THE DESIGN AND TESTING OF A SHORT HRT ATTACHED GROWTH PRE-FERMENTER SYSTEM: SCVFA PRODUCTION BY TWO MEDIA TYPES AND TWO FEED SOURCES

by

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ABSTRACT

A pilot-scale, short Hydraulic Retention Time (HRT), fixed film fermenter system was constructed to test for Short Chain Volatile Fatty Acids (SCVFA) production performance at various retention times. The performance of two media types, Ringlace® and Kaldnes media, was monitored at intervals of 20min, 40min and 60min provided by three 20min hydraulic retention time (HRT) reactors in each media series. The UBC waste water pilot plant was used as the waste source for all experiments. Two fermentation runs were completed; the first using primary effluent as feed and the second using screened raw wastewater.

The sampling and processing scheme addressed temporal variations in the following parameters: SCVFA concentration, total organic carbon (TOC), total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD) and suspended solids analysis. Turbidity analysis was attempted but was not continued for the duration of the research. Metabolic fingerprinting, using the BIOLOG plate assay, provided a snapshot of the biofilm's grouped microbial metabolic activities present at the time of sampling.

SCVFA production in the BF-system was near zero in the reactors containing no media during both the primary effluent and raw wastewater runs. Significant levels of SCVFA's were produced by

short HRT attached growth fermenters using both primary effluent and raw wastewater as feed. Both sets of media reactors tested produced more SCVFA's than the control reactors. TOC and sCOD analyses, which measure dissolved organics in the waste stream, indicated a net solubilization of filterable solids in both media series fed raw wastewater. Kaldnes media was deemed not suitable as a short HRT pre-fermentation media in the current BF-system configuration, due to an inability to handle the solids loading of the waste stream. Suspended solids analysis supported the choice of appropriate media for future research by demonstrating the tendency of Kaldnes media to become clogged with solids. Ringlace® media has proven itself as a suitable short HRT pre-fermentation media by resisting plugging under the solids loading conditions tested. Results of the two fermentation runs indicate that, a 60min HRT fermenter system fed raw domestic wastewater, is capable of producing significantly more SCVFA's than a system fed primary effluent, (11.2mg/l and 5.6mg/l, respectively).

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LIST OF ABBREVIATIONS

BF-system	:	Biological Fermentation System
BNR	:	Biological Nutrient Removal
BOD	:	Biochemical Oxygen Demand
C1,2,3,	:	Control reactors: 1,2,3,
COD	:	Chemical Oxygen Demand
EPS	:	Extracellular Polymeric Substances
GN	:	Gram Negative
HRT	:	Hydraulic Retention Time
K1,2,3,	:	Kaldnes reactors: 1,2,3,
N_2	:	Nitrogen gas
ORP	:	Oxidation Reduction Potential
PHB	:	poly-β-hydroxybuterate
PP	:	Polypropylene
PVC	:	polyvinyl chloride
R1,2,3,	:	Ringlace® reactors: 1,2,3,
sCOD	:	Soluble Chemical Oxygen Demand
SCVFA	:	Short Chain Volatile Fatty Acid
SRT	:	Solids Retention Time
SS	:	Suspended Solids
tCOD	:	Total Chemical Oxygen Demand
TOC	:	Total Organic Carbon
TSS	:	Total Suspended Solids
VFA	:	Volatile Fatty Acid
VSS	:	Volatile Suspended Solids

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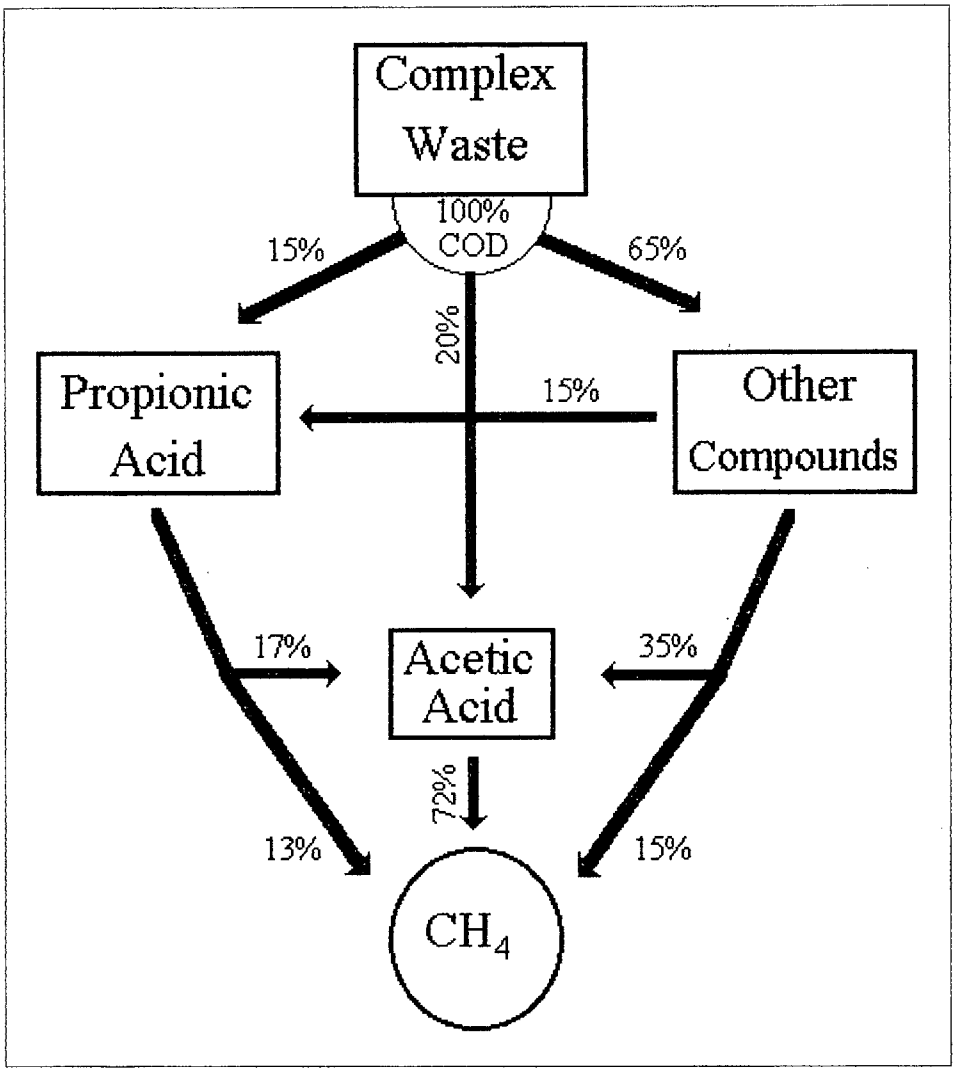
CHAPTER 1: INTRODUCTION

1.1 Fermentation

Anaerobic fermentation is the, microbially mediated, breakdown of carbohydrate and protein compounds, without the presence of oxygen as a terminal electron acceptor. The end products of complete fermentation are methane and carbon dioxide. Typical biochemical pathways, for the fermentation of waste water, are presented in Figure 1.1 (Barnard, 1992). The anaerobic degradation of complex organics follows a multi-step mechanism of both parallel and series reactions (Shin and Song, 1995). The degradation pathway can be divided into four distinct steps: Hydrolysis, Acidogenesis, Acetogenesis, and Methanogenesis. The first three steps are grouped as Acidification reactions, whereas the last is named Methanation.

Hydrolysis consists of the conversion of complex organics (proteins, carbohydrates, and lipids) to monomeric and oligomeric compounds (amino acids, sugars, and fatty acids). Acidogenesis consists of the degradation of the products of hydrolysis, yielding the following: volatile fatty acids, lactate and alcohols. The products of Acidogenesis are further metabolized in acetogenic reactions to yield acetate, carbon dioxide, and hydrogen. Methanogenesis reactions convert the acetate and hydrogen, produced in the previous step, to methane and carbon dioxide.

Figure 1.1: Bacterial Fermentation Pathways



Adapted from Barnard, 1994.

Pre-fermentation systems are designed to augment the acidification steps, and hence, produce volatile fatty acids. Retention times are kept short in pre-fermenters, to prevent methanation ( ). Methanation reactions would consume the VFA's produced in the pre-fermenter, negating the benefits of VFA addition (see section 1.2). Most current fermentation literature is focused on the methanation component of fermentation. Literature on the optimization of VFA production, outside

the BNR field, involves the optimization of the acidogenic stage of a two stage anaerobic sludge digester (Barnard and Rabinowitz, 1997).

1.2 Biological Nutrient Removal

Conventional biological treatment plants treat domestic wastewater by utilizing the metabolic capabilities of complex bacterial populations to oxidize organic components to carbon dioxide, water and biomass, which is then settled out of the treated waste water. Suspended solids are either physically removed in a primary clarifier or metabolized biologically. Dissolved organics are principally metabolized biologically. In addition to the damaging effects of suspended solids and oxygen demand of untreated domestic wastewater, it has been shown that the presence of nitrogen and phosphorus contribute to eutrophication of receiving waters (Argaman, 1991). Nitrogen can be removed from the system via biochemically processing the nitrogen containing compounds in a two step process, nitrification-denitrification. The end product, N_2 gas, evolves from the system and is therefore removed (Barnard, 1974). However, the nitrogen is not destroyed, it is simply converted to its gas form, which is environmentally non-damaging. Phosphorus, unlike nitrogen, cannot be converted to gas form and must be recovered from the waste stream as an inorganic precipitate or as phosphate bound within bacterial cells (Trevino et al., 1992; Lie et al., 1997).

Biological Nutrient Removal (BNR) was developed during the mid-1970's as it became clear that the nutrients, nitrogen and phosphorus, were still present in conventionally treated effluent. This effluent was contributing to the eutrophication of receiving waters by fertilizing algal growth blooms. Early BNR systems consisted of a modified activated sludge process, with the addition of

anoxic and anaerobic zones (Barnard, 1974). This type of system , called the "Bardenpho" process, utilized the ability of certain bacteria to take up excessive amounts of phosphate aerobically and store it. The phosphate-rich bacteria could then be removed from the system with the 2^0 clarifier sludge. Modern BNR plants are designed to treat wastewater by removing these nutrients from the waste stream as well as controlling traditional components such as suspended solids and Biochemical Oxygen Demand (BOD).

The first BNR plant in western Canada was built in Kelowna, British Columbia, in 1982 (Barnard et al.,1995). Two more BNR plants were built in British Columbia, Westbank and Penticton (Dawson et al., 1995). Several existing plants in western Canada have been retrofitted to BNR systems including the following: Calgary and Edmonton Alberta, Salmon Arm British Columbia (Wilson and Keller, 1995). The industrial sponsor for this research, Reid Crowther, Ltd., is currently involved with several BNR projects, world-wide, with an emphasis on improving the process efficiency and lowering the treatment costs. In addition, Reid Crowther Ltd. has been involved in various laboratory, pilot-scale and full-scale BNR research projects, in conjunction with the University of British Columbia, resulting in the exchange and implementation of knowledge between the university, engineering consultants, and treatment plant operators.

1.3 The Bio-Fermentation System

A pilot-scale, short Hydraulic Retention Time (HRT), fixed film fermenter system was constructed, at the University of British Columbia pilot plant site, to test for Short Chain Volatile Fatty Acid (SCVFA) production performance at various retention times, as a measure of acidogenic potential

and feasibility as a retrofit to existing BNR plants. The Bio-Fermentation (BF) system consisted of nine PVC reactors grouped as three series of three reactors. Two series represent test media fermenters and the third was a negative control with no media. The fermenter system tested two different media concurrently, Ringlace® and Kaldnes media, to determine which might be better suited for short HRT partial fermentation of primary effluent and raw wastewater. The media were judged mainly by their ability to produce SCVFA's, within a set HRT of the BF-system. Three reactors in series allow sampling at time intervals of 20min and 40min, as well as the 60min system HRT. The UBC waste water pilot plant was used as the waste source for all experiments. The system was designed as "up-flow" but was inverted to yield a "down-flow" system after preliminary testing. Two fermentation runs were completed; the first using primary effluent as feed and the second using screened raw wastewater.

1.4 Objectives

This research focused on three parameters in the BF system: fixed film media type, waste stream makeup, and HRT. Each variable was analyzed in terms of its effect on SCVFA production and system sustainability. After the initial startup period necessary to develop biofilm populations, and troubleshoot the operational protocol for the BF-system, two experimental runs were performed. The first assessed media performance, fermenting primary effluent, while the second assessed media performance, fermenting raw wastewater. Both runs employed the same HRT, media configurations and sampling design.

The following outlines the specific objectives of the two BF system sampling runs:

1. Compare the performance and suitability of two fixed film media types for the fermentation of primary domestic effluent to yield a significant increase in SCVFA levels.

2. Compare the performance and suitability of two fixed film media types for the fermentation of raw domestic wastewater to yield a significant increase in SCVFA levels.

3. Determine the feasibility of this reactor system as a producer of significant SCVFA's within the HRT constraints set up by Reid Crowther Ltd., the co-sponsor of this research project.

3. Determine the SCVFA production possible utilizing a short HRT prefermenter.

4. Describe the accumulated biofilm in each experimental condition metabolically.

CHAPTER 2: LITERATURE SEARCH

2.1 Biological Nutrient Removal

The focus of domestic wastewater treatment has shifted from treating individually targeted components of sewage to the understanding of nutrient interactions as they relate to overall waste treatability (Stensel et al., 1992). The goal of the BNR system is to remove nitrogen and phosphorus from the waste stream as well as BOD content (Stensel and Barnard, 1992). Biological Nutrient Removal systems (BNR) consist of a modified activated sludge process, with the addition of anoxic and anaerobic zones (Barnard, 1974). The basic principles of nitrogen and phosphorus removal, presented below, were adapted from a BNR design manual written by Randall, Barnard, and Stensel (1992).

Nitrogen, as ammonia-Nitrogen, is removed from the waste stream by a series of nitrification and denitrification reactions. Nitrification reactions involve the oxidation of ammonia-Nitrogen, in the presence of oxygen, producing nitrate and nitrite (equations 2.1 to 2.3).

Nitrosomas

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_20 \qquad \text{Equation 2.1}$$

Nitrobacter

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad \text{Equation 2.2}$$

Total reaction

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_20 \qquad \text{Equation 2.3}$$

Nitrification reactions occur in the aerobic zone of BNR systems. The reactions are mediated by two types of bacteria, *Nitrosomas* and *Nitrobacter*, which produce nitrite and nitrate, respectively. It has been shown that, the overall reaction (equation 2.3), is rate limited by the nitrite producing step.

Denitrification reactions involve the reduction of nitrate, in the absence of free oxygen, to nitrogen gas. These reactions require an electron donor, wastewater BOD, to provide energy for the reduction reactions. Various heterotrophic bacteria are responsible for denitrification reactions, including bacteria which can utilize oxygen as well as nitrate as the final electron acceptor. Denitrification reactions occur, predominantly, in the anoxic zone of BNR systems. However, recent studies have shown that, under low oxygen conditions, denitrification can also occur simultaneously with nitrification in the aerobic zone (Koch, 1996).

Phosphorus must be manually removed from the waste stream, as particulate matter, because it does not exist as a gas, as in the previous case of nitrogen (Argaman, 1991). In chemical removal systems, phosphorus is removed as a mineral precipitate. In BNR systems, the particulate matter is bacterial cells. BNR systems facilitate the removal of waste stream phosphorus through alternating anaerobic and aerobic conditions (Argaman, 1991). In the aerobic zone, bacteria capable of storing phosphate, use up some of the energy generated from metabolizing, both extracellular and stored carbon sources, storing phosphate as polyphosphate granules (Jardin and Pöpel, 1997). Under anaerobic conditions, these organisms extract energy from the release of stored phosphate, which gives them a biological advantage over "non-polyphosphate storing" bacteria. This energy is used for cell maintenance and for the uptake and storage of carbon sources, specifically acetates which are assorted as poly-β-hydroxybuterate (PHB) (Stensel and Barnard, 1992). Upon re-exposure to

aerobic conditions, "polyphosphate containing" bacteria metabolize the stored carbon source, and once again, take up excess phosphate. The stored phosphorus is removed from the waste stream via the wasting of sludge, collected from the aerobic zone, containing bacteria which have an excess accumulation of phosphate. The ratio of phosphorus removal to sludge removal has been calculated: 1g P to 3g SS sludge (Jardin and Pöpel, 1997).

The form of carbon, specifically Short Chain Volatile Fatty Acids, present in the influent sewage has been shown to increase nitrogen and phosphorus treatability levels in existing BNR systems (Lie et al., 1997; Munch and Koch, 1997; Oldham and Abraham, 1994; Rabinowitz and Barnard, 1997; Raper et al., 1997). Converting a larger fraction of the waste's carbon component to VFA's internally could increase this effect, and hence overall nutrient removal. The HRT of the anaerobic zone, of a BNR system, can be drastically reduced because of the VFA supplementation of sidestream primary sludge fermenters (Barnard et al.,1995). Facilities employing primary sludge fermenters have shown that if their fermenters are taken out of service, they can no longer meet their phosphorus discharge limits (Barnard and Rabinowitz, 1997).

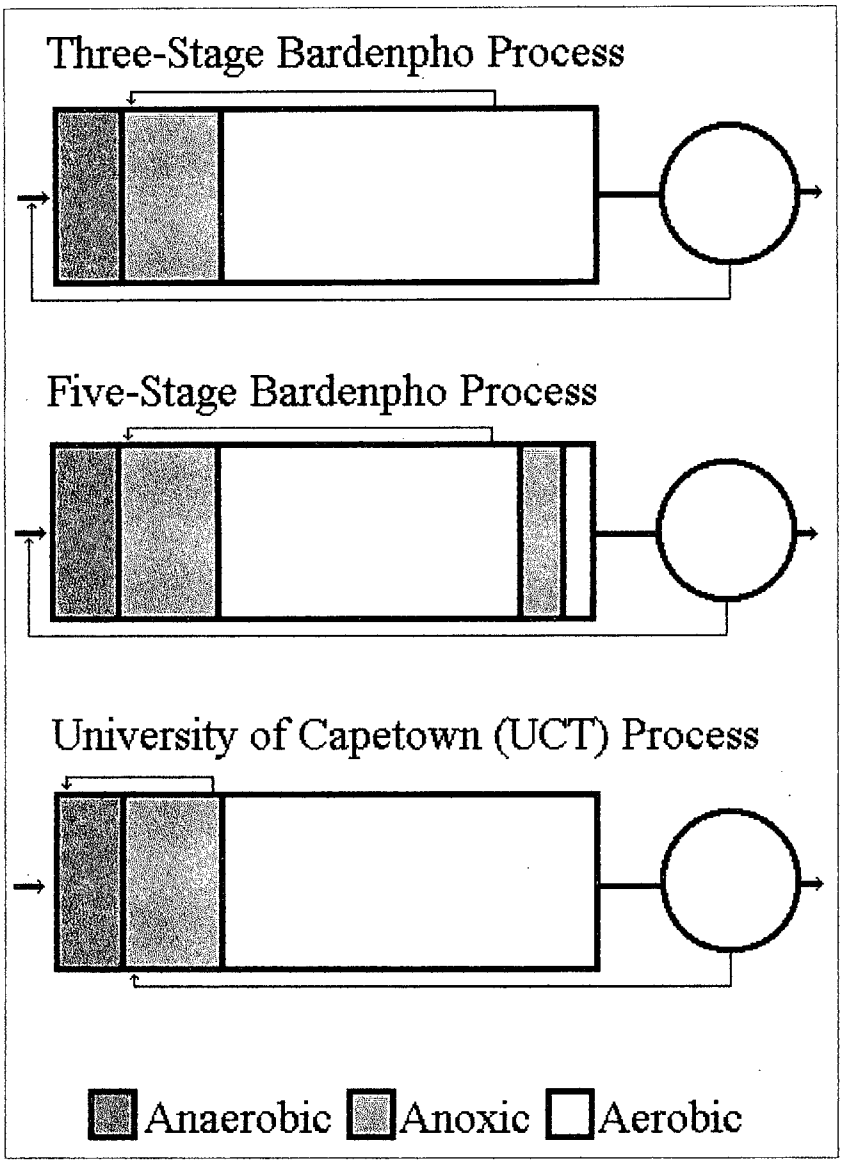
There is some discrepancy in the reported relationship between VFA content and phosphate removal. Reported values for the amount of VFA's necessary to remove one milligram of soluble phosphate range from 5mg (Barnard, 1998) to 20mg (Lie et al., 1997). However, it has been shown that, excessively high concentrations of acetic acid are inhibitory to phosphorus removal (Randall and Chapin, 1997). Lab-scale BNR experiments indicated that, acetic acid concentrations of up to 400mg/L were beneficial to the process. Above 400mg/L however, the performance of both phosphorus and COD removal dropped.

Three basic types of BNR systems are shown in Figure 2.1: Three-Stage Bardenpho, Five-Stage Bardenpho, and University of Capetown (UCT). The three-stage Bardenpho system employs anaerobic, anoxic, and aerobic reactors in series. A portion of the aerobic activated sludge is recycled to the anoxic zone to enhance denitrification. Secondary clarifier underflow is recycled to the anaerobic zone to stimulate biological phosphorus removal. A three-stage Bardenpho system first went into service in 1974 (Barnard, 1974).

The five-stage Bardenpho modification to the previous system was designed to increase both nitrogen and phosphorus removal (Argaman, 1991). The five-stage modification first went into practice in 1978 (Randall, 1992). Two extra reactors were added, anoxic and aerobic, to further enhance denitrification and ensure aerobic conditions in the secondary clarifier, respectively. Anaerobic conditions in the secondary clarifier must be avoided to prevent the bacterial release of phosphate. The five-stage design is predominantly used where the nitrogen removal regulations are stringent (Barnard and Rabinowitz, 1997).

The UCT system is identical to the three-stage Bardenpho, with the exception to the recycle configuration (Argaman, 1991). Activated sludge is recycled to the anoxic zone and then from the anoxic zone to the anaerobic zone. This configuration ensures that, no nitrates will enter the anaerobic zone (Barnard and Rabinowitz, 1997) and hence, no interference with SCVFA storage (Argaman, 1991). The UCT process first went into practice in 1984.

Figure 2.1: Three Basic Types of BNR Systems



(Adapted from Barnard and Rabinowitz, 1997)

2.2 Pre-Fermentation: Role in Nutrient Removal

It has been shown that, wastewater must be at least partially fermented, yielding volatile fatty acids, before it can fuel enhanced phosphorus removal (Randall, 1994). This "pre-fermentation" can occur in three ways:

- Fermentation can occur in sewer pipes leading to the waste facility.
- The anaerobic zone of a BNR plant can provide the fermentation.
- A pre-fermenter can partially ferment the waste stream, either raw wastewater or settled solids, and feed VFA's directly to the anaerobic zone of the BNR process.

