inhibitory to the methanogens. Methanogenic activity was recorded and accounted for 0.8% of the degradation of the dye. Biogas production was greater than in the controls, suggesting metabolism of the dye by other microbial populations, also resulting in the reduction of the initial COD by 48.2%. The initial yellow colour of the dye was reduced by 94.4% but a change in colour to purple/maroon was observed. This could be problematic in treatment of the dye. The change in colour could be attributed to degradation products bonding to form a different dye structure; or else it could be due to oxidation of the degradation products, during gas measurement and sampling.

Ponceau 4R Supra had an IC_{50} concentration of > 20 g/L. Biogas production, during the incubation period, was greater than that in the controls, suggesting utilisation of the dye. Methanogenic metabolism contributed to 1.64% of the degradation of the dye. COD reduction was measured at 48.5% and colour reduction at 86.9%. Thus, anaerobic treatment of this dye would be efficient, but it requires the co-operation of several bacterial populations. A similar deduction could be drawn for Black PN Extra, with 1.9% methanogenic utilisation of the dye. This case illustrates the discrepancy between the theoretical and measured COD values. Based on the theoretical dye COD, the methanogenic utilisation of the dye would have been 11.6%. However, the initial measured COD was that much greater to reduce the methanogenic efficiency to 1.9%.

Biogas production was lower than the controls in the Green S Supra bioassays. This was not expected since the methanogenic IC_{50} concentration was 19.6 g/L. The dye could have been inhibitory to other micro organisms in the biomass, or it could be that it is not readily utilised. Colour reduction was high at 94.9%. No reduction in colour was observed in the colour controls, therefore, it is assumed that decolourisation was due to adsorption to the biomass.

Inhibition was observed in the Patent Blue V Supra assays, where the biogas production was lower than in the controls. This verified the anaerobic toxicity assays results, which calculated the IC_{50} concentration at 2.15 g/L. The dye concentration added to the bioassays was, however, lower than this concentration. There was no methanogenic activity. Reduction in COD (61.8%) could have been due to adsorption of the dye to the biomass (33.6% reduction in colour). Biogas production in Brilliant Blue Supra bioasssays was greater than in the controls except that no reduction in colour was achieved. This was of interest because the dye manufacturer could not achieve chemical decolourisation of the dye either. The gas production and reduction in COD (54%) could be due to degradation of readily available side groups on the dye molecules, without actual break down the dye molecule itself.

Biogas production was the same as that in the controls, for Quinoline Yellow Extra, up to day 10, after which biogas production increased. This would suggest acclimation of the micro organisms to the dye. There was no methanogenic utilisation of the dye. There was also no decolourisation.

Biogas production was greater than in the controls for Erythrosine Supra, which was unexpected, due to the high toxicity data recorded in the anaerobic toxicity assays. The dye concentration added was lower than the IC_{50} concentration of 0.2 mg/L. However, there was still no methanogenic utilisation of the dye. The biogas production was due to activity by other microbial populations. Colour reduction was low at 0.8%.

The untreated dye effluent was more degradable at the lower concentration of 20% (v/v) than at 100% (v/v). Biogas production was greater than in the controls for the 20% concentration. There was no gas production in the 100% concentration until acclimation was achieved by ca. day 35. The biogas production rate was then 0.1 mL/d. Methanogenic utilisation of the dye was greater (2.2%) in the lower wastewater concentrations than in the undiluted wastewater (0.43% methanogenic utilisation). Methanogenic degradation of the chemically treated effluent was lower, 1.96% (for the 20% concentration) than in the untreated samples. Similar COD and colour reductions were achieved. There was no methanogenic utilisation of the undiluted wastewater.

The two leather tannery effluents exhibited degradation potential. Biogas production was always greater than the controls. In the tannery (1) effluent, the methanogenic degradation of the wastewater increased with increasing concentration. This was, however, not observed in the tannery (2) effluent where the methanogenic activity decreased with increasing wastewater concentration.

These bioassays have provided a more thorough understanding of the dye characteristics and degradation potential. This knowledge can then be used to predict the optimal treatment option. It must be noted, however, that these bioassays investigated the final products of the industry, i.e. the dyes. The factory effluent would contain concentrations of the dye precursors, from the synthesis processes. These could have a severe effect on the treatment process as several aromatic amines have been shown to be toxic or inhibitory.

Additional tests could be run with the supplementation of another carbon source, such as glucose or ethanol. The results presented for these biodegradability assays showed that the dyes were not readily utilised as a sole substrate, however, degradation of the dyes could be enhanced by co-metabolism with another substrate. These tests would also provide a faster indication of the decolourisation potential of a dye.

A COD method more conducive to the accurate measurement of solids, e.g. the open reflux method, should be used to determine the total COD and, thereby prove, or disprove, the assumption that the dyes, and their associated COD, are adsorbed to the biomass. Adsorption bioassays could quantify the amount of adsorption of a particular dye to anaerobic biomass.

Further batch tests could be used to evaluate the half lives of dyes, under varying conditions such as inoculum source, dye class, sulphide concentrations and the presence of redox mediators.

3.1.3 Conclusions

The objective of the toxicity assays was to determine the concentration at which each dye became inhibitory to the methanogenic biomass.

1. The toxicity assays were specific to the methanogenic populations of the anaerobic digester sludge.
2. A wide range of toxicity data were obtained with IC50 values ranging from > 20 g/L to as low as 0.2 mg/L.
3. The two most toxic dyes were Carmoisine Supra (IC50 of 0.25 g/L) and Erythrosine Supra (IC50 of 0.2 mg/L).
4. The IC50 concentration of tartrazine was 14.3 g/L.
5. The dye manufacturing effluent was relatively inhibitory to the methanogens with IC50 values of 22.5% and 15.9% (v/v), for the untreated and chemically treated effluents, respectively.
6. Further tests should be conducted to determine whether the methanogenic biomass could utilise the dyes as a substrate and acclimate to the inhibitory dyes.

The objective of biodegradability assays was to evaluate whether the anaerobic biomass could utilise the added dye as a sole substrate.

1. Generally, the methanogenic activity was low, suggesting that these dyes were not readily utilised by methanogenic populations.
2. Decolourisation due to adsorption or reduction of the azo bond was negligible for the majority of the dyes.
3. These bioassays have provided a more thorough understanding of the dye characteristics and degradation potential. This knowledge can be used to predict the optimal treatment option.

4. Additional tests could be run with the supplementation of a carbon source, to investigate co-metabolism of the dyes. Adsorption bioassays could quantify the amount of adsorption of a particular dye to anaerobic biomass.
5. A COD method more conducive to the accurate measurement of solids should be used to determine the total COD and, thereby prove, or disprove, the assumption that the dyes, and their associated COD, are adsorbed to the biomass.

3.2 TEXTILE DYES

Initial screening tests were undertaken so as to ensure that the concentrations of dyes used in the ABR were biodegradable to some extent and would not inhibit the methanogenic s.

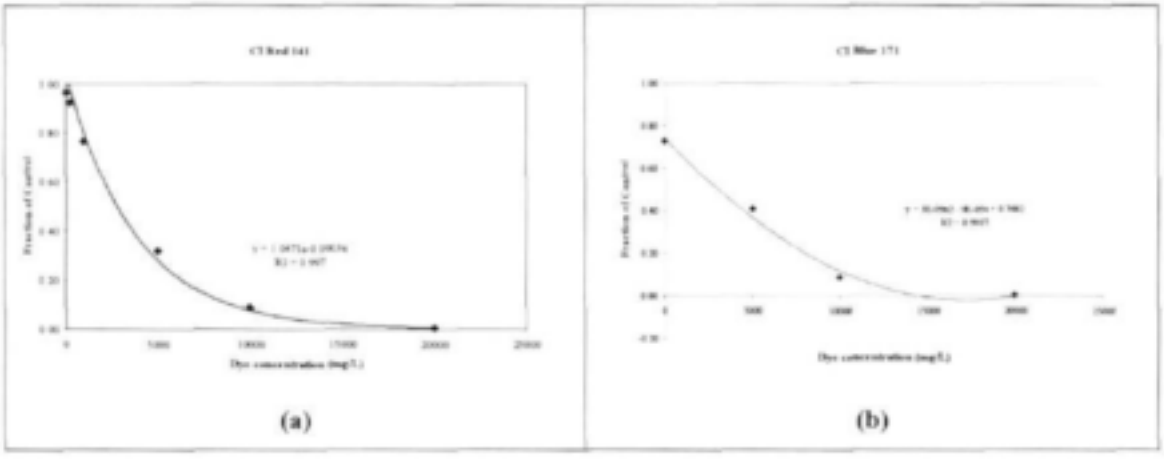
A series of serum bottle tests (Table 3-5) were undertaken using the same technique as in Section 3.1

Table 3-5 : Textile dye screening tests

Test	Spike	Substrate
Methanogenic toxicity	Acetate-propionate	Textile dyes
Acidogenic toxicity	Glucose	Textile dyes
Anaerobic biodegradability	-	Textile dyes
Anaerobic biodegradability	-	Textile dye degradation products

3.2.1 Anaerobic Toxicity Assays

Representative results are presented in Figure 3-2, the complete results can be found in the PhD dissertation (Bell, 2002). Figure 3-2 shows the methanogenic activity, as a fraction of that in the control, for each investigated dye concentration and the best-fit lines through the experimental data points, used to determine the IC_{50} concentration for each dye.



(a) (b)

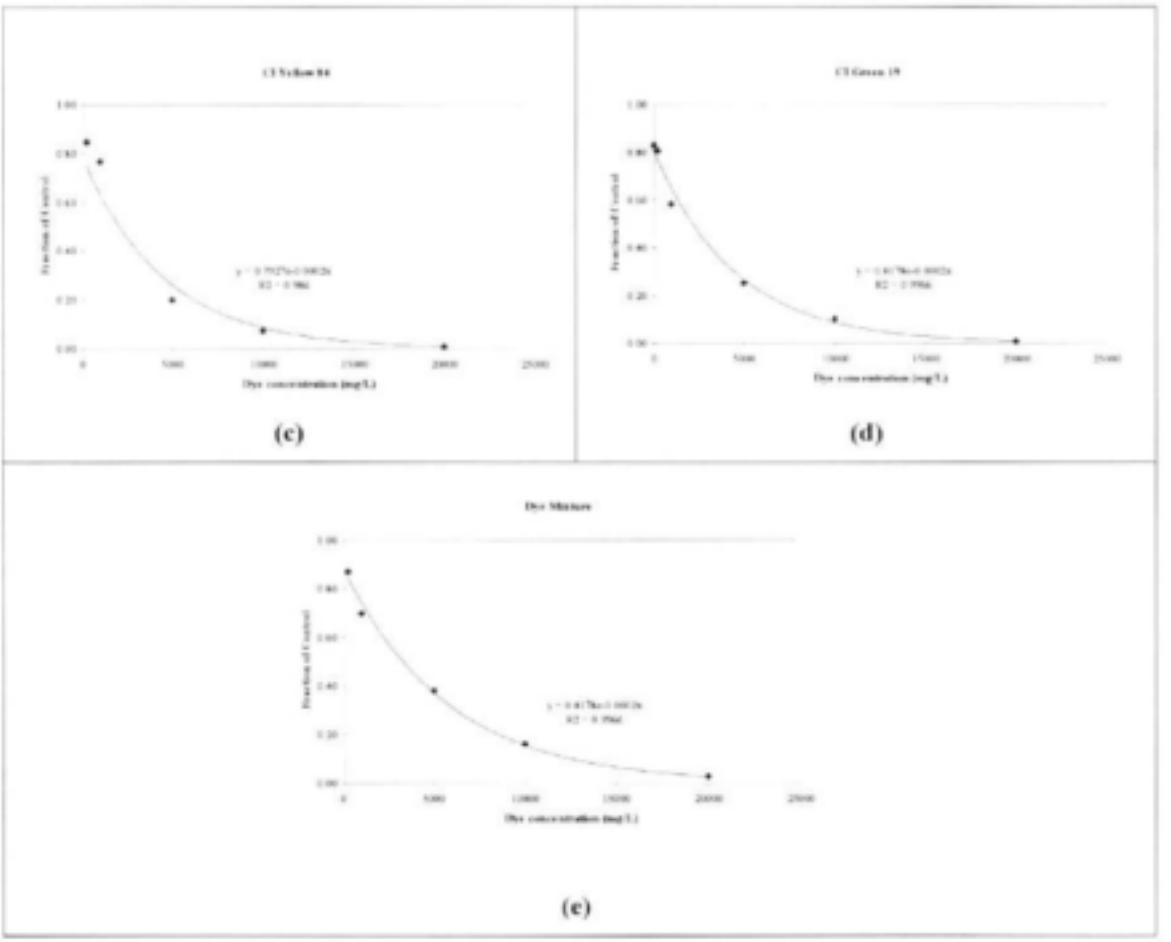


Figure 3-2 : Methanogenic activity at each dye concentration.

A typical gas production result is presented in Figure 3-3, the full results for each dye are given in Bell, 2002. The symbols represent the duplicate samples, and the line through the data points is the calculated mean biogas production. For each concentration, the gas production curve is shown relative to the gas production rate of the controls. A decrease in biogas production (line below that of the control) indicated inhibition of the methanogens due to addition of the dye. The calculated IC_{50} concentration for each dye is given in Table 3.6..

Table 3-6 : Calculated methanogenic IC_{50} values for the investigated textile dyes.

Dye	Methanogenic IC_{50} (g/L)
CI Red 141	2.46
CI Blue 171	3.07
CI Yellow 84	2.30
CI Green 19	2.46
Dye mixture	2.46

Figure 3-3 shows the cumulative biogas production for each concentration of the investigated textile reactive dyes, relative to the gas production measured in the controls (black line).

Discussion

The results of these toxicity assays showed that the four investigated dyes and the dye mixture were inhibitory to the methanogens, with all of the IC_{50} concentrations < 3.1 g/L. This indicates that these dyes could be problematic in the anaerobic treatment of dye wastewaters since they could cause inhibition of the methanogens present in the treatment system, resulting in reactor failure and inefficient treatment. Although it is unlikely that a normal dyehouse effluent would have a dye concentration > 3.1 g/L, it is not impossible. This could easily occur with wastage or washing procedures, resulting in a large volume of the dye in the final effluent.

The gas production for all concentrations of the investigated dyes, and the dye mixture, was lower than the gas production in the controls, indicating that all of the dyes were inhibitory to the methanogens, resulting in reduced methanogenic activity within the serum bottles. There were two exceptions, in the 50 and 250 mg/L concentrations of CI Reactive Red 141, the gas production curve was initially lower than the controls, but increased to equal the gas production in the controls, by day 5. This suggests acclimation of the biomass to the lower dye concentrations. In all cases, the degree of inhibition increased with increasing dye concentration. The most inhibitory dye was CI Reactive Yellow 84 (IC_{50} of 2.3 g/L).

Carliell *et al.* (1995) observed that CI Reactive Red 141 was inhibitory to methane production at concentrations over 100 mg/L using the toxicity assay methodology described by Owen *et al.* (1979). The results of this study indicate acclimation and methane production at a CI Reactive Red 141 concentration of 250 mg/L.

An interesting result was observed in the CI Reactive Yellow 84 assays; although equal volumes of biogas produced were measured for the first three days, after this the gas production in the 250 mg/L and 1 g/L assay bottles was marginally greater than in the 50 mg/L assay bottles. This would suggest acclimation and utilisation of the dye as a substrate, however, the gas production in all of these bottles was still lower than in the controls, indicating inhibition of the methanogens due to the addition of the dye. The same was observed in the dye mixture assays where the gas production in the 250 mg/L assay bottles was greater than in the 50 mg/L bottles.

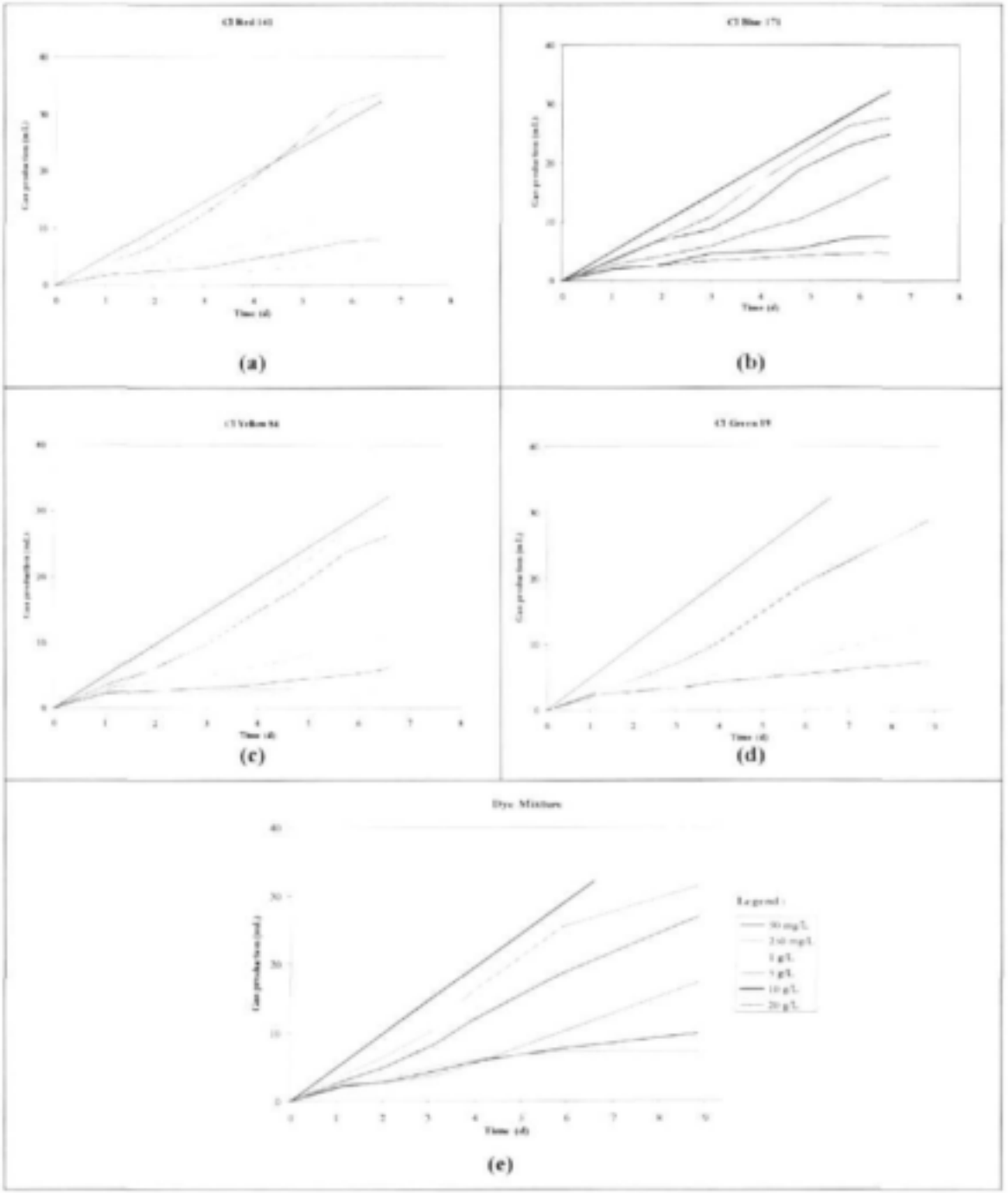


Figure 3-3 : Gas production plots for the methanogenic toxicity assays

Conclusions

1. The toxicity assays were specific to the methanogenic populations of the anaerobic digester sludge.
2. The results showed that the four investigated dyes, and the dye mixture, were inhibitory to the methanogens, with all of the IC50 concentrations < 3.1 g/L.
3. The CI Reactive Red 141 assays showed acclimation of the biomass to the dye, at the 50 and 250 mg/L concentrations.
4. For all dyes, the degree of inhibition increased with increasing dye concentration.
5. The most inhibitory dye was CI Reactive Yellow 84 (IC50 of 2.3 g/L).

3.2.2 Biodegradability Assays

The same four dyes were investigated as in the anaerobic toxicity assays (Table 3-7).

Table 3-7 : Bioassay conditions to assess anaerobic biodegradability of a range of textile dyes.

Dye	Methanogenic IC_{50} (g/L)	Theoretical COD (g COD/g dye)	Assay Dye Conc. (g/L)
CI Reactive Red 141	2.46	0.93	0.263
CI Reactive Blue 171	3.07	1.98	0.263
CI Reactive Green 19	1.16	1.98	0.263
CI Reactive Yellow 84	2.30	Unknown*	0.263
Dye Mixture	2.46	-	0.263 of each

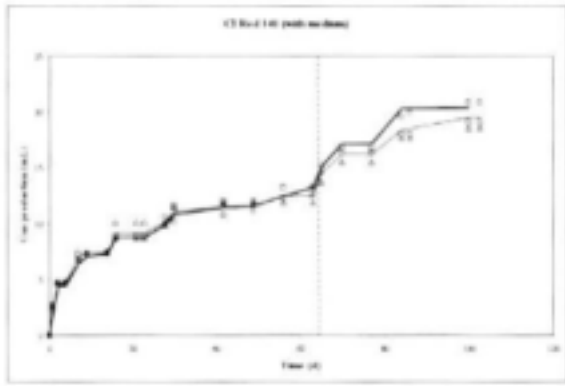
* The chemical structure of this dye was unknown

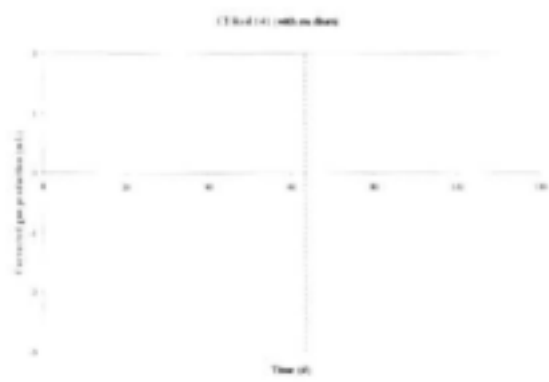
No additional carbon source or acetate-propionate solution was added.

Two variations of the CI Reactive Red 141 assays were set up; the one contained nutrient medium and the other did not. The purpose of this was to determine the difference in colour reduction in the presence and absence of the reducing agents in the anaerobic nutrient medium.

Measurements were taken and results calculated after 60 d of incubation at 35 °C. Representative results of the biodegradability assays are presented in Table 3.8 which shows the measured biogas production, relative to the biogas produced in the controls containing the nutrient medium and the inoculum sludge. Each plot is divided into two by a dotted line; the data on the left of the line represent the results of the initial dye biodegradability assays and the data on the right of the dotted line represent the results of the degradation product assays. The corrected gas production was also plotted for each dye. Here the amount of gas produced due to degradation of the dye alone is shown by subtraction of the control biogas from that measured in the samples. The symbols represent the triplicate samples, and the line through the data points, is the calculated mean biogas production. For each concentration, the gas production curve is shown relative to the gas production rate of the controls (solid black line).

Table 3-8 : Results of the biodegradability assay with CI Red 141 (with nutrient medium).





Biodegradability :

Dye concentration :	0.26 g/L	
Theoretical dye COD :	0.93 g COD/g dye	
Theoretical Assay COD (in 47.5 mL) :	11.625 mg COD	
Initial biogas production rate :	2.03 mL/ d	**Degradation Products**
Total gas production (37 °C) :	12.61 mL	6.9 mL
CH_4 production :	3.0892 mL	4.0763 mL
Net CH_4 production :	0 mL	0 mL
CH_4 – COD :	7.78 mg	10.3 mg

Methanogenic activity

Methanogenic activity :	26 mL CH_4/g VSS	32.2 mL CH_4/g VSS

COD balance

CODin : 117.2 mg (in 47.5 mL)	COD out :	26.3 mg (in 47.5 mL)
	CH_4 – COD :	7.78 mg
	Total CODout :	34.05 mg
Balance :	**29%**	
COD reduction :	78%	

Colour reduction

Measured colour reduction :	99% (545 nm)

The results of all the tests are summarised in Table 3.9.

Table 3-9 : Results of the textile dye anaerobic biodegradability assays (60 d).

Dye	Methanogenic Activity (mL CH_4/g VS)	COD Reduction (%)	Colour Reduction (%)
CI Reactive Red 141 (with nutrient medium)	26.0	78	99
CI Reactive Red 141 (without nutrient medium)	26.0	92	99
CI Reactive Blue 171	21.8	87	94
CI Reactive Green 19	24.3	89	96
CI Reactive Yellow 84	19.2	90	97
Dye Mixture	16.8	79	93

Discussion

The objective of these tests was to evaluate whether the anaerobic microbial populations would be able to utilise the added dye as a sole substrate.

A dye concentration of 0.26 g/L was added to each set of bottles. The dye concentration was lower than the calculated IC_{50} value for all of the dyes. The dye concentrations added were relatively low, thus the COD load in each bottle was low which would explain the low metabolic activity observed.

Gas production is indicative of metabolic activity, thus the shape of the gas production curve indicates the degree of degradability of a substrate. From the results, it was seen that the methanogenic activity was low, indicating that these dyes were not readily utilised by methanogenic populations.