A prefermenter ensures a plentiful supply of VFA's to the anaerobic reactor, independent of fluctuations in raw sewage flow (Randall, 1994). High VFA's in the anaerobic zone can improve the settleability of secondary sludge, by selecting for polyphosphate organisms in the BNR system. The settleability is increased because, polyphosphate bacteria form better flocs than non-polyphosphate bacteria, and the increased phosphorus content, in the flocs, increases the overall sludge density (Randall, 1994). Even under conditions where the sewage is partially fermented in the sewer system, the addition of a prefermenter, will produce higher and more consistent phosphorus removal rates (Barnard, 1994). The addition of a side-stream prefermenter, provides VFA's to the BNR system independent of the main system flow, and the current "health" of the activated sludge organisms (Barnard, Stevens, and White, 1995). A side-stream fermenter can supply a concentrated amount of VFA's anywhere in the BNR process, allowing more optimization options for nitrogen and phosphorus removal. The results of a BNR facility, Beckton Sewage Treatment Works, (in London, England) demonstrated that the average effluent phosphorus without prefermentation was 4mg/L. With the addition of a side-stream prefermenter, the effluent

phosphorus dropped to 1-2mg/L (Rabinowitz and Williams, 1995).

The size of the anaerobic reactor can be reduced as much as 95% because of the VFA contribution of a prefermenter (Randall, 1994). The combination of, a smaller anaerobic zone and prefermenter, has been shown to prevent the secondary release of phosphorus in BNR systems (Barnard, 1994). Secondary phosphorus release is defined as, the anaerobic release of phosphorus, without the associated uptake of nutrients. Without the accumulation of PHB in the anaerobic zone, there will be no enhanced phosphorus uptake in the aerobic zone, due to lack of bacterial energy stores.

There are four types of sludge fermenters currently employed in BNR systems: activated primary tank, complete mix fermenter, single stage fermenter/thickener, and 2-stage complete mix fermenter/thickener. Descriptions of each type, listed below, were adapted from a fermenter system summary by Barnard and Rabinowitz (1997).

An activated primary tank fermenter utilizes the sludge blanket, formed in the primary clarifier, as a source of fermentable solids for the BNR system. The primary clarifier underflow is recycled to the clarifier inlet, either directly or via a mixed holding tank. A fraction of the recycled sludge is wasted to the sludge-handling system to maintain a workable sludge blanket depth on the floor of the clarifier. VFA's, produced by the sludge blanket, are carried into the BNR process with the primary effluent. An advantage of using this type of fermenter is that, no additional construction is needed, since the system employs an existing clarifier as a reactor (Barnard, 1994) A complete mix fermenter is essentially a modified version of this system. The main operational differences between the two systems involve the location of sludge fermentation. In the later system, the

fermentation occurs in an enlarged mixed holding tank, whereas the primary clarifier sludge blanket is minimized.

The single and 2-stage fermenter systems separate the primary effluent flow from the VFA- enriched fermenter effluent. The single stage system utilizes an enlarged gravity thickener, which can operate at a flexible HRT and SRT because it is not directly dependant on the flow of raw sewage. One advantage of this type of system over the previous examples is that, VFA-rich thickener effluent is added directly to the BNR anaerobic zone, protecting the VFA's from metabolic activity in the clarifier. Thickener underflow is drawn off to the sludge handling system. The 2-stage system adds a complete mix fermenter in series between the primary clarifier and the gravity thickener. This design allows recycle from the thickener back to the mixed fermenter, allowing longer SRT.

2.3 Microbial Biofilms

Microbial biofilms can be defined as mixed or pure bacterial populations growing on an inert surface. It has been shown that almost any "wetted" surface is inhabited by a population of bacteria (Gjaltema and Greibe, 1995). The bacterial populations live in a layer of Extracellular Polymeric Substances (EPS) on the surface of the inert substratum. The EPS retains the bacteria and diffused nutrients providing anchorage and protection. It has been documented that a killed or inactivated biofilm surface is quickly re-colonized because of the EPS layer left behind from the previous population (Flemming and Melo, 1995). A developed biofilm layer protects its bacterial inhabitants from suspended disinfectants or poisons, which makes an established biofilm very difficult to kill (LeChevallier et al., 1988). The transport of any chemical from the bulk liquid to the biofilm layer

is highly rate limited. The result is that the required contact time for a suspended disinfectant to kill the resident population is much longer for a biofilm population than a suspended population.

The detailed structure of biofilms can only be theorized due to the lack of effective techniques for the in situ observation of biofilm development (Lazarova and Manem, 1995). Light microscopy has the advantage of simplicity and the ability to view the biofilm directly, without chemical or physical pre-treatment. Sophisticated monitoring techniques are not practical for use within current reactor designs (Gjaltema and Greibe, 1995). Confocal laser and electron microscopy have been used to study biofilm formation in micro-scale experiments (Wolfaardt et al., 1994). However, the biofilm analysis of larger systems occurs off-line, involving the removal of the biofilm from the substratum. The removed biofilm can then be characterized by physical or chemical parameters, such as: mass, thickness, TOC, COD, protein content, or VSS (Lazarova and Manem, 1995;Meraz et al., 1995). In practice, biofilm sampling is difficult because access to the interior of bioreactors is highly limited, especially in the case of anaerobic fermenters (Gjaltema and Greibe, 1995).

The accepted theory of biofilm structure involves a series of layers, each with unique bacterial and biochemical characteristics. Recent research has identified the development of a biofilm layer as the growth of microbial clusters which mushroom up from the substratum surface (Bishop and Rittmann, 1995). The result is a non-uniform, mountainous terrain, with channels between developing colonies of bacteria.

Biofilm heterogeneity can be described in terms of the following: diversity within populations, metabolic reactions, biofilm chemistry, variation in the amount of EPS and variation in the physical

structure (Bishop and Rittmann, 1995). Modeling biofilm development and performance is difficult and highly dependant on the specific reactor conditions. Bacterial growth dynamics in the biofilm layer have been studied to determine standardized growth models for biofilm systems (Cheng et al., 1992; Meraz et al., 1995). Models relating product inhibition, metabolic efficiency and nutrient flux into the biofilm, have been theorized (Bhaskar and Bhamidimarri, 1991; San et al., 1993). However, they are not ready to serve as guidelines for bioreactor design.

2.4 Attached Growth Media

In biological sewage treatment systems, biofilm reactors have higher volumetric productivity over suspended growth systems (Bhaskar and Bhamidimarri, 1991). In order to achieve this high treatment efficiency, it is important that a high concentration of microorganisms is retained in the process. An optimal way to obtain this is to use a suspended carrier, or attached growth, in the process to increase the Solids Retention Time (SRT) relative to system Hydraulic Retention Time (HRT) (Lessel, 1991). An effective attached growth media must offer a high specific surface area, a good biofilm holding capacity and avoid clogging due to increasing biomass. Once the media clogs, the available surface area is greatly reduced, thus lowering the efficiency of nutrient transfer from the waste water to the biofilm. Abrasion is used, in fluidized media systems, to control and limit biofilm development (Wilderer et al., 1995). The following desired properties of suspended carriers have been outlined by Tsubone (et al., 1992).

- The surface of the carriers must be rough, to ease biofilm colonization.
- The carriers must be easily fluidized. The fluidization should not require more aeration or

mixing than is necessary to sustain the biological reaction in the reactor.

- The carrier must be easy to separate from the effluent. The size of the carrier must be such that it is easy to screen out of the reactor outflow.
- The functional lifespan of the media must be long. The media should not be susceptible to mechanical and biological breakdown

In previous research, with both aerobic nitrifying reactors and anaerobic fermenters, a variety of media types have been employed (Table 2.1). The trend in media design is away from rigid media structures, due to plugging tendencies, towards fluidized carriers or other flexible media (Demerci et al., 1993; Lessel, 1991; Tsubone et al., 1992).

Table 2.1: Examples of Attached Growth Media

Researcher(s)	Media Type(s)	Media Description	Application
Cheng et al., 1992	ceramic particles	0.5mm diameter, 1.7g/cm3	Anaerobic fermenter
Demirci et al., 1993	pea gravel 3M-macrolite spheres PP-composite chips	 aluminum oxide PP+oat hulls, 2-3x3mm	Anaerobic fermenter
Hem, 1991	KMT media	Kaldnes media pre-cursor	Nitrifying reactor
Lessel, 1991	Bionet Linpor Ringlace	rigid polyethylene mesh floating foam cubes flexible PVC rope	Nitrifying reactor
Meraz et al., 1995	PE pellets	0.28-0.56mm diameter, 0.27g/cm3	Anaerobic fermenter
Ney et al., 1990	porous glass rings	25x26mm, 1.7g/cm3, 55% porosity	Anaerobic fermenter
Tsubone et al., 1992	porous PP carrier	3x3mm, 1g/cm3	Air-fluidized-bed

PE - polyethylene; PP - polypropylene

The research of each paper referenced in Table 2.1 is summarized in the following paragraphs.

Cheng (et al., 1992) performed methanation research with a pilot-scale Upflow Anaerobic Sludge Blanket (UASB) reactor, fed food processing wastewater. Ceramic carriers were fluidized, in a 21m tall tower, by a laminar flow of 25-37m/hr. System HRT was decreased from 24hr at startup to 1.5hr after methane production was established. Analysis of the carriers after one month revealed negligible bacterial growth. The biofilm development was limited to cracks in the media surface. The smooth surface, combined with high liquid velocity, was inhibiting biofilm colonization. Further analysis after two months revealed complete bacterial coverage of the carrier surface. Bacterial testing of the biofilm indicated that the biofilm consisted primarily of filamentous organisms. Cheng hypothesized that rods and cocci were absent because they were easily dislodged by the wastewater flow. Rods and cocci were present in suspension, supporting his theory.

Demirci (et al., 1993) tested three types of suspended carrier in a lab-scale anaerobic fermenter, fed glucose as a carbon source. The three media tested were: 3M-macrolite spheres, pea gravel, and composite polypropylene (PP) chips. The results indicated that only the composite PP chips produced a complete biofilm growth layer. The pea gravel and macrolite spheres produced only a partial bacterial covering, consisting predominantly of filamentous organisms. Further biofilm formation testing with a variety of composite materials demonstrated that, oat hulls and soy flour, combined with PP, produce the best carrier particles. Demirci and Pometto (1995) later hypothesized that, the organic component of the composite carriers increases the affinity for bacterial adhesion, by possibly providing a carbon and/or nitrogen source.

Hem (1991) performed biofilm research on fluidized attached growth nitrifying reactors, lab-scale then pilot-scale. He chose a, newly developed, cylindrical carrier called KMT as the experimental media. This media was a pre-cursor of the Kaldnes media tested in this research. The original KMT media carrier differs from the current Kaldnes design by its lack of fins along the carrier's outer circumference. Hem found that, even after the biofilm was established, there was little growth on the outside of the carrier. Biofilm on this surface is unprotected and can be easily dislodged by either physical contact with other carriers or internal reactor structures.

Lessel (1991) tested the performance of three media types in a full-scale nitrifying reactor. The media tested were the following.

- Bionet, a rigid bio-filter material fixed in cages.
- Linpor, porous cube-shaped suspended carrier media.
- Ring-lace, flexible rope media attached to rigid cages. The same material is currently known as Ringlace® media.

The experimental results indicated that both Bionet and Linpor media types were inefficient, as nitrifying media, due plugging and general solids buildup. Lessel recommended that very high shear was required to prevent clogging of the Bionet media, hence extra cost associated with aeration or mixing. Linpor media, once loaded with biofilm, tended to sink to the bottom of the reactor. Lessel found that, up to 50% of the Linpor media in the reactor had settled to the bottom. Ring-lace media maintained its biofilm layer, without clogging. Lessel concluded that, the swaying movement of the media in its cage, prevented excess accumulation of solids. In addition, Lessel demonstrated that, although the accumulation of biomass and solids by Ring-lace media was much lower than the solid

structure media and individual carrier media, the nitrification performance of all three was comparable.

Meraz (et al., 1995) assessed the immobilization dynamics of a downflow fluidized bed anaerobic reactor. The media, consisting of ground polyethylene pellets (0.28-0.56mm), was fluidized with a laminar flow of 9m/h. The reactor design was similar to that of the BF-system reactors filled with Kaldnes media. Two operational differences between the two designs were that, the reactor used by Meraz, was equipped with an external trap device to filter out media that had sunk to the bottom and a higher laminar flow (approximately 3m/h for the BF-system). Biofilm performance was measured as ability to degrade acetate. The results of batch testing at mesophilic (35 °C) and thermophilic (55 °C) temperatures indicated the following.

- Mesophilic biofilm activity increased for the first 70 days of the run. The amount of immobilized biofilm plateaued after 60 days.
- Thermophilic biofilm activity was very low throughout the experiment (120 days). The immobilized biofilm was also poorly developed.

Meraz hypothesized that, the poor thermophilic performance may have been due to the source of bacterial inoculum. The inoculum was mesophilic sludge, acclimated to acetate degradation. The thermophilic inoculum was incubated at 55 °C for two years prior to use. He noted that, during thermophilic incubation, the sludge lost its granular structure which may have hindered its ability to form a biofilm on the polyethylene pellets.

Ney (et al., 1990) performed anaerobic fermentation research on a lab-scale (20L), packed bed,

attached growth fermenter. The media chosen consisted of porous glass rings (25mm by 26mm), with a wall thickness of 4mm and pore size range of 60-300μm. HRT was adjusted from four days, at startup, to 12.5h after two weeks of operation. Methane production had reached a maximum after two weeks. The results indicated the following.

- 82% of the total biomass in the reactor was immobilized on the glass rings.
- The amount of attached biofilm was evenly divided between the outer surface of the rings and the pores inside the structure.
- After eight weeks, the system clogged with biomass.

The design of these reactors was quite similar to the BF-system reactors filled with Kaldnes media, which functioned more like a packed bed than a fluidized system, due to low laminar flow.

Tsubone (et al., 1992) assessed attached growth media performance in an Air-Fluidized-Bed-Bioreactor (AFBBR). Resin-based media, which entraps bacteria, was produced by combining concentrated activated sludge with a photopolymerizable resin. The carriers produced were approximately 3mm in diameter. Results comparing the performance of entrapped media with non-entrapped media indicated that, there is no advantage to entrapping biomass inside the media. Tsubone hypothesized that, most of the biologically-mediated reactions occur in the developed biofilm, not the entrapped organisms. Tsubone developed list of criteria for a suitable suspended carrier (see page 16). A cylindrically shaped, expanded polypropylene carrier (CEPPC) was developed which matched the listed criteria. The media, resembled cylindrical "cheerios" in appearance (3mm by 3mm cylinder with a 1.5mm inner diameter). This media was shown to be very effective at treating high BOD wastes in an AFBBR reactor.

2.5 BIOLOG Assay

The Biolog system (Biolog, Inc., Hayward, California) is a bacterial identification method which identifies pure isolates based on their sole source carbon utilization pattern. The microplates each contain 96-wells; 95 wells containing a single carbon sources and one well with no carbon source. Carbon source utilization (oxidation) is measured colorimetrically by the reduction of Tetrazolium violet dye from its colorless form to violet. The intensity of this color formation reflects the level of oxidation and can be measured either by manual color comparison of the test wells with the control or by using a MicroStation automated spectrophotometer unit marketed by Biolog Inc. GN-type (Gram Negative) plates were chosen for the assay.

The color patterns and resulting computer database based identifications (Microlog computer database) produced from BF-system samples cannot be interpreted as true identifications because the samples were not purified cultures. The Biolog system is designed for identification of pure cultures and hence the database reflects the biochemical diversity of individual organisms only. The assay will however, yield a map of biochemical activity for the mixed population. This map or fingerprint represents the prevalent biochemistry of the reactor populations as a group.

The Biolog system has been previously used for the purpose of defining the bacterial activity profile of soil populations and how this profile varies with habitat (Garland and Mills, 1991). The pattern of positive and negative reactions has been used to indicate the level of biodiversity in soil populations in terms of functional diversity and temporal development (Zak et. al.,1994). The extent and time required for dye formation has been monitored in several publications with dye intensity counts taken at various intervals for 20 hours (Haak et al., 1995) to 72 hours (Zak et.

al.,1994).

Functional diversity in this experiment is defined as the ability, as a group, to utilize a large number of substrates. Since the starting materials and populations were the same for all systems, differences in biodiversity indicate the effects of conditional differences within each media system. Once a pattern typical for a productive short HRT acidogenic fermenter is determined, it is hoped that Biolog testing of individual systems can be used to assess the overall health of fermenter's biofilm. In the BF-system the temporal pattern of positive reactions of each system can be used for future comparison with other measurable biochemical parameters, such as SCVFA production, to assist in the optimization of the fermenter system design. It has been shown that the detection of functional bacterial groups in fermenter systems is more valuable than the detailed description of species present (deBeer and Muyzer, 1995).

CHAPTER 3: MATERIALS and METHODS

3.1 Bio-Fermentation System Components

A bio-fermentation (BF) reactor system was developed to allow intensive monitoring of fixed film fermenters fed parallel waste streams. Because the goal was to assess SCVFA production within an HRT of one hour, triplicate reactors were connected in series for each experimental condition and operated as a single plug flow system with a total HRT of one hour. The flow through each reactor was adjusted to yield an HRT of twenty minutes. Figure 3.1 represents the original design implemented and Figure 3.2 represents the configuration employed for the two experimental runs. The system operated at ambient temperature for both runs. No attempt was made to control the temperature profile because both the two "media-containing series" and the control series were subjected to the same temperature variations. UBC Pilot plant temperature data indicates that the average seasonal temperature ranges during the two experimental runs are, 17-24 ℃ and 10-16 ℃ (Doucette, 1998).

Each component of the system is discussed individually and the total flow profile calculations are also included.

Figure 3.1: BF-System Initial Configuration

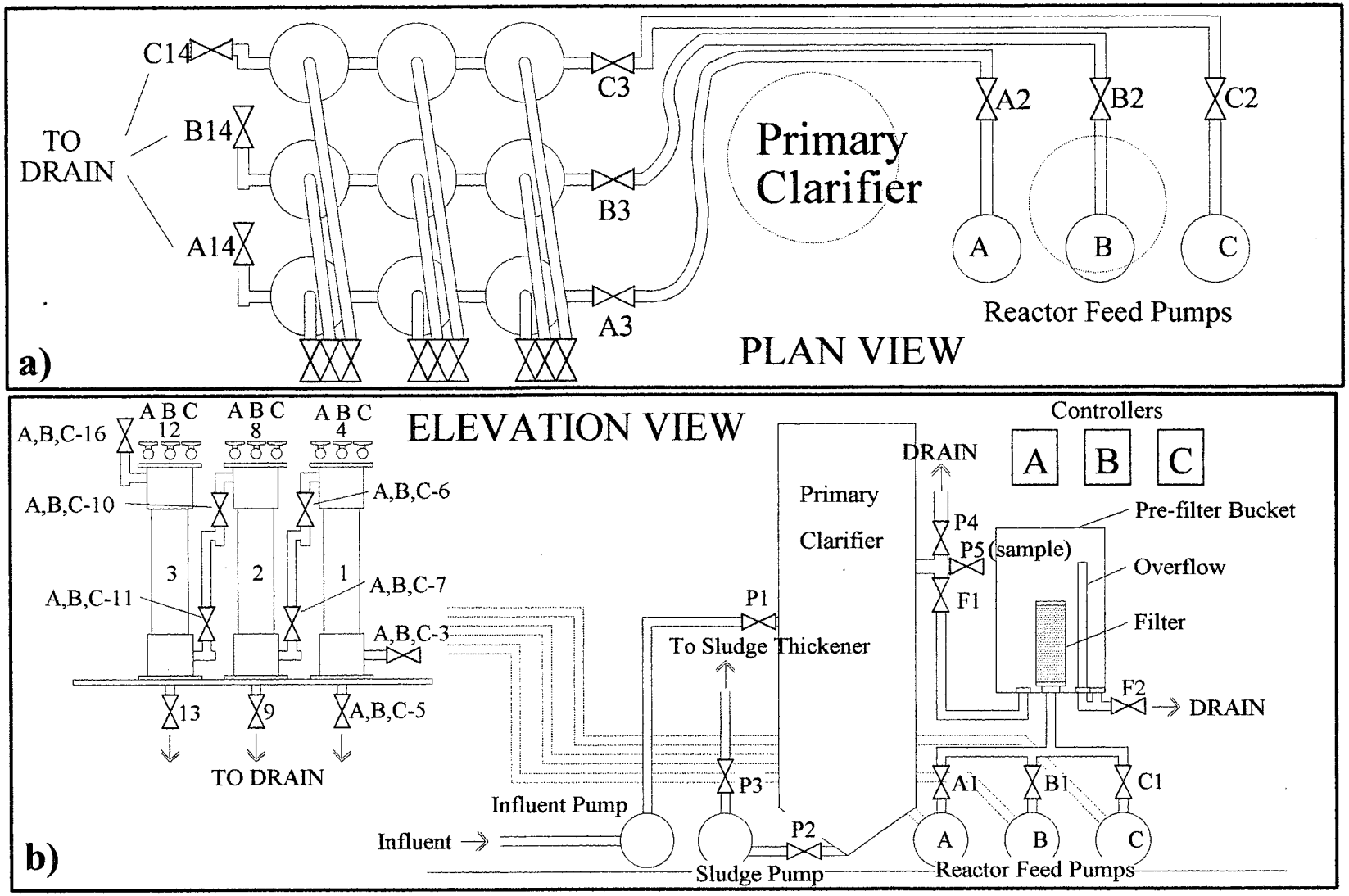
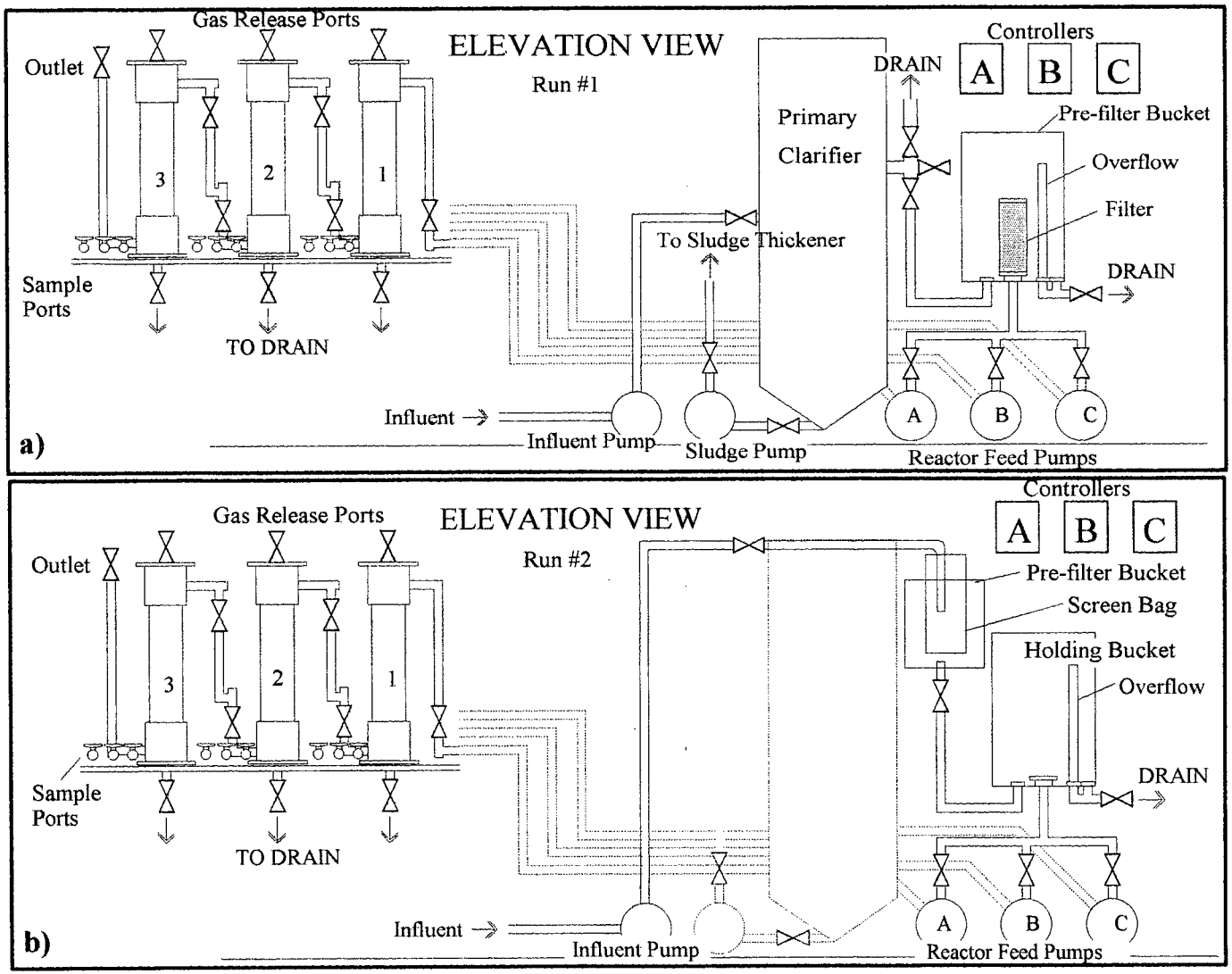


Figure 3.2: BF-System Configuration, Experimental Runs



3.1.1 Primary Clarifier

A cylindrical clarifier, operated with a functional volume of 600L, was plumbed upstream of the process for run #1 to provide primary effluent as feed. The operational HRT range of the clarifier was approximately 85-100min. The HRT cannot be calculated more accurately due to unaccountable flows within the system (due to a worn sludge pump and leaks between the outlet weir and main tank). The solids retention time (SRT) was minimized to reduce insitu fermentation by pumping out bottom solids frequently, resulting in a negligible sludge blanket. The effluent level within the clarifier outlet chamber was raised with a fifteen centimeter length of ½" pipe to just below the weir level, to reduce aeration of the primary effluent. Prior to the pipe addition, the effluent dropped fifteen centimeters over the weir as a thin film.