As with the food dye assays, there was a degree of inaccuracy associated with the data presented for the COD balances, resulting in the poor balances attained. The reason is unclear. The loss of COD may be attributed to adsorption of the dye (and its associated COD) to the biomass. Spencer (1999) provided possible reasons for inaccurate results and balances in batch screening tests. These include: (i) the inability to accurately measure the COD removed or COD contribution of the inoculum sludge, (ii) the presence of toxins, (iii) the lack of trace metals, (iv) poor acclimation of the inoculum sludge to the substrate, (v) faults in the methodology, particularly in ensuring strict anaerobic conditions in all seed and substrate transfers, and (vi) gas leaks in the system and the inaccuracy of gas measurement because of the small volumes of gas being measured (Spencer, 1999).

The biogas production in the CI Reactive Red 141 bioassays, with nutrient medium, was equal to or greater than the biogas production in the controls. The initial biogas production rate was 2.03 mL/d. The net methane production, or methane production due to degradation of the dye molecule, was zero. Reduction in COD and colour were relatively high at 78% and 99%, respectively.

In the CI Reactive Red 141 bioassays without the nutrient medium the biogas production was lower than in the controls and lower than in the assays with the nutrient medium. The COD and colour removal were high at 92% and 99%, respectively. Thus, it could be deduced that the nutrient medium did not enhance colour reduction since the same colour reduction was achieved in both assays.

The biogas production was low in the CI Reactive Blue 171 assays. The total volume of methane produced was 2.8 mL, with a methanogenic activity of 21.8 mL CH_4/g VS. A COD reduction of 87% was achieved and a colour reduction of 94%. These results suggest that the acidogenic populations, which were shown not to be inhibited by the dye, were responsible for the measured biogas production and the COD and colour reduction. The difference in biogas production between the assay samples and the controls was due to the inhibited, or reduced, methanogenic activity in the assay bottles due to addition of the dye.

Similar results were recorded for the CI Reactive Green 19 samples, where the methanogenic activity was low; total volume of methane produced was 3.1 mL with a methanogenic activity of 24.3 mL CH_4/g VS. There was an 89% reduction in COD and 96% reduction in colour. These results suggest inhibition of the methanogens in the anaerobic biomass but metabolism by the acidogens.

Similarly for CI Reactive Yellow 84, although the net methane production was zero, with a methanogenic activity of 19.2 mL CH_4/g VS, the COD was reduced by 90% and the colour by 97%. This metabolic activity was attributed to the acidogens.

Willetts (1999) investigated the decolourisation rate reactions of mixtures of dyes. These results showed that the two dyes in a mixture affected one another in terms of the kinetics of their decolourisation. The more easily reduced dye of the pair was decolourised at the same rate as when it was present alone. Reduction of the second dye, however, showed a slower start. First order kinetics were thus no longer adhered to, indicating that the supply of reducing equivalents became rate-limiting. The decolourisation did, however, still reach completion. An increase in the decolourisation rate of the second dye was evident at the point that the first dye was completely reduced and therefore, no longer consuming the reducing equivalents (Willetts, 1999). No sequential degradation was observed in the biogas production plot for the textile dye mixture. Methanogenic activity was inhibited in these assays with the 79% COD reduction and 93% colour reduction being attributed to the acidogenic bacteria.

The results presented for these biodegradability assays showed that the dyes were not readily utilised as a sole methanogenic substrate, however, the biogas production, COD and colour reduction suggests that the acidogenic populations were actively reducing the dyes. The methanogenic activity may have been enhanced by supplementation with an additional carbon source. Although, in similar tests conducted by Bell (1998), on CI Reactive Red 141, it was found that an increase in solids concentration and/or addition of a source of readily biodegradable COD (glucose) had little effect on the rate of decolourisation of CI Reactive Red 141 (Bell, 1998).

Conclusions

1. Although the bioassays showed efficient COD reduction and decolourisation, the methanogenic activity was low, indicating that the dyes were not readily utilised by methanogenic populations.
2. The acidogenic bacteria were responsible for the biogas production and the COD and colour reduction.
3. No significant VFA concentrations were detected; the samples would have contained aromatic amines from the reduction of the azo bonds by the acidogenic bacteria.
4. The bioassays provided a more thorough understanding of the dye characteristics and degradation potential.

3.3 BREWERY EFFLUENT

A company adjacent to the local Durban brewery, receives the spent grain effluent from the brewery, prior to fermentation, and removes the solids from this effluent. The solids are used as additives in animal feed. The effluent produced by this company is high in organic strength and thus expensive to dispose of. The effluent is currently collected by tanker and transported to the Durban Southern Wastewater Treatment Works for marine discharge. Pre-treatment of this effluent would reduce the COD and thereby facilitate direct discharge to sewer, with a significant reduction in tariffs.

Anaerobic toxicity assays were conducted as described in Bell, 2002.

3.3.1 Results and Discussion

Anaerobic Toxicity Assays : The cumulative gas production for each wastewater concentration, relative to the control gas production, are shown in Figure 3-4.

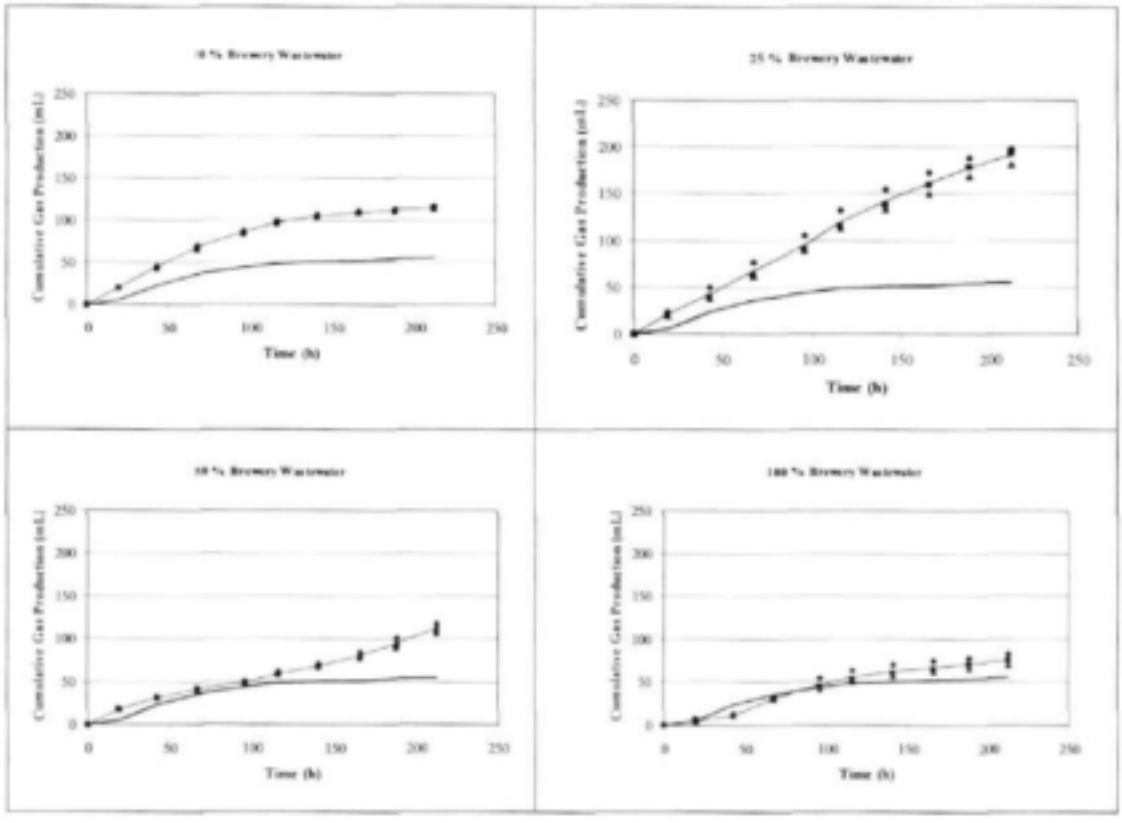


Figure 3-4. : Results of the anaerobic toxicity assay of the brewery wastewater, showing the cumulative gas production of each wastewater concentration, relative to the control gas production.

These results show that at a concentration of 10% (v/v) and at 25% (v/v), the brewery wastewater was not inhibitory to the anaerobic biomass. The cumulative gas production increased with an increase in concentration, which suggests that the micro organisms were metabolising the substrate. The initial gas production rate for the 10% (v/v) concentration was, however, higher (1.01 mL/h) than for the 25% (v/v) concentration (0.97 mL/h), thus the higher concentration was initially slightly inhibitory to the biomass. The cumulative gas production decreased with a further increase in concentration. The 50% (v/v) concentration was not inhibitory to the biomass since the amount of

gas produced was greater than that in the control. However, the amount of gas produced was smaller than in the 25% concentration and the initial gas production rate was lower, at 0.49 mL/h. These results suggest that the increase in substrate concentration results in an organic overload which inhibited the initial degradation rate. This was verified with the 100% (v/v) concentration, where the substrate was actually inhibitory to the biomass and the gas production only recovered, to be greater than in the control, after ca. 80 h incubation. The initial gas production rate was 0.27 mL/h, relative to 0.64 mL/h for the control.

These results represent total gas production. Plots of methane production will give a more definitive explanation of the effect of the increasing substrate concentration on the activity of the methanogens. It is evident from these results that the biomass may require a period of acclimation to the higher substrate concentrations; to recover from the *organic overload*.

CHAPTER 4

Laboratory-Scale ABR

Perspex laboratory-scale ABRs were set up in order to evaluate its performance on three industrial effluents (a segregated food dye – tartrazine, a mixed food dye manufacturing plant effluent and a pure textile dye)

4.1 TARTRAZINE DEGRADATION

Tartrazine, classified as Colour Index Food Yellow 4, is a monoazo synthetic organic colorant (Figure 4-1) with a molecular mass of 534.38 g/mole. Maximum absorption of the dye is at a wavelength of 430 nm. Tartrazine is a universally used lemon yellow colorant with excellent all round stability for all foodstuffs. In 1979, because of allergic reactions produced in humans, the identification of tartrazine, if contained in food and drugs, was required by name on the label. In 1987 tartrazine was certified by the FDA for use in food and beverages. At this time the dye was the third most commonly used food colorant (Collins et al., 1990).

The anaerobic degradation of tartrazine by intestinal microbes, particularly Proteus vulgaris has been studied extensively (Chung et al., 1978). It has been found that tartrazine is not readily degraded under anaerobic conditions (Haug et al., 1991). According to toxicological data, tartrazine is mildly toxic by ingestion, with a death concentration of 14 µg/kg. Ingested in high concentrations, it may cause peripheral nervous system effects and musculo skeletal effects

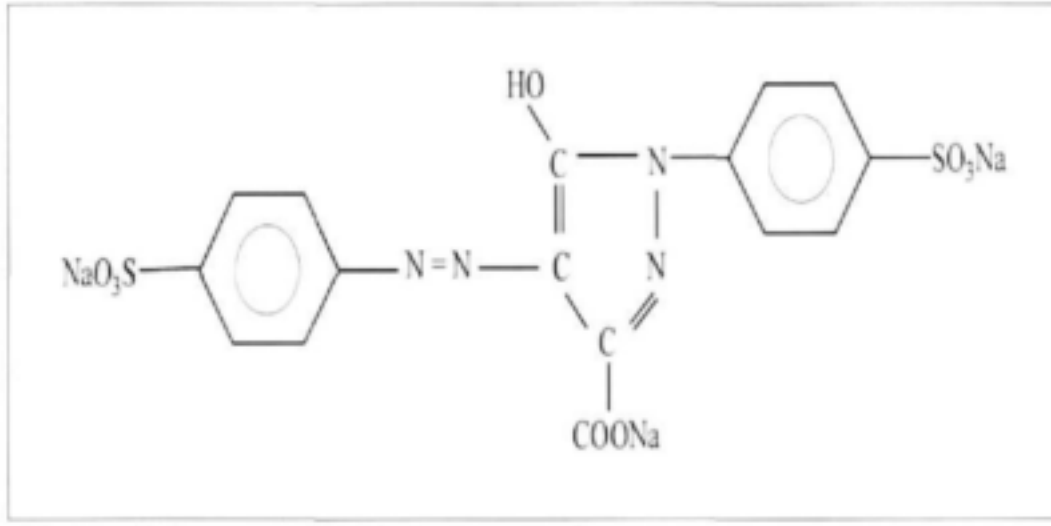


Figure 4-1 : Chemical structure of the Tartrazine molecule

Tartrazine was chosen for investigation in this study because the dye manufacturer produced large quantities of the dye. Tartrazine accounted for approximately 50% of the production from this factory. It was, therefore, assumed that tartrazine dye and its precursors would constitute a significant proportion of the final trade effluent. From discussions with the plant managers, it was ascertained that the tartrazine waste streams could be segregated on site, therefore, there was the potential for anaerobic treatment of the tartrazine waste alone, if treatment of the total effluent did not prove efficient.

Results of the anaerobic toxicity assays (**Chapter** 3) showed that tartrazine was not inhibitory to the methanogenic biomass, with an IC_{50} concentration of 14.3 g/L. In the biodegradability assays methanogenic utilisation of the dye was observed (0.8%). Biogas production was greater than in the controls, suggesting metabolism of the dye by other anaerobic microbial populations, also resulting in the reduction of COD and colour.

These results suggest the potential for anaerobic degradation of the tartrazine molecule. This may require acclimation of the biomass, or selection for particular populations, since tartrazine is not readily degraded. Problems may also be encountered with colour reduction and colour change. The objective of this experiment was to assess the efficiency of the anaerobic baffled reactor for the degradation, or treatment, of tartrazine.

4.1.1 Physical Decolourisation

Dye permeability through the cell membrane has been shown to be a rate-limiting factor in the biological treatment of dyes. Decolourisation of a wastewater, in a biological treatment system, may be attributed to adsorption of the dye to the anaerobic biomass, and not entirely to degradation or breakdown of the dye molecules. It is assumed that a saturation point would be reached where dye could no longer be adsorbed. A test was conducted to determine the extent of adsorption of tartrazine to the digested sludge, since this could contribute to the decolourisation potential in the ABR.

Materials and Methods : Anaerobic digester sludge was inactivated by autoclaving at 110 °C for 80 min. Once the sludge had cooled it was aliquoted into a series of serum bottles. The TSS of the inoculum sludge was measured. Five serum bottles were set up with 10, 20, 50, 75 and 90 mL autoclaved sludge, respectively. The working volume was 100 mL. The tartrazine dye stock solution was diluted to the required volume. The same dye concentration (250 mg/L) was added to each bottle. A control was set up for each bottle, containing the same amount of sludge but with no dye. The function of the controls was to evaluate the background absorbance of the sludge. The bottles were sealed and incubated in a water bath, at a constant temperature of 35 °C. The absorbance for each was measured at the start of the test and then periodically thereafter, for a period of 6 d.

The bottle contents were well mixed, prior to sampling. Samples (1.5 mL) were withdrawn by a syringe, through the rubber septa. The samples were sealed in Eppendorf tubes and centrifuged (4 000 rpm) for 5 min. The supernatant was withdrawn (1 mL) and diluted 10 x in distilled water. The absorbance of each was read at 430 nm. The background absorbance (control) was subtracted from the absorbance measured in the dye samples to give the absorbance of the dye alone. The results were plotted (Figure 4-2).

Results and Discussion : the biomass was autoclaved so as to inactivate the cells, thus, any observed decolourisation would be due to physical and not biological means. A drawback of this method is that autoclaving could damage the cell structure, resulting in a greater surface area for adsorption of the dye. Some cells may also be heat resistant and, therefore, not inactivated. A better method of inactivating the cells could be to treat them with an inhibitor such as sodium azide or irradiation.

The bottles were inoculated with increasing volumes of sludge to test for increased decolourisation with increased sludge volume, and therefore, increased surface area for adsorption. The TSS of the autoclaved sludge was measured at 26.3 mg/L.

The results did not follow the trend expected, i.e. increased decolourisation with increased sludge volume. Colour reduction between 90 and 100% was achieved in the bottles containing 50, 75 and 90 mL of sludge (Figure 4-2). Colour reduction was lower in the bottles with 20 mL sludge than in those with 10 mL sludge. The reason for this is unknown. The decolourisation in these bottles was due to adsorption to the biomass but the results may not be completely representative since the autoclaving may have increased the surface area available for adsorption by rupturing the cells. It is also unknown whether all of the biomass was inactivated by the autoclaving, therefore, some of the decolourisation may have been due to degradation or breakdown of the dye.

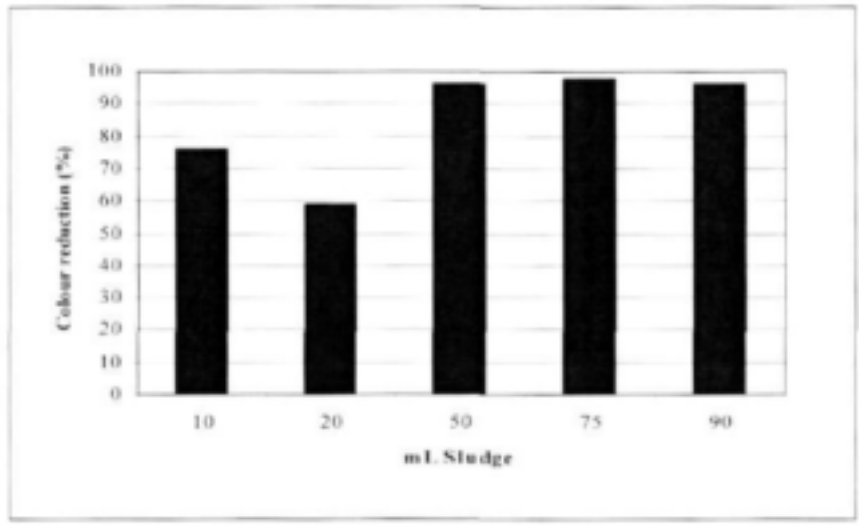


Figure 4-2 : Tartrazine decolourisation due to adsorption to increasing volumes of inactivated anaerobic sludge

The initial dye concentration added to each bottle was 250 mg/L, which was equivalent to 25 mg tartrazine dye in the 100 mL working volume. The TSS of the inoculum sludge was measured as 26.3 mg/L, thus, the amount of TSS added with each volume of inoculum sludge could be calculated. The tartrazine concentration in each serum bottle, at the end of the test period, was calculated from the final measured absorbance and the tartrazine calibration curve. A plot of dye removal as a function of TSS is shown in Figure 4-3.

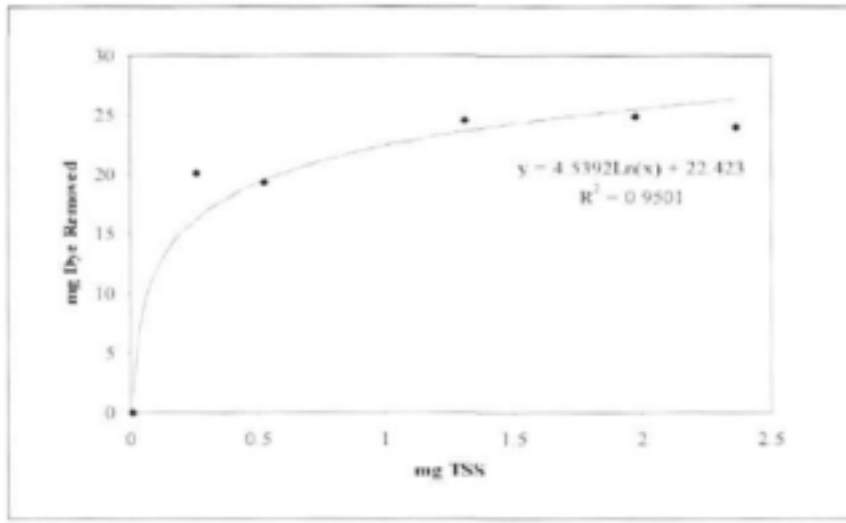


Figure 4-3 : Plot of Tartrazine adsorption.

It can be seen that the amount of decolourisation due to adsorption to biomass was a logarithmic (saturation) response. From the equation of the line it can be estimated that 22 mg tartrazine in solution is adsorbed per mg of total solids. From this experiment it can be concluded that adsorption does play a role in the decolourisation of the dye.

A tartrazine solution was fed to an ABR (designated ABR 1) to assess the efficiency of the reactor, and its configuration and separation of microbial populations, in the degradation of the dye.

4.1.2 Experimental Design

Two reactors were set up in a water bath, which was maintained at a constant temperature of 35 °C. The ABR used for tartrazine degradation was seeded with an original digested sludge of 18 g/L TSS of which 12 g/L were VSS. The sludge was allowed to settle for one week before feeding began. The feed connections for tartrazine degradation were set up as illustrated in Figure 4-4.

The feed solution was continuously pumped, by a variable speed Watson-Marlow peristaltic pump (model 101U/R), and diluted by distilled water pumped by a variable-speed peristaltic pump (model 505s). The two streams combined to form a single feed stream just before the inlet to the reactor.

The treated effluent passed through a glass U-tube for level control and a biomass trap before running to the effluent reservoir. Effluent samples were taken from the bottom of the U-tube.

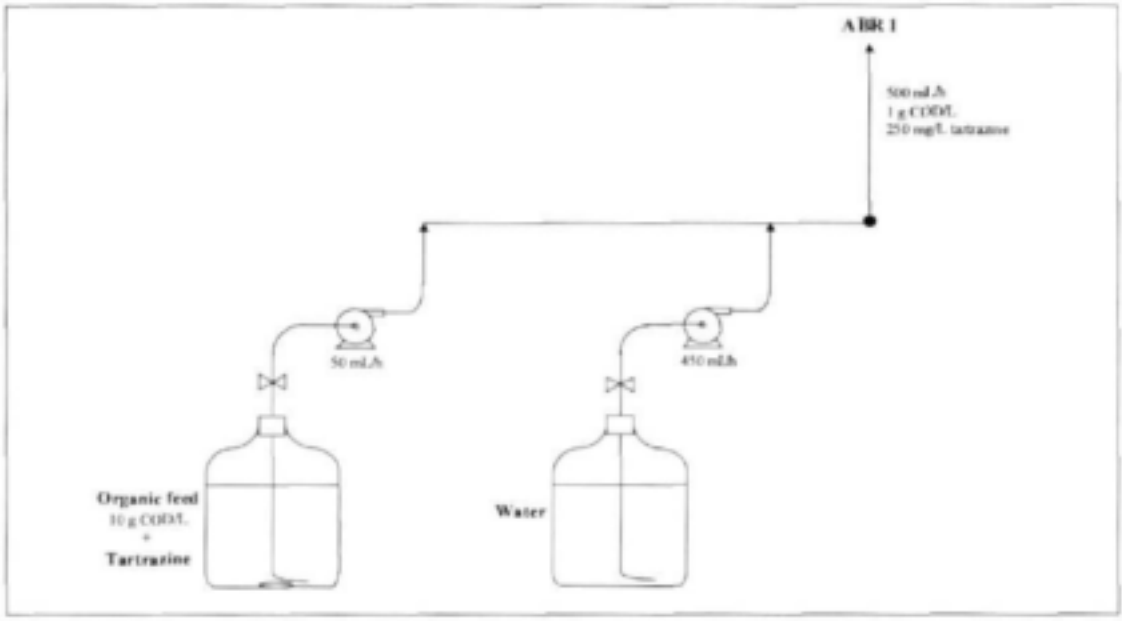


Figure 4-4 : Schematic diagram of the experimental layout

A standard feed solution was made up, as described in Bell, 2002. The feed and nutrients were autoclaved for 40 min at 110 °C (Astell Hearson Model AAB002 Autoclave). Feeding began, with the standard feed solution, at an organic loading rate of 1 g COD/L and an HRT of 40 h. The flowrate were gradually changed with a stepwise decrease in the HRT from 40 h to 35, 30, 25 and then 20 h. The HRT was maintained at 20 h and the reactors were run for approximately 2 months with the sugar/protein feed, at an organic load of 1 g COD/L.

Once the reactors had reached steady state, tartrazine dye was added to the feed solution of ABR 1. The dye powder (15 g) was diluted in 6 L of the sterilised feed solution (concentration of 10 g COD/L). The feed was diluted 10x by distilled water, such that the feed to the reactor contained a dye concentration of 250 mg/L. The organic load to the reactor was 1 g COD/L. The feed was concentrated 10x so that it would stay fresh longer at ambient temperature, and to prevent daily preparation which was time consuming.

4.1.3 Analytical Methods

Two to 3 times per week, samples were taken from the ABR and analysed. Each compartment was sampled (15 mL), through the sampling ports in the lid. Sampling began at the U-tube (effluent), and worked through the reactor, finishing at compartment 1. The samples were drawn through a long stainless steel needle and syringe, which were first used to mix the compartment contents by repeatedly plunging the syringe. When TSS and VSS were measured, larger sample volumes were taken. The samples were immediately sealed in centrifuge tubes. The pH of each was measured. The samples were then centrifuged (Heraeus Biofuge Stratos) at 10 000 rpm, for 15 min at 20 °C. The supernatant liquor was filtered through 0.45 µm filters (Sartorius) into plastic vials and sealed. This liquid was used to analyse COD, TOC, VFAs, and colour. The analytical techniques are detailed in Bell, 2002. The remaining sample supernatants were acidified and stored at –20 °C, for future reference.

Biogas samples (1 mL) were taken from the headspace of each compartment and injected into a GC, for composition analysis (Bell, 2002).

4.1.4 Fluorescent In Situ Hybridisation

Periodically, samples were taken from each compartment of the ABR and probed to identify the microbial populations, the predominant populations and microbial changes, or shifts in population, with time. The samples were fixed and probed according to the method outlined in Bell, 2003. A range of fluorescent in situ hybridisation (FISH) oligonucleotide probes, specific for the alpha, beta, and gamma subclasses of the Proteobacteria; for the Cytophaga-Flavobacterium cluster; for the gram-positive bacteria with a high mol%G+C in their DNA; for the sulphate reducing bacteria (SRB), the Bacteroides group, the Archaea and for the Eubacteria were used The oligonucleotide probes and their target groups are listed in Table 4-1.

Table 4-1 : Sequences, target sites and specificities of rRNA-targeted oligonucleotide probes used for whole-cell hybridisation.

Probe	5'-Sequence- 3'	Specificity	Reference
ARC915	GTGCTCCCCCGCCAATTCCT	Archaea	Stahl and Amann, 1991
EUB338	GCTGCCTCCCGTAAGGAGT	Bacteria	Stahl et al., 1989
ALF1b	CGTTCGGYTCTGAGCCAG	Proteobacteria (alpha, delta)	Manz et al. 1992
BET42a	GCCTTCCCACTTCGTTT	Proteobacteria (beta)	Manz et al. 1992
GAM42a	GCCTTCCCACATCGTTT	Proteobacteria (gamma)	Manz et al. 1992
SRB385	CGGCGTCGCTGCGTCAGG	Proteobacteria (delta)	Amann et al., 1990
CF31Ga	TGGTCCGTGTCTCAGTAC	Cytophaga-flavobacteria	Manz et al. 1992

BAC303	CCAATGTGGGGGACCTT	Bacteroides (CFB phylum)	Manz et al. 1992
HGC6Ga	TATAGTTACCACCGCCGT	High mol%G+C gram-pos.	Roller et al., 1994
LGC354	TGGAAGATTCCCTACTGC*	Low mol%G+C gram-pos.	Meier, 1998
LGC354	CGGAAGATTCCCTACTGC		
LGC354	CCGAAGATTCCCTACTGC		

* Probes specific for gram-positive bacteria LGC (not Clostridia and mycoplasma). Made up an equimolar mixture of the three probes.

Since the majority of these groups are classes within the Eubacteria, the samples were probed with the probe for the class of interest, as well as the general EUB338 probe such that counting would give the relative proportion, of the class of interest, of the total Eubacteria. A control was placed in the first well of each slide. E. coli was used as a control for the Eubacteria. When probing for the Archaea, the Eubacteria were also probed to give a relative comparison in each of the reactor compartments. DAPI stains were used to identify, and count, all living cells as this stain is specific for the DNA

4.1.5 Results and Discussion

The experimental results are presented and discussed in two parts: results of reactor analyses, i.e., assessment of the tartrazine degradation; and results of the probing experiments, describing the observed population dynamics.

Tartrazine Degradation : Tartrazine was added to the feed after 68 d of operation of the reactor (indicated by the arrow on the graph). The pH (Figure 4-5) was not greatly affected by the change in feed, except that the pH in compartment 1 increased slightly. The pH of compartment 1 was observed to increase slightly after the addition of the tartrazine. This suggested that the tartrazine might have been slightly inhibitory to the acidogens since pH increased. However, the pH increase was very slight, Eubacterial activity was detected by probing techniques and the reactor efficiency was not affected, therefore, this observed increase in pH was not thought to be significant.

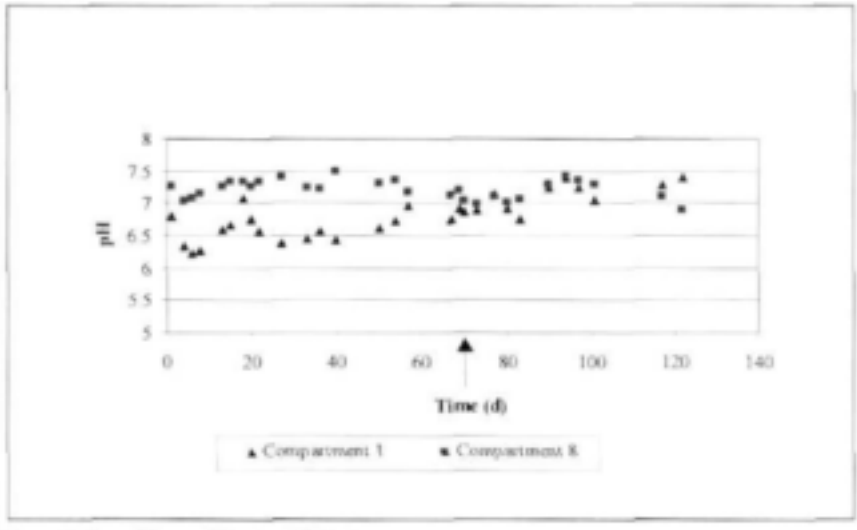


Figure 4-5 : Plot of the pH profiles in ABR 1

Total suspended solids (TSS) and VSS were measured periodically. Results of the measurements are shown in Figure 4-6. The most obvious result is that addition of the tartrazine resulted in washout of solids in compartments 4 and 8. A decrease in TSS and VSS, measured in compartments 4 and 8, after addition of the tartrazine, suggest

washout of the biomass. Biomass activity remained high in Compartment 1. The reason for the washout could have been due to higher gas production in compartment 1, with the additional feed.

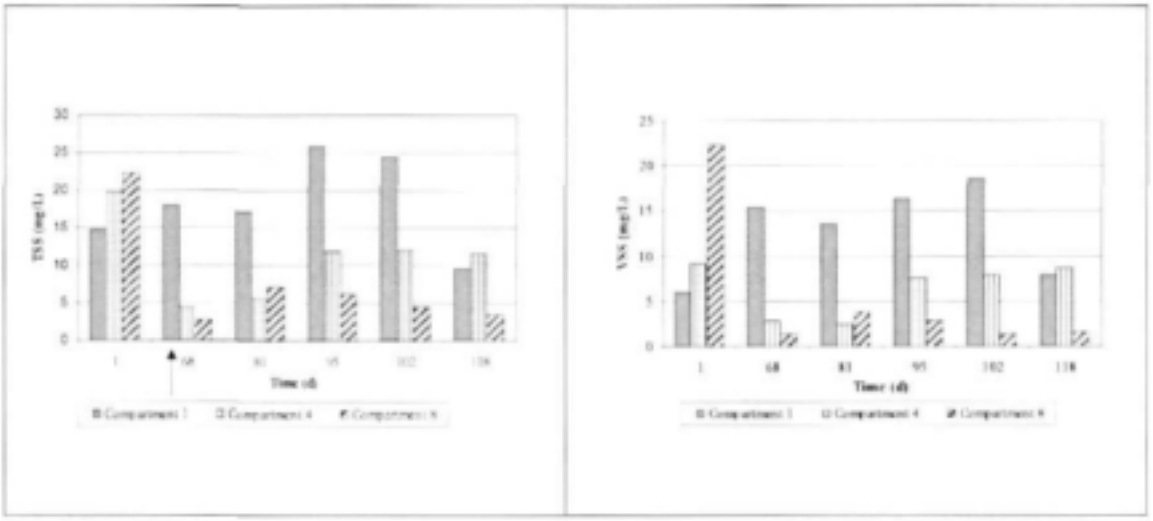


Figure 4-6 : Plots of the TSS and VSS measured in compartments 1, 4 and 8 of ABR 1

The reduction in COD was measured during the operation of the reactor. Figure 4-7shows the influent and effluent CODs over time, as well as COD profiles, throughout the reactor, at three different time periods. These results show that COD reduction decreased with addition of the tartrazine to the reactor.

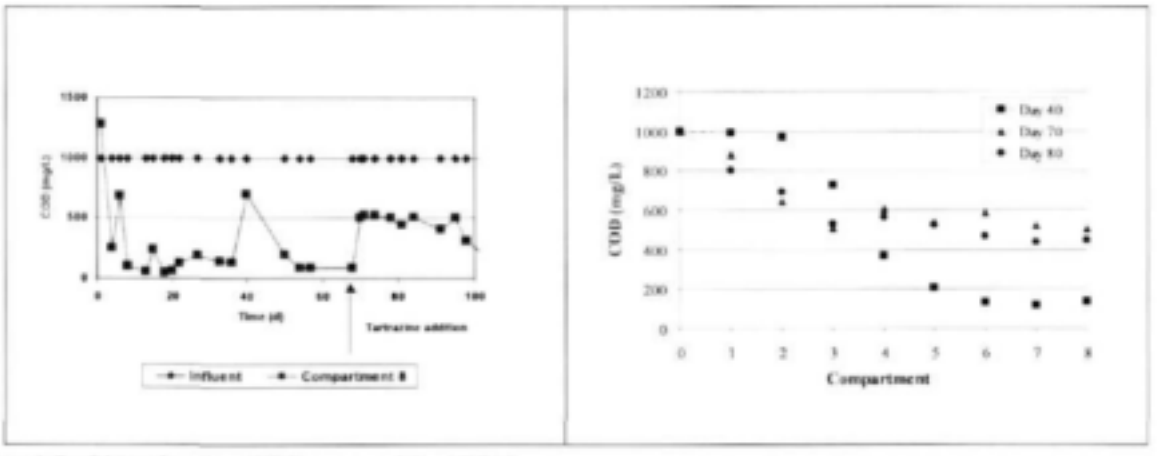


Figure 4-7 : Plots showing COD removal in ABR 1

Reduction in chemical oxygen demand (COD) decreased after addition of the tartrazine (Figure 4-7). The initial fluctuations were during the start-up of the reactor and can be attributed to technical problems with the peristaltic pump, resulting in inaccurate flowrate. These were stabilised and the inlet COD stabilised at 1 000 mg/L. At steady state, COD reduction was between 80 and 95%. The plot showing the COD profile through the reactor (COD in each compartment) illustrates the efficient reduction in COD, before addition of the tartrazine (day 40), where the effluent COD was ca. 100 mg/L. After addition of the tartrazine, the COD profiles fluctuated. The COD in the effluent increased since there was only a 50 to 60% reduction of the inlet COD. These results suggest that the addition of the tartrazine had an inhibitory or negative effect on the micro organisms, resulting in less efficient degradation of the waste. COD reduction did improve with time as can be seen in the comparison profiles for days 70 and 80. It is possible that the micro organisms required a period of acclimation to the dye.

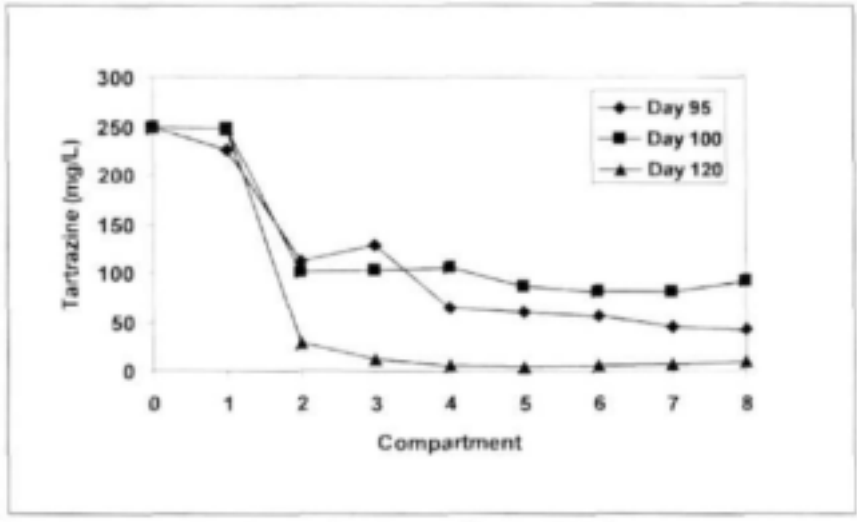


Figure 4-8 : Graph showing the colour reduction profiles in ABR 1

Difficulty was encountered in accurately measuring the colour in the reactor. Although the solution in the reactor was visibly yellow, during the sampling procedure and preparation of the samples for absorbance measurements, the samples tended to change to a maroon/brown colour. The reason for this could be that the degradation products became oxidised, resulting in a colour change. Another explanation could be that degradation products are binding to form another dye structure. Other authors have experienced problems with auto-oxidation during sample preparation (Knapp and Newby, 1995). Huag et al. (1991) overcame this problem by using gas-tight cuvettes for colour measurement. The method was improved and accurate colour measurements obtained. For this reason, results are only provided from day 95. Figure 4-8 shows the colour profiles in the reactor, on three different days. The plot shows increased colour removal with time, suggesting acclimation of the biomass to degradation of the tartrazine. By day 120, the tartrazine concentration in the effluent was down to 12 mg/L. However, the effluent was still highly coloured because even a very small amount of dye in solution results in visible colour. According to the results, most of the colour reduction was achieved in the first compartment of the ABR.

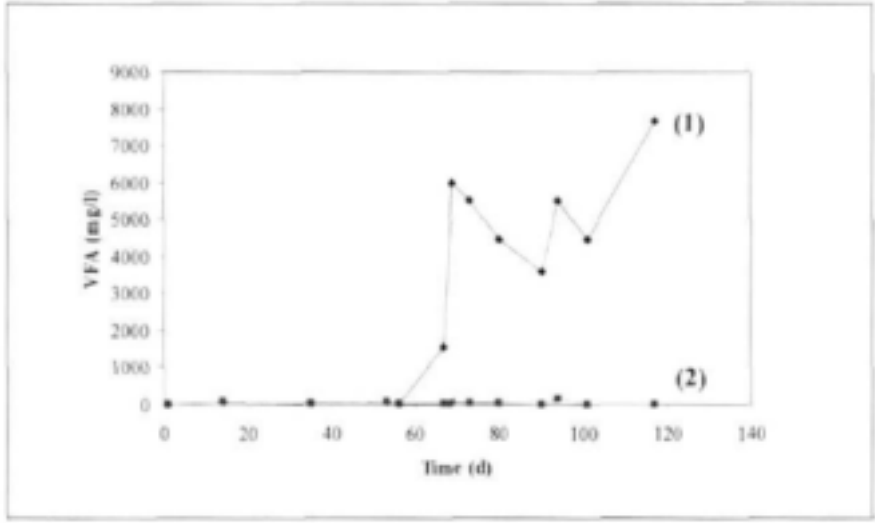


Figure 4-9 : Total volatile fatty acids in the effluent of ABR 1, as a function of time, showing (1) the high concentration as propionate and (2) the high concentration as a dye degradation product

After addition of the tartrazine, high concentrations of VFAs were detected in the reactor, specifically high propionate concentrations. However, since other measured parameters did not indicate VFA accumulation, i.e. the reactor pH did not decrease and reactor performance did not change significantly, it was thought that the tartrazine or its degradation products, which were obviously accumulating in the reactor due to adsorption and slow degradation, was being detected at a retention time similar to that at which propionate is usually detected. Figure 4-9 illustrates the two scenarios. The first is where the high concentration is plotted as propionate and contributes to the total effluent VFA concentration. This results in VFA concentrations greater than 5 000 mg/L, which would not satisfy a COD balance since only 1 000 mg/L COD was being added in the feed. The second scenario plotted shows the total VFA in the effluent, without the measured propionate concentrations. Here, the VFA concentration remains below 150 mg/L and is thought to be more representative of the reactor conditions.

The measured VFA profiles (data not shown) showed that the acetate concentrations in the reactor effluent were below 50 mg/L, thus indicating efficient conversion to methane and carbon dioxide. The propionate profiles showed the significant increase in propionate concentrations after the addition of the tartrazine. These levels remained relatively constant over the reactor and increased with time. If these concentrations were representative of tartrazine it would account for the increasing concentrations since the tartrazine was not readily degraded and towards the end of the test when the tartrazine was degraded, these values may be representative of degradation products or accumulated dye in the biomass. Iso-butyrate was not often detected in the reactor and remained at concentrations below 10 mg/L. The formate profiles showed relatively constant levels throughout the reactor, however, these concentrations were always below 30 mg/L.

Biogas production was monitored throughout the operation of the reactor, particularly for detection of methanogenic activity. The plots in Figure 4-10 show that the methanogens were present throughout the reactor.

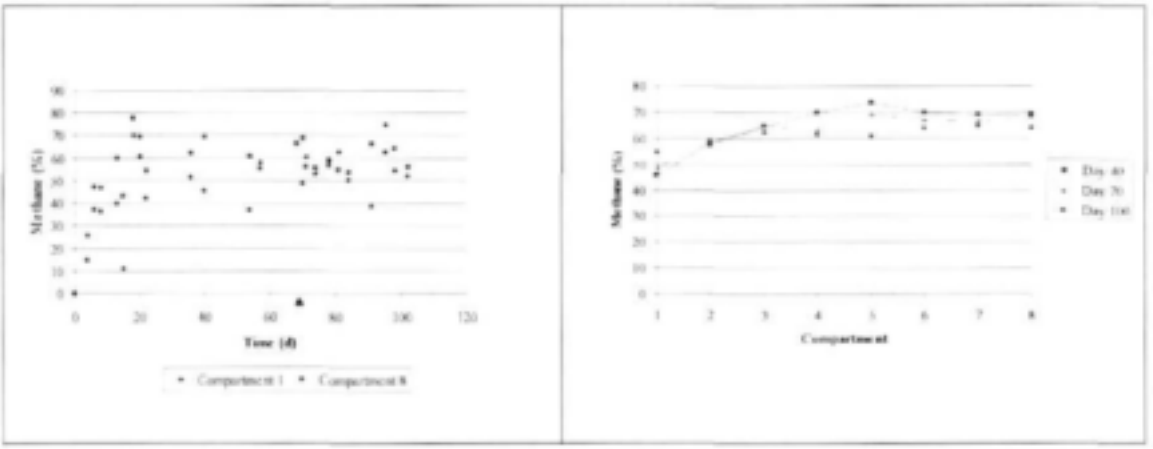


Figure 4-10 : Plots showing the biogas composition in ABR 1

The general assumption of the ABR is that the methanogens predominate towards the back of the reactor, where the VFAs, produced in the first compartments are converted to methane. Biogas monitoring showed that methanogenic activity was present even in the first compartment. This activity can be attributed to readily biodegradable substrates, which are easily broken down and converted to methane. The more recalcitrant compounds, like tartrazine, would be converted much slower, if at all, and account for the methanogenic activity in the last compartments of the reactor. The profiles in Figure 4-10 show that methane production was indeed lower in the first 3 compartments and then increased and remained relatively constant throughout the remainder of the reactor.

From these results, it can be concluded that tartrazine is not readily degraded by anaerobic digestion. In batch experiments, Haug et al. (1991) assessed the degradation of a number of different azo dyes. For tartrazine, 94% of the initial dye remained after 3 d of anaerobic incubation, whereas the majority of the other azo dyes had been decolourised. Glucose was added, resulting in complete metabolism of several of the azo dyes but only 16% of the tartrazine was degraded. These results verify that tartrazine is not readily biodegradable. The increased reduction in COD and colour do, however, suggest some acclimation of the biomass to the dye and indicate the potential for more efficient treatment. The reactor will continue to run on a tartrazine feed to evaluate whether the degradation efficiency improves.

As stated above, the ultimate objective of treating a coloured wastewater cannot be removal of the colour alone since many of the aromatic amines, which could be present in the effluent as degradation products, are extremely toxic. Benzidine is a carcinogenic aromatic amine present in tartrazine. The concentration in tartrazine is limited, by the FDA, to 1 ng/g (Prival et al., 1993). Benzidine contained in tartrazine most commonly originates as an impurity in the sulfanilic acid used for the synthesis of the dye. Aniline is a precursor in the synthesis of sulfanilic acid, and benzidine is a possible oxidation product of aniline. Any benzidine present in the sulfanilic acid could diazotise and couple during manufacture of tartrazine. The reduction in colour, in the ABR, proves that there is degradation of the tartrazine, however, degradation products in the effluent have not yet been identified.

Population Characterisation : Reactor biomass samples were fixed and probed, however, accurate counts could not be taken due to high fluorescence caused by dye molecules present in the samples. The bright areas visible in Figure 4-11 are not actually cells but are thought to be interference caused by the dye.

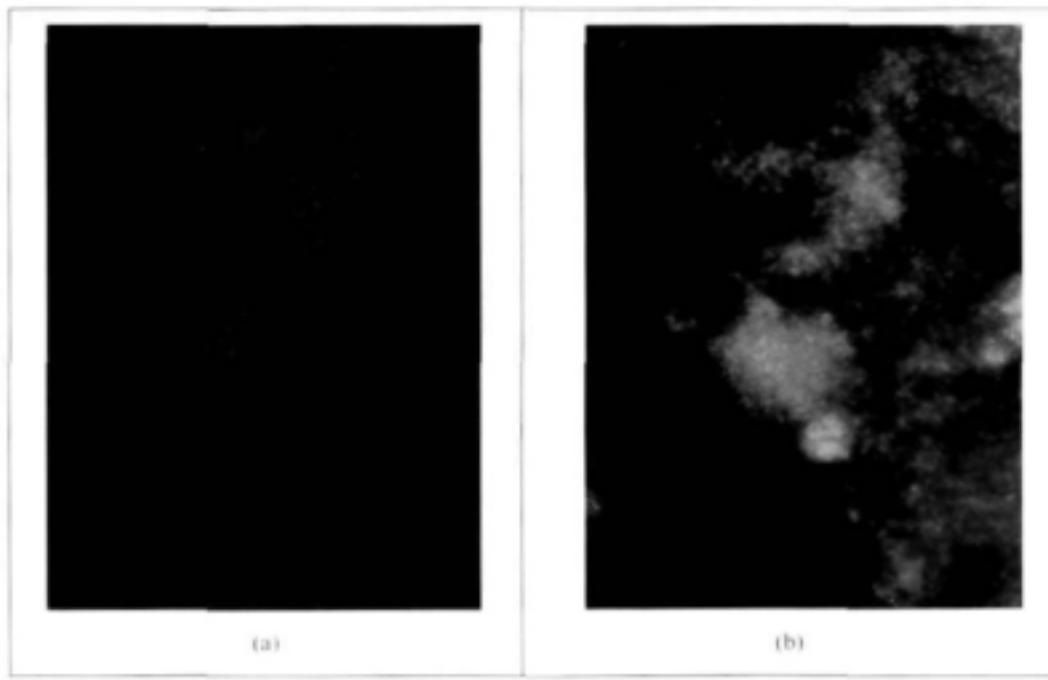


Figure 4-11 : Epi-fluorescence microscopy data of a sample taken from compartment 1 of ABR1, probed with EUB338 (a) and DAPI (DNA) stain (b)

Figure 4-11 (a) is a field of a EUB338-probed sample from compartment 1, of ABR 1. Few cells fluoresce, relative to the DAPI (DNA) stain (b) of the same field. The brightly fluorescing regions in the field are due to interference by the dye.

Probing of samples from ABR 1 resulted in brightly fluorescing objects, making it impossible to focus on, and count, surrounding cells. Initially, it was thought that the bright areas were clumps of dye and because the fluorescent probes were detected at a similar wavelength to the tartrazine, that the presence of the dye was affecting the visibility. To prove this hypothesis and to try and view the cells associated with the dye clusters, samples were DAPI stained but not probed. It was hoped that the dye would still be visible but that the cells associated with them could be detected, due to the DAPI stain, and give some indication of the cell morphology and numbers. However, no bright dye regions were observed. It was therefore, deduced that the bright regions were formed during probing; it is possible that the probe, or the amino linker in the probe label attaches to the dye (has a greater affinity for the dye than for hybridisation to the microbial rRNA) and actually probes the dye, resulting in the brightly fluorescing regions. This hypothesis is substantiated in Figure 4-11 where (a) is a sample probed with EUB338, and (b) is the same field, with the DAPI stain. In Figure 4-11 (a) only a few, very bright regions are visible and no definite cells can be seen, relative to the numbers of cells that are actually present, shown in (b). It is concluded that the tartrazine dye, associated with the biomass interferes with the probe hybridisation resulting in the probes binding to the dye and not to the associated biomass. This has prevented counting and characterisation of microbial populations in the reactor. Possible solutions to the problem are being evaluated.

4.1.6 Conclusions

1. Tartrazine, classified as Colour Index Food Yellow 4, is a monoazo synthetic organic colorant, with a maximum absorbance at 430 nm.
2. Adsorption to anaerobic biomass plays a role in the decolourisation of the dye.
3. Addition of tartrazine to the ABR feed resulted in a 50 to 60% reduction of COD.
4. Colour removal increased with time, suggesting acclimation of the biomass to degradation of the tartrazine. After approximately 2 months, the tartrazine concentration in the effluent was as low as 12 mg/L (95% reduction).
5. Most of the colour reduction was achieved in the first compartment of the reactor.
6. There was methanogenic activity in the first compartment of the ABR.
7. Tartrazine is not readily degraded by anaerobic digestion, however, degradation may be improved with acclimation of the biomass.
8. *It is important that the tartrazine degradation products are identified.*
9. The tartrazine dye, associated with the biomass interferes with probe hybridisation resulting in the probes binding to the dye and not to the biomass.