3.1.2 Storage Bucket

Wastewater during both runs was gravity fed to a 20 L storage bucket fitted with an overflow, rinsing drain and the BF system inlet for the duration of both runs. All samples representing system influent were taken from this bucket once it was deemed that samples taken directly from the reactor inlet pipe were not representative. During run #1, the BF system inlet was fitted with a cylindrical metal mesh filter (3mm pore size) to prevent entry of larger fragments from entering the system. This mesh was removed prior to the second run due to severe mesh plugging when employed to screen raw wastewater. An inner mesh with a larger pore size (5mm) was retained for the raw sewage run. A mixer was added to the bucket to reduce solids settling along the bottom. The bucket had an HRT of about 3.5min, assuming the designed overflow rate was maintained during a 24hr cycle. An overflow rate of 10% of the designed influent flow rate was maintained to reduce scum

layer formation at the bucket surface. A low fluid level sensor was added for run two, which turned off the BF-system pumps if the influent level in the bucket was less than one third.

3.1.3 Pumps and Plumbing

Three identical screw pumps (Moyno 500) were employed to provide continuous pressurized flow, one for each of the three reactor lines of the BF-system. Pump speed was controlled by three controller units (SP500) allowing individual adjustment. One pump pressurized each reactor series, allowing fine tuning of flows, as well as the ability to maintain different specific HRT settings on parallel reactor series within a single run in the future. Each pump was adjusted to deliver a flow of 1.4 L/min through each reactor line. A rigid ½" PVC head works plumbed from a single storage bucket outlet split the flow three ways and into each pump. Downstream of the pumps, each reactor line had a separate and, for the most part, parallel feed line constructed of ½" PVC and ½" flexible PVC hose.

Ball valves and unions were installed between each reactor to allow a variety of sampling and maintenance options as well as the removal of an individual reactor without disassembling the rest of the system. An outlet manifold was mounted at the end of each series, fifteen centimeters above the outlet level prior to dropping the effluent to a communal 2" PVC drain. The raised outlet assured that the system would flow under positive pressure and the reactors would remain full. Gas buildup was periodically released through a valve at the top of each reactor. BF system effluent was wasted directly to drain for both runs. Designed flow settings were calculated for a reactor volume of 28L, to account for interior plumbing and minor construction differences between the reactors. Actual flows were measured and optimized manually at the outlet of each series, to assure similar flows

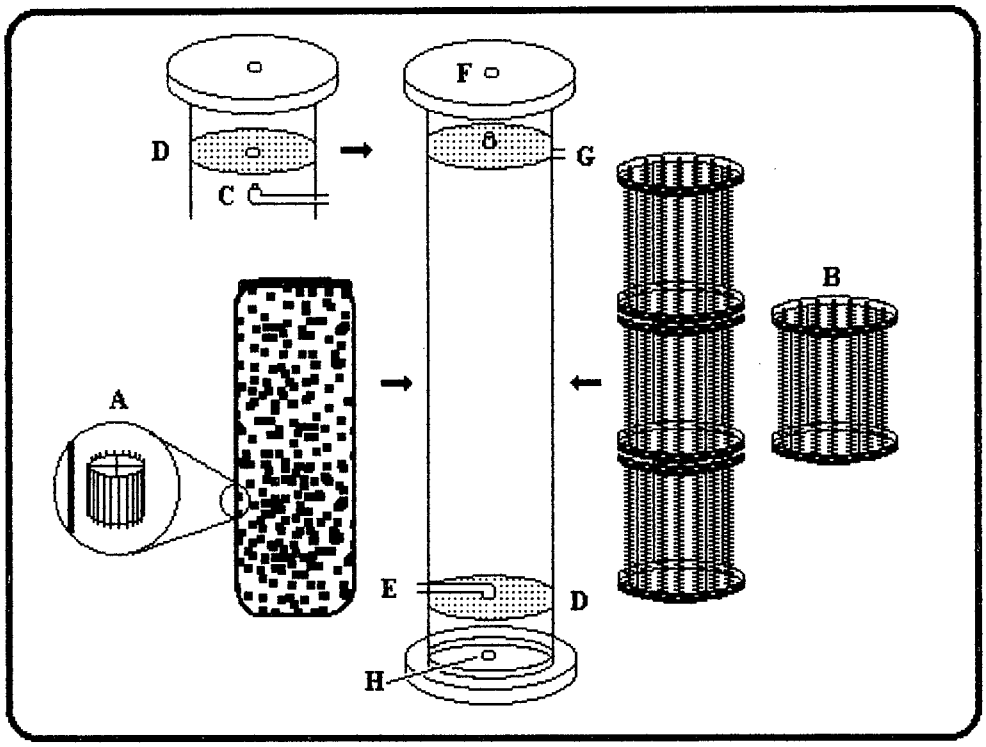
for each series.

Two other pumps (Moyno 33101) were used to operate the BF-system; one to pump system feed and a second to pump out primary sludge from the clarifier. System feed flowed continuously at 6-7L/min into the primary clarifier during run one and 5-6L/min into the filter screen bucket during run two, depending on the sewage levels in the pilot plant storage tanks. The sludge pump was operated intermittently to maintain a minimal sludge blanket in the clarifier; 1.5min on, 2.5min off.

3.1.4 Reactors

The reactor system consisted of nine reactors connected in three series of three reactors and designed to be fed equal portions of feed water. The three series (K, R and C) were similar with respect to hardware and designed flow. Each series contained a different attached growth media: series K contained Kaldnes media; series R contained Ringlace® media; and series C contained no media. The two media types are characterized later in this chapter (section 3.2). Series C was the control or baseline for the system, reflecting chemical changes to the influent due to insitu fermentation by the naturally occurring suspended and fixed biomass. This parallel reactor design allowed for a true control reactor component, since each system within a run was fed the same waste stream to compensate for periodic influent make up fluctuations.

Figure 3.3: BF-System Reactor Components



A - Kauldness media **B** - Ringlace® media **C** - intake jet
D - Diffuser plate **E** - outlet / sample port **F** - vent / sample port
G - inlet port **H** - drain

Each reactor had a total volume of 30L and was divided into three compartments each separated by a diffuser plate: inlet, media , and outlet chambers (Figure 3.3). Mixing chambers were added to the reactor design to separate the plug flow active reactor zone from the turbulent entry zone of each reactor. Prior to the BF system assembly, dye tests were performed with a larger scale clear PVC reactor to determine the mixing and flow characteristics of the mixing chambers. The results were used to determine design specifications of the mixing chamber, diffuser plate and nozzle size of the inlet ports. Each chamber was defined as the area between the end surface of the reactor and a diffuser plate with 1/4" holes drilled at ½" centers. The holes were chamfered to 3/8" on both sides of the plate to provide better flow. The outlet chamber and inlet mixing chamber were identical,

except for a 3/8" diameter jet adapter, which was added to the inlet pipe of the inlet mixing chamber. The dye tests had indicated that a jet smaller than ½" was necessary to stimulate turbulence within the mixing chamber.

The media zone was defined as the large central area between the diffuser plates and was designed as plug flow. The two media types resided in this zone either in bags (Kaldnes) or on PVC racks (Ringlace®). Kaldness media was encased in 1/4" mesh sacks, 20-21" in length, during the primary effluent run. However, for the raw wastewater run, it was loaded without sacks in an attempt to reduce plugging within the bags. Cylindrical PVC cages were constructed to provide a removable support structure for the Ringlace®. The units consisted of two 1" long cylindrical sections mounted on four 10" by ½" hollow spacers by 11" long steel rods (Figure 3.4). By sizing the cages to yield multiple units per reactor, destructive biofilm sampling was possible without disturbing the bottom ⅔ of each reactor. In addition to sampling considerations, the small unit size allowed the option of running the system with different amounts of media per unit of reactor volume. Although the nine reactors were designed to be identical, there were some structural and plumbing differences which have resulted in non-identical volumes (Table 3.1). For all HRT calculations, the volumes of the upper mixing chamber, lower mixing chamber and media zone are defined as 1L, 1L and 28L respectively.

Figure 3.4: Ringlace® Cage Detail

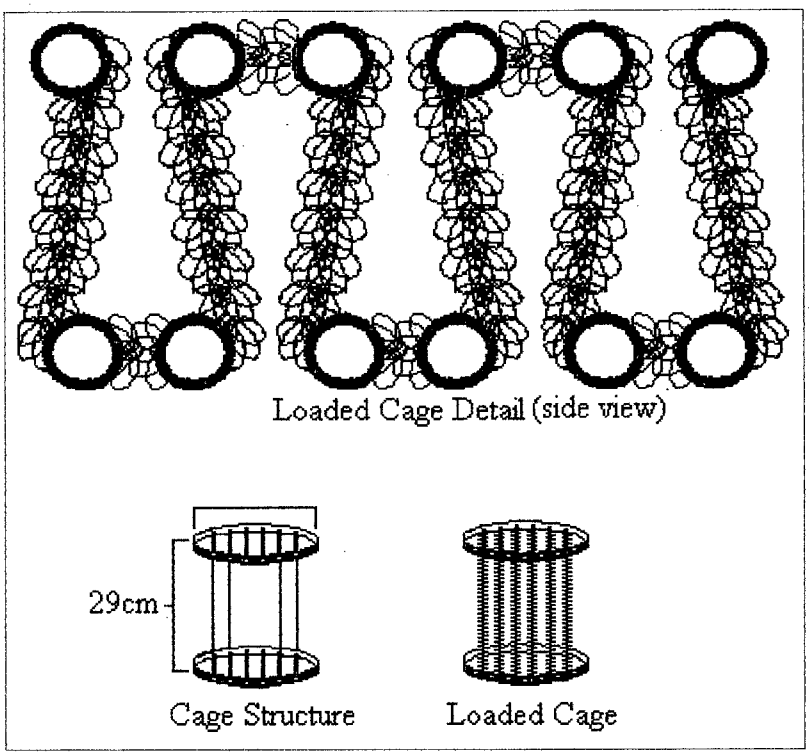


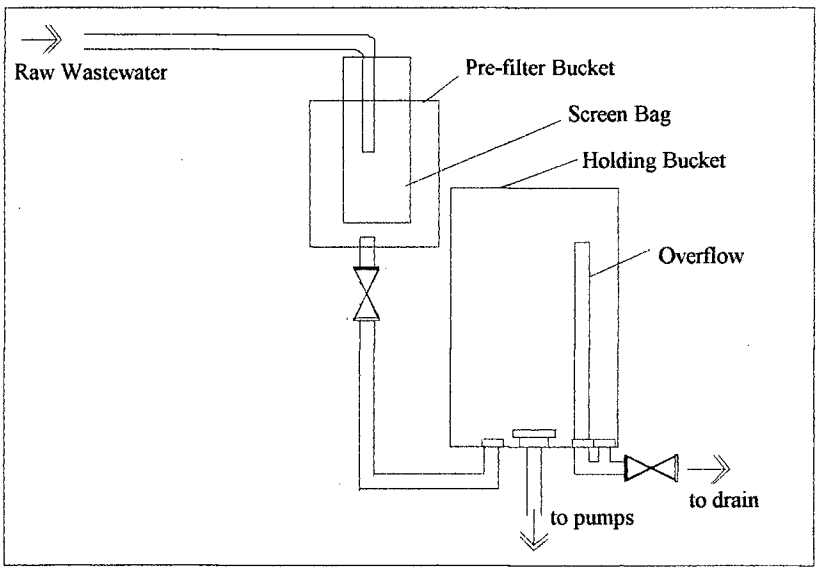
Table 3.1: BF-System Reactor Working Volumes

Media	Kaldnes	Ringlace	Control
Reactor #1 (L)	21.3	26.2	28.4
#2	22.0	26.3	27.5
#3	22.5	26.1	28.5
Average (Mean)	21.9	26.2	28.1
Media Volume	6.2	1.9	na
Media / reactor	28%	7%	na

3.1.5 Pre-Filter Bucket

A second reservoir (Figure 3.5) was placed upstream of the original filter bucket to house a screen bag (4mm pore size) to catch the larger solids particles when the system feed was switched to raw wastewater for run two. This reservoir was designed to run almost empty to reduce the retention time within it, and hence, reduce insitu fermentation. The disposable screen bags were changed periodically when they filled up with solids and impeded flow. Within one day, the 4mm bags were shown to be inadequate and were replaced with larger pore size bags,7mm.

Figure 3.5: Run #2 Pre-filter Bucket



3.1.6 BF-System Flow Profile

Each experimental run utilized a different pool of equipment. As a result, the flow profile of each run is unique and is discussed separately. For a schematic view of the flow profile for runs one and two, see Figures 3.6 and 3.7, respectively. Definitions of each flow component are listed below.

X_1	raw sewage into the system	X_{4ABC}	BF-system influent flow
X_2	clarifier sludge waste flow to the thickener	X_5	bucket overflow
X_3	primary effluent flow	X_6	pre-filter bucket overflow

Figure 3.6: BF-System Flow Definitions, Run #1

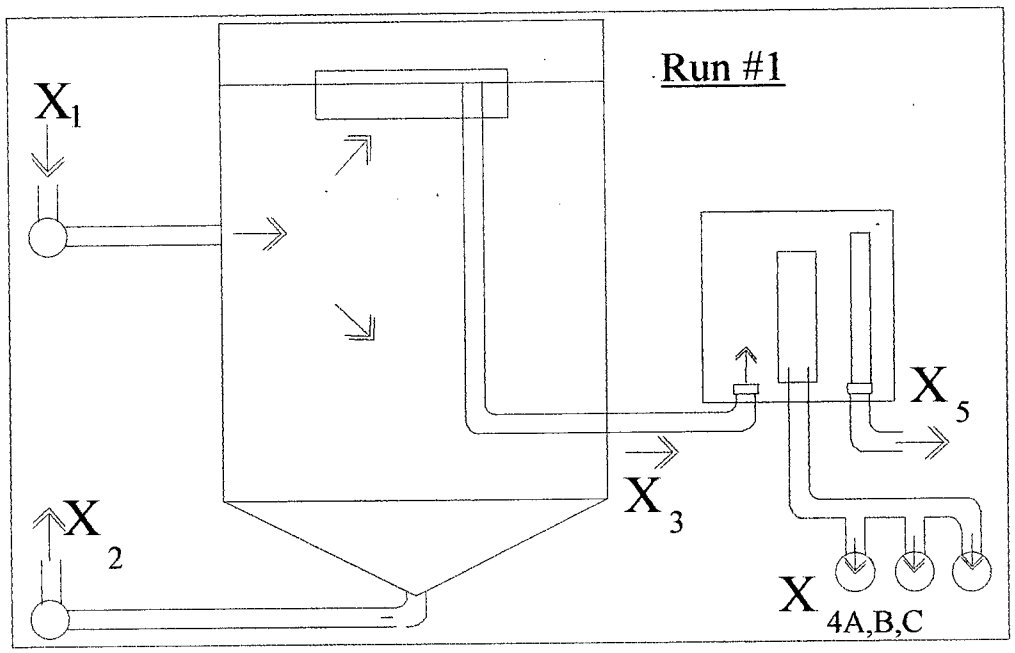
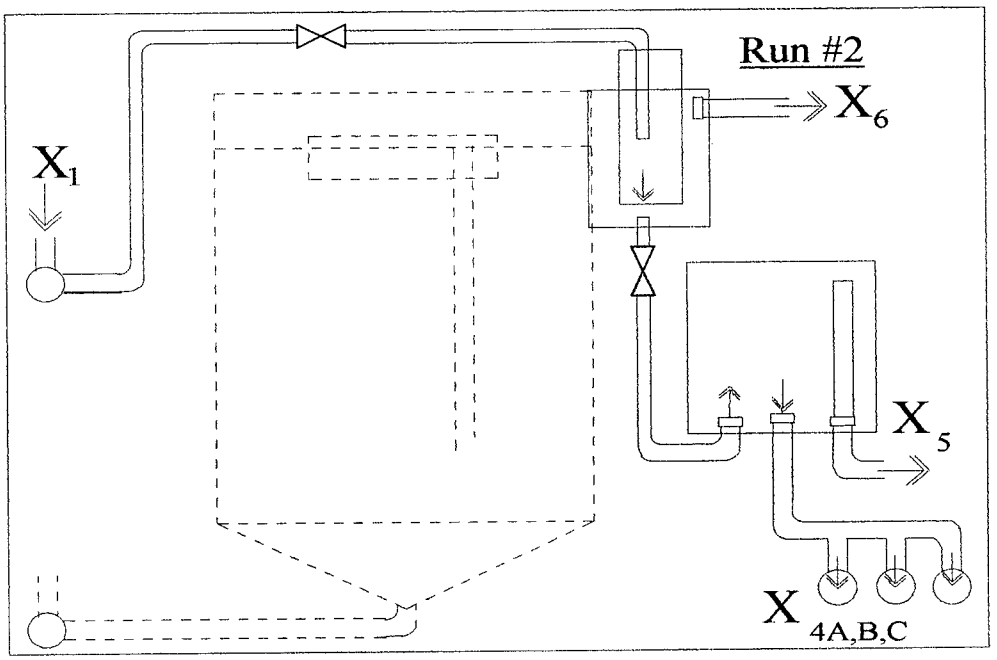


Figure 3.7: BF-System Flow Definitions, Run #2



3.1.6.1 Run #1

The design flow for each reactor series was 1.5L/min (X_{4ABC})which translates to a minimum of 4.5L/min entering the influent pump head works. After trial and error testing, it was determined that the clarifier sludge waste flow (X_2) had to be maintained at 1.2L/min at a 15% duty cycle. In order to regain a full bucket each duty cycle , the minimum raw sewage flow (X_1) was calculated to be 6.5L/min. Periodic overflow (X_5) on each duty cycle served the purpose of scum removal from the top of the bucket reservoir. Flows were checked daily to account for flow loses caused by hardware inefficiencies and waste stream supply fluctuations.

3.1.6.2 Run #2

The design flows for run two were similar to run one so few changes were made to the system flows. Without the sludge pump running, X_1 only needed to exceed the X_{4ABC} flow requirements to ensure some scum removal with the bucket overflow (X_5). The raw sewage flow was operated between 5-6L/min for the duration of the run.

3.2 Media

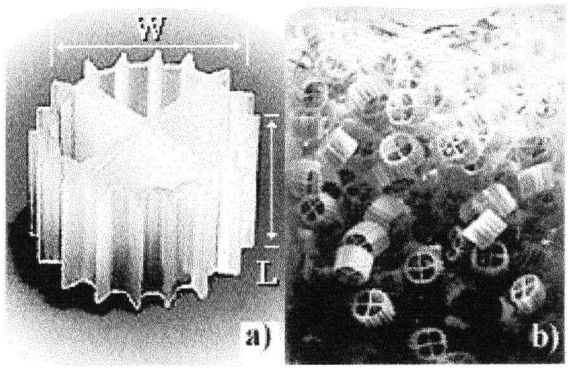
Biomass in a fixed film system is attached to the media surface, and not floating freely as in activated sludge systems. As a result, the fixed biomass has a greater tendency to remain in the system and reduce the effluent solids level. Thus the total amount of sludge retained by the system during normal operations is significantly increased (Lessel, 1991). Fixed media vary with respect to design but share the following goal: to provide a high surface area per unit volume attachment surface for bacterial growth. The large amount of surface area provided by the media allows for

development of a population of biomass with more "active surface" and hence greater metabolic capability than conventional floc solids systems. This enables the organic waste material to be broken down and digested much more quickly than a typical activated sludge process. Decreased retention time translates to smaller system size and therefore, significant cost savings. Both media types tested are being used aerobically in full-scale waste treatment systems (Sen et al, 1993; Hem, 1991).

3.2.1 Kaldnes Media

Kaldnes media was developed as a neutrally buoyant attached growth media with the goal of increased nitrification efficiency in aerobic reactors (Hem, 1991), similar to that of Ringlace®. The Kaldnes media for this research was supplied by Anglian Water (Cambridge, England). The patented Kaldnes Process is based on the biofilm which grows on the surfaces of free-floating plastic carriers made of polyethylene (PURAC Engineering Inc, 1998). The carriers, as shown in Figure 3.8, consist of plastic cylinders, 7mm by 9mm, with internal cross-braces and ridges around their circumference. The ridges were designed to protect surface biofilm from abrasion in a fluidized reactor (Hem, 1991).

Figure 3.8: Kaldnes Media



a) W= 9mm, L= 7mm b) Media in CSTR (PURAC Engineering Inc.)

Kaldnes carriers have a density, without biofilm, of just under 1g/cm^3,which allows them to be easily suspended by a reactor's aerators, in an aerobic process, or by mechanical mixers in non-aerated systems. Research with a polypropylene carrier similar to Kaldnes stated that, because of the protected nature of the interior of the carrier, a biofilm layer was established in three days (Tsubone et el., 1992). The exterior surfaces required twelve days for biofilm establishment. The manufacturer (Kaldnes Miljoteknologi AS, Norway) recommends a maximum media loading of 67% of the reactor volume. This gives an effective media surface area loading of approximately. 350 m^2/m^3 reactor.