4.2 DEGRADATION OF AN INDUSTRIAL DYE WASTEWATER

The industrial partner in this experiment was a food dye manufacturer, based in Northumberland, England. The factory operates continuously, producing an average volume of 310 m^3 of effluent per day. The main organic components of the effluent are azo dyes, sub-dyes and unchanged raw materials (dye precursors). The effluent does not contain any major inorganic components. The pH range of the effluent is 7.9 to 8.1. The average salt content is

3% NaCl, sulphate concentrations average 1 040 mg/L and COD, 620 mg/L. As much as 50% of the production of the factory is dedicated to tartrazine production, resulting in high concentrations of this dye, and its precursors, in the final effluent.

Currently the effluent is chemically treated with sodium dithionite, which forms a precipitate, thus removing a significant amount of the colour from the wastewater. The purpose of this treatment is to achieve compliance with the discharge optical consent levels, for discharge to sewer and treatment at a local wastewater treatment works. Chemical treatment is, however, not favourable because of the cost associated with the chemicals, the problem of disposal of the precipitate that is formed, and because the company is still charged high tariffs for effluent discharge, based on volume, organic content and settleable solids.

Due to the wide range of dyestuffs produced at the factory, the composition of the effluent varies considerably depending on which dyes are being synthesised and the amount of washing and cleaning of machinery and pipes. A pre-treatment system, such as an ABR, on-site, has the potential of reducing running costs for the company as it would alleviate the need for chemicals, for chemical treatment, and reduce the discharge tariffs since the wastewater would be more stabilised.

The objective of this experiment was, therefore, to assess the efficiency of the ABR for treatment of the dye effluent.

4.2.1 Experimental Design

An ABR (designated ABR 2) was dedicated to the treatment of the dye effluent. The reactor set-up and starting conditions were the same as those described for ABR 1. The inoculum sludge had a TSS of 27 g/L, of which 19 g/L were VSS, i.e., the sludge was more concentrated than in ABR 1. The reactor was started up in the same manner as detailed above. The experimental set-up for ABR 2 is illustrated in Figure 4-12.

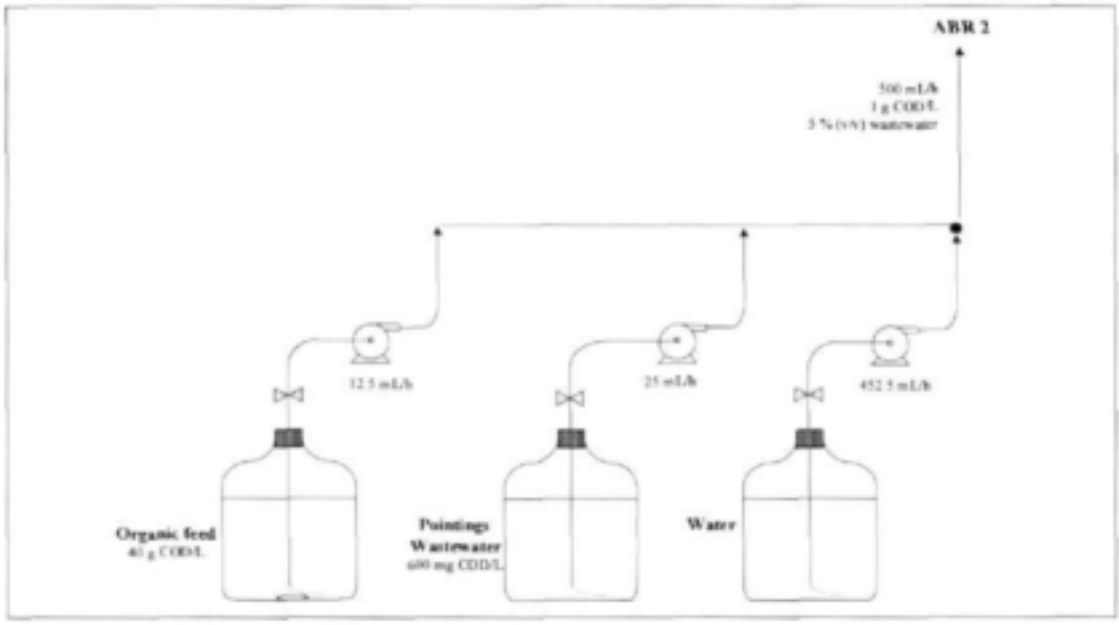


Figure 4-12 : Schematic diagram of the experimental layout

Each of the two feed streams were continuously pumped, by variable speed peristaltic pumps (Watson-Marlow model 101U/R), and diluted by tap water, also pumped by a variable-speed peristaltic pump (model 505s). The three streams combined to form a single feed stream just before the inlet to the reactor.

Once the reactors had reached steady state, the wastewater was added to the feed stream of ABR 2. The anaerobic toxicity assays showed the wastewater to be relatively inhibitory, therefore, feeding commenced with a wastewater concentration of 5% (v/v). The sterilised feed solution (concentration of 40 g COD/L) was diluted with one part wastewater and 37 parts tap water, such that the organic load to the reactor was 1 g COD/L, with an HRT of 20 h. On day 95, the wastewater feed concentration was increased to 10% (v/v).

4.2.2 Analytical Methods

The sampling technique and analytical methods were the same as those described in Section 4.1.3, except for the COD measurement. The high salt content in the wastewater reacted with the COD reagents resulting in the formation of precipitates in the COD tubes and inaccurate optical COD data. To overcome this problem, the total organic carbon was measured (Bell, 2002) to provide an indication of the reduction of organic content in the reactor and the reduction profile, through the compartments.

The maximum absorbance wavelength for the wastewater was 500 nm.

4.2.3 Fluorescent In Situ Hybridisation

Reactor samples were taken, from each compartment, on day 65, day 81 and day 103. The samples were hybridised (Bell, 2002) with the fluorescent-labelled oligonucleotide probes listed in Table 4-1 to identify the microbial populations, the predominant populations and microbial changes, or shifts in population, with time.

4.2.4 Results and Discussion

Degradation of Industrial Dye Wastewater : The wastewater (5% v/v) was added to the feed after 68 d of operation. On day 95, the concentration was increased to 10% (v/v). No significant effects were observed in the pH of the reactor effluent (Figure 4-13), even when the wastewater concentration was increased to 10% (v/v). This suggests that the reactor was not stressed due to the addition of the wastewater. The pH was maintained within the range required for anaerobic digestion.

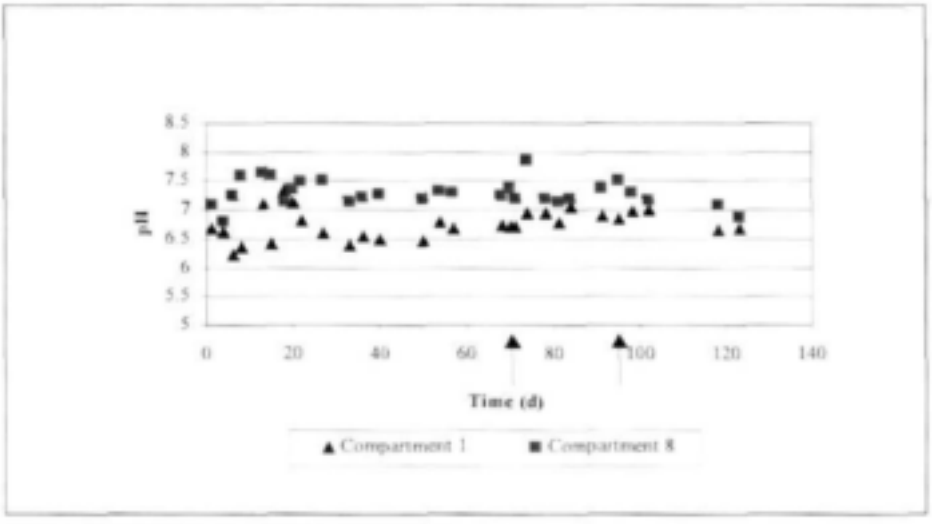


Figure 4-13 : Plot of the pH profiles in ABR 2

Total suspended solids (TSS) and VSS were measured periodically. Results of the measurements are shown in Figure 4-14. The plots show that the amount of solids, in compartment 1, decreased after the addition of the wastewater. There was also a reduction in solids after day 95, when the wastewater concentration was increased to 10% (v/v). This indicates that there was a degree of washout of the biomass. It is common during the start-up of a reactor to experience some biomass loss. This is usually due to the increased activity of the micro organisms, resulting in increased biogas production and subsequent floating and loss of biomass. Foaming was observed in the first 3 compartments, when the wastewater was initially added and then again when the wastewater concentration was increased to 10% (v/v). The biomass washout can, therefore, be attributed to increased gas production, possibly as a stress response to the addition of the new influent or else due to increased metabolism of the readily biodegradable substrates in the influent. This activity settled after approximately 2 d.

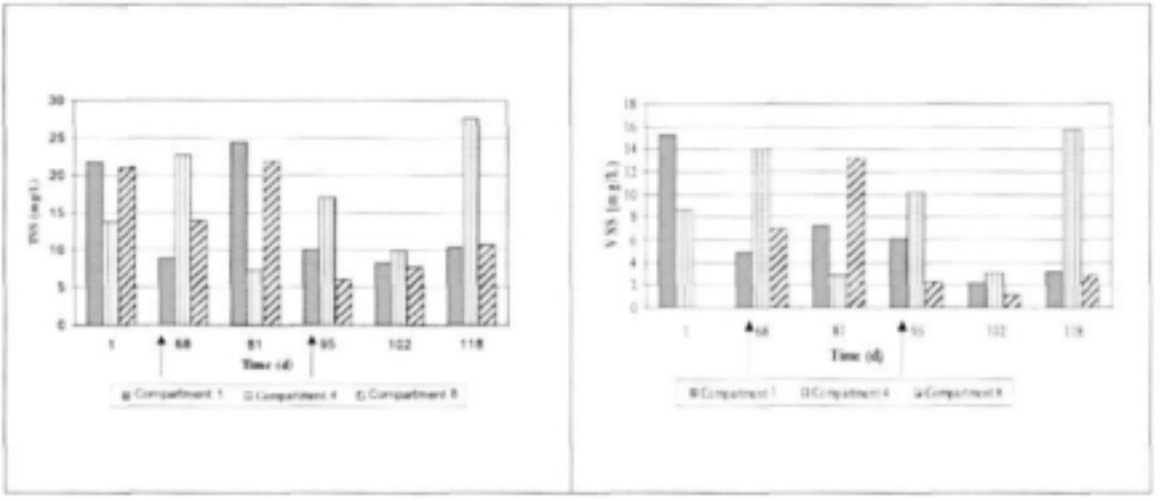


Figure 4-14 : Plots of the TSS and VSS measured in compartments 1, 4 and 8 of ABR 2

The reduction in COD was measured until the addition of the wastewater to the feed stream. The total organic carbon was then measured on the reactor samples. Figure 4-15 shows the initial influent and effluent CODs during start-up of the reactor and during steady state. The reactor COD profiles are also shown, including a profile on

day 70, after the addition of the dye wastewater, showing the inaccurate COD measurements. Figure 4-16 is a plot of the measured TOC reduction during the operation of the reactor.

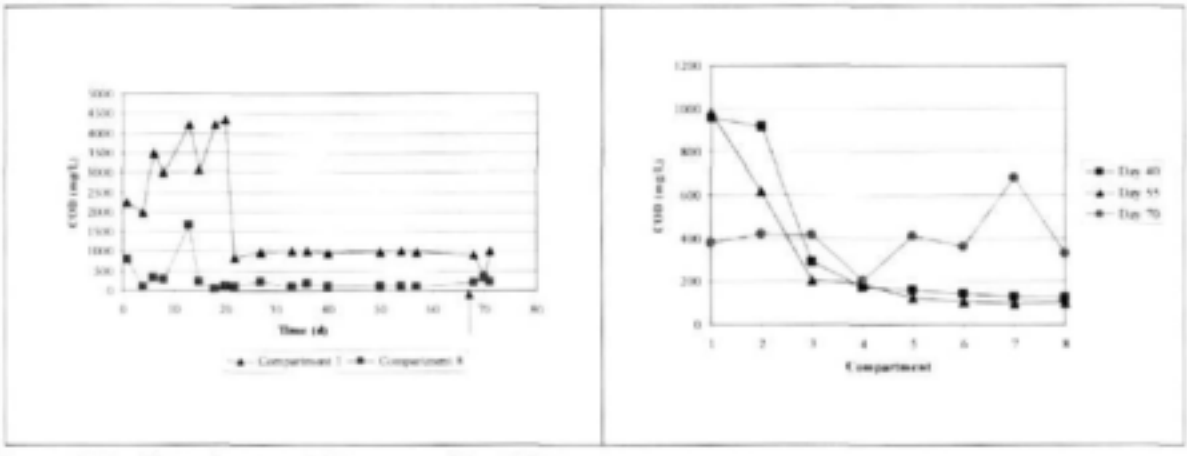


Figure 4-15 : Plots showing COD removal in ABR 2

The inlet and effluent COD fluctuated during the first 20 d of start-up (Figure 4-15). The levels then stabilised and the reactor reached steady state, with a consistent COD reduction of ca. 90%. The reactor profiles in Figure 4-15show that the majority of the COD was degraded in the first 4 compartments of the ABR. The plot includes a profile for day 70, which was 2 days after the addition of the 5% (v/v) wastewater concentration. The fluctuation of this profile indicates the inaccuracy of the COD measurements caused by the high salt concentration in the wastewater. The salt was thought to react with the COD reagents, resulting in precipitates forming. The organic content was then determined by measurement of the TOC in the reactor samples (Figure 4-16). The reduction in TOC was consistent between 70 and 80%.

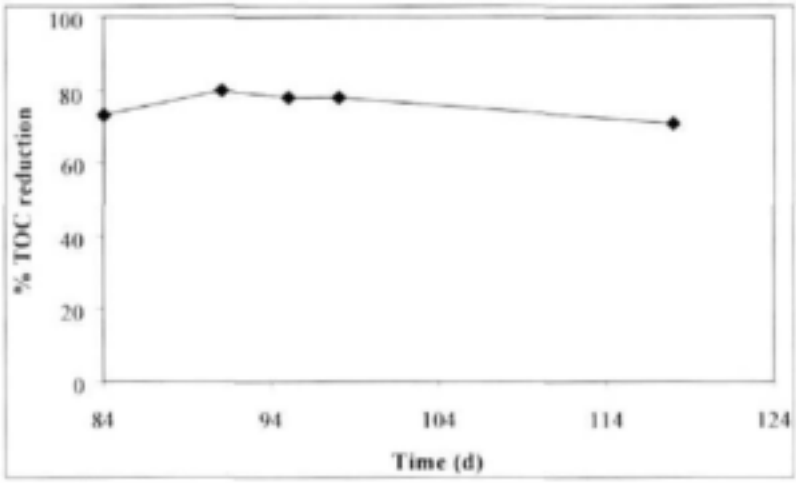


Figure 4-16 : Plot of the measured total organic carbon (TOC) reduction in ABR 2

The colour reduction measurements are shown in Figure 4-17. A colour calibration curve was plotted for the wastewater, however, due to the variability in the composition of the wastewater, it was found that this curve was not always an accurate estimation of the wastewater concentration, thus, colour reduction was plotted as a reduction in absorbance and not as a concentration. The results in Figure 4-17 show that the colour reduction in the ABR was significant and that most of the reduction occurred in the first compartment. The profile on day 120 indicates an

increase in the effluent colour. The reason for this was that that wastewater sample, received from the factory, had a distinct green colour, caused by a high concentration of a particular dye that was obviously recalcitrant since the green hue was still evident in the reactor effluent. This illustrates the difficulty in treating real industrial wastewaters, because of their variability. The biomass would need time to acclimate to the specific dye.

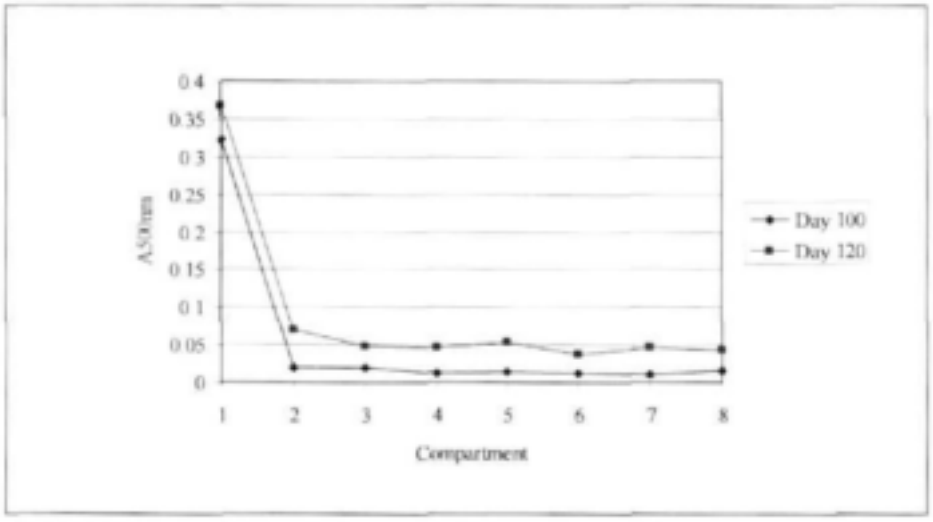


Figure 4-17 : Graph showing the colour reduction profiles in ABR 2

The total volatile fatty acids measured in the effluent of ABR 2 are plotted in Figure 4-18. As in the results for ABR 1, the data labelled (1) represents the VFA levels incorporating the high propionate concentrations. Data (2) assumes that the detected VFA is not actually propionate, but may be tartrazine or one of its degradation products. With this assumption, effluent VFA levels remained below 200 mg/L.

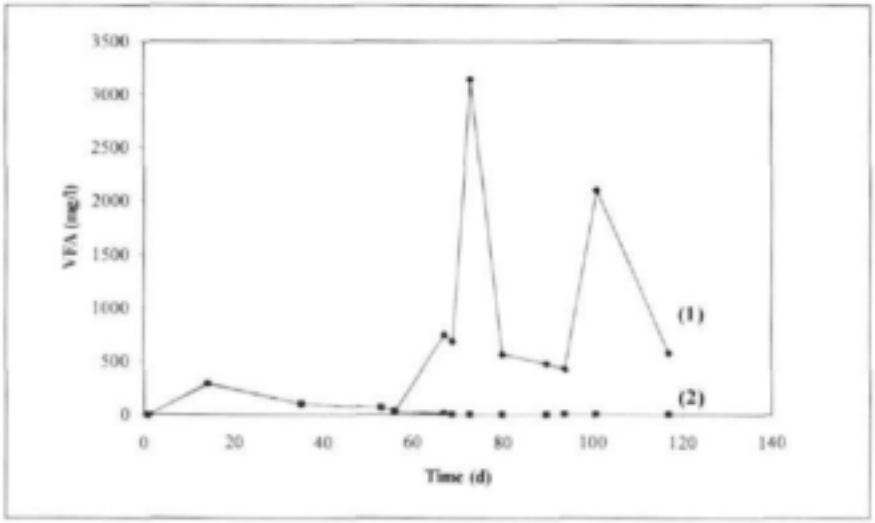


Figure 4-18 : Plot of the total volatile fatty acids in the effluent of ABR 2, as a function of time, showing (1) the high concentration as propionate and (2) the high concentration as a dye degradation product

Since tartrazine is a major component of the industrial effluent, the same explanation is given for the high VFA concentrations measured in these samples. The apparent propionate concentrations increased with the addition of the wastewater to the reactor. Again, other operating parameters did not support the accumulation of VFAs in the

reactor, thus, it was thought that the tartrazine, or a dye degradation product, had been detected on the HPLC column at a similar residence time to that at which propionate is usually detected. The total VFA concentrations were much lower, ca. 3 000 mg/L, than those in ABR 1 which can be explained by the fact that less tartrazine was being fed to the reactor because it was diluted by the other effluent components. The plot in Figure 4-18, where the high propionate concentrations are omitted from the final effluent VFA concentration, then the highest measured VFA concentration in the effluent was 200 mg/L. These results suggest efficient operation of the reactor.

Biogas production was monitored throughout the operation of the reactor, particularly for detection of methanogenic activity. The data in Figure 4-19 show that the methanogens were present throughout the reactor and that methanogenic activity increased with increasing concentrations of the wastewater. Evidence of methanogenic activity is critical in an anaerobic reactor because it proves that the reactor is running efficiently, that there is no accumulation of acids and that the organics being fed to the reactor are being mineralised to methane and carbon dioxide. The profiles of methane production, in each compartment, show that before the addition of the wastewater (day 40), methanogenic activity was lower in the first compartments of the reactor. However, after addition of the wastewater, methane production increased and remained relatively constant throughout the reactor. The production of methane increased further, up to 70%, when the wastewater concentration in the feed was increased to 10% (v/v). These results suggest that the effluent contained readily biodegradable substrates resulting in methane production throughout the reactor. The presence of methanogens in each compartment was verified by the population characterisation studies.

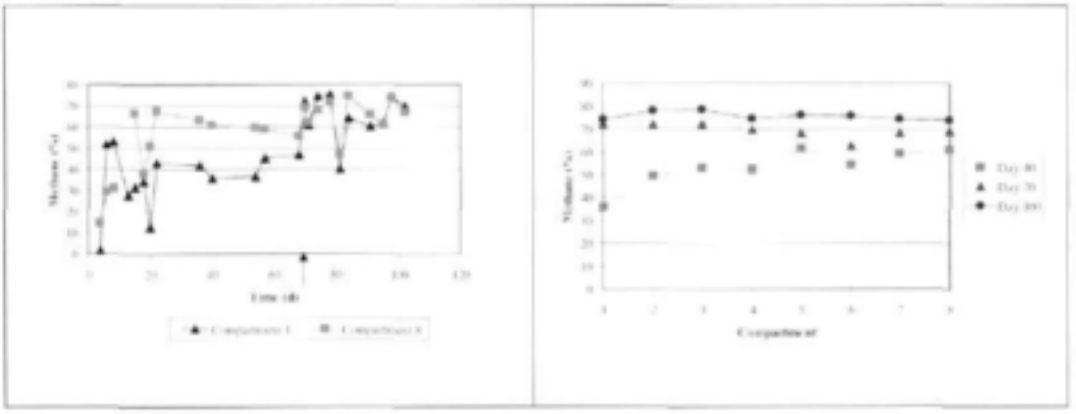


Figure 4-19 : Plots showing the biogas composition in ABR 2

The results of this experiment verified that anaerobic digestion has potential as an efficient treatment option for the dye wastewater, contrary to the initial results of the anaerobic toxicity assays. Methanogenic activity was high in the reactor, the organic content of the influent was reduced by ca. 70% and colour was reduced by almost 90%. A potential problem is the variability of the wastewater, which could result in less efficient degradation and variations in the effluent quality.

Population Characterisation : Reactor samples were taken at three different time period during the operation of the ABR. The first set were taken on day 65, which was before the dye wastewater was added to the feed. Subsequent samples were taken on day 81 and day 103, to detect population shifts due to the addition of the wastewater. The wastewater concentration was increased from 5% (v/v) to 10% (v/v) on day 99, thus any immediate population changes would be evident in the samples taken on day 103.

The oligonucleotide probes that were used for whole cell hybridisation are listed in Table 4-1. The complete counts of the gamma subclass of the proteobacteria and the sulphate reducers are shown in the following plots.

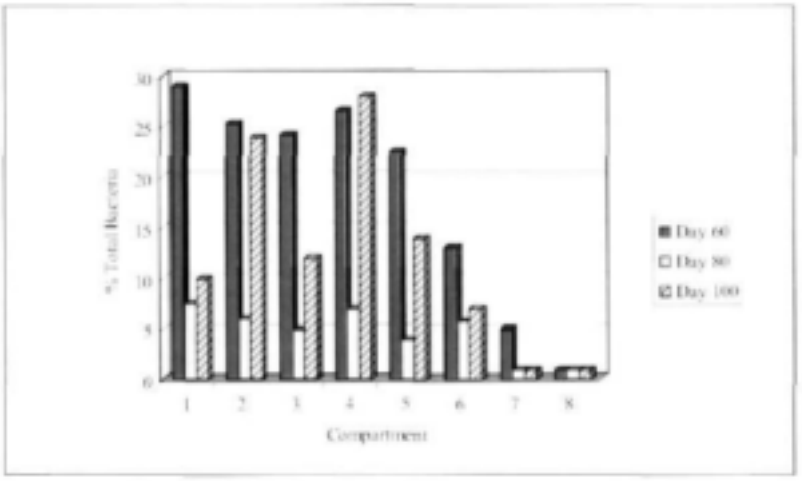


Figure 4-20 : Plot of the proportion of gamma proteobacteria in each compartment of ABR 2, at three time periods

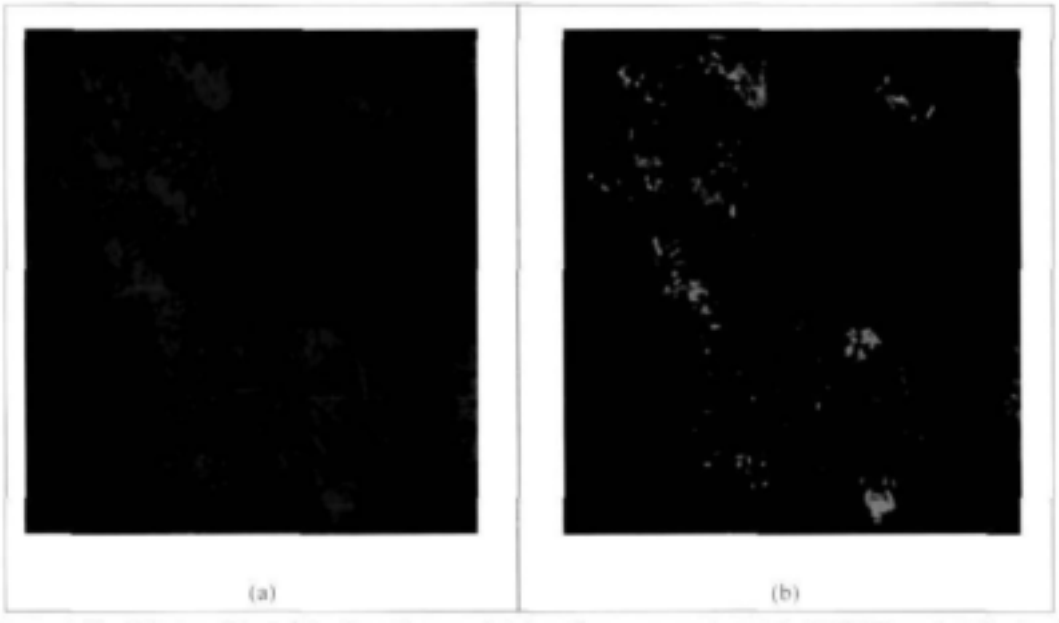


Figure 4-21 : Whole cell hybridisation of a sample taken from compartment 1 of ABR 2 on day 65, showing the same field probed with EUB338 (a) and GAM42a (b)

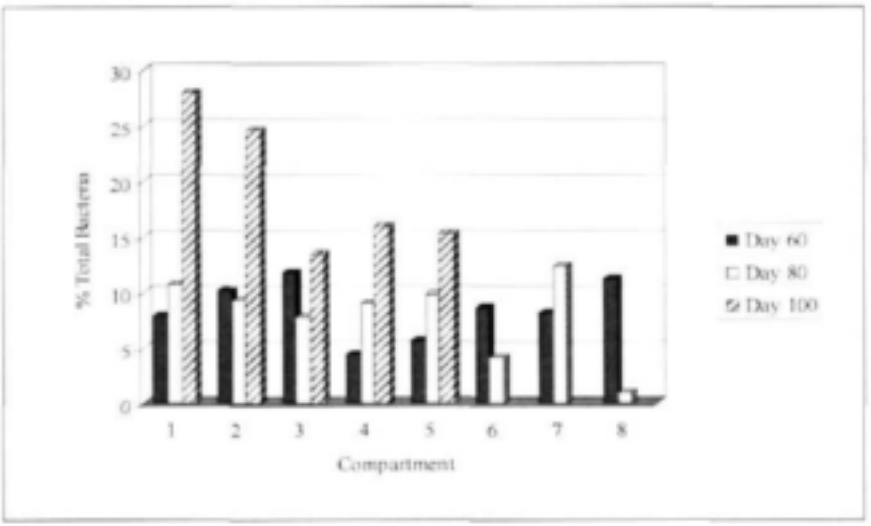


Figure 4-22 : Plot of the proportion of sulphate reducing bacteria in each compartment of ABR 2, at three time periods

The following figure highlights the Methanosarcina species observed in compartment 1 of the reactor.

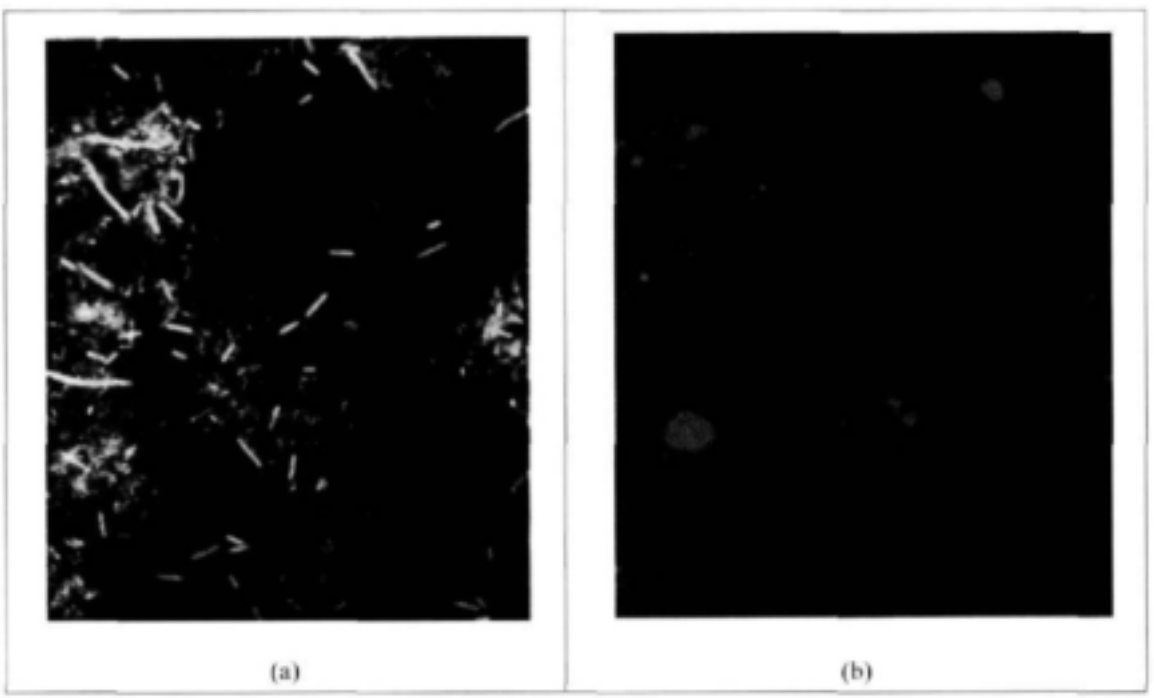
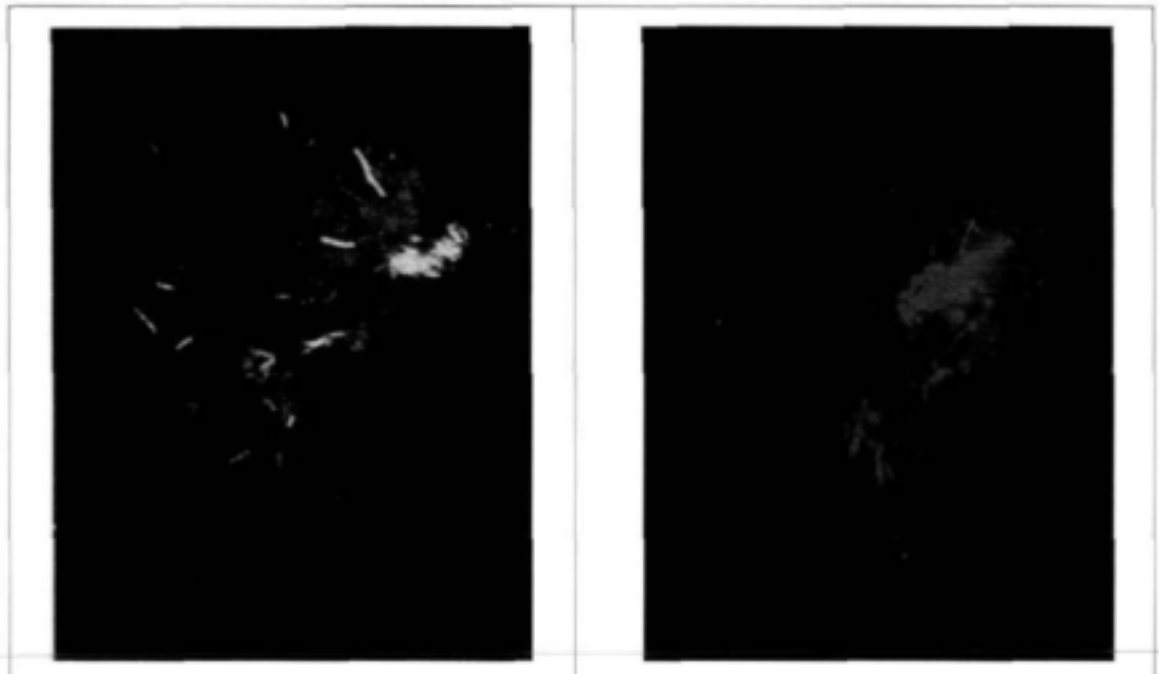


Figure 4-23 : Whole cell hybridisation of a sample taken from compartment 1 of ABR 2 on day 81, showing the same field probed with EUB338 (a) and ARC915 (b)

The following figure shows the morphology of the Methanosaeta species which were more predominant in the later compartments of the reactor.



(a) (b)

Figure 4-24 : Whole cell hybridisation of a sample taken from compartment 2 of ABR 2 on day 103, showing the same field probed with EUB338 (a) and ARC915 (b)

With time, changes in the general morphology of the Eubacteria were observed. They were predominantly rod-shaped, however, the samples taken when the reactor the reactor was being fed 10% (v/v) wastewater were distinctly broader than those observed before the addition of the wastewater. It also appeared that some of the cells had formed endospores, which probed brightly relative to the rest of the cell. This response of some of the microbial populations was attributed to the addition of the wastewater. Eubacteria numbers decreased significantly from compartment 3 to compartment 8 verifying that most of the acido- and acetogenic activity occurred in the first compartments and mostly methanogenesis in the back compartments.

The beta subclass of the proteobacteria were probed in the day 103 samples. The numbers were very low (< 1% of the total population), thus these organisms were not active in the degradation of the wastewater.

Figure 4-20 shows the changes in numbers of the gamma subclass of the proteobacteria. The numbers decreased after addition of the wastewater then seemed to gain in number again after some time of being fed the wastewater. This suggests that the initial response of the micro organisms, to the wastewater, was of inhibition, or inactivity, with a gradual acclimation and activity resumed. Figure 4-21 illustrates the proportion of gamma proteobacteria making up the total eubacteria, in compartment 1 of the ABR. The photograph also shows the rod-shape morphology of the cells.

The SRB385 probe was specific for the sulphate reducing bacteria (SRB); mostly members of the delta subclass of the proteobacteria and a few gram-positive bacteria. The results (Figure 4-22) showed a general increase in the numbers of sulphate reducers in the reactor, with time, after addition of the wastewater. This would be due to the sulphate content of the wastewater, thus the numbers of bacteria would increase with increasing concentrations of

wastewater. The results show the SRB to be active throughout the reactor; they are known competitors of the methanogens and therefore can survive in all compartments.

The Archaea, or methanogens, were observed throughout the reactor. The two populations detected were the Methanosarcina (coccoid clusters) and the Methanosaeta (filaments). The Methanosarcina were dominant in the first few compartments whereas Methanosaeta, although it was observed in the first compartments, was predominant toward the back of the reactor. This spatial arrangement, or separation, can be attributed to the growth kinetics of each. Methanosaeta are known scavengers and are thus able to survive in the back compartments of the reactor, where VFA concentrations would be much lower than in the front compartments, where Methanosarcina, which is a poor scavenger with a K_s of 400 mg/L, is the predominant Archaea population. Figure 4-23 and Figure 4-24 show the relative proportions of Archaea to Eubacteria, in a particular sample. The Methanosarcina can be seen in Figure 4-23, however, the morphology cannot be clearly seen because of the brightness of the probing and the planes of focus. Methanosaeta filaments are illustrated in Figure 4-24.

Hybridisation conditions for a number of the probes needed to be optimised to obtain a result for accurate counting. The BAC303 probe (specific for the bacteroides cluster of the cytophaga-flavobacterium phylum) hybridised to the amino groups in the gelatine coating of the slide. The hybridisation had to be redone without gelatine. With a 20% formamide concentration in the hybridisation buffer, binding of the ALF1b probe was non-specific and accurate counts of the alpha subclass of the proteobacteria could not be obtained. The hybridisation conditions needed to be modified by increasing the amount of formamide and thereby improve the stringency. Non-specificity was also observed with the LGC and CF31Ga probes.

The majority of the colour reduction was achieved in compartment 1. During sampling, a definite change in the consistency of the sludge was observed in this compartment. The sludge became very slimy. This change in consistency is attributed to changes in the microbial populations, in response to the addition of the wastewater. These results indicate definite population shifts in response to the addition of the wastewater. There was selection for the micro organisms best adapted to utilise the wastewater as a substrate and thus facilitate efficient degradation in the reactor. These results verify the importance of molecular studies to facilitate a more holistic understanding of the biological processes occurring within a reactor.

4.2.5 Conclusions

1. The objective of this experiment was to assess the efficiency of the ABR for treatment of the dye effluent.
2. Anaerobic degradation of the wastewater was efficient.
3. Methanogenic activity was high in the reactor, the organic content of the influent was reduced by ca. 70% and colour was reduced by almost 90%.
4. Most of the colour reduction was achieved in compartment 1.
5. Efficient degradation may be dependent on the composition of the wastewater, which is variable and may upset the degradation process.
6. Whole cell oligonucleotide probe hybridisation showed definite shifts in the microbial populations due to the addition of the wastewater to the reactor. The general morphology of the Eubacteria changed as did their numbers, which decreased significantly in the back reactor compartments. Methanogens were

observed throughout the reactor with Methanosarcina clusters dominant toward the front of the reactor and the scavenging Methanosaeata species dominant towards the back.

4.3 DEGRADATION OF A TEXTILE DYE (CI RED 141)

The base study of the labile sucrose feed, showed the applicability of fluorescent *in situ* hybridisation for the characterisation of the evolving microbial populations in the ABR compartments. In this next phase of the investigation, a well-defined and previously researched reactive textile dye, CI Reactive Red 141, was added to the sugar/protein feed to a laboratory-scale reactor. Batch screening tests Indicated that (i) the reactive dyes were not inhibitory to the acidogenic populations; (ii) the biogas production increased with increasing dye concentration which suggests that the dyes were being actively metabolised by the acidogens; (iii) reactive dye compounds, present in textile dye wastewaters, did not adversely affect the anaerobic degradation process; (iv) Methanogenic activity was inhibited by the dye degradation products; and (v) there was no further reduction in COD or colour. The chemical and molecular results from the laboratory-scale ABR treating the synthetic CI Reactive Red 141 waste stream, are presented and discussed.

4.3.1 Treatment in the Anaerobic Baffled Reactor

A laboratory-scale ABR was set up in a constant temperature room at 35 °C. The reactor was seeded with 7.68 L (0.96 L/compartment) of screened digester sludge taken from Umbilo Sewage Works (TS = 28 g/L; VS = 20 g/L). This gave an inoculum of 19.2 g biomass per compartment, or 153.6 g biomass in the reactor. The sludge was allowed to settle for one week before feeding began. The feed connections for CI Reactive Red 141 degradation were set up as illustrated in Figure 4-25

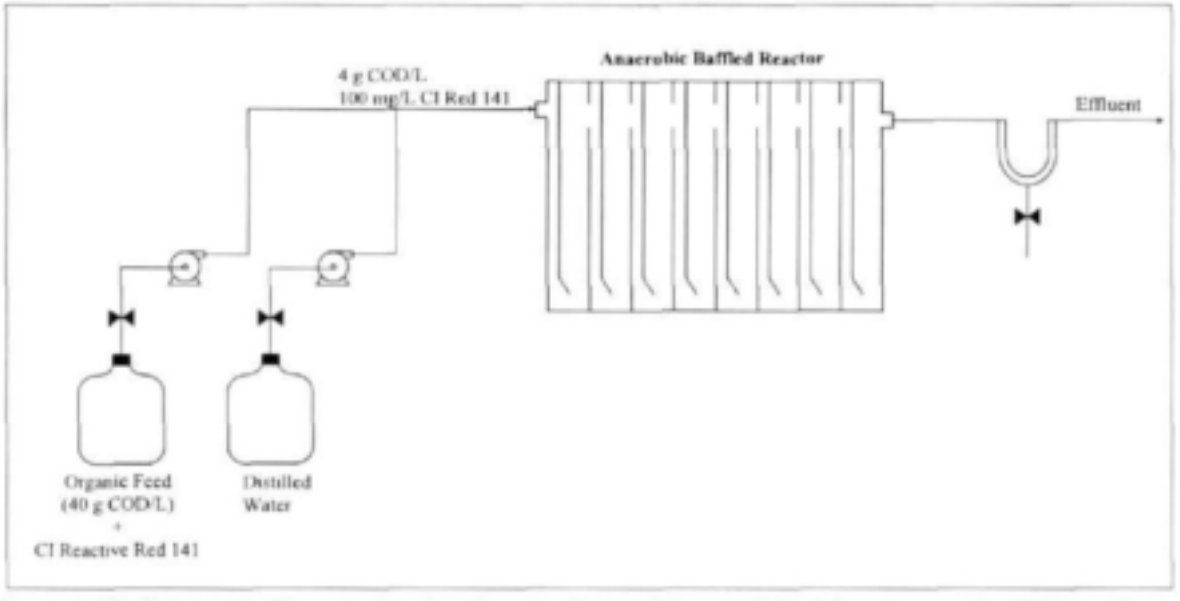


Figure 4-25 : Schematic diagram showing the experimental layout of the laboratory-scale ABR treating a synthetic CI Reactive Red 141 stream (not to scale).

The operating conditions are outlined in Table 4-2. Once the reactor had reached steady state, at a 20 h HRT, the CI Reactive Red 141 dye was added to the feed solution. The dye powder (2 g) was diluted in 2 L of the sterilised feed solution (concentration of 40 g COD/L). The feed was diluted 10x with distilled water, such that the feed to the

reactor contained a dye concentration of 100 mg/L. The COD concentration to the reactor was maintained at 4 g COD/L. To achieve acclimation, the concentration of CI Reactive Red 141 was increased stepwise from 100 mg/L (0.12 g/L.d), to 250 mg/L (0.3 g/L.d) on day 96, to 500 mg/L (0.6 g/L.d) on day 127. Throughout the experimental period, the reactor was supplied with a constant COD loading of 4.8 g COD/L.d of the synthetic feed co-substrate. On day 155, a dye shock load (1 g/L) was fed to the reactor for one HRT. The feed dye concentration was then reduced to 100 mg/L to determine the ability of the reactor to return to stable operation after the shock load.

Table 4-2 : Summary of the operating conditions.

Day	HRT (h)	Organic Loading Rate (g COD/L.d)	CI Reactive Red 141 (mg/L)
1	80	1.2	0
16	60	1.6	0
30	30	3.2	0
50	20	4.8	0
65	20	4.8	100
96	20	4.8	250
127	20	4.8	500
155/156	20	4.8	1 000
156	20	4.8	100

4.3.2 Reactor pH

The measured pH values of compartments 1 and 8 are shown, to assess the effect of the increasing dye concentration on the reactor pH.

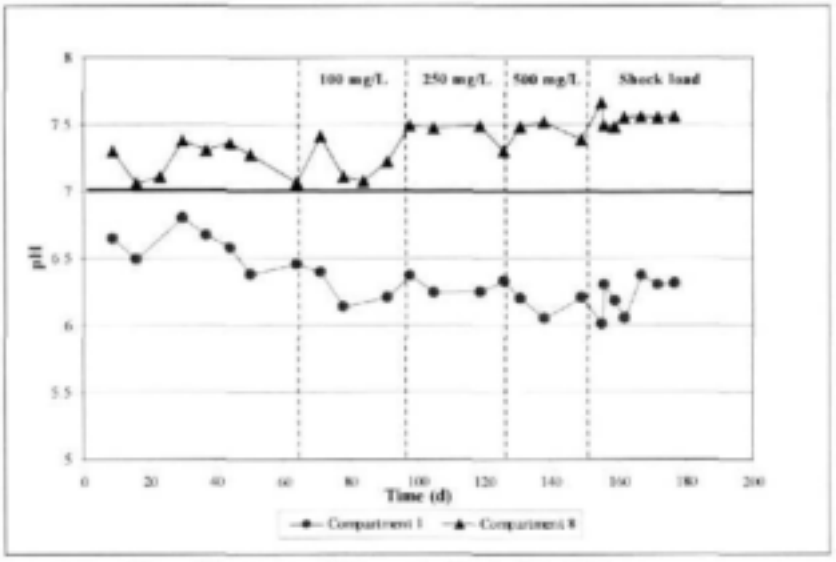


Figure 4-26 : Plot of the pH profiles in the CI Reactive Red 141 ABR.

Analysis of the data indicates that changes in the dye concentration had a slight effect on the reactor pH. These results also illustrate the horizontal separation of acidogenesis and methanogenesis through the ABR. In Figure 4-26 the bold line indicates pH 7 and the changes in dye concentration are indicated as dotted lines.

The pH was quite variable during the start-up of the reactor but stabilised to an average pH of 6.33 in compartment 1 and 7.36 in compartment 8. When the dye was first introduced to the reactor, at a concentration of 100 mg/L on day 65, the pH in compartment 1 dropped to 6.14 and the pH in compartment 8 was variable over 4 data points. The pH in compartment 1 dropped again when the dye concentration was increased to 500 mg/L, however, the pH in compartment 8 was not affected which illustrates the ability of the compartmentalised reactor to protect the more sensitive methanogenic species, in the later compartments, from inhibitory components or concentrations in the feed stream. During the dye shock load, when the dye concentration was increased to 1 g/L for a period of 1 HRT, the pH in compartment 1 was variable but it did not drop below pH 6. The pH in compartment 8 was not affected by the shock load. The dye concentration was reduced to 100 mg/L on day 156 and by day 167 the pH in compartment 1 was stable at ca. pH 6.3. The fact that the pH did not drop below pH 6 with the shock load indicated that the acidogenic process would not have been inhibited and, therefore, the anaerobic digestion process in the ABR would not have been adversely affected by the dye shock load.

4.3.3 Reactor Solids

Figure 4-27 is a plot of the cumulative solids washed out of the reactor. The measure of volatile solids was taken as an indication of the biomass concentration. These results show that there was biomass washout with each change in dye concentration and that the total amount of solids lost was relatively high (3.37 kg of which 1.47 kg were volatile solids). Only ca. 50% of the solids washed out of the reactor were volatile solids.

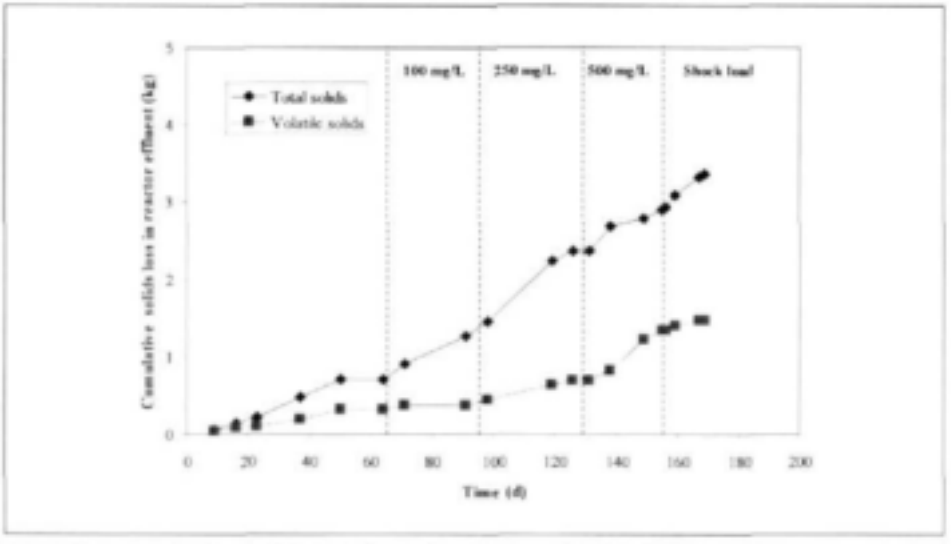
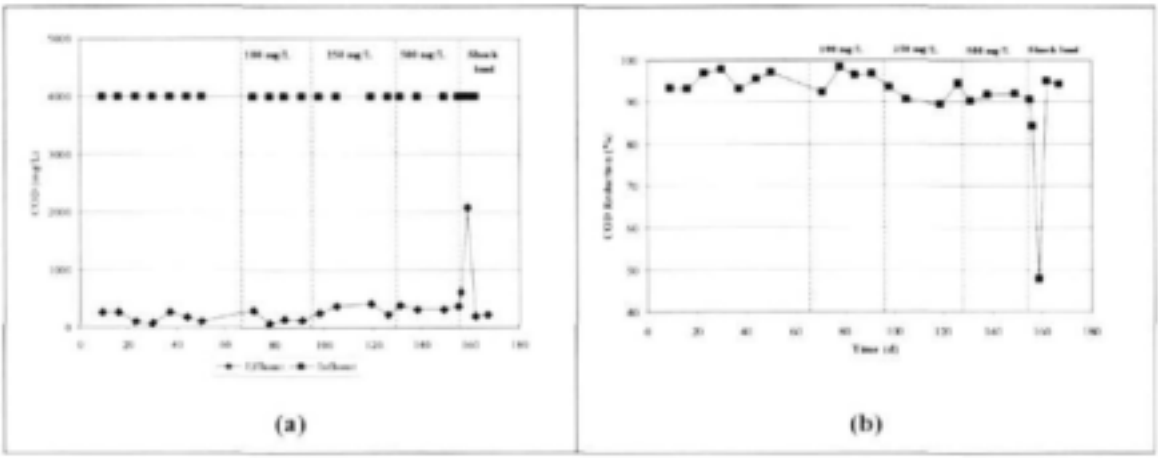


Figure 4-27 : Plot of the cumulative solids lost from the CI Reactive Red 141 ABR

4.3.4 Reactor Chemical Oxygen Demand

Figure 4-28 and Figure 4-29 depicts the soluble COD removed by the reactor over time. The COD removal during start-up, or before the addition of the dye to the feed stream, averaged 95%, or an effluent COD of 189 mg/L.