Kaldness media was encased in 1/4" mesh sacks to ease insertion into the reactors and reduce the chance of plugging at the diffuser plate, by wedging up against the chamfered holes. Bags were used during the primary effluent run only. The Kaldnes media was loaded without sacks during the raw wastewater run in an attempt to reduce plugging within the bags. The loaded sacks were 20-21" in length, roughly ⅔ the height of the media zone. Although this ratio was suggested by the manufacturer for fluidized systems, it was chosen as the ratio for our plug-flow system. The media

and bags had a displacement volume of approximately 4.4 L

3.2.1.1 Kaldnes Surface Area and Media Loading

All surfaces on the Kaldnes media, including edges, were included in the surface area calculations performed. The surface area calculated is an indicator of the theoretical maximum area possible. Once a biofilm is formed, the areas between ridges and the interior of the cylinder, fill up with growth. Therefore, a better approximation of the "working" surface area is between the above calculation and that of a solid cylinder. Equation 3.1 calculates the surface area of the media's cylindrical walls.

Cylinder Edge	+	outside Cylinder	+	Inside Cylinder	=	Kaldnes Cylinder Surface area	(Equation 3.1)
(pi*4.52)-(pi*42)	+	(2*pi*4.5*7)	+	(2*pi*3.75*7)	=	376mm2	

The cross-braces inside the Kaldnes cylinder and the external ridges are accounted for in Equation 3.2.

Cross sides	+	Cross ends	+	Ridges	=	Kaldnes Ridges Surface area	(Equation 3.2)
8*(7*3.75)	+	2[(0.5*8)+(0.5*7.5)]	+	18(2*0.5*7)	=	352mm2	

The two solutions above are combined, in Equation 3.3, to yield the maximum surface area possible per Kaldnes particle.

Cylinder Surface area	+	Ridges Surface area	=	Kaldnes Media total Surface area	(Equation 3.3)
376mm2	+	352mm2	=	728mm2 7.3cm2	

The calculations for the minimum working surface area for the Kaldnes media, representing clogged media, is shown in Equation 3.4.

Cylinder wall	+	Top and Bottom	=	Kaldnes Full Cylinder Surface area	(Equation 3.4)
(2 x pi x 4.5 x 7)	+	(2 x pi x 4.5 x 4.5)	=	32.1 mm2	

The surface area loading per reactor was calculated by, counting the number of Kaldnes units in a 1L container, and then extrapolating to determine the total number per reactor. The counting results, conducted by a fellow researcher, indicated that approximately 925 Kaldnes carriers occupy the volume of 1L. By considering the maximum individual surface area and bulk media volume, the relative surface area loading per volume of media was calculated, 676m^2 media/m^3 media. The Kaldnes media bags occupied approximately 63% of the reactor volume, resulting in a reactor loading of 430m^2/m^3. If the clogged media model is considered, the calculated minimum reactor

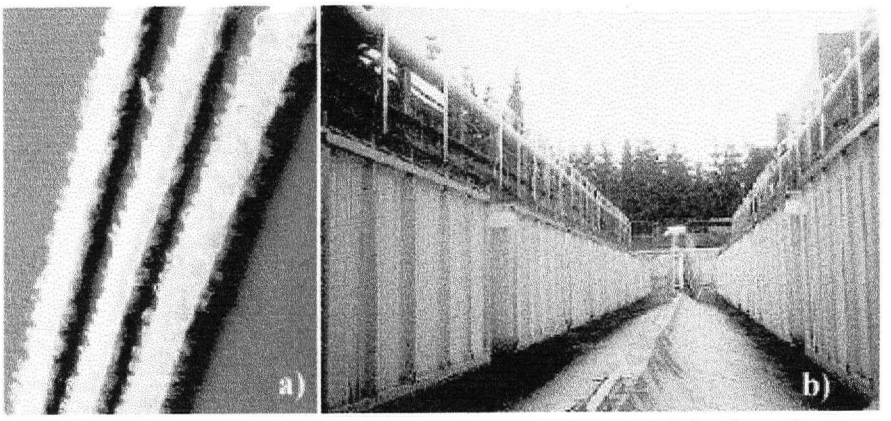
loading would be reduced to $19m^2/m^3$.

The previous calculations refer to media loading as a function of biofilm surface area. However, since one of the goals of attached growth systems is to increase the SRT, this researcher also calculated the volume loading of the media. Media volume loading was defined as the volume of solids retained by the media per unit volume of the reactor. This value reflected a maximum value, and assumed that the media carriers are completely full of solids. It was assumed that the volume of the media structure is negligible in its impact on the maximum calculated volume of solids retained by each carrier, $445mm^3$. The Kaldnes media volume loading, in the BF-system, was calculated to be $0.4m^3/m^3$. The above calculations do not include the solids trapped between adjacent carriers, only the solids attached to a free carrier such as in a fluidized system.

3.2.2 Ringlace® Media

Ringlace® biomedia was developed in Japan in the 1970's and became available in the United States in 1990. Ringlace® Products, Inc., the supplier of the media for this project, has been the exclusive distributor for North America since 1992. Ringlace® rope media was first used in sewage reactors by Lessel (1991) in an attempt to upgrade the nitrification performance of an aeration tank. He found that the use of such an attached growth media effectively increases the sludge age relative to a suspended growth system with the same HRT.

Figure 3.9: Ringlace® Media.



a) Ringlace Strands. b) Full-scale Ringlace Cages (Ringlace® Products Ltd., Website)

Ringlace® media consists of polyvinyl chloride plastic fibers knitted into continuous strands containing many loops arranged in rows along a 6mm wide ribbon. The ribbon is wound in a tight spiral with the loops on the outside, resulting in the appearance of a rope (Figure 3.9a). The media must be mounted to a fixed lattice, such as the full-scale cages pictured in Figure 3.9b, as Ringlace® media is not self supporting. Once mounted, it is stretched to 110-115% of its relaxed length to allow some strand movement but to reduce the chance of entanglement between parallel sections as suggested by the manufacturer (Ringlace® Products Ltd., Oregon). The established ratio of media to reactor volume for Ringlace® is 18 - 28% to maintain homogeneity (Lessel, 1991). However, this range is designed for aerobic conditions and should not apply to this research.

Full-scale Ringlace® installations have been in operation world-wide since 1978, with over 300 Ringlace® installations in Japan (www.Ringlace®.com). All of these systems employ Ringlace® in an aerobic environment, this research however, focuses on Ringlace® performance in an anaerobic environment.

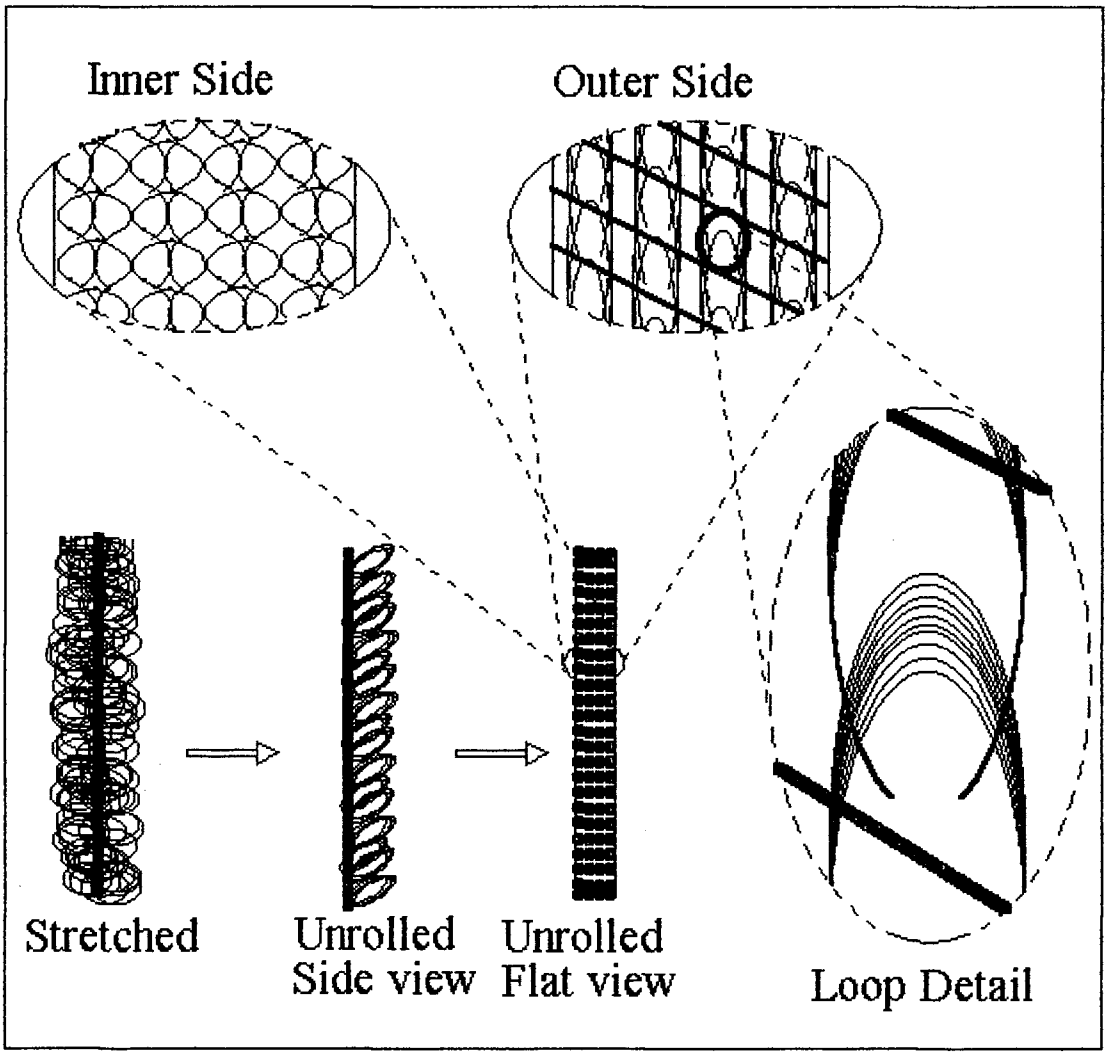
Ringlace® media requires a rigid mounting surface to maintain the strand spacing recommended by its manufacturer. Custom fabricated cylindrical cages were constructed from PVC and stainless steel which fit, length-wise, three deep into each of the three Ringlace® media reactors (Figure 3.3). Each cage housed thirty-eight strands mounted from six PVC rungs on each end plate for a total length of eleven meters of Ringlace®. Strands were aligned parallel to the plug flow and affixed at one inch (2.5cm) centers with PVC zap straps. Previous research used 4.5cm spacing between strands of Ringlace®, mounted inside nitrification/denitrification reactors (Lessel, 1991). Total length per reactor was therefore 33m and total length per 60min HRT of 99m. The media loading for the Ringlace® was 3500 m/m^3. This is much higher than the recommended media loading range, which is 250-500m Ringlace®/ m^3 cage volume (Lessel, 1991). The recommended loading values refer to an aerobic system, which requires aeration between strands. One of the goals of this research was to maximize the biofilm contact, so a tighter packing distance than the recommended value was chosen. Pictures of the cage structure loaded with media are displayed in Appendix C.

3.2.2.1 Ringlace® Surface Area and Media Loading

The Ringlace® media strand was unrolled to yield a flat 6mm wide ribbon, woven from both loop material and other support fibers. Four bundles of loop strands are woven in across the width of the ribbon (Figure 3.10). The loop bundles each consist of ten similar length loop strands and protrude from only one surface of the ribbon. When the ribbon is allowed to form a tight helix, the loop-side faces outward. The Ringlace® rope was stretched to approximately 110% of its static length and several five inch sections were marked with a felt pen. Each section was unrolled and re-measured

to determine the "ribbon to rope" length ratio. The ratio was found to be approximately 1.1:1, ribbon to rope, respectively. Several loop bundles were cut off, as close to the ribbon surface as possible, to determine the average length.

Figure 3.10: Ringlace® Media, Surface Detail



For the purpose of surface area determination, the following assumptions were made:

- The active biofilm matrix forms on the loops only, therefore only the loop area was counted.
- Loops contribute to the surface area equally and are of identical functional length.
- Area was calculated based on the media being stretched to 110% of its natural length.
- 1cm of stretched Ringlace® unrolls to 1.1cm of ribbon.
- The strand thickness is 0.1mm.

The following equations were used to calculate the surface area per centimeter of Ringlace® stretched on the cage lattice. Equation 3.5 relates the total individual strand length of each fiber in 1cm of stretched Ringlace® media.

loop length (cm)	x	number of loops per bundle	x	number of bundles per cm	x	Stretch correction	=	Strand Length per cm	(Equation 3.5)
2.6cm	x	10	x	20	x	1.1	=	572cm 5720mm	

Equation 3.6 relates the total strand surface area in 1cm of stretched Ringlace® media.

2	x	pi	x	Strand Radius	x	Strand Length per cm	=	Strand Surface area per cm	(Equation 3.6)
2	x	3.1416	x	0.05mm	x	5720mm	=	1797mm2 18 cm2	

Since each reactor contains 33m of Ringlace®, the surface area per reactor can also be calculated. The maximum Ringlace® surface area per reactor was calculated to be approximately 5.9 m^2. The loading per cubic meter of reactor was 208 m^2/m^3.

At the cessation of the runs, it was noted that the Ringlace® media resembled a solid rope surface, although not smooth. Therefore, the functional surface area and media loading, of this media fall somewhere between the above calculation and the calculated minimum surface area of the media, shaped like a cylinder, with a 9mm radius. The calculated minimum surface area for Ringlace® media in the current BF-system configuration is 67 m^2/m^3 (1.9 m^2/reactor).

The media volume loading of Ringlace® media was calculated, in a similar manner as the minimum surface area calculation above, and assumed that the media is shaped like a rope with a 9mm diameter when full of solids. The calculated maximum volume of solids retained by Ringlace® media was $2.5x10^{-4}m^3/m$. The maximum media loading for Ringlace®, in the current BF-system configuration, was $0.3m^3/m^3$.

3.3 Analytical Procedures

The sampling and processing scheme was designed to address temporal variations in the following parameters: VFA concentration, total organic carbon(TOC), total chemical oxygen demand(tCOD), soluble COD components and turbidity.

3.3.1 Sampling

Initially, all sampling was done to coincide with the HRT of the system, in order to better represent real changes to the primary effluent. Samples were taken "forward" starting from the system influent and ending with the effluent of the third reactor in each series. This sampling scheme was designed to overcome temporal variations in sewage stream makeup by sampling from the "same" slug of material by timing the sampling to coincide with the HRT of each reactor. However, this sampling scheme was abandoned due to the disruptive effects of sampling on system flow continuity. Each reactor sampling port was allowed to run for 30 to 60 seconds prior to sample collection which channeled the system flow away from the downstream reactors.

The sampling scheme was reversed for the experimental runs. Samples were collected from the outlets of the third reactors and last from the reservoir bucket. This method resulted in no downstream disruptions occurring prior to sampling. Unless otherwise noted, the pre-filter bucket and each reactor outlet flows were sampled for each parameter.

The crude sample consisted of 40-50ml aliquots of liquid. Samples were extracted from each sample port, following flushing, to ensure that the samples represented the makeup of the active process stream not the idle liquid present in the sampling pipe. Each sample was processed and preserved

at the pilot plant within twenty minutes of sampling. Sub-samples requiring unfiltered aliquots were dispensed directly from the crude sample bottles and preserved, with the exception of suspended solids samples (which require no preservative).

Approximately 10-20ml of each crude sample was vacuum filtered through 1.5μm glass fibre filters (Whatman 934-AH,). The resulting filtrate was dispensed in multiple 1-3ml aliquots for laboratory analysis. All samples were chemically preserved and refrigerated to prevent degradation before analysis. Since the 24 hour sampling experiment of the pilot plant influent revealed regular temporal changes, sampling was done once during each sampling day during a specified time period when the influent make-up was constant. Samples were taken three times per week during both runs, except where operational problems prevented regular sampling. The following section lists and discusses relevant sampling and maintenance changes made during the BF-system runs.

Reactor flushing protocol was constantly adjusted to try to optimize solids removal while reducing media exposure to oxygen. The importance of a strict regimen of flushing became evident during run #2 because of the high solids loading of a BF-system using raw wastewater as feed. Details of this progression and an outline of the best protocol are included in the following section.

3.3.1.1 Preliminary experiments (June-July, 1996)

- BF-system was operated as an up-flow system.
- Initial sampling window was set to 8-9:30am due to the results of the 24h sampling.
- Samples were taken from all sampling points at once and processed at the UBC Pilot Plant. Sampling order was as follows: K3, R3, C3, K2, R2, C2, K1, R1, C1, storage bucket and primary effluent.
- A four inch impeller type mixer was added to the storage bucket after it was noted that a sludge blanket (2-5cm) formed on a daily basis.
- The up-flow design was shown to result in the accumulation of fine solids within the reactors. Within eight weeks of operation, several of the BF reactors were completely filled with fine solids.

3.3.1.2 Run #1 (August-November, 1996)

- BF-system re-configured as a down-flow system.
- Due to results of the particle analysis experiment, the Whatman no.4 filter was dropped in favor of Whatman glass fiber filters for all analyses.
- Sampling order was adjusted to reduce the time interval between sampling and processing each sample. Samples were sampled and processed in four groups as follows: 60min samples, 40min samples, 20min samples and finally the bucket and primary effluent samples.
- Sampling window was moved to 3-5pm (September 12,1996) due to personal inefficiencies with early morning sampling.

3.3.1.3 Run #2 (December, 1996-February, 1997)

- Began flowing raw wastewater December 10,1996
- Switched filter bags from 4mm to 7mm pore to alleviate plugging
- Reactor A1 plugged (December 17), cleaned out Kaldness media by hand.
- Noted that the raw influent contained few solids for approximately one week due a pilot plant reservoir mixer breakdown (December 26 - January 4).
- As of the January 14 sampling, a "bucket" sample is taken by scooping material directly from the storage bucket.
- Reactor K1 plugs often and must be force flushed with tap water forwards and backwards under high pressure to release built up solids (January 23 and 30).

3.3.1.4 BF-System Reactor Flushing Protocol

- Keep pumps on running during flushing.
- Open drain into a bucket, with valve to top and next reactor closed
- Momentarily open the top valve to introduce SOME air into top (also speeds flow out and helps dislodge solids.)
- Periodically close the drain valve for 10-20s to build some pressure and help dislodge solids, only after some air has entered the mixing chamber at the top of the reactor.
- After repeating above until the drain effluent is no longer opaque, open valve to next reactor and close the drain.
- Bleed out top air by opening the top valve while closing the valve from the downstream reactor
- Repeat the above series for each downstream reactor.

3.3.2 Analysis

Details of each analytical technique utilized during this research is discussed in this section individually.

Preserved BF-system samples were refrigerated for up to three weeks and processed in batches at the Civil Engineering laboratory, either by this researcher or the resident technicians, with the exception of TOC; this assay was performed at the Bio-Resource Engineering laboratory. In addition to regular liquid sampling, samples of each media were removed at the end of each run to assess biofilm activity by determining its crude microbial fingerprint. Metabolic fingerprinting utilized the BIOLOG plate assay.

3.3.2.1 Chemical Oxidation Demand

Chemical Oxidation Demand (COD) analysis of the BF-system attempted to describe variations in components of the system feed as it is processed by the reactors. Total COD (tCOD) is defined as the colorimetrically determined COD in an aliquot of the whole, unfiltered, BF-system sample. Soluble COD (sCOD) is defined as the COD which passes through a 1.5μm glass fiber filter. Particulate COD is defined as the difference between total and soluble COD measurements for the purposes of these experiments. Both tCOD and sCOD were measured to determine which feed components are being metabolized to yield VFAs. Two techniques were employed for the processing of COD samples (Table 3.2). Both methods were chosen from the established COD protocol of the Environmental Engineering Laboratory at UBC (Hach method, digestion at 150 ^{0}C for 2h). Total COD samples were processed by the "high range" method and soluble COD by the "low range".

Preliminary samples were processed with both methods to determine the working range of each sample and select the appropriate method. COD sampling was done concurrently with VFA sampling during startup and experimental runs.

Table 3.2: COD Protocol

	Hi Range (20-900)	Low Range (0-200)
Wavelength used	600 nm	420 nm
Standards (mg COD/L)	0, 50, 100, 200, 400, 800	0, 50, 100, 200
Zero"	0	200
COD reagent : - volume dispensed	(10.217g $K_2Cr_2O_7$) + (167ml H_2SO_4) + (833ml H_2O) 1.2 ml	(2.27g $K_2Cr_2O_7$) + (167ml H_2SO_4) + (833ml H_2O) 1.2 ml
Acid reagent : - volume dispensed	(5.5g Ag_2SO_4 / 1kg H_2SO_4) 2.8 ml	(5.5g Ag_2SO_4 / 1kg H_2SO_4) 2.8 ml
Sample volume	2 ml	2 ml

3.3.2.2 Suspended Solids

Solids analysis of the BF-system was limited to suspended solids, specifically components filterable by glass fiber filter (Whatman 934-AH, 1.5μm). Results are defined by the following parameters.

- TSS was defined as the fraction of total sample solids filterable by glass fiber filter which does not evaporate after heating to 105°C.
- DSS was defined as the fraction of total sample solids which are not filterable by glass fiber filter and pass through with the filtrate. DSS was not measured during the BF-system experiments.
- FSS was defined as the fraction of filterable suspended solids which remains after heating to 550°C.
- VSS was defined as the fraction of the TSS which oxidizes and is driven off in gaseous form by heating to 550°C. This value is obtained by the subtraction of FSS from TSS for each sample.

A separate 250ml bottle of BF-system sample was taken at each sampling point and refrigerated at the pilot plant, prior to processing for suspended solids in the Environmental Engineering laboratory. A volume of each sample, between 10ml and 100ml, was vacuum filtered using pre-dried and weighed glass fiber filters. All filtrate was discarded and the filters were dried for 12-24 hours at 105°C to determine TSS, then flamed in a muffle furnace for one hour at 550°C to determine FSS. SS analysis was performed during part of run #1 only.

3.3.2.3 Total Organic Carbon

Total Organic Carbon (TOC) analysis was added near the completion of run one and continued throughout run two. The chosen TOC sampling and processing protocol resulted from verbal consultation with Environmental Engineering laboratory staff. Disposable 4ml Pipettes were modified to serve as both the transfer devices and storage chambers for the 3ml filtered TOC samples. Approximately 2cm of the tapered open end of each pipette was removed to enlarge the aperture sufficiently to allow easier insertion of the preservative, 0.1ml of 0.1N sulfuric acid using a pipettman. The sample aliquot is drawn up by suction prior to addition of preservative. Each filled plastic pipette was sealed by melting approximately 1cm of the open end and pinching it off with pliers. The preserved samples were refrigerated until being processed in two or three week batches.

3.3.2.4 Turbidity

Turbidity measurements were taken to assess temporal variations in suspended solids and color at each stage of the system. The goal was to determine the correlation between turbidity and SS using preliminary data and to replace SS analysis for the duration of the experiments in favor of the quicker of the two analyses, turbidity. However, early in run one, turbidity measurements were dropped from the protocol due to inconsistency of results.

3.3.2.5 Short Chain Volatile Fatty Acids

For the purposes or the BF-system experiments, only short chain volatile fatty acid (SCVFA) concentration was measured. SCVFA results are expressed as total acetic acid equivalents and were calculated from the measured concentrations of propionic acid (CH_3COOH) and acetic acid (CH_3CH_2COOH) by the following equation:

$$\text{acetic acid concentration} + \left(0.81 \times \text{propionic acid concentration}\right) = \text{acetic acid equivalent} \qquad \text{Equation 3.7}$$

Aliquots for VFA analysis were taken from the vacuum filtrate of all BF-system samples. Each 1ml sample was preserved with 0.1ml of 2-3% Phosphoric acid, sealed in glass ampules and refrigerated until being processed in weekly batches by Environmental Engineering laboratory personnel. Samples during preliminary testing were taken from the pre-filter bucket and the sampling ports of the third reactor in each series only. During the actual runs, SCVFA samples were taken from all reactor sampling ports, as well as the pre-filter bucket and raw influent. Sampling was done once per week during the start up period of run one and three times per week during both runs.

3.3.2.6 BIOLOG Assay

Samples from the third reactor in each of the tested media series were removed at the cessation of both the first and second runs. These samples, consisting of biofilm and media, were processed to release a portion of the affixed population into suspension. Samples of media were shaken to release biofilm components into suspension. In an attempt to separate the microbial biomass from nutrients

in suspension, the samples were centrifuged, washed and re-suspended in sterile distilled water, prior to inoculation of the BIOLOG plates. The procedure resulted in significant solids present in the final dilutions so the protocol was modified for the second run. The media was removed prior to centrifugation step in run two to reduce solids transfer to the dilution series. Some effort was made to preserve an anaerobic environment for the samples but the bulk of the analyses were performed aerobically, according to the recommended modified Biolog analysis for anaerobic organisms in the published user manual (Marello and Bochner, 1989).

The modified protocol requires inoculation of a higher concentration of organisms than with aerobic samples to compensate for lack of growth by anaerobic cultures under aerobic conditions. One difference between the recommended protocol and the protocol of this researcher was the reliance on turbidity standards and strict controls on inoculate concentration. In an attempt to reduce exposure to oxygen, this author chose to use several dilutions for each sample, rather than adjust the turbidity to the recommended range.

The second series of Biolog analysis employed the use of an anaerobic chamber for incubation and part of the sample processing to reduce the samples' exposure to oxygen. It was hoped that these modifications would increase the biochemical response of anaerobic populations.

In addition to manual notation of well pigmentation, the photography of incubated BIOLOG plates was added to the protocol for run two. Two light sources were used; a fluorescent light box was used to back light the plates for all time points and natural sunlight was used for the last time point only.

The first analytical run consisted of six gram negative (GN) type plates (Table: 3.3). Three plates

were used for each media type: Ringlace® and Kaldness media. Three different concentrations of each sample were inoculated and processed for 24hrs. All of the plates were observed at 4, 8, and 24hrs; distinguishable well patterns were noted. The second analytical run consisted of fourteen GN type plates were inoculated with three concentrations of sample suspensions and processed for 36hrs; seven plates were used for each media type. All of the plates were observed at 17, 24, and 36hrs; distinguishable well patterns were noted. Pigment intensity present in each of the 95 test wells on the Biolog plate relative to the control well intensity was recorded by manual observation and assigned a number between "0-4" representing the range from control well intensity to strongly pigmented wells. Photographs were also taken of all plates throughout run two.

Table 3.3: GN Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	water	a-cyclodextrin	dextrin	glycogen	tween 40	tween 80	N-acetyl-D-galactosamine	N-acetyl-D-glucosamine	adontitol	L-arabinose	D-arabitol	cellobiose
B	I-erythritol	D-fructose	L-fructose	D-galactose	gentiobiose	a-D-glucose	m-inositol	a-lactose	a-D-lactose lactulose	maltose	D-mannitol	D-mannose
C	D-melibiose	B-methyl D-glucoside	psicose	D-raffinose	L-rhamnose	D-sorbitol	sucrose	D-trehalose	turanose	xylitol	methyl pyruvate	mono-ethyl succinate
D	acetic acid	cis-aconitic acid	citric acid	formic acid	D-galactonic acid lactone	D-galacturonic acid	D-gluconic acid	D-glucosaminic acid	D-glucuronic acid	a-hydroxy butyric acid	B-hydroxy butyric acid	G-hydroxy butyric acid
E	p-hydroxy phenylacetic acid	itaconic acid	a-keto butyric acid	a-keto glutaric acid	a-keto valeric acid	D,L-lactic acid	malonic acid	propionic acid	quinic acid	D-saccharic acid	sebacic acid	succinic acid
F	bromo succinic acid	succinamic acid	glucuronamide	alaninamide	D-alanine	L-alanine	L-alanyl-glycine	L-asparginine	L-aspartic acid	L-glutamic acid	glycyl-L-aspartic acid	glycyl-L-glutamic acid
G	L-histidine	hydroxy L-proline	L-leucine	L-omithine	L-phenyl alanine	L-proline	L-pyro glutamic acid	D-serine	L-serine	L-threoline	D,L-carnitine	G-amino butyric acid
H	urocanic acid	inosine	uridine	thymidine	phenyl ethylamine	putrescine	2-amino ethanol	2,3-butanediol	glycerol	DL-a-glycerolphosphate	glucose-1-phosphate	glucose-6-phosphate
	1	2	3	4	5	6	7	8	9	10	11	12

3.3.3 Experiments

3.3.3.1 Preliminary Experiments

Prior to establishing a sampling schedule, a 24 hour sample of raw influent and primary effluent was characterized to determine the validity of grab samples or necessity of sampling within a daily time window for consistent results. The sampling was performed by Frederic Koch, Research Associate, Jason White, summer student and this author on July 4,1997 from 7am until 6am July 4. Raw wastewater and primary effluent were sampled hourly for a 24hr period and processed to reveal daily fluctuations in the following: suspended solids, nitrates, phosphates, COD and VFA's (data presented in Appendix A). Sampling windows were established at times when the makeup of the influent was most consistent: 8-10am and 3-5pm.

3.3.3.2 Main Experiments

The first run compared the performance of Ringlace® and Kaldness as fixed film media for the acidogenic fermentation of primary domestic effluent in terms of VFA production and resistance to plugging. The HRT was set to 20min per reactor, yielding a one hour HRT for each reactor series. The first two series were Kaldness and Ringlace® containing reactors respectively and the third series was the control; this series contains no media and reflected changes to the influent resulting from time and naturally occurring biological activity present on the plumbing surfaces and in the bulk liquid.

The second run focused on VFA production performance using screened raw wastewater as feed and utilized the same media as the previous run. The BF-system headworks had to be re-worked to

accept the raw wastewater. Other than feed related hardware changes, the second run was designed to mimic the conditions in the later half of run one: down-flow design and afternoon sampling.

3.3.3.2.1 Run #1

The first run was initiated on June 6,1997 and operated for several weeks as an up-flow system, without sampling to establish the biofilm and standardize operating protocol. After a false start on June 30, the first sample was processed on July 4. All subsequent samples were obtained between 0800hr and 0945hr until September 12. In the hope of processing fresher sewage, the sampling time interval was moved to late afternoon, 1600hr to 1830hr, for the rest of the run. Because the principal filling time for the raw waste water reservoirs was 1000hr, it was decided that an afternoon sampling would yield results of fermenting fresher sewage than the original morning samples. Run one was halted on July 28, when severe solids buildup was noted within the reactors. The BF system design was changed from up-flow to down-flow, to circumvent the solids build up. The re-configured down-flow system was re-started on July 31 with cleaned media. Since all media and reactors were washed during the stoppage, no BF-system samples were taken until Aug 16. Samples were taken approximately bi-weekly until October 15.

3.3.3.2.2 Run #2

The renovated BF-system was started December 13 (a Friday) but was halted within 24hr due to pre-filter plugging. The filtering effect of packed Kaldnes media resulted in a dense mass of solids accumulating in the K1 reactor within 24 hours of the start-up of run two. Modifications to the

protocol and system head works, mentioned in the sampling section (3.3.1.3), only delayed the inevitable plugging, resulting from running raw wastewater through a packed Kaldnes media bed. The Kaldnes series was operated with a positive pressure of 20-30 psi for most of the second run.

The system was operated on and off as modifications were being made during the run itself, until December 30, 1996. During this period plugging was rampant and resulted in two system modifications: usage of a larger pore pre-filter bag and removal of the Kaldnes media bags from within the reactors. Samples for the second run were taken tri-weekly from January 5 to February 17 with the exception of the first week. Due to the distinct possibility of complete plugging, five samples were taken during the first week.

3.3.4 Statistical Analysis

All statistical calculations were performed using Quattro Pro spreadsheet software (version 6.0). Statistical analysis of this research addressed the following aspects of the experimental results.

Firstly, the sampling and processing error for the SCVFA samples was considered. Samples taken January 5th (1997), were collected and processed in duplicate to test variability in the sampling collection procedure, as well as the variability in sample processing.

Secondly, Student t-tests were conducted to compare the two test media SCVFA production trends, against the control series production (0.95%CL). In addition, Student t-tests were utilized as the vehicle for the comparison of SCVFA production differences between the media types. Sequential

t-tests were conducted with increasing "hypothesized mean difference" values, until the obtained "t" value was negative, to determine the maximum, statistically valid, difference in SCVFA production. Table 4, in Appendix B, provides an example of the testing method, beginning with a hypothesized difference of 0mgHAc/L and ending with a negative "t" value at 18mgHAc/L. The t-tests were conducted on the data sets from both experimental runs.

Finally, linear regression analyses were performed to compare the SCVFA production trends, with the trends in TOC, sCOD and tCOD production. Correlation between the data sets was expressed numerically, in terms of the calculated Pearson Coefficient, and graphically by considering the calculated least squares line. The Pearson Coefficient is a dimentionless indicator of overall correlation between two data sets, with a value range of zero to one. The correlation between two data sets in the least squares method is represented by the slope of the calculated least squares line: a positive slope implies positive correlation, a negative slope implies an inverse correlation, and a slope of zero implies no correlation. The regression analyses were performed on data from both media types, but only from the results of experimental run #2.

CHAPTER 4: RESULTS AND DISCUSSION

Each experimental parameter assayed during the research is discussed individually in the following sections. Trends affecting more than one parameter are expressed with regard to the main goal of the research, SCVFA analysis of the BF system. Tabulated data is included with each section, where appropriate. Data for all experiments is located in Appendix A.

4.1 Chemical Oxygen Demand

Variability in COD analysis results of both experimental runs was large. Tables 4.1 and 4.2 summarize the averaged total production values of sCOD and tCOD for both experimental runs. The disparity between the mean and median values of each tCOD data set illustrate the skewed nature of the sample sets. Much of this variability can be attributed to the varying solids load to each reactor, due to system plugging and general solids building up through both runs. Due to the inconsistent results, tCOD analysis was not chosen as an indicator of either the BF system or individual media performance. The high variance number reported for all three reactor systems, in both runs, demonstrates the inconsistency of the tCOD data.

Soluble COD data, while still somewhat erratic, provided useful information of BF system performance. Run one sCOD total production indicated a net loss of the measurable sCOD component from the waste stream. This may be due to adsorption and metabolic degradation by the insitu biofilm flora. The results from the control fermenters indicate that, when fed primary wastewater, the sCOD degradation pattern was independent of media within the reactors; hence, the

increased working biofilm surface area, provided by the growth media made no detectable sCOD profile changes to the waste stream.

Table 4.1: Total sCOD Production (minus bucket)

	Media type	Kaldnes	Ringlace	Control
Run #1	Mean (mg/L)	-10.3	-8.8	-9.4
	Standard Error	4.9	5.0	4.1
	Median	**-6.0**	**-2.5**	**-6.5**
	Standard Deviation	21.8	22.5	18.5
	Variance	475.6	504.6	341.2
	Range	96.0	87.0	75.0
	Minimum	-83.0	-68.0	-52.0
	Maximum	13.0	19.0	23.0
	Count	20	20	20
	Confidence Level(0.95)	9.6	9.8	8.1
Run #2	Mean (mg/L)	21.7	10.2	-1.6
	Standard Error	2.2	2.9	2.6
	Median	**21.0**	**11.0**	**-1.0**
	Standard Deviation	8.9	12.2	10.7
	Variance	80.1	147.8	114.0
	Range	32.0	55.0	50.0
	Minimum	5.0	-29.0	-33.0
	Maximum	37.0	26.0	17.0
	Count	17	17	17
	Confidence Level(0.95)	4.3	5.8	5.1

Table 4.2: Total tCOD Production (minus bucket)

	Media type	Kaldnes	Ringlace	Control
Run #1	Mean (mg/L)	98.3	-22.8	-0.1
	Standard Error	52.1	30.4	32.8
	Median	**28.0**	**-1.0**	**-5.0**
	Standard Deviation	226.9	132.4	143.1
	Variance	51487.3	17531.3	20477.8
	Range	1020.0	594.0	736.0
	Minimum	-122.0	-315.0	-261.0
	Maximum	898.0	279.0	475.0
	Count	18	18	18
	Confidence Level(0.95)	104.8	61.2	66.1
Run #2	Mean (mg/L)	127.6	-54.5	149.3
	Standard Error	53.2	24.3	53.7
	Median	**106.0**	**-53.0**	**42.0**
	Standard Deviation	219.3	100.3	221.6
	Variance	48094.8	10068.8	49091.1
	Range	779.0	396.0	689.0
	Minimum	-133.0	-252.0	-43.0
	Maximum	646.0	144.0	646.0
	Count	17	17	17
	Confidence Level(0.95)	104.2	47.7	105.3

Run two sCOD total production results indicated increased levels in both media reactor streams. Increased sCOD levels indicate a net increase of dissolved organics in the waste stream at each stage of the fermentation. The control reactor series demonstrated similar trends to the control series fed primary effluent in run one, ie, a net loss of dissolved organics. The comparison of control series performance during the two runs indicates that, increasing the feed strength and solids content alone, does not increase the rate of particulate organics breakdown in a short HRT fermenter. Results from the "media-containing series" indicate that, approximately 10mg/l to 20mg/l of sCOD can be produced from the fermentation of raw wastewater for sixty minutes in a BF system, depending on the attached growth media, Ringlace® or Kaldnes media, respectively.

4.2 Suspended Solids

Suspended solids analysis, consisting of TSS and VSS measurements, was performed during part of run one, from August 9 to September 9. Results were found to be highly sensitive to differences in BF-system maintenance procedures, specifically, flushing routines. The solids results could be adjusted up or down, respectively, by increasing or decreasing the volume of wastewater flushed each sampling day. The assay results, while varied, do illustrate the behavior of attached media fermenters relative to excessive solids retention. Figure 4.1 expresses the VSS results for the third reactor of all series. VSS samples from mid August demonstrate the effects of poor system maintenance on solids content in the reactors. Sustainable system maintenance protocols were still being determined during this time interval and the large VSS peaks in all reactor series prove that the SRT of reactors was not being tightly controlled.

Figure 4.1: VSS Analysis of the 60min Reactors

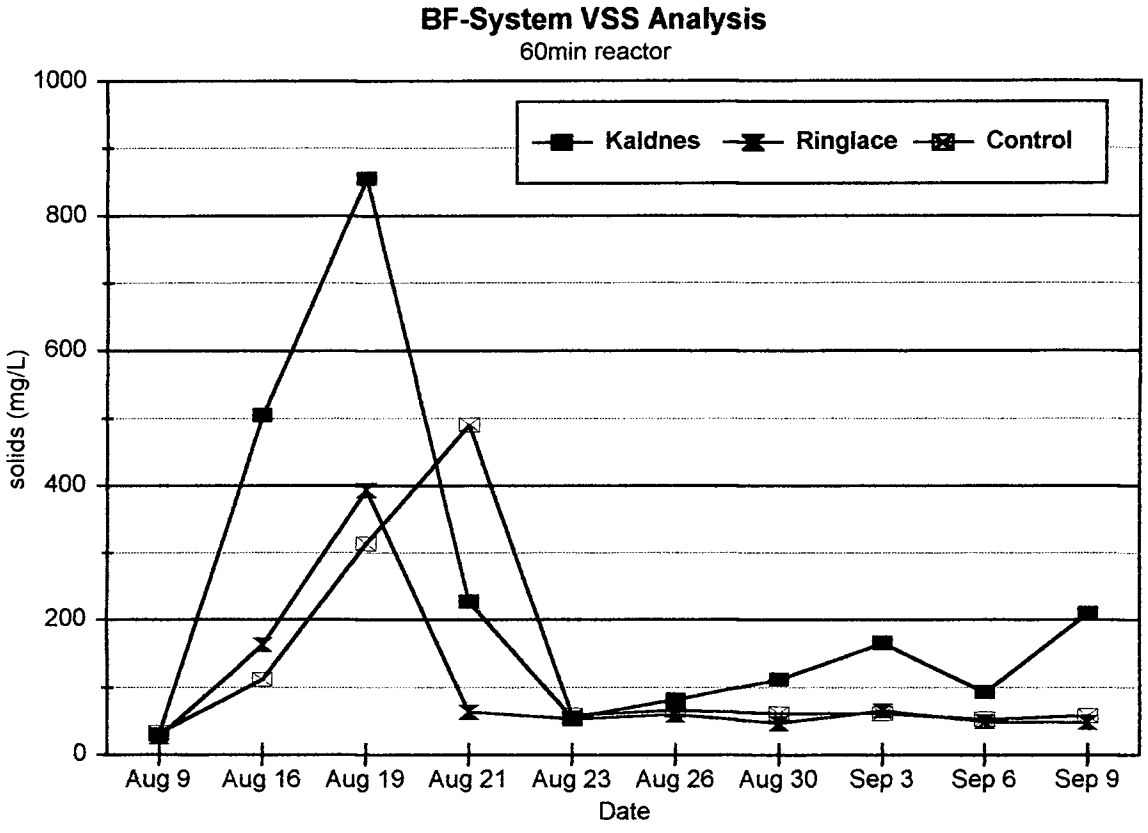
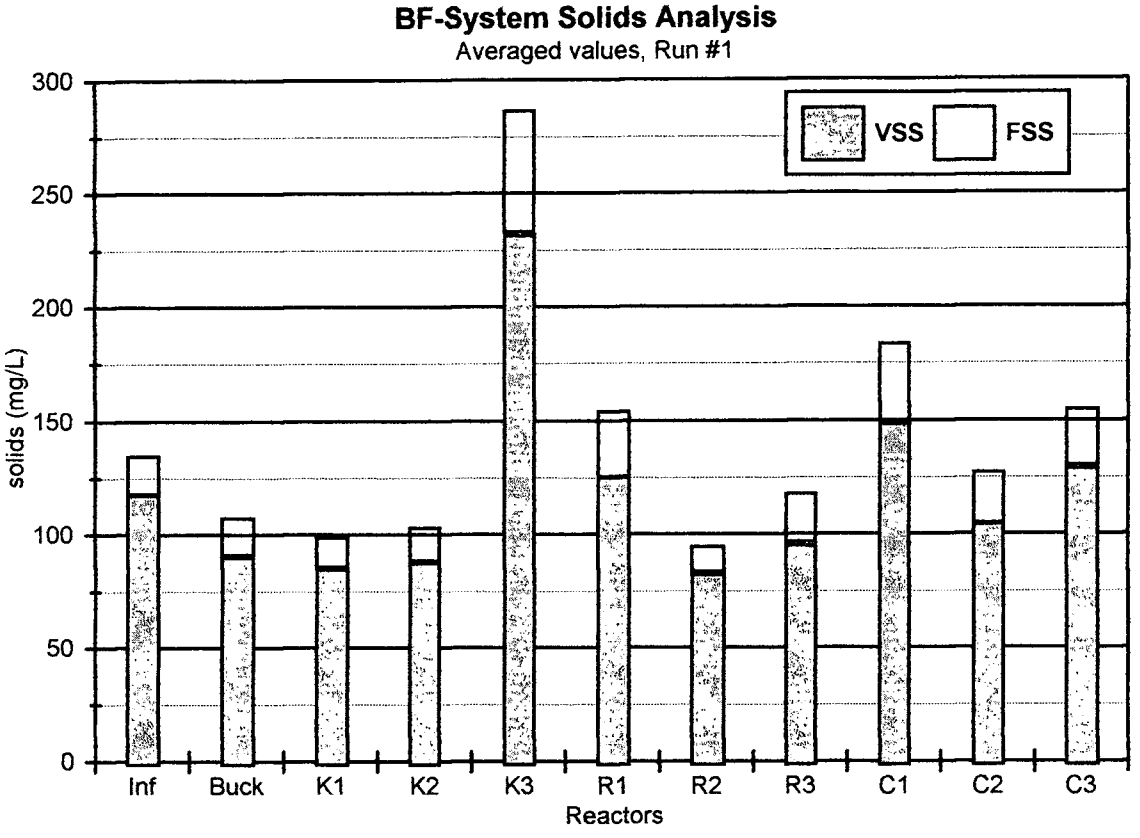


Figure 4.2: Averaged Total Solids Analysis



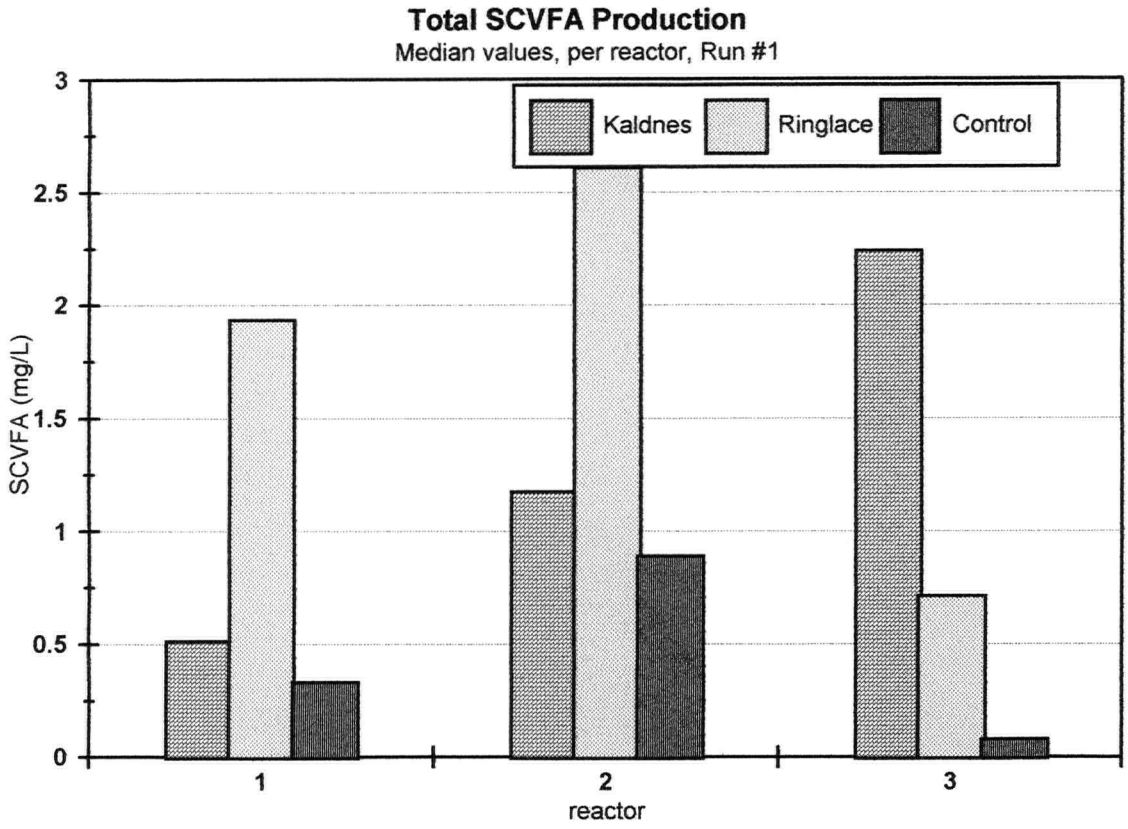
The second half of the SS samples processed demonstrate more stability in the solids profile of the system and a much lower SRT. The two media series responded differently to solids throughput. The Ringlace® series SS profile matched that of the control reactors. The VSS graph reporting averaged VSS for all reactors (Figure 4.2), shows the similarity between Ringlace® reactors and their corresponding control counterpart. The third Kaldnes reactor yielded twice the SS content of the other two series. This result is consistent with the finding that, by the cessation of run one, the K3 reactor was almost completely full of solids.