(a) (b)

Figure 4-28 : Plots showing (a) the influent and effluent CODs and (b) the COD reduction in the laboratory-scale ABR.

CI Reactive Red 141 was added to the feed stream at a concentration of 100 mg/L, on day 65. This resulted in a slight decrease in the COD reduction with an effluent COD of 303 mg/L. The COD reduction stabilised within 3 HRTs to give an effluent COD concentration of 163 mg/L. The average COD reduction for the 250 mg/L dye concentration was 92% and 91% for the 500 mg/L concentration. Thus, there was a slight decrease in the COD removal efficiency with each increase in the dye concentration. The dye shock load (1 g/L CI Reactive Red 141) resulted in a sharp decrease in the COD removal, to 47.8%. It is thought that the high dye concentration caused a temporary inhibition of microbial metabolism, resulting in the substrate and intermediate acids not being completely metabolised. These results correlate with the VFA results. The biomass recovery was almost immediate with an effluent COD of 198 mg/L attained within 3 HRTs of the shock load. Thus, these results indicate that addition of the dye to the ABR feed stream did not have a long-term adverse or inhibitory effect on the anaerobic degradation process, except that the shock load caused a temporary inhibition of the microbial metabolism.

Figure 4-29 shows the COD profiles through the reactor, at different time periods during the experiment. On the plot, compartment 0 represents the reactor feed and compartment 9 represents the reactor effluent. The profiles show that the majority of the COD was reduced in the first three compartments of the reactor, due to the horizontal separation of acidogenesis and methanogenesis. The COD profiles indicate that the COD reduction decreased with each increase in dye concentration. This could be attributed to reduced metabolic activity of the methanogens (degrading the intermediates from the sucrose in the feed) since they were shown to be inhibited by CI Reactive Red 141 and its degradation products. The profiles also show very little COD reduction in the last three compartments of the reactor; this was substantiated by the biogas results and the population characterisation experiments, which showed low metabolic activity in these compartments.

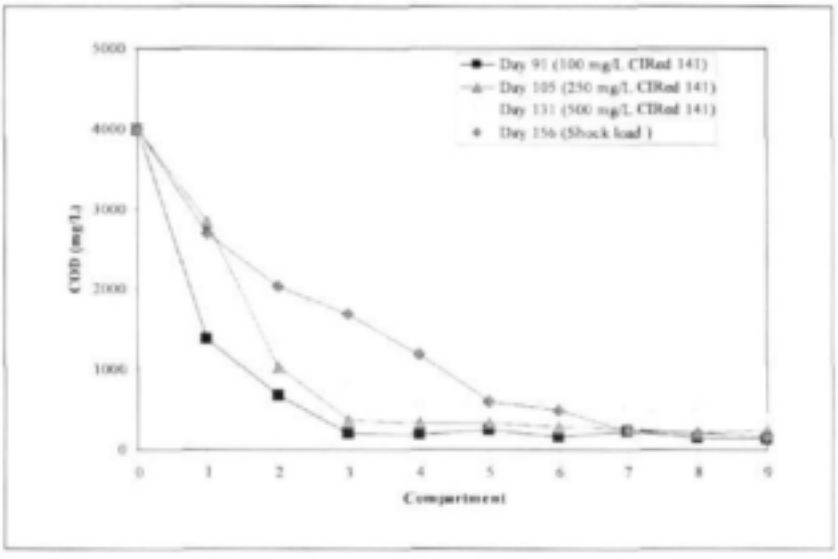


Figure 4-29 : Plot showing the COD profiles through the laboratory-scale reactor at different times during the experimental period.

The general response of anaerobic processes to a shock load can be characterised by an increase in VFAs, a decrease in removal efficiency, a decrease in methane content and higher effluent suspended solids. Consequently, the highest concentration shock load causes the lengthiest deterioration of effluent quality in terms of peak soluble COD and VFA concentrations (Nachaiyasit and Stuckey, 1997a). This increase in soluble COD in the effluent will consist of both unmetabolised VFAs and SMPs. The degree of deterioration in performance depends on the duration and magnitude of the shock and the rate of adaptability of the micro organisms; hence, the function of higher biomass concentrations in anaerobic reactors is usually to enhance their stability rather than improve COD removal (Nachaiyasit and Stuckey, 1997a).

4.3.5 Reactor Colour

The dye concentration in each compartment and in the final effluent was measured by absorbance. The concentration of CI Reactive Red 141 in the influent was increased periodically after at least 20 hydraulic retention times (HRT of 20 h) and when more than 75% removal of the dye and the co-substrate (sucrose in the synthetic feed) COD had been achieved. Sugar, in the form of glucose or sucrose, is deemed a model wastewater substrate necessary for providing the electrons for the reduction of azo compounds (Donlon, Razo-Flores *et al.*, 1997). Sucrose was added to the synthetic feed and acted as a co-substrate for the azo dye reduction. Figure 4-30 shows the colour reduction with time. The dotted lines indicate the changes in dye concentration in the feed.

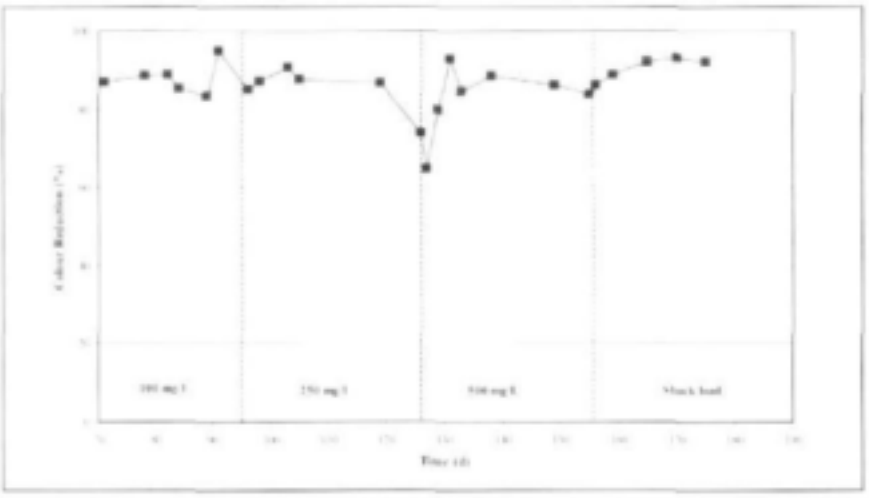


Figure 4-30 : Plot showing the colour reduction achieved in the laboratory-scale ABR treating a synthetic C1 Reactive Red 141 waste stream

The colour reduction averaged 87% with a 100 mg/L dye concentration in the feed and 85% for the 250 mg/L concentration. The colour removal dropped to 65% when the dye concentration was increased to 500 mg/L (day 127). This was the lowest colour removal achieved throughout the duration of the test, and had increased to 80% within one HRT. The dye shock load of 1 g/L did not inhibit the anaerobic process and a colour reduction of 85% was achieved during the shock load. When the dye concentration was reduced back to 100 mg/L, to assess the ability of the reactor to return to stable operation after the shock load, colour reduction stabilised at ca. 90%. Thus, these results show that colour removal was efficient with an average colour removal of 86% over the whole test period. The minimum dye concentration achieved in the effluent was 5 mg/L, on day 91, however, this concentration is still significant since colour is visible at concentrations ≥ 1 mg/L. The effluent would require further treatment before discharge to a water source. If this colour reduction was achieved by pre-treatment at the factory, farther aerobic reduction of the aromatic amines could be achieved by conventional treatment.

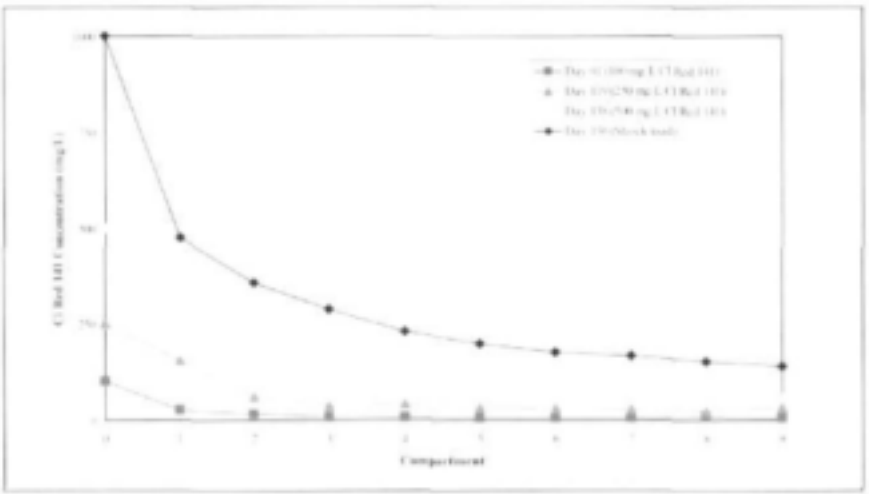


Figure 4-31 : Plot showing the colour reduction profiles in the laboratory-scale ABR treating a synthetic C1 Reactive Red 141 waste stream

The colour reduction profiles (Figure 4-31) show that, for all investigated dye concentrations, at least 50% of the colour was removed in the first two compartments of the reactor. This verifies the theory that in the context of the ABR, and the horizontal separation of acidogenesis and methanogenesis, reduction of the azo bond, with concurrent decolourisation, would occur in the first compartments of the reactor. Therefore, it would be the acidogenic populations that would be exposed to the dye compounds, and the methanogenic populations in the later compartments that would be exposed to the dye degradation products, or intermediates. The toxicity assays showed that the acidogens were not inhibited by CI Reactive Red 141 and this was verified by the significant colour removal achieved in the reactor. The final effluent concentration was < 150 mg/L for all investigated dye concentrations.

The results of the adsorption assays indicated that adsorption would contribute significantly to the decolourisation of the CI Reactive Red 141 waste stream. If decolourisation was only achieved by adsorption, i.e. there was no reduction of the dye molecules, then the biomass would have reached a saturation point where no more dye molecules could be adsorbed and a break-through of dye would have been observed in the reactor effluent. This was not observed, therefore, it was concluded that there was also metabolic reduction of the dye molecules contributing to the decolourisation of the dye waste.

The literature shows that azo reduction exhibits different reduction potentials in different environments. Many redox reactions occur simultaneously in a biological system and the reduction potentials vary for each reaction. Therefore, the reduction potential of the azo bond in a biological system will be the apparent reduction potential of the bulk solution. Many authors have reported non-specific decolourisation of single dyes, mixtures of dyes or real textile effluent, in the presence of anaerobic or facultative mixed cultures acquired from varying sources ((Brown and Laboureur, 1983; Banat et al., 1996) In contrast to pure culture studies, the reduction of dyes by mixed cultures appears independent of the structure of the dye molecule involved. Hence, the bulk of decolourisation would appear to occur extracellularly and be dependent on the redox potential of both the bulk phase and the dye. Carliell *et al.*, (1995) noted that the presence of other electron acceptors in the bulk phase, such as nitrate, caused a lag phase before dye reduction, during which time the nitrate was reduced. This preference was simply explained by the lower redox potential required for reduction of azo dyes as compared with nitrate (Carliell et al., 1995; Wisjnuprapto et al., 2001). The reduction potential was not measured in this investigation. Carliell *et al.* (1995) showed that the redox potential of an anaerobic system decreased from ca. -375 mV (addition of CI Reactive Red 141) to ca. -475 mV by the end of a 5 h decolourisation period (Carliell *et al.*, 1995). Wisjnuprapto *et al.* (2001) also concluded that, in order to achieve colour removal, a redox potential of – 375 mV was required. Although the precise redox potential for optimum decolourisation is not known, it can be concluded that strictly anaerobic conditions are conducive to decolourisation. Bell (1998) found that the rate and extent of decolourisation of CI Reactive Red 141 was affected by the presence of oxygen in the anaerobic system. It was concluded that the presence of oxygen increased the bulk oxidation reduction potential (ORP) of the solution thus inhibiting the reduction of the dye molecule (Bell, 1998).

4.3.6 Reactor Volatile Fatty Acids

Figure 4-32 shows the total VFAs found in the reactor effluent over time. There was an initial VFA peak in the effluent, during start-up, after which the measured VFA concentration in the effluent was constant at 0 mg/L. When the reactor was exposed to the dye shock load, the VFA concentration in the effluent increased to 735 mg/L. It is thought that the high dye concentration caused a temporary inhibition of microbial metabolism, resulting in the

VFAs being present in the effluent. However, recovery was almost immediate with the effluent VFA concentration returning to < 100 mg/L.

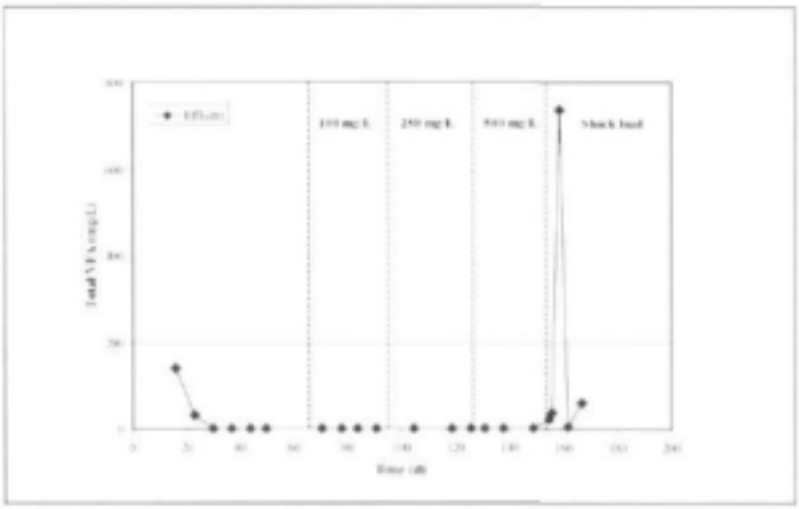


Figure 4-32 : Plot of the total VFAs in the laboratory-scale ABR effluent.

The concentrations of each of the individual acids in compartment 1 and the reactor effluent are presented in Bell, 2003. Consistent with the hypothesis of acidogenesis occurring in the first compartments, there was a relatively high concentrations of acetic acid in compartment 1 and low, or almost negligible, concentrations in the effluent. Similarly, for the other acids, the concentrations were higher in compartment 1 than in the effluent; most were not detected in the effluent.

These results indicate that the addition of CI Reactive Red 141 to the ABR feed stream did not have an adverse effect of the anaerobic digestion process. The only observed response to the dye was when the concentration was increased to 1 g/L, as a dye shock load to the reactor. The results show that the response to this was temporary inhibition but recovery to stable operation within 5 HRTs.

4.3.7 Population Characterisation

Reactor samples were taken, from each compartment, on days 64, 96, 126 and 150 of operation. The samples were hybridised (Bell, 2002) with the fluorescent-labelled oligonucleotide probes listed in Table 4-1 (except MS5 and MB4) to identify the microbial populations, the predominant populations and microbial changes, or shifts in population, with time and in response to changes in the dye concentration.

Initial hybridisations with the universal eubacteria (EUB338) and universal archaea (ARC915) probes revealed an abundance of members of both in the first compartment, at each sampling date. This correlated with the analytical data from the reactor operation, where it was evident that there was methanogenic activity in the first compartments and that the methanogenic activity increased with each increase in dye concentration. The relative ratio of eubacteria to archaea in each compartment, at each dye concentration, was determined and the results are presented in Figure 4-33.

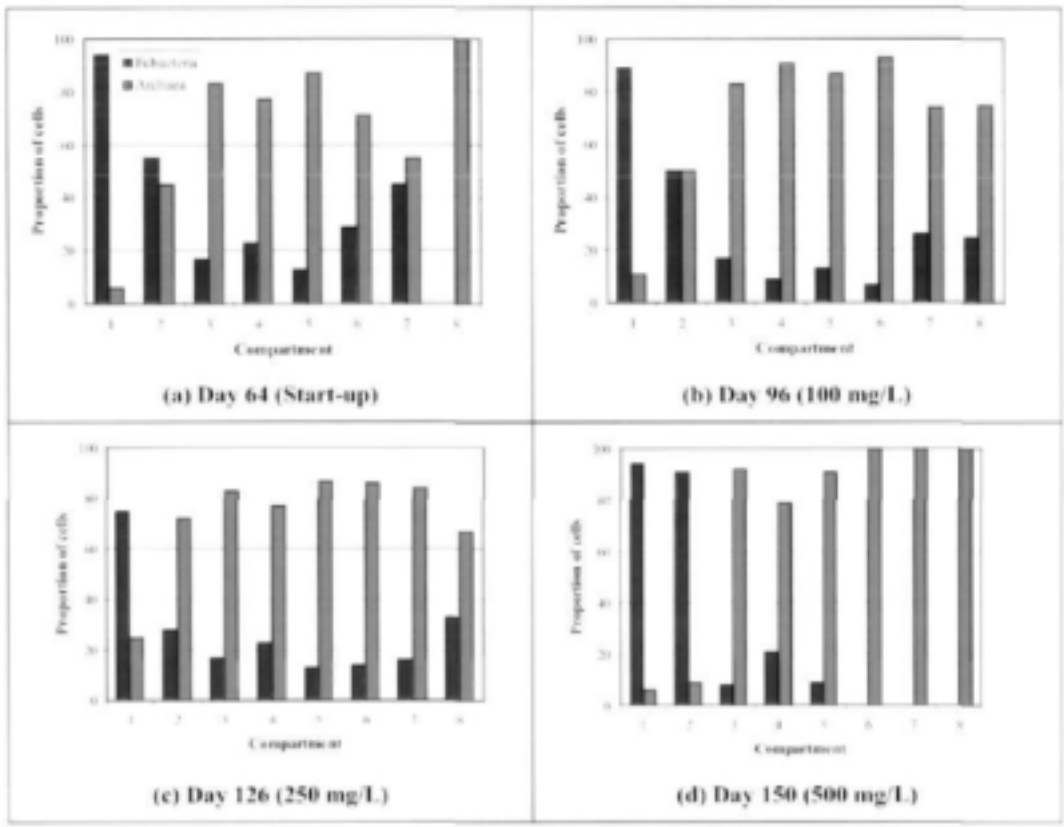


Figure 4-33 : Ratios of Eubacteria (EUB338-hybridised) to Archaea (ARC915-hybridised) in each compartment of the ABR, for each investigated CI Reactive Red 141 concentration

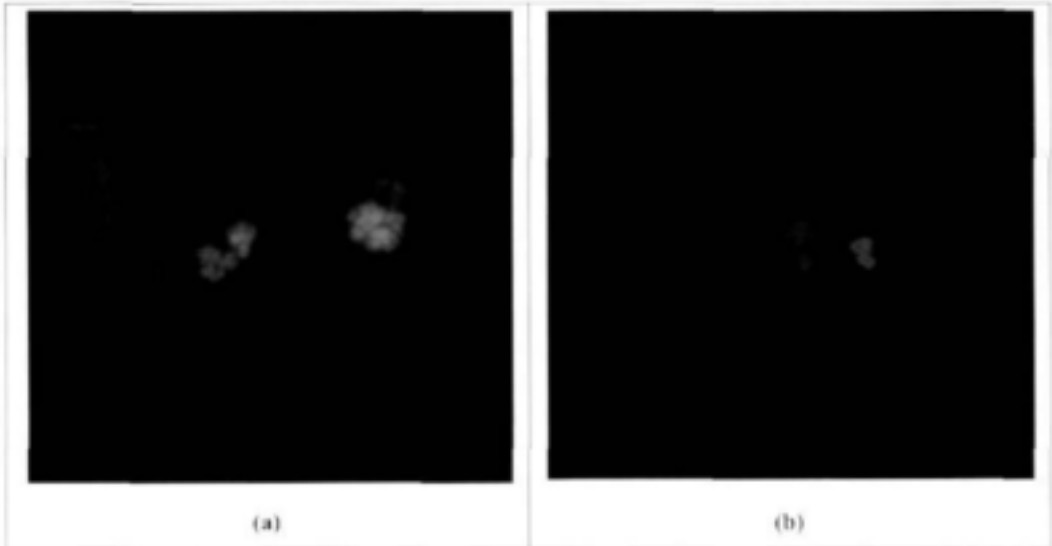
Analysis of samples taken from compartment 4 onwards revealed a decline in the ratio of bacterial cells to archaeal cells. The characteristic morphology of Methanosaeta (long sheathed filaments) was visualised using ARC915, and confirmation of the identity of these filaments using the genus specific probe MX825 was obtained. Another morphotype observed hybridising to ARC915 included Methanospirillum-like shorter filaments, which dominated the archaeal populations in the first four compartments, but did not hybridise with either the MX825 or MG1200 probes. Detailed results for each sample set are given below.

Day 64 (Start-Up) : The samples taken on day 64 were representative of the reactor biomass during start-up. The biomass had been exposed to a stepwise decrease in the HRT, from 80 h to 60, 30, then 20 h, with the synthetic sugar/protein feed. The components of the feed were readily biodegradable, thus there was a predominance of the eubacteria (Figure 4-34) in compartment 1 (94%) and equal populations of eubacteria and archaea in compartment 2. The archaea were the dominant micro organisms making up the microbial populations from compartment 3, through the rest of the reactor.

Figure 4-33 shows a predominance of archaea in compartments 7 and 8, however, the fluorescent signal emitted by these hybridisations was very faint, indicating low metabolic activity. This correlates with the biogas and COD data. All of the archaea in these compartments hybridised with the MX825 probe, i.e. scavenging Methanosaeta spp., which are able to survive at low acetate concentrations.

Figure 4-34 : FISH image of bacterial cells in compartment 1 of the CI Reactive Red 141 ABR, hybridised with EUB338

Figure 4-36 shows the plots of the archaeal community analysis of the ABR compartments 1 to 8, sampled at each investigated dye concentration, showing counts obtained using family- and genus-specific probes expressed as a fraction of the total archaeal counts achieved using probe ARC915. On day 64, 32% of the archaeal population in compartment 1 was made up a short filamentous species. Methanosarcina cells were observed in compartment 2; they made up 28% of the archaea and hybridised with the MS821 probe (Figure4-35).



(a) **(b)**

Figure4-35 : FISH images of *Methanosarcina* cells, from compartment 2 of the CI Reactive Red 141 ABR, hybridised with (a) ARC915 and (b) MS821

The remainder of the compartments, i.e. compartment 3 onwards, were dominated by the MX825-hybridised Methanosaeta spp. No MG1200 hybridisations were observed in these samples.

Day 96 (100 mg/L CI Reactive Red 141) : The biomass samples taken from the reactor on day 96 represented the sludge which had been treating a CI Reactive Red 141 concentration of 100 mg/L for 31 d. Figure 4-33 shows that 89% of the microbial population in compartment 1 was made up of eubacteria; the hybridisations emitted bright fluorescent signals indicating high metabolic activity. These organisms were responsible for the majority of the COD and colour reductions. The relatively low OLR did not require an extended acidogenic phase, thus the microbial population in compartment 2 was composed of ca. 50:50 eubacteria to archaea. The archaea dominated the populations from compartment 3 onwards. Compartments 7 and 8 contained very few, undefined cells. The fluorescent signals emitted by the hybridisations in these compartments were very faint, indicating low metabolic activity, which correlates with the biogas and COD results.

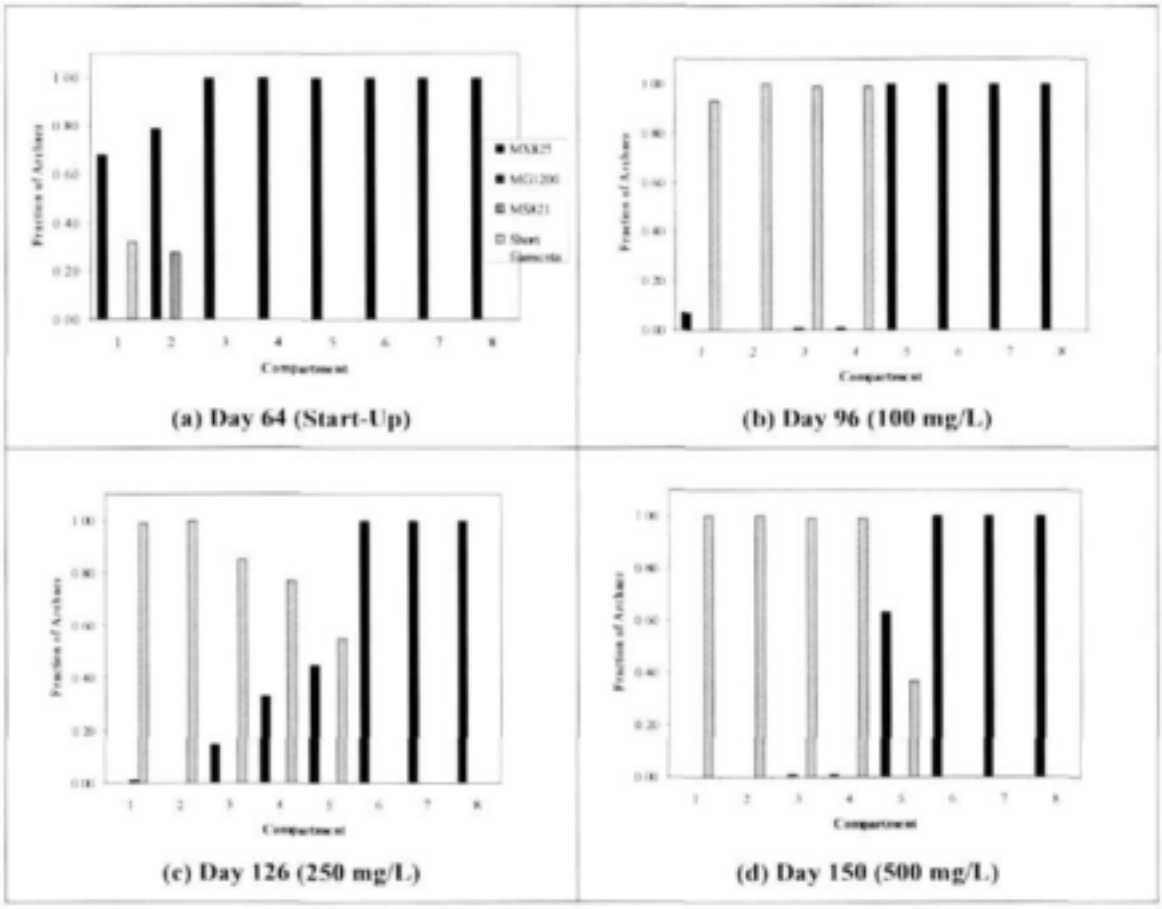
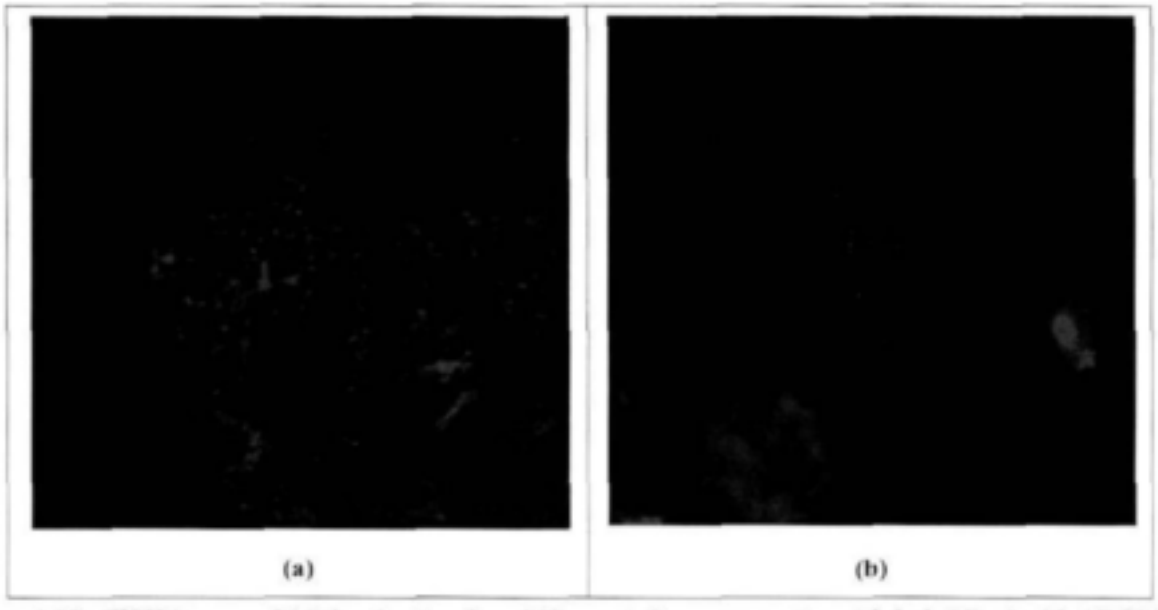


Figure 4-36 : Archaeal community analysis of ABR compartments 1 to 8, sampled at each investigated dye concentration.

Figure 4-36 **(b)** illustrates the composition of the archaeal population on day 96. Compartments 1 to 4 were dominated by a short filamentous species (Figure 4-37 **(a)**). Although these cells were similar in morphology to those observed in **Section 4.1**, they did not hybridise with the *MG1200* probe. It was thought that they were *Methanosaeta* spp., however, they did not hybridise with the MX825 probe either. It was evident that these cells had proliferated due to the addition of the dye. They were not inhibited by the dye or the dye degradation products since the bright fluorescent signals indicated high metabolic activity. These micro organisms would not have been present in the anaerobic toxicity assay sludge; the results suggest that selection occurred due to the addition of the dye. Further identification of these micro organisms would require extraction and sequencing of the DNA.



(a) (b)

Figure 4-37 : FISH images of (a) the short archaeal filaments from compartment 2, hybridised with ARC915 and (b) the long *Methanosaeta* filaments from compartment 6, hybridised with MX825.

A portion (7%) of the archaeal community in compartment 3 hybridised to the MG1200 probe. The *Methanosaeta* spp. dominated the archaeal populations from compartment 5 through the remainder of the reactor. These organisms hybridised with the MX825 probe (Figure 4-37 **(b)**). The metabolic activity of the *Methanosaeta* spp. was relatively low, as can be seen by the faint fluorescent signal emitted in Figure 4-37 **(b)**. This corresponded with the reactor biogas and COD data which showed low methanogenic activity in the final reactor compartments.

Day 126 (250 mg/L CI Reactive Red 141) : The biomass samples taken from the reactor on day 126 represented the sludge which had been treating a CI Reactive Red 141 concentration of 250 mg/L for 30 d. The EUB:ARC ratios in Figure 4-33 show that although the microbial population in compartment 1 was dominated by the eubacteria (75%), there was a larger fraction of archaea in this compartment, compared to previous samples. This was unexpected since the anaerobic toxicity assay showed inhibition of the methanogens by the dye, thus it was expected that increasing the dye concentration would result in lowering the methanogenic activity. However, the biogas results showed increased methanogenic activity in the first compartments of the reactor, with increased dye concentration. The FISH experiments verified this with hybridisation of the short filamentous archaeal micro organism which showed high metabolic activity in compartments 1 to 4. The archaea dominated the communities from compartment 2, through the remainder of the reactor (Figure 4-36 **(c)**). *Methanosarcina* spp., hybridised with MS821, comprised ca. 1% of the archaeal community in compartment 1.

Day 150 (500 mg/l CI Reactive Red 141) : The biomass samples taken from the reactor on day 150 represented the sludge which had been treating a CI Reactive Red 141 concentration of 500 mg/L for 23 d. The eubacteria dominated in compartment 1 (94%) and compartment 2 (91%). The reason for the increased eubacterial activity could be that the methanogens were inhibited by the higher dye concentration, although the biogas results (Figure 4-32) showed an increase in the methanogenic activity with the dye concentration increase to 500 mg/L. The results of the FISH experiments (Figure 4-36 **(d)**) verified these showing that the short filamentous archaeal species was predominant and active in the first four compartments. The EUB338 hybridisations emitted a bright fluorescent signal, indicating that these organisms were metabolically active and obviously involved in the colour reduction of

the dye and COD reduction of the waste stream. The main component of the archaeal community, from compartment 5 onwards was the MX825-hybridised *Methanosaeta* spp. Very few cells were present in compartments 7 and 8 and the MX825 hybridisations in these compartments emitted very faint fluorescent signals, indicating low metabolic activity.

This investigation showed an unexpected result, namely the abundance of the short filamentous archaeal micro organism, which did not hybridise with either the MG1200 or MX825 probes. This species became metabolically active after the addition of the dye to the ABR feed stream, thus, it was deduced that there was selection for the organism. The bright fluorescent signals, together with the measured increased methane production suggest that these organisms were actively involved in the colour reduction of the dye and the COD reduction of the waste stream, in the first four compartments of the reactor. There was a horizontal separation through the reactor of this short filamentous species and the MX825-hybridised *Methanosaeta* spp. Metabolic activity was low in compartments 7 and 8.

4.3.8 Conclusions

1. The results of the physical decolourisation tests suggested significant decolourisation due to adsorption to the biomass, however, it is possible that the dye chromophores were reduced due to the low redox potential environment within the test bottles. No dye break-through, due to adsorption saturation, was observed during operation of the reactor.
2. COD reduction was consistently > 90%, except for the period during the dye shock load. Colour reduction averaged 86%.
3. The biomass showed acclimation to the dye, with increased methanogenic activity with each increase in dye concentration.
4. The inaccurate biogas measurement resulted in the poor COD balance of 18.2%.
5. The reactor operation was stable, even with increases in the dye concentration. The only observed response to the dye was when the concentration was increased to 1 g/L (shock load), resulting in temporary inhibition but recovery to stable operation within 5 HRTs.
6. This investigation has shown that successful treatment of a highly coloured wastewater is possible in the ABR. The design of the ABR facilitates efficient treatment of concentrated textile dye wastewaters by protecting the sensitive methanogens from the inhibitory dye molecules and promoting efficient colour and COD reduction, primarily by the acidogens in the first compartments.
7. Metabolic activity was low in the final three compartments of the reactor.
8. There was a definite shift in the microbial populations through the ABR, with a predominance of eubacteria in the first compartments (acidogenesis) and archaea (methanogenesis) in the later compartments.
9. The use of molecular approaches provided useful descriptions of the methanogens actively involved within each compartment. These results showed a horizontal separation of a short filamentous archaeal micro organism and the long sheathed filamentous Methanosaeta spp.
10. The short filamentous archaeal micro organism proliferated after addition of the dye to the reactor and was metabolically active in the first four compartments of the reactor. Identification of the micro organism will require DNA extraction and sequencing.

11. The application of molecular techniques to the ABR process improved the understanding of the metabolic processes occurring within each compartments and the micro organisms involved in these reactions.

CHAPTER 5
Pilot Plant Design and Performance

One of the original aims of the current project was to design, install and operate a pilot scale ABR to treat an industrial effluent. As discussed in **Section 1.2**, the focus of the research effort subsequently shifted to treatment of wastewater from dense peri-urban settlements and consequently it was decided to test the pilot scale ABR on domestic wastewater. The details of the pilot plant study, include design, commissioning and operation have therefore been presented in WRC Report K5/1248/06 Evaulation of the Anaerobic Baffled Reactor for Sanitation in Dense Peri-urban Settlements (Foxon et al., 2006). This chapter provides an overview of some of the important results obtained which are also relevant to the design and operation of ABRS for industrial wastewater treatment. **Section 5.1** presents the results of a computational fluid dynamics (CFD) analysis of the hydraulic design of the ABR while **Section 5.2** presents a summary of the results obtained from the pilot plant works at Umbilo Wastewater Treatment Plant, which was treating a 50:50 mixture of industrial and domestic wastewater.

5.1 COMPUTATIONAL FLUID DYNAMICS

The pilot reactor design was based on the laboratory-scale reactors. Two aspects of the laboratory-scale reactor were investigated on the CFD program, FLUENT, namely, the position of the baffle in the compartment and the width of the slot between the compartments. When using CFD, it is important to start with the simplest model. Since the reactor is symmetrical along the longitudinal axis and all eight compartments are identical, only half of a single compartment was modelled (Figure 5-1). Since information on the properties of the reactor solids was not available, a single phase model was set-up on FLUENT. The lamina phase model was selected and the properties of water were entered. Surface effects on the gas liquid interface were ignored and a frictionless surface was specified.

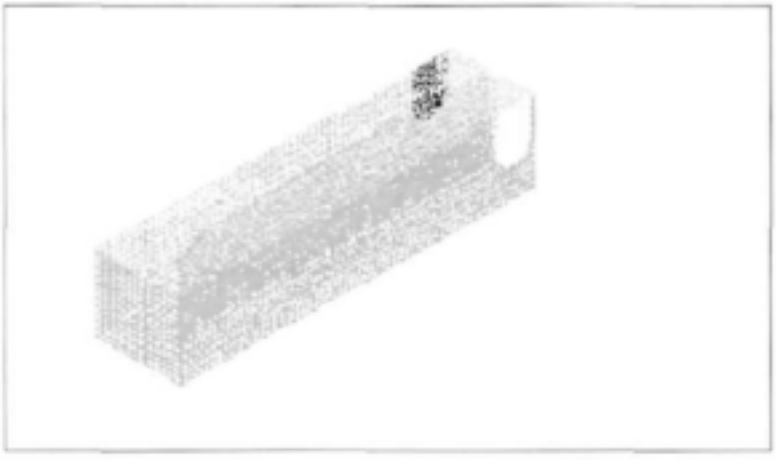


Figure 5-1 : Grid generated on FLUENT to predict flow patterns in the laboratory-scale ABR

5.1.1 Baffle Spacing

Two grids were set-up on the programme PreBFC. The first grid had the baffle in the centre of the compartment, and in the second grid, the baffle was placed such that the up flow to down flow area ratio was 3:1 (Figure 5-2). One of the main features of the ABR is the retention of solids in the system. In order to achieve this, low up flow velocities

need to be maintained in the individual compartments. As expected, a greater surface area for the up flow region resulted in lower up flow velocities. However, increasing the up flow surface area also resulted in greater volume of dead space.

Figure 5-2 : Baffle positioning for the CFD investigations on the ABR

The velocity vector profiles, along a transverse plane, for the two baffle positions are presented in Figure 5-3. The magnitude of the velocity is presented as a function of length, i.e., the longer the arrow, the greater the velocity. A uniform distribution of flow was attained with configuration B. Increasing the up flow area resulted in a further increase in channelling and dead space in the up flow region.

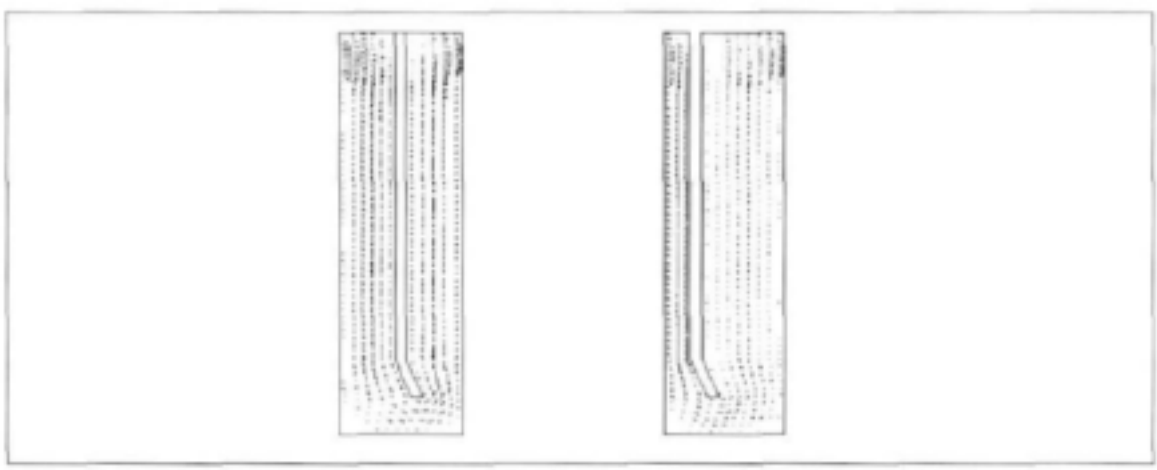


Figure 5-3 : Velocity vector profiles obtained for a 20 h HRT on FLUENT

The height of the baffle above the bottom of the reactor is another important factor to be considered in the design. Flow at this region has to be sufficiently high in order to reduce clogging. Higher velocities can be obtained by reducing the distance between the bottom of the baffle and the reactor bottom. Very low areas would, however, also promote clogging.

A CFD model of a compartment in the pilot-scale reactor was also generated on FLUENT. The straight baffle configuration was compared with an angled baffle system. It was found that the angled baffle resulted in better flow patterns and a reduced dead space. The results are presented in Figure 5-4.

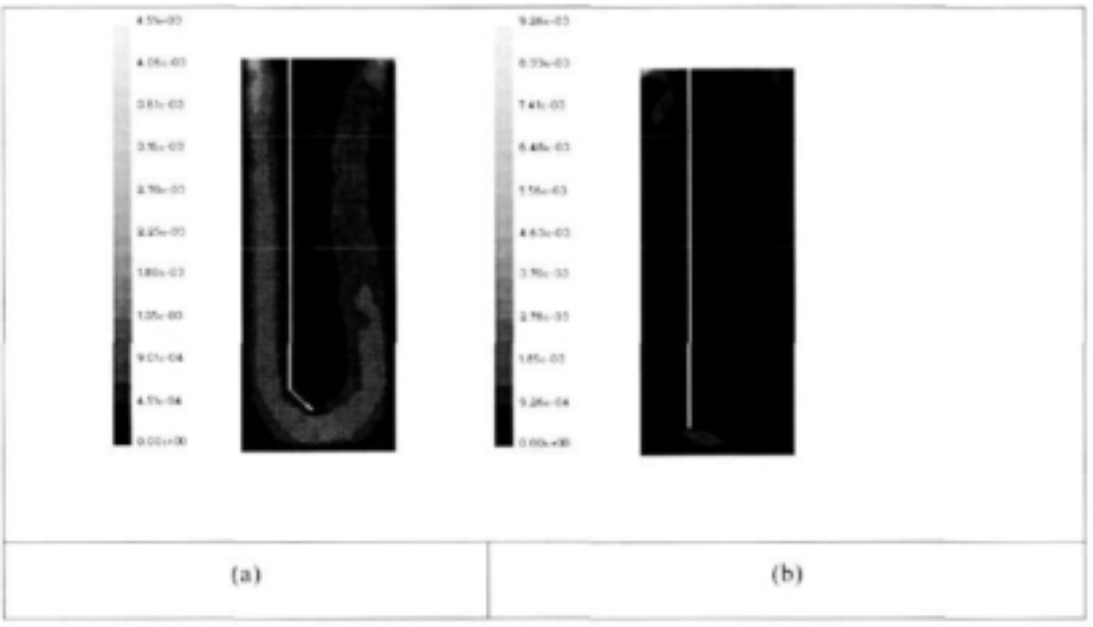


Figure 5-4 : Longitudinal section through an ABR compartment illustrating the CFD velocity *contours for the two different* baffle configurations: (a) angled baffle, (b) straight baffle

5.1.2 Slot Width

Dye tracer tests and CFD modelling on the laboratory-scale reactor revealed that the shape of the slot, between adjacent compartments, resulted in a constriction to flow. A CFD model with a wider slot was generated and the results showed a reduction in the amount of dead space and cycling. A comparison of flow patterns obtained with a 57 mm slot and an 87 mm slot are show in Figure 5-5.

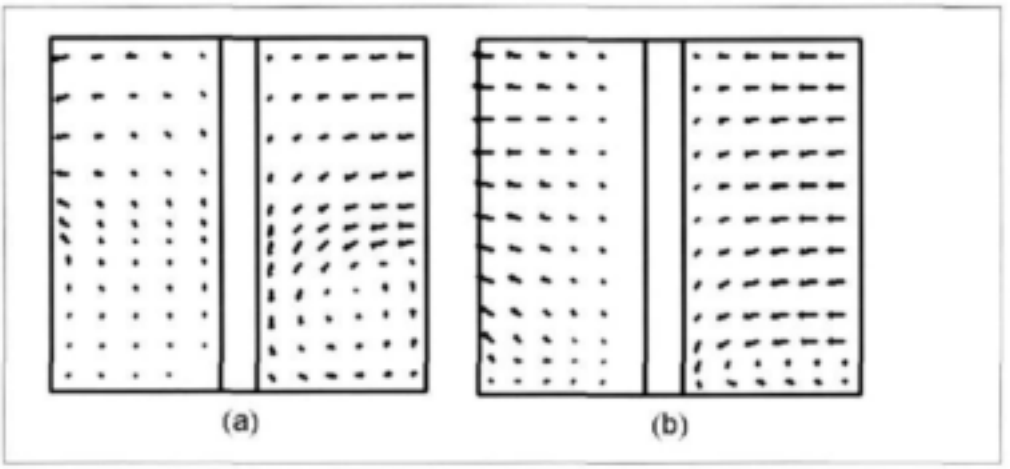


Figure 5-5 : Transverse section through the compartment of the ABR showing the velocity vectors with a slot width of (a) 59 mm and (b) 87 mm.

The pilot reactor was built such that the slot expanded across the width of the reactor as an over flow weir. The hanging baffles were attached to the top of the reactor to separate the gas pockets between the compartments. This was done to enable gas measurement from individual compartments. The heights of the standing baffles were

reduced across the reactor to facilitate an ease of flow through the reactor. A schematic diagram of the pilot scale reactor is shown in Figure 5-6.

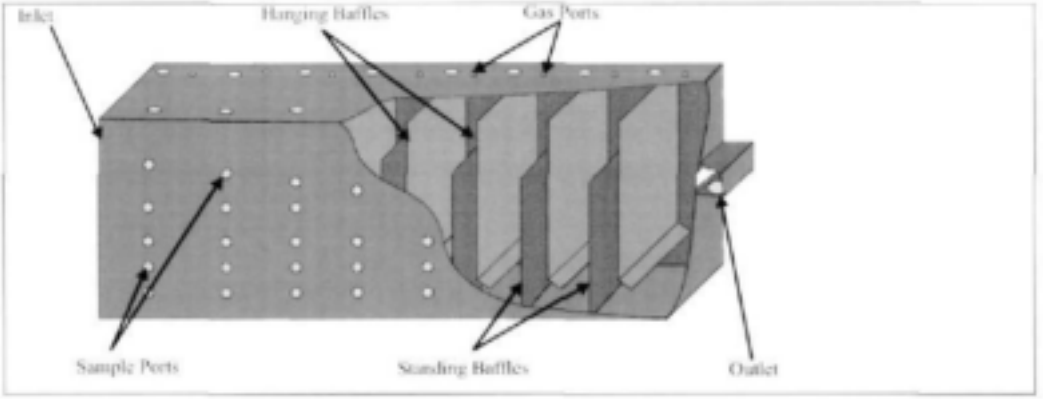


Figure 5-6 : Schematic diagram of the pilot-scale ABR with a cutaway to give an indication of the baffle configuration

5.2 *SUMMARY OF RESULTS FROM THE UMBILO WWTP PILOT PLANT STUDY*

This section presents a summary of the main results and conclusions emanating from the pilot plant work at Umbilo Wastewater Treatment. This plant treats a 50:50 mixture of domestic and industrial wastewater. The industrial component consists primarily of effluent from local textile industries. The reader is referred to WRC Report K5/1248/06 Evaluation of the Anaerobic Baffled Reactor for Sanitation in Dense Peri-urban Settlements (Foxon et al., 2006) for the full presentation of this work.

The 3 000 ℓ pilot ABR was operated for 409 days from July 2000 to August 2001 at Umbilo WWTP at target hydraulic retention times of 60 h, 32 h and 20 h. Key results and conclusions included the following:

- The pilot ABR was initially seeded with only 10 ℓ of anaerobic digester sludge, and therefore took more than a year to develop stable biomass loads. Seeding of an ABR was identified as the critical factor in reducing the length of the system start-up period.
- Fairly stable operation of the pilot ABR was obtained in all three operating periods (where each operating period corresponded to a specific hydraulic retention time target) despite occasional variations in flow and load, and biomass washout incidents.
- The pilot ABR exhibited COD removal in all operating periods, even during start-up where biomass concentrations in the reactor were very low. The ABR reduced the COD of the feed from a range of 600 to 1 000 mg/L to a range of 550 to 50 mg/L.
- No nutrient removal is obtained in an anaerobic treatment system. Ammonia concentrations in the pilot ABR increases as a result of liberation of organically bound nitrogen during digestion of complex organic material, and phosphorus concentrations were largely unaffected. Small sulphate concentrations in the influent were reduced by sulphate reducing bacteria to H2S. Alkalinity increased due to generation of bicarbonate and carbonate during digestion.

- Significant removal was obtained for all pathogen indicator organisms tested viz. total coliforms, Escherichia Coli, coliphages and Ascaris eggs.
- The ABR effluent was not suitable for direct discharge to water courses or groundwater. Further treatment is required.

Based on the experience gained from the pilot ABR, detailed design guidelines for full scale applications were developed. These guidelines are presented in Chapter 9 of WRC Report K5/1248/06. While these guidelines are specifically for peri-urban sanitation applications, the general principles and calculations are also applicable to industrial applications.

CHAPTER 6
Conclusions and Recommendations

The conclusions incorporate the information gained during screening tests (toxicity assays and biodegradability assays), laboratory-scale ABRs, a comparative study of an ABR and a fully mixed reactor and the operation of a pilot scale ABR at Umbilo WWTP.