4.3 Short Chain Volatile Fatty Acids

The BF system attached growth reactors produced significant SCVFA's with both primary effluent and raw wastewater as feed. The control series in both run one and two produced less than 1.5mg/L and 2.5mg/L SCVFA as acetic acid, respectively. This indicates that the increased bacterial concentration present in attached growth systems are beneficial for the production of SCVFA's under short HRT fermentation conditions.

The primary effluent run produced more SCVFA's, after 60 minutes of fermentation, in both "media-containing" series than in the control. However, the production profiles differed for each media (Figure 4.3). The Ringlace® media series produced a total of 5.6mg/L of SCVFA's, but almost 90% of the production occurred in the first 40 minutes. This demonstrates that a reactor designed with Ringlace® media as the attached growth media and a 40 minute HRT is capable of producing 5mg/L of SCVFA's, using primary domestic effluent as feed. The Kaldnes media series produced

Figure 4.3: Total SCVFA Production per Reactor (Run #1)



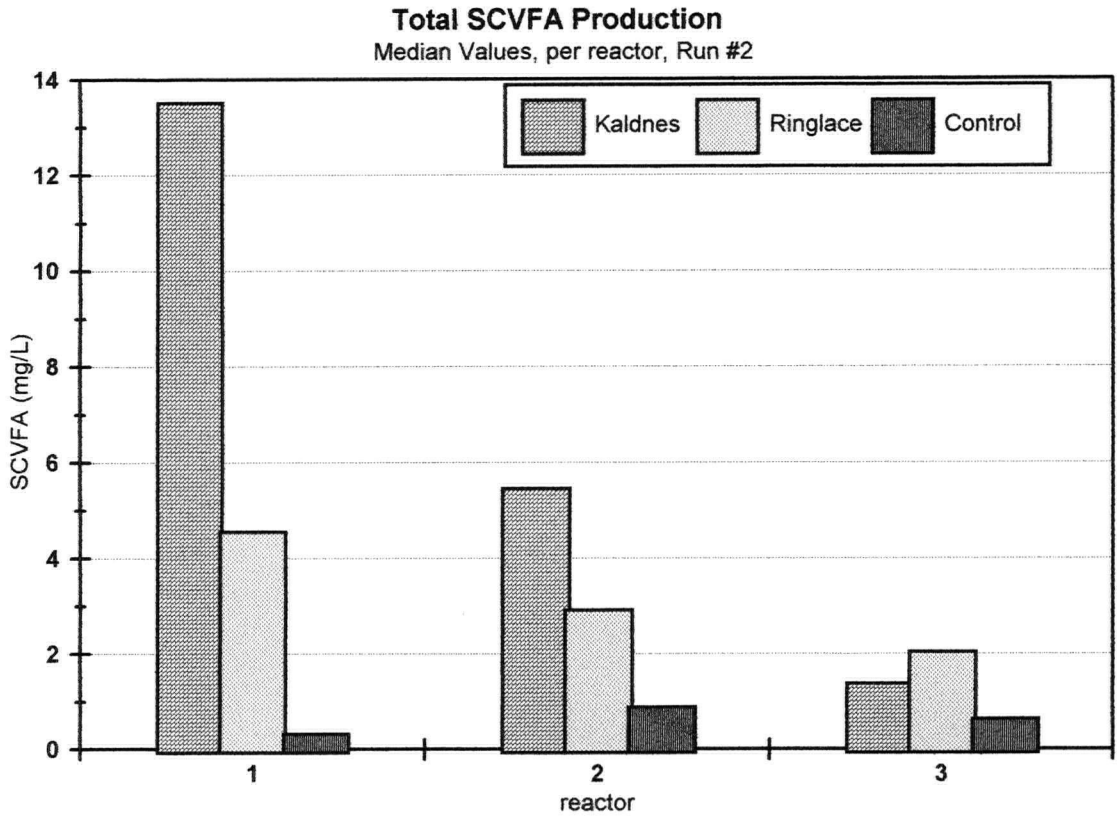
3.7mg/L after 60 minutes, but less than 50% was produced in the first 40 minutes.

Solids analysis performed during the first third of run one indicated that the organic solids content in reactor K3 was the highest among the reactors. At the cessation of solids sampling, it appeared that the VSS content in the K3 reactor was double the amount in any of the other media reactors. The effects of twice as much VSS in this reactor may have been inflated the SCVFA production numbers. The correlation between increased reactor VSS and increased VFA production has been documented by Anderson et al. (1994). VFA production in their acidogenic pre-fermenter rose 400% following a 30% increase in system VSS.

The two highest production values for reactor K3, 11.4mg/L and 7.8mg/L (Table 4.3), coincide with high solids contents at up to five times the VSS levels noted later in the run. Similar peaks occurred in the other reactor series, although only the "media-containing series" displayed noticeable increases in SCVFA production. Reactor R3 displayed both a solids peak from August 16th to 19th, at up to two times the normal solids content, and SCVFA production up to ten times higher than its calculated median value for the remainder of the run. Statistical testing of the median production values for both media, in comparison with the control, revealed significant differences of 3mgHAc/L and 4mgHAc/L for Kaldnes and Ringlace® medias, respectively (Tables 3 & 5, Appendix B).

The raw sewage feed run, run two, produced substantially more SCVFA's in all reactor series than in run one (Figure 4.4). The Ringlace® and control series produced approximately double the SCVFA's produced in run one after 60 minutes, 11.2mg/L and 2.5mg/L respectively.

Figure 4.4: Total SCVFA Production per Reactor (Run #2)



However, the Kaldnes series produced over six times the SCVFA's produced in run one, yielding 20mg/L after 60 minutes. Statistical testing of the median production values for both media, in comparison with the control series, revealed significant differences of 17mgHAc/L and 8mgHAc/L for Kaldnes and Ringlace® medias, respectively (Tables 4 & 6, Appendix B). Of the SCVFA production in the Kaldnes reactors, almost 70% of the acid production occurred in reactor K1 (Table 4.4). Similar to the K3 reactor in run one, solids buildup in the K1 reactor contributed to the high 20 minute acid production in the second run. The production rate was lower in each subsequent reactor, 5.4mg/L and 1.4mg/L, for K2 and K3, respectively.

The K2 acid production level is significant for a 20 minute HRT fermentation, even though the waste stream was essentially filtered by the K1 reactor. With this reactor, some solids buildup was present, but certainly not the sole source of the acids production. Reactor K2 did not require the high influent pressure necessary for K1, indicating much less clogging. Therefore, the production in reactor K2 represented attached biofilm activity, not the result of suspended sludge floc fermentation.

Table 4.3: Run #1 SCVFA Production (minus control)

HAc mg/L	Kaldnes			Ringlace		
Date	K1	K2	K3	R1	R2	R3
July 11	2.9	0.1	2.5	1.4	-5.4	7.8
July 17	-0.1	3.7	-0.3	-1.3	0.7	-2.4
Aug 16	3.5	-5.3	15.7	7.0	-5.2	8.4
Aug 19	-5.0	5.9	6.7	-1.1	0.1	5.1
Aug 21	1.2	1.0	4.2	-0.0	3.4	-0.1
Aug 23	-7.7	9.3	1.6	-7.1	11.1	-0.3
Aug 26	-0.2	-2.2	3.6	1.5	-0.2	2.1
Aug 30	4.8	-0.3	-3.0	0.7	3.0	-0.7
Sep 3	1.6	1.1	8.3	4.1	0.1	5.9
Sep 6	-0.2	1.1	-0.0	1.2	1.8	-0.0
Sep 9	-1.9	2.2	1.1	0.6	1.4	-0.7
Sep 12a	-0.4	-0.3	4.4	2.1	0.5	0.3
Sep 12b	2.0	-0.2	3.2	3.3	1.1	0.7
Sep 20	-0.4	0.1	4.8	1.4	1.4	2.6
Sep 23	0.9	-0.3	1.4	2.2	0.9	0.5
Sep 25	-0.8	0.8	0.4	0.9	2.7	-0.3
Sep 27	0.3	-1.3	1.6	2.3	4.7	-1.8
Oct 6	0.3	0.0	-1.2	3.8	-2.1	1.1
Oct 7	-0.8	-1.2	0.7	-1.3	3.0	1.3
Oct 8	0.2	-0.9	-0.5	3.2	1.0	0.3
Oct 15	-0.2	-0.6	0.1	3.5	-0.2	1.2
Mean	**-0.2**	**0.5**	**2.8**	**1.5**	**1.5**	1.4
Standard Error	0.6	0.7	1.0	0.7	0.7	0.6
Median	**-0.2**	**-0.2**	**1.6**	**1.5**	**1.1**	**0.5**
Standard Deviation	2.7	3.0	4.2	2.9	3.2	2.6
Variance	7.4	9.1	17.5	8.1	10.0	6.6
Range	12.5	14.6	18.7	14.1	16.2	10.2
Minimum	-7.7	-5.3	-3.0	-7.1	-5.2	-1.8

Table 4.4: Run #2 SCVFA Production (minus control)

HAc mg/L	Kaldnes			Ringlace		
Date	K1	K2	K3	R1	R2	R3
Jan6	8.2	6.1	1.1	2.8	0.5	3.3
Jan7	9.5	1.0	6.8	2.1	-1.1	3.8
Jan9	9.4	3.3	3.6	4.7	1.7	3.7
Jan10	8.3	1.7	4.5	5.1	0.1	4.1
Jan14	6.4	4.3	1.2	2.8	0.1	-1.2
Jan15	12.2	3.5	-3.3	3.8	5.1	-4.5
Jan17	15.9	6.1	-1.8	7.5	4.2	1.1
Jan20	12.8	10.7	-1.5	2.9	6.9	3.5
Jan22	12.1	8.0	0.4	8.3	0.8	3.8
Jan24	10.3	6.8	3.3	0.4	6.2	3.6
Jan27	10.9	2.0	3.5	2.5	-3.3	4.3
Jan29	9.0	15.4	-4.7	4.2	6.2	0.3
Jan31	19.9	1.0	-0.4	13.8	-5.4	-1.1
Feb3	17.0	2.2	-1.1	3.5	0.8	1.0
Feb5	14.5	3.5	8.0	3.8	1.2	2.2
Feb7	12.1	7.0	-1.2	6.2	0.6	3.4
Feb10	14.5	1.1	0.8	3.0	7.2	-3.7
Feb12	14.8	2.8	-1.3	1.6	5.7	2.0
Feb14	12.3	6.8	-1.7	3.2	4.5	-2.1
Feb17	14.3	4.2	-1.3	3.5	2.4	7.1
Feb19	15.3	2.1	0.1	3.8	2.7	1.9
Mean	12.4	4.7	0.7	4.3	2.2	1.7
Standard Error	0.7	0.8	0.7	0.6	0.7	0.6
Median	**12.2**	**3.5**	**0.1**	**3.5**	**1.7**	**2.2**
Standard Deviation	3.3	3.6	3.2	2.9	3.4	2.9
Variance	11.2	12.8	10.4	8.1	11.4	8.4
Range	13.5	14.4	12.7	13.4	12.6	11.5
Minimum	6.4	1.0	-4.7	0.4	-5.4	-4.5

The Ringlace® series produced 2 - 4.5mg/L of SCVFA's in all reactors. Approximately 50% of the total acids was produced in the R1 reactor, the first 20 minutes of fermentation. Subsequent reactors demonstrated production rate drops of 36% and 31%, respectively, for R2 and R3, yielding 2.9mg/L and 2mg/L production. Other published research, utilizing lab-scale, acetogenic stirred batch-fermenters, indicated that a VFA production peak occurs after one to four hours of fermentation, dependant on the process temperature (Kozuchowska and Evison, 1995). Kozuchowska and Evison identified the most efficient temperature for the fermentation of synthetic coffee waste as 45 ℃, with an HRT of one to three hours, producing a maximum of 358mg VFA/l. Because the BF-system operated at ambient temperature, direct comparison of SCVFA production is impossible. The above results support the theory that short HRT acidogenic fermentation efficiency relies on the presence of readily degradable components in the waste stream to fuel large SCVFA production. As these feed components are metabolized, and hence removed from the waste stream, the SCVFA production rates drop. The results of run #2 indicate that a BF-system, consisting of Ringlace® media reactors fueled by raw wastewater, will lose half its SCVFA production efficiency if operated at an HRT of over 20 minutes.

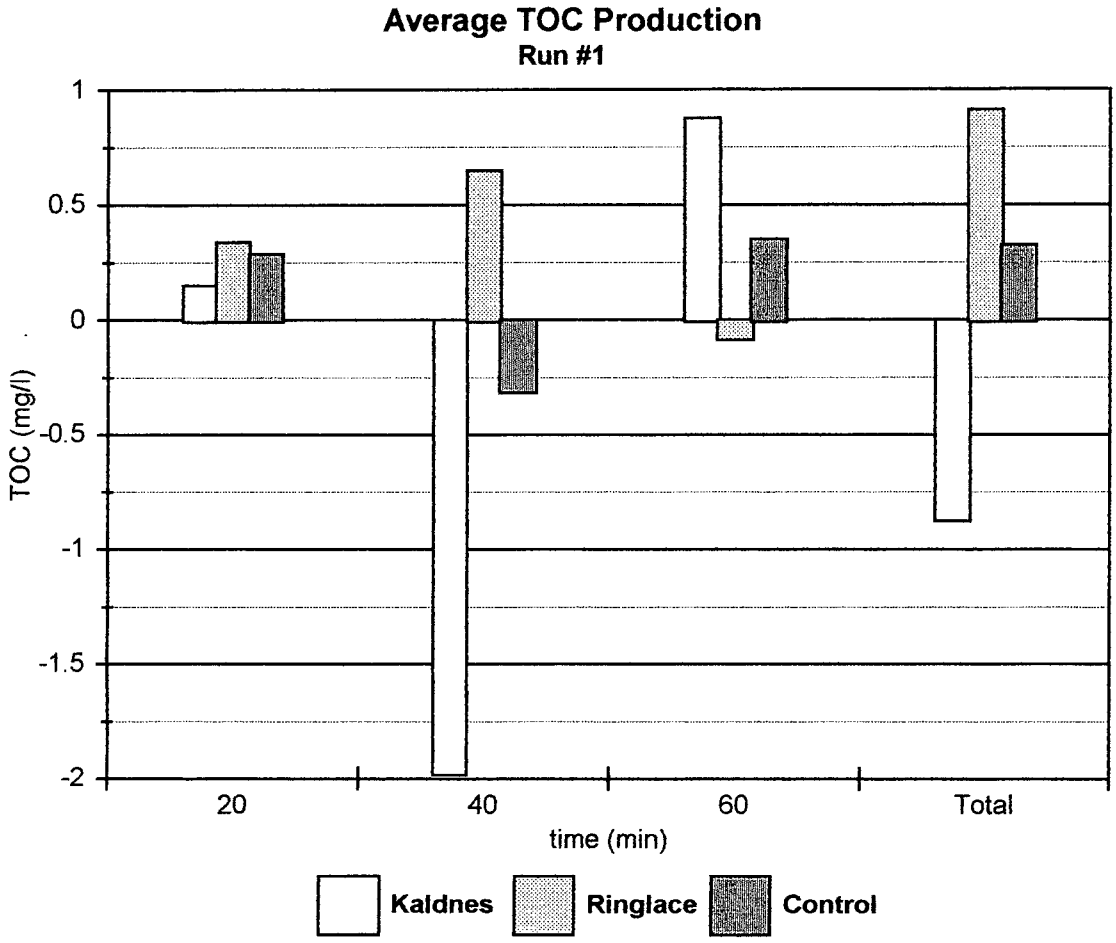
Error analysis was done to determine variability in SCVFA sample collection and processing. The results indicated that the error associated with collecting duplicate samples was ±3% and the error associated with processing duplicate samples was ±7%.

4.4 Total Organic Carbon

The results of Total Organic Carbon (TOC) analysis of the BF-system samples can best be interpreted by assessing either production or consumption of measurable TOC, at each stage of the process. Since SCVFAs are also measured as TOC, one would have expected to see a net production of TOC at each stage of the process and to link this production to that of SCVFAs. TOC samples represent dissolved and fine colloidal organic carbon in the system because they were filtered prior to processing. The net production of TOC, in the BF-system, represents the fermentation of suspended organics, adsorbed into the biofilm, and processed by the established flora to yield dissolved organic products.

Run one TOC levels remained approximately the same in the Ringlace® and control reactor series (Figure 4.5). However, the Kaldnes reactor series demonstrated high variability and a net loss of approximately 2mg/l in the K2 reactor. This indicates a net loss of free dissolved organics, probably by bacterial metabolism. Reactor K3 results indicate the opposite metabolic conditions, ie, a net production of TOC, due to the metabolic breakdown of suspended organic solids directly extracted from the bulk liquid or stored organics adsorbed to the attached biofilm. Solids buildup in the K3 reactor, noticed near the end of run one, may explain the increase in TOC production. By effectively increasing the solids loading to that reactor, more stored nutrients were provided for the biofilm to break down. TOC sampling early in the operation of run one could have answered this question by indicating K3 production levels prior to solids buildup.

Figure 4.5: Average TOC Production, Run #1



Run two "averaged" TOC production levels were much higher than the production in run one, due to the higher nutrient loading of a raw wastewater feed with respect to primary effluent as feed (Figure 4.6). The control reactor series produced the least increase in measurable TOC of the three reactor series, 2mg/l at sixty minutes (Table 4.5). The Kaldnes reactor series produced the greatest increase in TOC concentration at approximately six times the control increase. The Ringlace® reactor series produced approximately two times the control increase. Production rates were highest in the first reactor of each media series. The third reactor in each series yielded a net loss of TOC in all but the control reactor. This indicates that, after approximately forty minutes of fermentation, the biofilm populations prefer to metabolize the dissolved organics in the waste stream than the remaining particulate organics. Both media systems consumed approximately 1mg/l of measurable organic carbon between forty to sixty minutes of fermentation. Conversely, the control series results indicated no significant change in the TOC of reactor C3 relative to reactor C2, probably due to the lower nutrient consumption in the previous reactors, relative to the "media-containing" series.

The ideal fermentation HRT indicated by the results of the BF-system, relative to TOC production, is less than forty minutes for a system fed raw wastewater. No ideal fermentation HRT for a system fed primary effluent can be derived from the first run, due to the lack of visible trends in the data.

Figure 4.6: Average TOC Production, Run #2

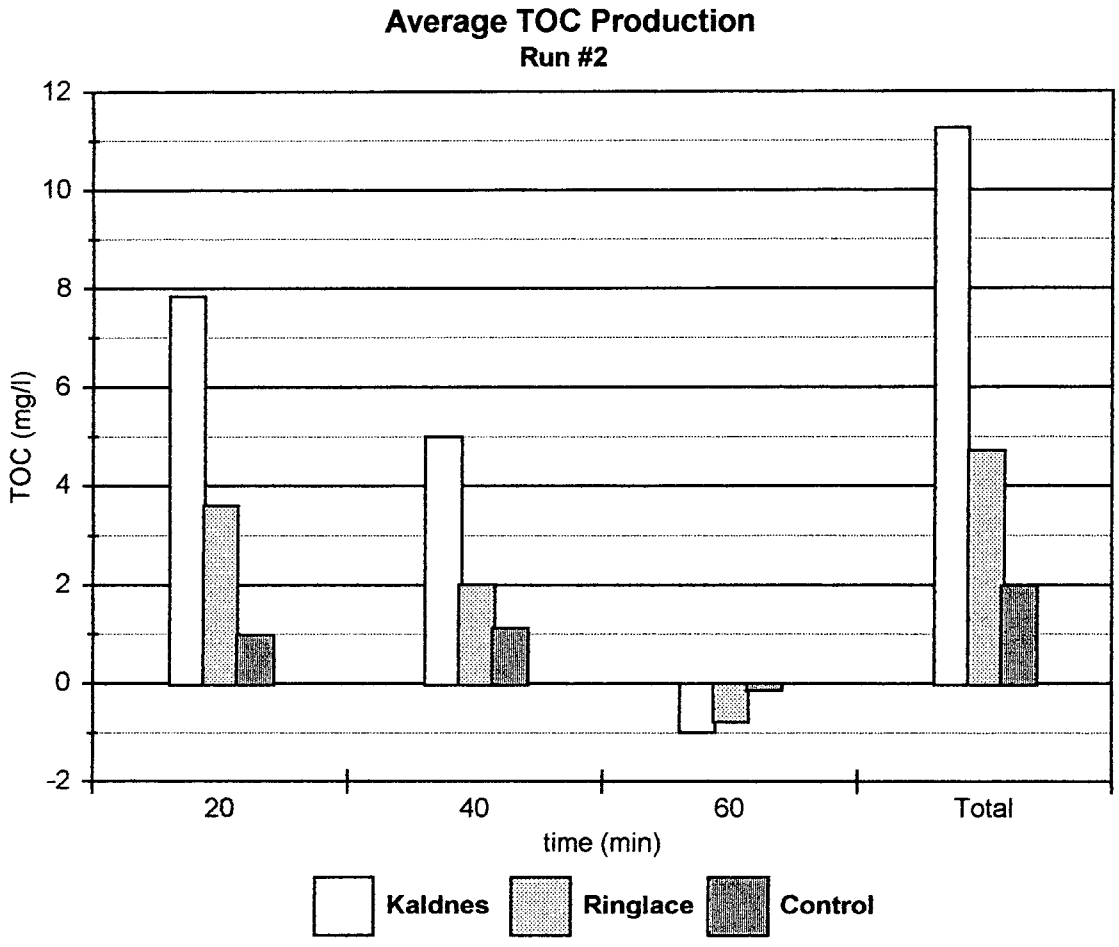


Table 4.5: Averaged TOC Production, Runs #1 and #2

	mg/L	Kaldnes	Ringlace	Control
Run #1	20min	0.15	0.34	0.29
	40	-1.97	0.65	-0.31
	60	0.87	-0.07	0.35
	Total	-0.86	0.91	0.33
Run #2	20min	7.41	3.40	0.98
	40	4.72	1.90	1.12
	60	-0.93	-0.68	-0.10
	Total	11.24	4.71	2.00

4.5 BIOLOG Assay

The experiments successfully produced carbon source utilization patterns on Biolog GN plates. The patterns produced from the first experimental analysis using samples from reactors K3 and R3 at the end of run one showed little correlation between media types and within dilutions of the same sample. These results reflect sample handling and general protocol faults. A significant amount of biofilm solids were present throughout the plating dilution series; this is believed to have affected both, the ability to monitor pigment formation in the undiluted plates, and the intensity of pigment generated. The latter effect resulted from the utilization of biofilm solids as a carbon source in the plate wells, in addition to or instead of the packaged specific carbon source. Adjustments to the protocol for the second experiment, using samples from reactors K3 and R3 at the end of run two, produced reproducible patterns, both within each plate dilution series and between the two media types.

The averaged well intensities for all plates processed during both experiments is presented in Table 4.6. The results of the first experiment were not considered representative of the BF-system biofilm, due to the high variance in pigment intensity between the two media and within each dilution series of a single media. The patterns produced in the second experiment show strong similarities within each dilution series, as well as between the patterns produced by each media type. A correlation analysis was performed with the cumulative plate data from the two media types tested. The resulting correlation coefficient, 0.914, shows the strong correlation between the two sets of patterns across the 95 test wells.

Table 4.6: Averaged Biolog Plate Data

a) Kaldnes, Run #1 (24hr)

	1	2	3	4	5	6	7	8	9	10	11	12	
A		1			3	3							A
B	/	/			1		1				/		B
C	1	1		1									C
D			1		1		2	2					D
E	/	/	1			1			1	1			E
F	1	/		/	1	1		2	2	2			F
G	/	/		/		1	1	2	2				G
H	1	2	1	1		1	1	1	2	2	1	2	H
	1	2	3	4	5	6	7	8	9	10	11	12	

b) Kaldnes, Run #2 (36hr)

	1	2	3	4	5	6	7	8	9	10	11	12	
A			2	3	/	/	2	4		1		2	A
B		1		2	1	1		2		1	1	1	B
C	2	3	/	1	/	2	3	3			1		C
D		1	2		1	3	3		1				D
E	1			/		2				/		/	E
F	/							1	2	1	/	/	F
G	/							2	2			1	G
H	/	1	/						2	1	2	3	H
	1	2	3	4	5	6	7	8	9	10	11	12	

c) Ringlace®, Run #1 (24hr)

	1	2	3	4	5	6	7	8	9	10	11	12	
A		1	1	1	2	2	1	1	1		1	1	A
B		1		/	/	/	/	1	1	/	/	1	B
C	1	1		/		1	1	1	/				C
D		1	/			1	1	1					D
E	/		1	/		/			/	1		/	E
F	1	/						1	1	1	/	/	F
G	1	/						1	1				G
H	/	1	1	/					1	/	/	/	H
	1	2	3	4	5	6	7	8	9	10	11	12	

d)Ringlace®, Run #2 (36hr)

	1	2	3	4	5	6	7	8	9	10	11	12	
A		/	3	4	/	/	1	4		1	/	3	A
B		2		1	/	1		1		2	2	1	B
C	2	4	/	2	/	3	4	4			2		C
D		2	2	/	2	4	4		1				D
E	/			/		3			/	1		2	E
F	1				/	/		2	1	2	/		F
G	/	/						3	3			/	G
H	/	2	/			/			1	/	1	2	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Metabolic fingerprinting of the mixed population from the BF-system, using the Biolog identification system, can yield consistent patterns within a sampling run. However, repetition of the second experiment is necessary before any conclusions can be reached about the validity of the obtained metabolic fingerprint. Future microbiological research is necessary to isolate, purify and re-test the sample organisms, to determine if the metabolic variation is consistent within a given fully functional system. In addition, testing must be done with both aerobic and anaerobic components of an existing sewage system to compare the fermenter biochemical fingerprints with fingerprints produced by other sewage treatment environments. Long term studies are necessary to study the variations in the nutrient fingerprint produced under both ideal performance conditions and conditions which result in low overall performance. Temporal microbial analysis of the prevalent organisms, thriving in the studied fermenter system under varying conditions, would link the BIOLOG results to actual population trends within the reactor and parallel temporal changes in other measurable biochemical components such as volatile fatty acids.

The development of a standardized quantitative technique for assessing the pigment producing trends of mixed bacterial populations would allow the sharing of information between facilities that are geographically isolated. If a technique can be developed using scanned images of incubated BIOLOG plates processed by readily available imaging software, the pool of information would be larger than techniques involving costly colorimetric equipment. A technique such as this would also allow the operators of full-scale sewage fermenters to assess the ongoing biochemical health of their systems by comparing "nutrient fingerprints".

4.6 Media Comparison

The two media tested, Kaldnes and Ringlace®, were compared physically and in terms of SCVFA production.

The results of the surface area analysis revealed that, the physical differences between the two media types, translate to differences in usable surface area. The calculations revealed that the maximum and minimum surface area loading possible, in the current BF-system configuration, were significantly different between the two media (Table 4.7).

Table 4.7: Test Media Surface Area Loading.

Media Type	Kaldnes	Ringlace
Maximum Surface Area Loading	430 m^2/m^3	208 m^2/m^3
Minimum Surface Area Loading	19 m^2/m^3	67 m^2/m^3

The media volume loading calculations indicated that, in the current configuration, the Kaldnes reactors had the capability to retain, or entrap, 25% more solids than the Ringlace® media reactors. During run #2, this figure was certainly much higher for reactor K1, due to solids entrapment

between Kaldnes carriers. Suspended solids analysis, performed during run #1, revealed that the Ringlace® series SS profile matched the control reactor series profile. Therefore, Ringlace® does not contribute to excessive solids deposition in BF-reactors. Conversely, the third reactor in the Kaldnes series SS content was twice that of the corresponding control (and Ringlace®) reactor at the cessation of SS sampling. This researcher believes that the solids buildup in reactor K3 originated from "sluffed" off biofilm from previous reactors, not solids in the primary effluent. Primary effluent solids would have been entrapped in the K1 reactor first; this pattern was evident during run #2 where raw waste solids clogged the K1 reactor. The results indicate that Kaldnes media is not practical as an attached growth media in the BF-system, due to clogging tendencies.

When the BF-system was fed primary effluent, neither media type had any effect on the sCOD concentration of the waste stream. Conversely, when fed raw wastewater, the Kaldnes and Ringlace® reactor series produced 10mg/l and 20mg/l of sCOD, respectively. The control series in the two runs demonstrated similar trends, indicating that the sCOD production was a direct product of the attached biofilm of both media types.

The two media differed in production of SCVFA's, not only in the amount produced, but also in the timing of production within the 60min HRT. The Ringlace® series, fed primary wastewater, produced 5mg/l of SCVFA's in the first 40min. By comparison, the Kaldnes series produced less than 2mg/l of SCVFA's in 40min. Statistical testing revealed that, the difference in median production values between Kaldnes and Ringlace®, was not statistically significant (Table 1, Appendix B). Run #2 SCVFA data for Kaldnes data, specifically the K1 reactor, was not considered in the media performance comparison because the excessive solids buildup contributed much of the

reactor fermentation. Statistical comparison of the median production values for run #2, revealed a significant difference of 9mgHAc/L between the two media. Correlation analyses were performed between the SCVFA production of the control series, and the two media series, for both experimental runs. The Pearson Coefficients, calculated in Appendix B, were significantly higher for Ringlace® media (0.825 and 0.638), than the values calculated for Kaldnes media (0.450 and 0.499). These calculations indicate that, variability in the Ringlace® series production values followed the variability trends in the control series, more closely than did the Kaldnes series.

Ringlace® has proven itself to be the better media for the BF-system in the current plug-flow configuration, especially when the system was fed raw wastewater. The Ringlace® series displayed double the SCVFA production in run #2 compared with run #1, producing almost 5mgHAc/L in 20min.

4.7 Summary

SCVFA production in the BF-system was near zero in the reactors containing no media during both the primary effluent and raw wastewater runs. Significant levels of SCVFA's were produced by short HRT attached growth fermenters using both primary effluent and raw wastewater as feed. Both sets of media reactors tested produced more SCVFA's than the control reactors. TOC and sCOD analyses, which measure dissolved organics in the waste stream, indicated a net solubilization of filterable solids in both media series fed raw wastewater.

The correlation analysis of the SCVFA production, of both media types, with the other measured

parameters, yielded both positive and negative results. Least squares lines calculated for the correlation of "soluble" parameters, sCOD and TOC, with SCVFA production revealed positive correlations (Figures 1-4, Appendix B). Conversely, the results of the tCOD correlation analyses revealed a slightly negative correlation (Figures 5 & 6, Appendix B). However, since the Pearson Coefficients generated for the tCOD correlations were low in both runs, one cannot justify the negative correlation. Therefore, the tCOD data was concluded to have no correlation with the SCVFA production data.

Kaldnes media was not suitable as a short HRT pre-fermentation media in the current BF-system configuration, due to inability to handle the solids loading of the waste stream. Suspended solids analysis supported this researcher's choice of appropriate media for future research by demonstrating the tendency of Kaldnes media to become clogged with solids. Ringlace® media has proven itself as a sustainable short HRT pre-fermentation media by resisting plugging under all solids loading conditions tested. Results of the two fermentation runs indicate that, a short HRT fermenter system fed raw domestic wastewater, is capable of producing significantly more SCVFA's than a system fed primary effluent. SCVFA production in the BF-system, by Ringlace® media, would have a significant impact on the phosphorus removal efficiency of a BNR facility.

The BF-system was designed as an inline prefermenter, which results in little dilution of the SCVFA's produced, relative to the production of a side-stream prefermenter system. A typical side-stream fermenter is designed to provide 5-10% of the flow of raw sewage influent. For example, a production of 100mg SCVFA/L in a side-stream fermenter, operating at 5% of the system flow, is equivalent to the production an inline prefermenter, such as the BF-system, producing 5mg

SCVFA/L. The two BF-system fermentation runs produced 6mg SCVFA/L*h and 9mg SCVFA/L*h, fermenting primary effluent and raw wastewater, respectively. If it is assumed that, 5mg SCVFA/L are needed to remove 1mg/L of phosphorus (Barnard, 1998), the effect of an inline BF-system on phosphorus removal, in downstream BNR facility, may be estimated at an extra 1-2mg/l, fermenting primary effluent and raw wastewater, respectively. However, it is important to consider that, the retrofit of a BF-system to an existing BNR facility, may result in the removal in the "last" milligram of phosphorus in the waste stream. As phosphorus effluent regulations tighten, the removal of the "last" milligram of phosphorus, will be of paramount importance.

The design of a short HRT prefermenter system can significantly reduce the costs associated with reactor construction and operation, relative to a longer HRT facility (Demirci and Pometto, 1995). The BF-system design combines the space savings, associated with a short HRT system, with the low operating costs of a reactor with no moving parts. Considering the additional savings associated with reducing the size of the anaerobic zone, permitted by the VFA contribution of a prefermenter system (Randall, 1994), the retrofit of a BF-system to an existing BNR facility is a cost effective alternative to sidestream sludge fermentation.

CHAPTER 5: CONCLUSIONS and RECOMMENDATIONS

5.1 Conclusions

The main objective for this thesis was to assess the SCVFA production performance of two fixed film media types in the BF-system, fed primary effluent and raw wastewater in separate fermentation runs. After reviewing the results of the two experimental runs, the following conclusions were made.

BIOLOG analysis was found to produce a reproducible metabolic fingerprint of the prefermenter biofilm. It is therefore possible to qualify the metabolic activity of the fermentation organisms in the BF-system. However, no determination of the bacterial species, active within the BF-system, can be performed using this technique because all samples represent mixed cultures.

The BF-system, attached growth fermenters series, produced more SCVFA's than the suspended growth control series. This indicates that a short HRT prefermenter design benefits from the addition of suspended growth media, to entrap solids and stimulate biofilm formation.

The two media tested, Kaldnes and Ringlace®, produced 3.7mg SCVFA/L*h and 5.6mg SCVFA/L*h, respectively, during the primary effluent fermentation run. However, the results were shown to be statistically similar.

Soluble COD and TOC analyses demonstrated a net increase in dissolved organics over a 60min HRT, during the raw wastewater run. This indicates a net conversion of particulate matter, in the waste stream, to dissolved matter.

Kaldnes media demonstrated plugging problems at the cessation of the primary effluent run and early during the raw sewage run. Because of the solids loading problem, Kaldnes was deemed unsuitable for use in a plug-flow, BF-system. A Kaldnes media system must therefore, be fluidized to avoid clogging, either due to entrapped solids or excessive biofilm growth.

Ringlace® media produced 5mg HAc/l after 20min and 11.2mg HAc/l after 60min when the system was fed raw effluent. The SCVFA production rates of the BF-system, configured as an inline prefermenter, would have a significant impact on the phosphorus removal performance of a BNR system. The BF-system, with an HRT of 60min, could theoretically provide enough SCVFA's to treat 1-2mg/L of phosphate, dependant on the type of feed.

The production of significant SCVFA's with an HRT of 60min or less, translates to a large savings in construction costs associated with prefermenter design. The shorter HRT design requires a smaller reactor and hence, a smaller footprint at a treatment facility.

5.2 Recommendations

Future pre-fermentation research should focus on the following areas:

Future research on Kaldnes media as a suitable carrier should be performed in a mixed, or otherwise fluidized, system to prevent the plugging problems encountered in this research.

A reactor system for short HRT pre-fermentation research should be designed to accommodate the solids loading of the waste-stream, without the need for "maintenance flushing".

In order to answer the question of biofilm behavior, particle adsorption vs particle entrapment, biofilm analyses of both media types should be performed. This would pertain to biofilm thickness, biofilm formation rate, and particle analysis.

Analysis of "truly" soluble COD by the FLOC-SOL method should be performed to better understand the flow of nutrients, from suspended to dissolved organics, in a prefermenter system.

Long term microbiological studies are necessary to study the BIOLOG generated nutrient fingerprint produced under both ideal fermenter performance conditions and conditions which result in low overall performance. This would link the BIOLOG results to actual population trends within the reactor and ideally parallel temporal changes in other measurable biochemical components, such as volatile fatty acids.

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LIST OF APPENDICES

APPENDIX A: RESULTS

1.1 Analytical Data

Table 1: sCOD, Raw Values

Run #1	Influent	Buck	K			R			C		
	mg/l		1	2	3	1	2	3	1	2	3
Aug 9		233			150			165			201
Aug 30	142	162			147			143			137
Sep 3	188	205			187			157			153
Sep 6	136	139	142	141	152	126	141	137	140	142	143
Sep 9	138	146			151			148			149
Sep 12a	149	147			133			143			138
Sep 12b	148	150			144			148			142
Sep 20	148	152			150			163			152
Sep 23	167	159			156			156			144
Sep 25	147	146			155			158			159
Sep 27	150	147	142	154	157	158	152	163	157	151	153
Oct 6	178	153			146			148			147
Oct 7	145	143			137			162			145
Oct 8											
Oct 15	148	147			139			157			141
Average	155	163	155	156	152	167	158	154	162	164	153

RUN #2	Influent	Bucket	K			R			C		
	mg/l		1	2	3	1	2	3	1	2	3
Jan5	165	168			173			173			160
Jan6	143	147			158			168			158
Jan7	143	144	155	149	163	142	148	160	156	163	151
Jan9	142	154			176			165			150
Jan10	167	164	179	183	190	170	182	185	168	173	165
Jan14	167	174			188			171			167
Jan15	150	156	168	158	169	157	166	162	155	149	155
Jan17	152	155			182			171			155
Jan20	157	163			190			179			163
Jan22	148	159	167	165	180	162	164	170	152	153	154
Jan24	157	153			190			179			155
Jan27	185	173			206			187			190
Jan29	168	174			190			183			173
Jan31	169	167	187	217	202	189	178	177	174	178	173
Feb3	175	174			202			187			163
Feb5	179	189			203			160			156
Feb7	nd	190			211			200			189
Average	151	165	171	174	187	164	168	175	161	163	163

Table 2: sCOD, Corrected for Bucket Level

RUN#1	Influent		K			R			C	
	mg/l	1	2	3	1	2	3	1	2	3
Aug 9	-233			-83			-68			-32
Aug 30	-20			-15			-19			-25
Sep 3	-17			-18			-48			-52
Sep 6	-3	3	2	13	-13	2	-2	1	3	4
Sep 9	-8			5			2			3
Sep 12a	2			-14			-4			-9
Sep 12b	-2			-6			-2			-8
Sep 20	-4			-2			11			0
Sep 23	8			-3			-3			-15
Sep 25	1			9			12			13
Sep 27	3	-5	7	10	11	5	16	10	4	6
Oct 6	25			-7			-5			-6
Oct 7	2			-6			19			2
Oct 8										
Oct 15	1			-8			10			-6
Average	-18	-12	-11	-11	-0	-9	-9	-5	-4	-10

RUN #2	Influent		K			R			C	
	mg/l	1	2	3	1	2	3	1	2	3
Jan5	-3			5			5			-8
Jan6	-4			11			21			11
Jan7	-1			19			16			7
Jan9	-12			22			11			-4
Jan10	3	15	19	26	6	18	21	4	9	1
Jan14	-7			14			-3			-7
Jan15	-6	12	2	13	1	10	6	-1	-7	-1
Jan17	-3			27			16			0
Jan20	-6			27			16			0
Jan22	-11	8	6	21	3	5	11	-7	-6	-5
Jan24	4			37			26			2
Jan27	12			33			14			17
Jan29	-6			16			9			-1
Jan31	2	20	50	35	22	11	10	7	11	6
Feb3	1			28			13			-11
Feb5	-10			14			-29			-33
Feb7	-190			21			10			-1
Average	-14	14	19	22	8	11	10	1	2	-2

Table 3: tCOD, Raw Values

RUN #1	Influent	Bucket	K			R			C		
1996	mg/l		1	2	3	1	2	3	1	2	3
Aug 16		202	360	420	1100	220	279	481	258	294	330
Aug 19	400	308	335	468	650	410	415	323	332	412	262
Aug 21		625	318	315	620	315	293	310	1300	1400	1100
Aug 23	410	291	262	310	298	580	316	300	635	313	295
Aug 26	340	315			355			301			300
Aug 30	312	298			405			279			270
Sep 3	382	305			500			340			300
Sep 6	403	281	292	290	361	703	319	280	292	299	292
Sep 9	352	542			606			282			281
Sep 12a	386	281			nd			318			281
Sep 12b	422	422			300			288			265
Sep 20	369	301			503			350			272
Sep 23	375	315			270			250			282
Sep 25	400	339			297			235			264
Sep 27	522	320	270	337	286	335	317	263	295	284	302
Oct 6	420	342			370			353			388
Oct 7	420	280			282			422			286
Oct 8											
Oct 15	372	280	270	290	332	290	231	261	297	297	275
Average	393	336	301	347	419	408	310	313	487	471	336

RUN #2	Influent	Bucket	K			R			C		
	mg/l		1	2	3	1	2	3	1	2	3
Jan5	380	525			460			520			482
Jan6	390	363			317			325			525
Jan7	326	362			468			241			402
Jan9	640	500			440			248			658
Jan10	462	460			585			500			442
Jan14	430	442			555			295			443
Jan15	425	400	690	1000	900			372			850
Jan17	450	385			560			340			612
Jan20	375	390			352			302			515
Jan22	340	498			365			315			540
Jan24	340	318			400			265			301
Jan27	398	380			312			308			482
Jan29	378	357			735			355			355
Jan31	420	400			645			278			408
Feb3	398	360			612			483			1000
Feb5	393	354			1000			498			1000
Feb7	391	408			365			331			425
Average	408	406			534			352			555

Note: shaded values were originally shown as ">900 or >1000" etc.

Table 4: tCOD, Corrected for Bucket Values

RUN #1	Influent	K			R			C		
	mg/l	1	2	3	1	2	3	1	2	3
Aug 16		158	218	898	18	77	279	56	92	128
Aug 19	92	27	160	342	102	107	15	24	104	-46
Aug 21		-307	-310	-5	-310	-332	-315	675	775	475
Aug 23	119	-29	19	7	289	25	9	344	22	4
Aug 26	25			40			-14			-15
Aug 30	14			107			-19			-28
Sep 3	77			195			35			-5
Sep 6	122	11	9	80	422	38	-1	11	18	11
Sep 9	-190			64			-260			-261
Sep 12a	105			nd			37			0
Sep 12b	0			-122			-134			-157
Sep 20	68			202			49			-29
Sep 23	60			-45			-65			-33
Sep 25	61			-42			-104			-75
Sep 27	202	-50	17	-34	15	-3	-57	-25	-36	-18
Oct 6	78			28			11			46
Oct 7	140			2			142			6
Oct 8										
Oct 15	92	-10	10	52	10	-49	-19	17	17	-5
Average	67	-29	18	98	78	-20	-23	157	142	-0

RUN #2	Influent	K			R			C		
	mg/l	1	2	3	1	2	3	1	2	3
Jan5	-145			-65			-5			-43
Jan6	27			-46			-38			162
Jan7	-36			106			-121			40
Jan9	140			-60			-252			158
Jan10	2			125			40			-18
Jan14	-12			113			-147			1
Jan15	25	290	600	500			-28			450
Jan17	65			175			-45			227
Jan20	-15			-38			-88			125
Jan22	-158			-133			-183			42
Jan24	22			82			-53			-17
Jan27	18			-68			-72			102
Jan29	21			378			-2			-2
Jan31	20			245			-122			8
Feb3	38			252			123			640
Feb5	39			646			144			646
Feb7	-17			-43			-77			17
Average	2			128			-54			149

Table 5: TOC, Raw Values

RUN #1	Influent	Bucket	K			R			C		
	mg/l		1	2	3	1	2	3	1	2	3
Sep 20	29.6	31.2	31.7	25.3	28.4	28.4	33	27.6	26.7	33.7	28.1
Sep 23	29.3	30.9	30	30	29.3	30.8	32.5	31.7	30.6	30.1	29.7
Sep 25	28.5	28.7	29.3	26.6	29.1	28.8	30.7	29.7	29.3	28.3	31
Sep 27	36.6	37.1	36.4	40	40.9	39.3	41	41.9	43.1	41.3	39.8
Oct 6	51	46.5	45	41.4	40.8	46.3	44.7	43.8	47.5	42.4	41.6
Oct 7	36.6	40.2	41.7	38	40.9	41.3	40.9	43.7	40.4	39.4	43.5
Oct 8	40.4	37.4	38.7	36.7	nd	39.6	36.8	40.5	36.8	36.5	39.7
Oct 15 **	26.5	25.2	25.6	24.6	23.5	25.4	25.5	25.6	25.1	25.3	26.4
Average	34.81	34.65	34.8	32.83	29.11	34.99	35.64	35.56	34.94	34.63	34.98