6.1 LABORATORY SCREENING TESTS

Laboratory-scale screening tests were conducted on several food and textile dyes as well as industrial wastewaters. The objective of the *toxicity assays* was to determine the concentration at which each dye became inhibitory to the methanogenic biomass. The toxicity assays were specific to the methanogenic populations of the anaerobic digester sludge. A wide range of toxicity data were obtained with IC_{50} values ranging from > 20 g/L to 0.2 mg/L. The two most toxic dyes were Carmoisine Supra (IC_{50} of 0.25 g/L) and Erythrosine Supra (IC_{50} of 0.2 mg/L). The IC_{50} concentration of tartrazine was 14.3 g/L. The dye manufacturing effluent was relatively inhibitory to the methanogens with IC_{50} values of 22.5% and 15.9% (v/v), for the untreated and chemically treated effluents, respectively. The objective of the *biodegradability assays* was to evaluate whether the anaerobic biomass could utilise the dye as a sole substrate. Generally, the methanogenic activity was low, suggesting that these dyes were not readily utilised by the unacclimated methanogenic populations. Decolourisation due to adsorption or reduction of the azo bond was negligible for the majority of the dyes. These bioassays provided a more thorough understanding of the dye characteristics and degradation potential. This knowledge can be used to predict the optimal treatment option. Additional tests could be run with the supplementation of a carbon source, to investigate co-metabolism of the dyes. Adsorption bioassays could quantify the amount of adsorption of a particular dye to anaerobic biomass.

6.2 LABORATORY-SCALE ANAEROBIC BAFFLED REACTORS

Continuous laboratory-scale experiments on a 10 L ABR were undertaken using well defined feeds and batches of factory effluent. Comparative tests with a completely mixed anaerobic reactor were undertaken with domestic wastewater.

6.2.1 Tartrazine Food Dye

Several laboratory-scale ABRs were operated under varying conditions. One investigated the decolourisation of a pure food dye, tartrazine. Tartrazine, classified as Colour Index Food Yellow 4, is a monoazo synthetic organic colorant, with a maximum absorbance at 430 nm. Adsorption to anaerobic biomass played a role in the decolourisation of the dye. The COD of a tartrazine feed to the ABR resulted was reduced by 50 to 60%. Colour removal increased with time, suggesting acclimation of the biomass to degradation of the tartrazine. After approximately 2 months, the tartrazine colour removal was 90%. Most of the colour reduction was achieved in the first compartment of the reactor. There was methanogenic activity in the first compartment of the ABR. Tartrazine was not readily degraded by anaerobic digestion, however, degradation may be improved with acclimation of the biomass. The use of molecular techniques (FISH) to identify the bacteria associated with the degradation of the

tartrazine dye was unsuccessful as the dye interfered with probe hybridisation, resulting in the probes binding to the dye and not to the biomass.

6.2.2 Food Dye Manufacturing Effluent

The second laboratory-scale ABR investigated the degradation and decolourisation of the trade effluent from a food dye manufacturer. Anaerobic degradation of the wastewater was efficient. Methanogenic activity was high in the reactor, the organic content of the influent was reduced by ca. 70% and colour was reduced by almost 90%. Most of the colour reduction was achieved in compartment 1. Efficient degradation may be dependent on the composition of the wastewater, which was variable and could upset the degradation process. Whole cell oligonucleotide probe hybridisation showed definite shifts in the microbial populations due to the addition of the wastewater to the reactor. The general morphology of the Eubacteria changed as did their numbers, which decreased significantly in the last compartments of the reactor. Methanogens were observed throughout the reactor, with Methanosarcina clusters dominant in the first compartments of the reactor and the scavenging Methanosaeata species dominant in the last compartments.

6.2.3 CI Reactive Red 141

A well-defined and previously researched reactive textile dye, CI Reactive Red 141, was added to the sugar/protein feed to a laboratory-scale reactor. Batch screening tests Indicated that (i) the reactive dye was not inhibitory to the acidogenic populations; (ii) the biogas production increased with increasing dye concentration which suggests that the dyes were being actively metabolised by the acidogens; (iii) reactive dye compounds, present in textile dye wastewaters, did not adversely affect the anaerobic degradation process; (iv) Methanogenic activity was inhibited by the dye degradation products; and (v) there was no further reduction in COD or colour. The results of the physical decolourisation tests suggested significant decolourisation due to adsorption to the biomass, however, it is possible that the dye chromophores were reduced due to the low redox potential environment within the test bottles. No dye break-through, due to adsorption saturation, was observed during operation of the reactor. The COD reduction was consistently > 90%, except for the period during the dye shock load. Colour reduction averaged 86%. The biomass showed acclimation to the dye, with increased methanogenic activity with each increase in dye concentration. The inaccurate biogas measurement resulted in the poor COD balance of 18.2%. The reactor operation was stable, even with increases in the dye concentration. The only observed response to the dye was when the concentration was increased to 1 g/L (shock load), resulting in temporary inhibition but recovery to stable operation within 5 HRTs. This investigation has shown that successful treatment of a highly coloured wastewater is possible in the ABR. The design of the ABR facilitates efficient treatment of concentrated textile dye wastewaters by protecting the sensitive methanogens from the inhibitory dye molecules and promoting efficient colour and COD reduction, primarily by the acidogens in the first compartments. Metabolic activity was low in the final three compartments of the reactor. There was a definite shift in the microbial populations through the ABR, with a predominance of eubacteria in the first compartments (acidogenesis) and archaea (methanogenesis) in the later compartments. The use of molecular approaches provided useful descriptions of the methanogens actively involved within each compartment. These results showed a horizontal separation of a short filamentous archaeal micro organism and the long sheathed filamentous Methanosaeta spp. The short filamentous archaeal micro organism proliferated after addition of the dye to the reactor and was metabolically active in the first four compartments of the reactor. Identification of the micro

organism will require DNA extraction and sequencing. The application of molecular techniques to the ABR process improved the understanding of the metabolic processes occurring within each compartments and the micro organisms involved in these reactions.

6.2.4 Comparison of an ABR with a Fully-Mixed Reactor

A laboratory-scale investigation was conducted by R Mudunge to compare the performance of an ABR with a completely mixed anaerobic reactor. It was found that the ABR was more stable and could withstand a greater shock load than the completely mixed reactor (Mudunge, 2002).

6.3 PILOT SCALE ABR

On a feed of 50% domestic wastewater and 50% industrial effluent and at hydraulic retention times ranging from 60 to 20 h, a 3 200 L pilot ABR was able to reduce the COD of the feed from a range of 600 to 1 000 mg/L to a range of 550 to 50 mg/L. There was some removal of pathogens (3 logs) but no removal of nutrients (nitrogen and phosphorus). The final effluent was not suitable for discharge to water courses without further treatment.

6.4 RECOMMENDATIONS

In the South African context, the ABR could be implemented for on-site for pre-treatment of agro-industrial wastes, with high COD contents or those with xenobiotic components, which prevent conventional treatment at a wastewater treatment works. Further research should focus on the following:

1. Implementation of a pilot- or full-scale ABR for pre-treatment of a high-strength or toxic industrial effluent.
2. Extend the use of molecular techniques to further improve understanding of the microbial population dynamics within the various compartments.

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APPENDIX A

CAPACITY BUILDING REPORT

TREATMENT OF HIGH-STRENGTH AND TOXIC ORGANIC INDUSTRIAL EFFLUENTS IN THE ANAEROBIC BAFFLED REACTOR

J BELL, P DAMA, R MUDUNGE and CA BUCKLEY

Pollution Research Group

School of Chemical Engineering

University of KwaZulu-Natal

Durban

Head of Pollution Research Group : Professor C A Buckley
Project Leader and Researcher : Dr J Bell

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Capacity Building

When the proposal was written for this project, capacity building was not a pre-requisite. Joanne Bell was awarded a PhD for research undertaken during the course of this project and Reginald Mudunge was awarded a MScEng. However, during the course of the project, the project team realised the importance of capacity building and incorporated it into the project, through collaboration with other institutions and incorporating undergraduate students into the project. These actions are detailed below.

A.1. COLLABORATIVE RESEARCH WITH IMPERIAL COLLEGE, LONDON

Prior to commencement of this project, the Pollution Research Group and Imperial College realise a common interest in anaerobic digestion and the ABR. In March 1998, Ms Joanne Bell (née Sacks) was awarded a FRD/British Council scholarship to attend Imperial College, where she investigated the treatment and decolourisation of food dyes in the ABR. Researchers in the Department of Chemical Engineering and Chemical Technology, at Imperial College, London, had conducted extensive investigations on the ABR. It had been shown to be efficient in treating soluble, non-toxic effluents (Grobicki and Stuckey, 1992). The hydrodynamics of the reactor had been investigated (Grobicki and Stuckey, 1991), as well as the effect of shock loads on reactor performance and microbial response to environmental changes within the reactor (Nachaiyasit and Stuckey, 1997).

A.1.1. Research

Investigations at this time included the efficiency of treatment of high solids wastes; low temperature treatment of low-strength wastes; comparison of the efficiency of suspended and granulated sludges in treatment efficiency; the role of hydrogen as a control parameter in anaerobic digestion; sulphate and nitrate reduction in the ABR; and the identification and treatment of soluble microbial products (SMPs) which are detected in the effluent as recalcitrant COD. A new investigation was to assess the efficiency of the ABR to decolourise dye effluents from textile, leather tanning and food industries.

In the South African context, the ABR could be implemented on-site for pre-treatment of agro-industrial wastes, with high COD contents or those with xenobiotic components, which prevent conventional treatment at a wastewater treatment works.

Thus, the purpose of the collaboration was the acquisition of knowledge and technology. Experience gained from the research conducted on the ABR was transferred to South Africa. This included the acquisition of knowledge on the design, operating procedures and functional aspects of the ABR; and the utilisation of fluorescent *in situ* hybridisation to elucidate the microbial population dynamics within the reactor compartments. Also became familiar with previous investigations on the ABR and received literature to return to South Africa.

A.1.2. Technical Visits

During this period, Ms Bell attended a 10 day International Course on Anaerobic Waste Water Treatment at IHE Delft in The Netherlands. Apart from comprehensive lectures and assignments on all aspects of anaerobic digestion, the course also included visits to the Wageningen Agricultural University laboratories and excursions to see anaerobic processes in place at the Heineken Brewery and Shell Moerdijk.

She also visited the Department of Civil Engineering at Loughborough University, where extensive research was being carried out on anaerobic digestion and water supply and sanitation.

She attended and presented a paper at the IAWQ Fourth International Symposium on Waste Management Problems in Agro-Industries, held in Istanbul, Turkey.

A.2. CENTRE FOR WATER AND WASTEWATER RESEARCH, TECHNIKON NATAL

The research group at the Centre for Water and Wastewater Research, at Technikon Natal, were using molecular techniques in the WRC Project No. K5/1191: *Microbial Characterisation of Activated Sludge Mixed Liquor Suspended Solids*. They had the necessary equipment for FISH (epifluorescent microscope and camera). A working arrangement and joint learning experience was established with them.

A.3. SADC PARTICIPATION

The Pollution Research Group collaborated with Dr Remi Zvauya, of the Department of Microbiology at the University of Zimbabwe. One of Dr Zvauya's students, Reginald Mdunge, completed an MScEng degree with the PRG. The intention was to have further exchange of students, however, this did not occur.

A.4. POLITECNICO DI MILANO

Luca Morganti, a student from the Politecnico di Milano, used the biosensor, ANITA, for methanogenic activity tests and toxicity tests with textile effluent components. This work was carried out in the School of Chemical Engineering at the University of Natal. This research was the result of collaboration with Prof. Alberto Rozzi, who visited South Africa in January 1999. David D'Ambrosio, also a student from the Politecnico di Milano, completed a research project on methanogenic activity tests using ANITA, at the University of Natal.

As a result of this interaction, an application for a research grant under the joint Italy/South Africa Science and Technology Agreement was submitted in April 2001. The project proposal is entitled *Titration Biosensors to Monitor Polluted Water*.

This collaboration will facilitate further exchange of research students between the two institutions and allow researchers on the ABR project to visit a full-scale ABR plant, treating domestic wastewater in Bologna, Italy.

A.5. UNDERGRADUATE PROJECTS

Undergraduate research projects were developed to allow students to experience being involved in a research project, with the aim of selecting suitable students for future postgraduate studies. The undergraduate projects that stemmed off the current project are summarised below.

Table A-1: Summary of Undergraduate Student Projects

Name	Gender	Race	Status	Duration of project	Current Position
L Murugen	F	Indian	4th year student	Vacation work Lab project	Industry
M Govender	F	Indian	4th year student	Vacation work	Industry

S Singh	F	Indian	4th year student	Lab project Lab project	Industry
L Morganti	M	White	International student	6 months	Post-grad
D D'Ambrosio	M	White	International student	6 months	Industry
P Pather	F	Indian	4th year student	Vacation work Lab project	Ind. Research
D Pillay	F	Indian	4th year student	Vacation work Lab project	Industry
S Pillay	M	Indian	4th year student	Vacation work Lab project	Post-grad
K Govender	M	Indian	4th year student	Vacation work Lab project	Under-grad
Meghendran	M	Indian	2nd year student	Research Assist Vacation work	Under-grad
A Bisunder	M	Indian	4th year student	Vacation work Lab project	Industry
Amal	M	Indian	4th year student	Lab project	Industry
D Mzulweni	M	Black	4th year student	Lab project	Durban Metro

Lee-Ann Murugen and Moganie Govender, were both offered MScEng positions but, due to financial circumstances, chose a position in industry. Shashona Singh realised that research was not for her and chose a career in industry.

Luca Morganti, a student from the Politecnico di Milano, completed his research project with the Pollution Research Group was awarded his degree *magna cum laude*. He completed a Masters degree at the Politecnico di Milano and is currently furthering his post-graduate studies in the USA. David D'Ambrosio, also a student from the Politecnico di Milano, completed his research project with the Pollution Research Group. He was awarded his degree and is currently working in industry, in Italy.

Prenusha Pather, a Mintek bursar, continued with a career in research at Mintek. Dhineshree Pillay Adhir Bisunder and Amal chose positions in industry due to financial circumstances.

Sarushen Pillay is currently a post-graduate student with the Pollution Research Group. Kuvarshan Govender and Meghendran are both completing their undergraduate studies..

David Mzulweni is working in research for the Durban Metro Water Services.

APPENDIX B

TECHNOLOGY TRANSFER REPORT

TREATMENT OF HIGH-STRENGTH AND TOXIC ORGANIC INDUSTRIAL EFFLUENTS IN THE ANAEROBIC BAFFLED REACTOR

J BELL, P DAMA, R MUDUNGE and CA BUCKLEY

Pollution Research Group

School of Chemical Engineering

University of KwaZulu-Natal

Durban

Head of Pollution Research Group : Professor C A Buckley
Project Leader and Researcher : Dr J Bell

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Technology Transfer

Technology transfer was achieved through publications in peer reviewed journals, conference papers and posters and reports. There was interaction with other academic institutions (both local and international). The Business Partners for Development initiative took an interest in the project and assisted in creating closer links with Durban Metro. An international firm of consulting engineers were interested in licensing the technology. There was interaction with other WRC projects. Undergraduate and post graduate students gained research experience through the project

B.1. PUBLICATIONS

Publications consisted of technical papers which published in peer reviewed journals, conference papers and posters and reports

B.1.1. Journal Articles

1. J Sacks, CA Buckley, E Senior and H Kasan. (1999). An assessment of the feasibility of anaerobic digestion as a treatment method for high strength or toxic organic effluents. Water Science and Technology. 39 (10-11): 347-351.
2. J Sacks and CA Buckley (1999). Anaerobic Treatment of Textile Size Effluent. Water Science and Technology. 40 (1): 177-182.
3. J Bell, JJ Plumb, CA Buckley, DC Stuckey. (2000). Treatment and Decolourisation of Dyes in an Anaerobic Baffled Reactor. Journal of Environmental Engineering. 126 (11): 1026-1032.
4. JJ Plumb, J Bell and DC Stuckey. (2001). Microbial Populations Associated with Decolourisation and Treatment of an Industrial Dye Effluent Using an Anaerobic Baffled Reactor. Applied and Environmental Microbiology. 67 (7): 3226-3235.

B.1.2. Conference Proceedings

1. J Sacks and CA Buckley (1998). Anaerobic digestion of a textile size effluent. WISA'98 Cape Town, South Africa. .
2. J Sacks, CA Buckley and DC Stuckey (1998). Treatment of high-strength or toxic organic effluents in the anaerobic baffled reactor (ABR). WISA '98 Cape Town, South Africa. .
3. J Sacks and CA Buckley (1998). Anaerobic treatment of textile size effluent. IAWQ Fourth International Symposium on Waste Management Problems in Agro-Industries. Istanbul, Turkey.
4. J Sacks, CA Buckley, DC Stuckey and JJ Plumb (1999). Treatment and decolourisation of food dyes in the anaerobic baffled reactor. African International Environmental Protection Symposium. Pietermaritzburg, South Africa.
5. LH Murugen, M Govender, J Sacks, CA Buckley, A Rozzi and S Frestel. (1999). Comparison of Methods to Measure Methanogenic Activity. African International Environmental Protection Symposium. Pietermaritzburg, South Africa.

6. P Dama, J Bell, CJ Brouckaert, CA Buckley and DC Stuckey. (2000). The Design of an Anaerobic Baffled Reactor with the Aid of Computational Fluid Dynamics. BioY2K Combined Millenium Meeting. Grahamstown, South Africa..
7. LH Murugen, M Govender, J Bell, CA Buckley, A Rozzi and S Frestel. (2000). Comparison of Methods for Measuring Methanogenic Activity. BioY2K Combined Millenium Meeting. Grahamstown, South Africa.
8. J Bell, CA Buckley, DC Stuckey, P Dama and E Senior. (2000). Laboratory-Scale Investigation of the Anaerobic Baffled Reactor. BioY2K Combined Millenium Meeting. Grahamstown, South Africa.
9. D D'Ambrosio, CA Buckley, A Rozzi, J Bell and V Naidoo. (2000). Evaluation of the Effect of Microbial Population Composition on Methanogenic Activity. BioY2K Combined Millenium Meeting. Grahamstown, South Africa. .
10. J Bell, P Dama, CA Buckley, DC Stuckey and E Senior. (2000). Pre Scale-Up Laboratory Investigation of the Anaerobic Baffled Reactor. WISA 2000, Sun City, South Africa.
11. J Bell, CA Buckley, DC Stuckey and JJ Plumb. (2000). Degradation of Food Dyes in the Anaerobic Baffled Reactor. WISA 2000, Sun City, South Africa. .
12. P Dama, J Bell, CJ Brouckaert, CA Buckley and DC Stuckey. (2000). Computational Fluid Dynamics: Application to the Design of the Anaerobic Baffled Reactor. WISA 2000, Sun City, South Africa.
13. D D'Ambrosio, CA Buckley and A Rozzi. (2000). Estimation of the Kinetic Constants for an Anaerobic Sludge. WISA 2000, Sun City, South Africa.
14. J Bell, P Dama, CA Buckley, DC Stuckey and E Senior. (2000). Treatment of Industrial Wastewater in the Anaerobic Baffled Reactor. SAIChE 2000, Secunda, South Africa.
15. P Dama, J Bell, CJ Brouckaert, CA Buckley and DC Stuckey. (2000). Computational Fluid Dynamics: Application to the Design of the Anaerobic Baffled Reactor. SAIChE 2000, Sun City, South Africa.
16. J Bell, P Dama, K M Govender, C A Buckley, and D C Stuckey (2001). Performance Characterisation and Microbial Populations Associated with the Start-Up of a Laboratory-Scale and a Pilot-Scale Anaerobic Baffled Reactor. AD 2001, Antwerpen, Belgium.
17. Priyal Dama, Joanne Bell, Valerie Naidoo, Katherine Foxon, Chris Brouckaert, Chris Buckley and David Stuckey (2001). The Anaerobic Baffled Reactor for the Treatment of Domestic Wastewater in Dense Peri-Urban Communities. AD 2001, Antwerpen, Belgium.
18. Priyal Dama, Kuvarshan Govender, Jenny Huang, Katherine Foxon, Joanne Bell, Chris Brouckeart, ChrisBuckley, Valerie Naidoo, David Stuckey (2001). Flow Patterns in an Anaerobic Baffled Reactor. AD 2001, Antwerpen, Belgium.
19. Priyal Dama, Joanne Bell, Katherine Foxon, Chris Brouckaert, Jenny Huang, Chris Buckley, Valerie Naidoo and David Stuckey (2001). Pilot-Scale Study of an Anaerobic Baffled Reactor for the Treatment of Domestic Wastewater. IWA Conference on Water and Wastewater Management in Developing Countries, Kuala Lumpar, Malaysia.
20. K.M. Foxon, P. Dama, J. Bell and C.A. Buckley (2001). Application of Aquatic Modeling to Design and Start-Up of an Anaerobic Baffled Reactor. IWA Conference on Water and Wastewater Management in Developing Countries, Kuala Lumpar, Malaysia.

21. K.M. Foxon, C.A. Buckley and A. Rozzi (2001). Substrate Characterization using an Anoxic Titration (pHstat) Biosensor. IWA Conference on Water and Wastewater Management in Developing Countries, Kuala Lumpar, Malaysia.

B.1.3. Reports

1. J. Sacks (1998). Investigation of the Treatment and Decolourisation of Food Dyes in the Anaerobic Baffled Reactor. Report on work completed during a 7 month academic exchange in the Department of Chemical Engineering and Chemical Technology, Imperial College of Science, Technology and Medicine, London

B.2. AUXILLARY PROJECTS

During the course of the project external bodies expressed an interest in the project.

B.2.1. Business Partners for Development

Under the auspices of the World Bank, a world-wide programme is underway called Business Partners for Development. The objective is to explore new ways or methods of providing service and sustainable development, in particular to deprived urban communities, through a more active association involving business, civil society and government at all levels.

Through an active association involving Durban Metro Water Service (DMWS), Vivendi Water, Mvula Trust, the Water Research Commission (WRC) and Umgeni Water (UW), the objective was to provide sustainable community-focused water and sanitation services in the dense peri-urban settlements of Bhambayi, Amatikwe and Ntuzuma G. The proposal involved the setting up of pilot projects in the Inanda-Ntuzuma area, building on and co-ordinated with the work underway in the pilot zones, with the aim that, after a two to three year trial period, the schemes are sustainable.

The BPD steering committee selected the BAR as being a suitable sanitation system. A pilot-scale (3.2 kL) ABR was financed by the BPD and built in April 2000. It was commissioned at the Umbilo Wastewater Treatment Works, in July 2000. This investigation is continuing as WRC Project No. 1248: *The Evaluation of the Anaerobic Baffled Reactor for Sanitation in Dense Peri-Urban Settlements* (2001-2003). Additional funding is being sought through the AtlantIC Alliance.

B.2.2. WS Atkins Water

A research project, *Anaerobic Baffled Reactor for Domestic Wastewater Treatment*, was initiated by WS Atkins Water, in collaboration with Imperial College, and was funded by 5 water companies: North West Water, Thames Water, Wessex Water, South West Water and Water Service Northern Ireland. The PRG/WRC was invited to join the initiative as a full partner and receive a royalty. The research commenced in April 1999 and continued for a 2 year period. A pilot-scale (30 kL) ABR was constructed and commissioned at the Ellesmere Port Wastewater Treatment Works. Five separate reactors were constructed, a simulating a baffled zone, with a header and exit tank at either end of the plant. The PRG/WRC were invited to join and an agreement between the potential partners was prepared, however, ultimately, the PRG/WRC declined to participate, opting instead to join the BPD project.

B.3. INTERACTION WITH OTHER WRC PROJECTS

The project has facilitated interaction with the following current WRC projects:

1. WRC Project No. 1075: Computational Fluid Dynamic Support to Water Research Projects.
2. WRC Project No. K5/1074: Co-Digestion of High-Strength/Toxic Organic Effluents in Anaerobic Digesters at a Wastewater Treatment Works.
3. WRC Project No. K5/1191: Microbial Characterisation of Activated Sludge Mixed Liquor Suspended Solids. Shared molecular biology techniques and equipment with Natal Technikon.
4. The current project resulted in WRC Project No. 1248: The Evaluation of the Anaerobic Baffled Reactor for Sanitation in Dense Peri-Urban Settlements.

B.4. UNDERGRADUATE RESEARCH PROJECTS

1. WRC Project No. 1075: Computational Fluid Dynamic Support to Water Research Projects.
2. Shashona Singh, a 4th year Chemical Engineering student, investigated the ABR tracer tests and CFD modelling.
3. Luca Morganti, a student from the Politecnico di Milano, used ANITA for methanogenic activity tests and toxicity tests with textile effluent components. This work was carried out in the School of Chemical Engineering at the University of Natal. He was awarded his degree magna cum laude. This research was the result of collaboration with Prof. Alberto Rozzi, who visited South Africa in January 1999.
4. David D'Ambrosio a student from the Politecnico di Milano, used ANITA for methanogenic activity tests. This work was carried out in the School of Chemical Engineering at the University of Natal.
5. Prenusha Pather and Dhineshree Pillay, both 4th year Chemical Engineering students, used anaerobic serum bottles to investigate the anaerobic toxicity and biodegradability of a range of textile dyes to anaerobic biomass.
6. Sarushen Pillay and Kuvarshan Chetty, both 4th year Chemical Engineering students, investigated the kinetics of the two acetate-utilising methanogen genera, Methanosarcina and Methanosacta.
7. Meghendran, a second year Chemical Engineering student assisted with the operation of the laboratory-scale reactors for his vacation work.
8. Adhir Bisunder and Amal, both 4th year Chemical Engineering students, investigated the hydrodynamics of the laboratory-scale reactor using computational fluid dynamics.
9. David Mzulweni, a 4th year Chemical Engineering student, performed a COD balance over the pilot-scale ABR

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