**Sample processed in January

RUN #2	Influent	Bucket	K			R			C		
	mg/l		1	2	3	1	2	3	1	2	3
Jan5	43.55	40.5	nd	nd	41.35	nd	nd	42.2	nd	nd	36.45
Jan6	32.9	33.9	43.6	47.1	45.9	39.9	39.8	33.7	36.3	41.2	39.8
Jan7	26.6	26.3	29.8	33.1	35.7	27.1	30.2	29.5	28.5	37	28.9
Jan9	26.7	29.5	33.1	35.7	36.4	30	32.7	30.7	28.4	28.5	22.6
Jan10	27.9	29.6	34.9	37.9	41.1	33.1	35.5	38.4	30.5	32.6	40.6
Jan14	31.1	35.9	43.4	43.6	43.1	32.1	34.6	35.5	32	34	40.6
Jan15	nd	41.6	46.4	44.9	49.1	43.8	43.8	45.2	41.4	38.4	45.7
Jan17	40	40	50.6	50.8	51.9	36.5	45.3	46.3	41.6	41	29.2
Jan20	27.1	39.5	49	52.6	51.9	44	46.1	47	32.3	46.1	41.1
Jan22	35.1	37.5	37.9	42.2	46.5	36.3	39.5	43.5	26.5	35.6	37.1
Jan24	28.5	28.4	35.4	46.5	42.3	40.9	41.3	37.5	39.9	36.5	38.6
Jan27	45.5	46.9	55.8	58.5	59.1	50	47.5	48.1	46.8	50	51.4
Jan29	42.2	45.7	53	68.1	54.9	45.1	49.4	53	47.5	45.7	50.4
Jan31	39.6	34.3	55.5	65.7	53.9	56	50	48.6	49.9	33.4	40.1
Feb3	35.2	43.8	51.3	62.5	58.7	41.1	50.8	51.1	47.7	41.3	45.7
Feb5	41.6	45.3	53.5	56.1	53.5	45	49.2	31.2	33.1	42	33.4
Feb7	47.2	38.5	46.9	58.4	60.5	54.5	52	53.8	51.2	49.8	49.7
Feb10	28.3	29.8	39.7	41	43.5	32.3	34.2	36.5	30.6	31.2	31.6
Feb12	31.6	28.3	43	41.4	40.5	31.9	34.2	37.6	31	30.1	29.7
Feb14	29	42	50.1	40.1	51.1	29.6	33.5	35.3	40.6	30.1	30.2
Feb17	40.4	41.7	52	56.8	50.2	45	45.6	49.6	42.9	42.1	43.1
Feb19	41.8	44.7	50.7	54.9	54.4	45.1	46.7	48.1	42.8	40.2	35.9
Average	33.72	37.44	43.44	47.18	48.43	38.15	40.09	41.93	36.43	36.67	38.27

Table 6: TOC, Corrected for Bucket Values

RUN #1	Influent	K			R			C		
	mg/l	1	2	3	1	2	3	1	2	3
Sep 20	-1.6	0.5	-5.9	-2.8	-2.8	1.8	-3.6	-4.5	2.5	-3.1
Sep 23	-1.6	-0.9	-0.9	-1.6	-0.1	1.6	0.8	-0.3	-0.8	-1.2
Sep 25	-0.2	0.6	-2.1	0.4	0.1	2	1	0.6	-0.4	2.3
Sep 27	-0.5	-0.7	2.9	3.8	2.2	3.9	4.8	6	4.2	2.7
Oct 6	4.5	-1.5	-5.1	-5.7	-0.2	-1.8	-2.7	1	-4.1	-4.9
Oct 7	-3.6	1.5	-2.2	0.7	1.1	0.7	3.5	0.2	-0.8	3.3
Oct 8	3	1.3	-0.7	nd	2.2	-0.6	3.1	-0.6	-0.9	2.3
Oct 15 **	1.3	0.4	-0.6	-1.7	0.2	0.3	0.4	-0.1	0.1	1.2
Average	0.1625	0.15	-1.825	-0.863	0.3375	0.9875	0.9125	0.2875	-0.025	0.325

RUN #2	Influent	K			R			C		
	mg/l	1	2	3	1	2	3	1	2	3
JAN5,97	3.05			0.85			1.7			-4.05
JAN6,97	-1	9.7	13.2	12	6	5.9	-0.2	2.4	7.3	5.9
JAN7,97	0.3	3.5	6.8	9.4	0.8	3.9	3.2	2.2	10.7	2.6
Jan9	-2.8	3.6	6.2	6.9	0.5	3.2	1.2	-1.1	-1	-6.9
Jan10	-1.7	5.3	8.3	11.5	3.5	5.9	8.8	0.9	3	11
Jan14	-4.8	7.5	7.7	7.2	-3.8	-1.3	-0.4	-3.9	-1.9	4.7
Jan15	-41.6	4.8	3.3	7.5	2.2	2.2	3.6	-0.2	-3.2	4.1
Jan17	0	10.6	10.8	11.9	-3.5	5.3	6.3	1.6	1	-10.8
Jan20	-12.4	9.5	13.1	12.4	4.5	6.6	7.5	-7.2	6.6	1.6
Jan22	-2.4	0.4	4.7	9	-1.2	2	6	-11	-1.9	-0.4
Jan24	0.1	7	18.1	13.9	12.5	12.9	9.1	11.5	8.1	10.2
Jan27	-1.4	8.9	11.6	12.2	3.1	0.6	1.2	-0.1	3.1	4.5
Jan29	-3.5	7.3	22.4	9.2	-0.6	3.7	7.3	1.8	0	4.7
Jan31	5.3	21.2	31.4	19.6	21.7	15.7	14.3	15.6	-0.9	5.8
Feb3	-8.6	7.5	18.7	14.9	-2.7	7	7.3	3.9	-2.5	1.9
Feb5	-3.7	8.2	10.8	8.2	-0.3	3.9	-14.1	-12.2	-3.3	-11.9
Feb7	8.7	8.4	19.9	22	16	13.5	15.3	12.7	11.3	11.2
Feb10	-1.5	9.9	11.2	13.7	2.5	4.4	6.7	0.8	1.4	1.8
Feb12	3.3	14.7	13.1	12.2	3.6	5.9	9.3	2.7	1.8	1.4
Feb14	-13	8.1	-1.9	9.1	-12.4	-8.5	-6.7	-1.4	-11.9	-11.8
Feb17	-1.3	10.3	15.1	8.5	3.3	3.9	7.9	1.2	0.4	1.4
Feb19	-2.9	6	10.2	9.7	0.4	2	3.4	-1.9	-4.5	-8.8
Average	-3.72	8.2095	12.129	10.993	2.6714	4.7	4.4864	0.8714	1.1238	0.825

Table 7: SCVFA, Preliminary and Run #1

	1° Effluent			Bucket			K1			K2			K3			R1			R2			R3			C1			C2			C3		
Prelim	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot
July 4th			nd	13	2	15	19	4	22				19	3	22	24	4	27				21	4	24	21	4	25				16	3	18
July 11			nd	23	4	26	29	5	33	29	4	32	31	4	35	28	4	31	25	3	28	29	5	33	27	4	30	29	4	32	26	4	29
July 17			nd	20	3	23	25	3	28	32	4	36	30	4	33	24	3	26	29	4	32	24	3	26	25	4	28	29	4	32	27	4	29
RUN #1																																	
Aug 16			nd	27	3	29	27	nn	27	26	2	27	34	6	38	28	3	30	28	3	31	32	4	35	23	nn	23	27	2	29	25	nn	25
Aug 19	28	3	30	27	3	29	29	3	31	32	5	36	38	6	44	31	5	35	30	5	34	36	5	40	33	4	36	32	4	35	32	5	36
Aug 21			nd	29	4	32	28	4	31	28	4	31	31	6	36	27	4	30	29	5	32	29	5	32	27	4	30	26	4	29	26	4	29
Aug 23	25	3	28	28	4	31	28	5	32	29	5	33	29	6	34	28	6	33	31	6	36	30	6	34	36	5	40	28	4	32	27	5	31
Aug 26	28	3	31	28	4	32	29	4	32	29	5	33	31	6	35	30	5	34	32	6	37	33	6	37	29	4	32	32	5	36	31	4	34
Aug 30	22	4	26	26	2	28	27	3	30	30	5	34		2		24	3	26	30	4	34	27	4	30	23	2	25	28	3	30	25	2	27
Sep 3	29	6	33	35	6	40	32	6	37	37	7	42	39	8	46	34	6	39	38	7	44	39	7	45	31	5	35	35	5	40	33	3	35
Sep 6	27	2	28	29	3	32	29	4	32	31	4	34	33	5	37	31	4	34	33	4	36	36	4	39	30	4	32	30	4	33	33	4	36
Sep 9	21	3	23	31	3	34	32	3	35	35	4	38	40	5	44	34	4	37	36	5	40	40	5	44	34	3	37	35	4	38	40	4	43
Sep 12a	15	4	18	25	4	28	23	4	26	24	4	27	27	5	31	25	4	29	27	5	31	27	5	31	24	4	27	25	3	28	25	3	28
Sep 12b	19	3	21	22	3	25	21	3	24	23	3	25	24	4	27	22	4	25	24	4	28	24	4	28	20	3	22	21	3	23	20	3	23
Sep 20	17	2	19	17	2	18	18	2	20	18	2	20	22	2	24	19	3	22	21	3	23	23	3	25	18	2	20	18	2	20	18	2	20
Sep 23	15	1	16	16	2	17	16	2	17	16	2	17	18	1	19	17	2	18	18	2	20	18	2	20	15	2	16	15	2	17	15	2	17
Sep 25	15	2	17	17	2	18	16	2	18	17	2	18	18	2	20	17	2	19	20	3	22	20	3	22	17	2	18	16	2	18	17	2	19
Sep 27	11	2	12	11	2	13	14	2	15	14	nn	14	16	nn	16	15	2	17	20	3	22	18	3	21	13	2	15	14	2	15	14	2	16
Oct 6	7	nn	7	10	2	12	12	3	14	14	3	16	15	2	16	15	3	18	16	3	18	17	3	20	12	3	14	14	3	16	15	3	17
Oct 7	5	nn	5	10	2	12	13	2	15	15	2	17	18	2	20	13	2	15	18	3	20	21	4	24	14	3	16	16	3	19	18	4	21
Oct 8	13	nn	13	12	nn	12	13	nn	13	13	nn	13				14	2	16	15	2	17	16	2	18	13	nn	13	13	nn	13	14	nn	14
Oct 15	18	0	18	19	2	20	19	2	20	19	2	20	20	2	21	20	2	22	20	2	22	22	2	24	19	2	20	19	2	21	20	2	22
Average	17	2	18	22	3	24	22	3	25	24	3	26	25	4	30	23	3	26	26	4	29	27	4	30	23	3	25	23	3	26	23	3	26

Table 8: SCVFA, Run #2

	Raw ww			Bucket			K1			K2			K3			R1			R2			R3			C1			C2			C3		
RUN #2	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot	HA	Pr	Tot
Jan5	8	2	10	11	3	13	nd	nd		nd	nd		25	8	31	nd	nd		nd	nd		19	6	23	nd	nd		nd	nd		15	5	19
Jan6	14	2	16	14	3	16	21	7	26	27	9	35	29	10	37	17	4	21	19	5	24	22	7	28	15	4	18	16	5	20	17	6	21
Jan7	11	2	12	13	2	14	19	6	24	21	8	28	25	9	32	15	2	17	15	4	18	17	3	19	13	2	15	15	3	17	12	3	14
Jan9	10	3	12	13	4	16	18	7	24	22	8	28	24	8	30	16	4	19	18	5	22	19	5	24	12	3	15	12	4	15	11	3	13
Jan10	17	3	19	17	4	20	23	7	29	27	9	35	30	11	39	21	6	26	24	7	30	26	10	33	18	4	21	21	5	25	20	6	24
Jan14	18	2	19	18	2	20	22	5	26	26	7	31	27	8	34	21	3	23	21	3	24	21	3	24	18	2	20	19	2	21	20	3	22
Jan15	11	1	12	13	2	14	20	7	26	23	8	29	24	9	31	15	3	18	19	4	23	20	5	23	12	2	14	12	2	14	16	3	19
Jan17	20	0	20	20		20	28	9	36	32	12	42	32	12	42	23	5	27	25	8	31	28	9	35	20		20	20		20	19	3	22
Jan20	13	0	13	15		15	24	9	31	31	12	41	31	11	40	18	4	21	22	6	27	25	8	32	18		18	17		17	16	3	18
Jan22	14	0	14	14		14	21	6	26	26	10	34	27	10	35	18	5	22	19	5	23	21	7	27	14		14	14		14	14		14
Jan24	9	0	9	12		12	19	6	24	24	9	30	26	10	33	14		14	17	4	20	20	5	23	14		14	13		13	13		13
Jan27	17	2	18	12	2	13	23	9	30	27	12	36	30	13	40	19	3	21	20	3	23	24	5	28	17	2	19	20	4	23	20	5	24
Jan29	14	2	16	11	2	13	19	9	27	28	12	38	25	12	35	18	4	22	19	6	24	20	8	26	16	3	18	11	3	14	13	3	15
Jan31	10	2	12	15	3	17	24	11	33	24	14	36	25	14	36	19	10	27	18	6	23	17	7	23	11	3	13	12	4	15	12	4	16
Feb5	23	3	25	25	4	28	30	10	38	33	12	43	36	13	47	23	5	27	25	6	29	24	6	28	20	3	23	21	4	24	18	4	21
Feb7	19	2	21	17	2	18	28	8	34	34	11	42	32	11	41	25	4	28	26	5	30	29	6	33	20	2	22	21	2	23	21	2	23
Feb10	16	0	16	19	2	20	27	9	34	28	9	35	33	10	41	19	4	22	26	5	30	27	6	31	17	2	19	18	2	20	23	3	25
Feb17	17	2	19	18	2	20	28	9	35	32	11	40	31	11	39	21	4	24	24	5	28	29	8	35	19	3	21	20	3	22	20	3	22
Feb19	17	2	18	16	2	18	26	9	33	29	11	38	30	11	38	18	4	21	23	5	27	24	7	29	16	2	18	18	3	21	18	3	21
Average	14	2	16	15	2	17	22	8	30	26	10	36	28	10	37	18	4	22	20	5	25	22	6	27	15	2	18	16	3	18	16	4	19

Figure 1: 24hr Sample, COD Analysis

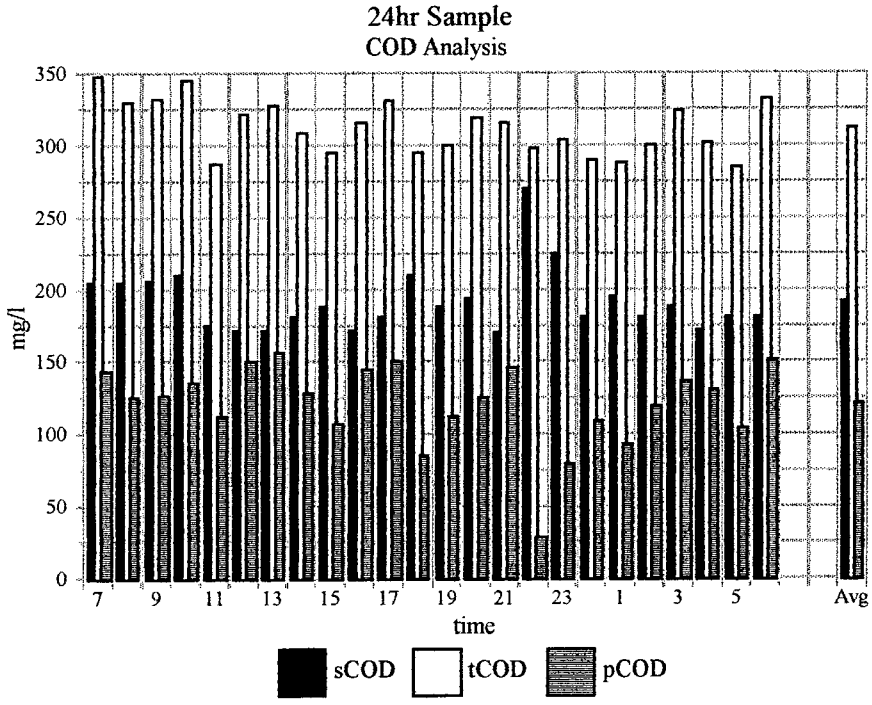


Figure 2: 24hr Sample, Ortho-P Analysis

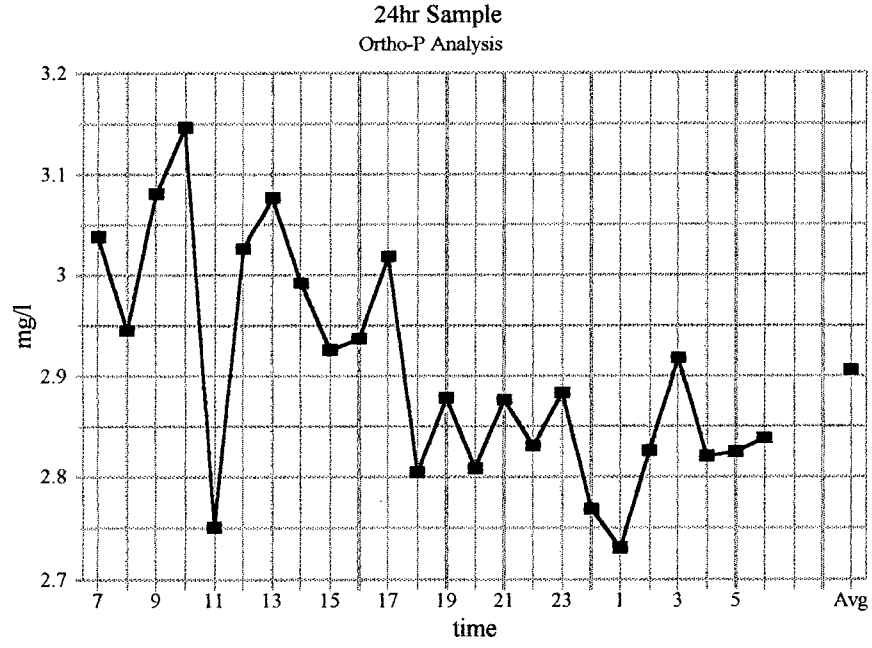


Figure 3: 24hr Sample, Ammonia Analysis

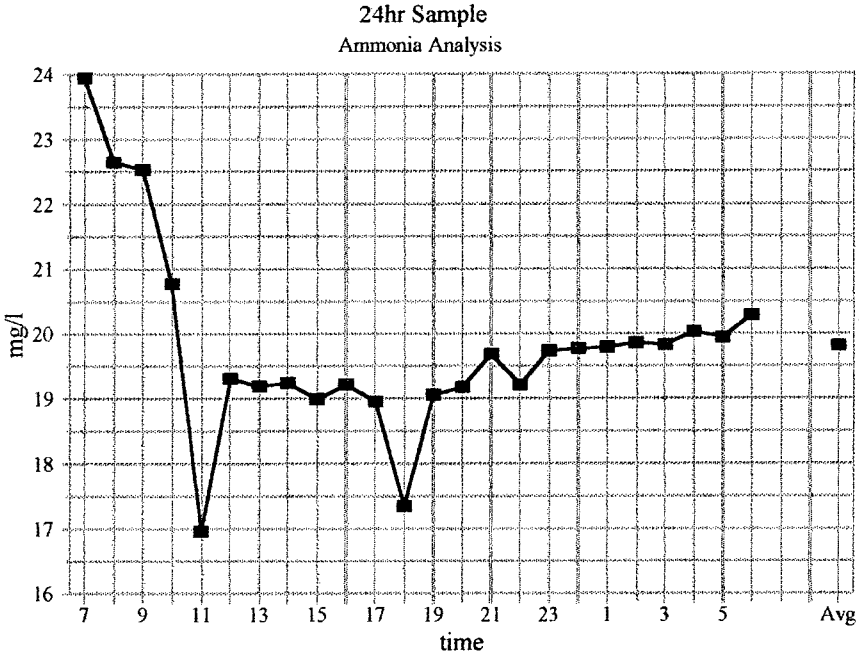
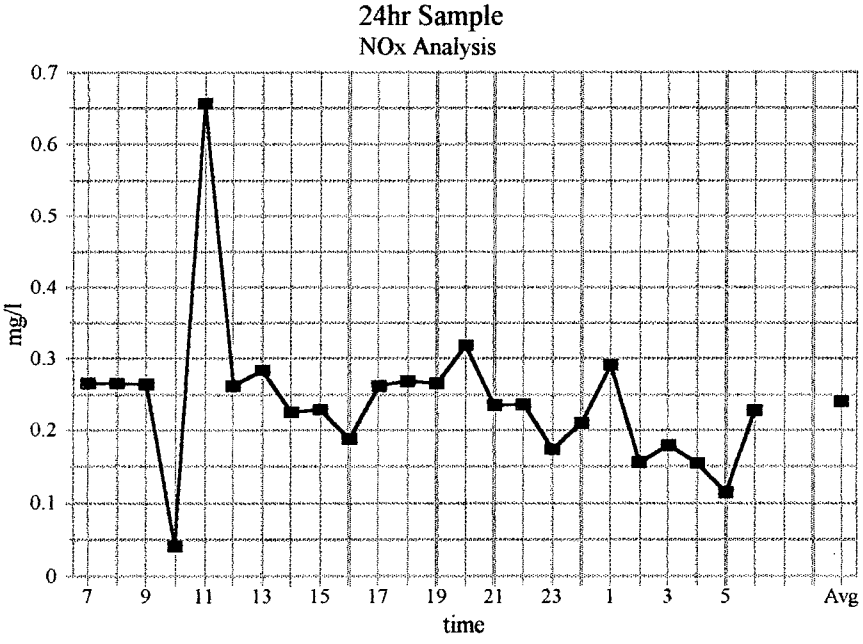


Figure 4: 24hr Sample, Ammonia Analysis



APPENDIX B: STATISTICAL ANALYSIS

Table 1: Media SCVFA Production Comparison, Run #1

Run #1	Ringlace	Kaldnes
Date	total	total
Aug 16	6.1848	9.8324
Aug 19	11.1837	14.707
Aug 21	0.3256	3.4013
Aug 23	3.2158	2.726
Aug 26	5.7412	3.4788
Sep 3	4.7458	5.6109
Sep 6	7.3143	5.1754
Sep 9	10.1985	10.3962
Sep 12a	2.9214	3.7017
Sep 12b	2.9612	2.8073
Sep 20	6.7968	5.8902
Sep 23	2.7302	1.2022
Sep 25	4.1613	1.2813
Sep 27	8.1754	3.6011
Oct 6	7.8913	4.216
Oct 7	12.4383	8.1034
Oct 15	3.381	0.557

t-Test: Paired Two-Sample for Means	**Kaldnes**	**Ringlace**
Mean	5.90392	5.09931
Variance	11.10831	14.02844
Observations	17	17
Pearson Correlation	0.73602	
Pooled Variance	12.56838	
df	16	
Hypothesized Mean Difference	0	
t	1.27587	
P(T<=t) two-tail	0.22021	
t Critical two-tail	2.11991	

Table 2: Media SCVFA Production Comparison, Run #2

Run #2	Kaldnes	Ringlace
Data	total	total
Jan5	18.169	10.73
Jan6	20.551	11.697
Jan7	17.489	4.934
Jan9	14.34	8.058
Jan10	18.408	13.141
Jan14	14.646	4.515
Jan15	16.946	9.006
Jan17	22.644	15.333
Jan20	25.834	17.137
Jan22	20.657	13.089
Jan24	21.895	11.745
Jan27	27.129	14.249
Jan29	21.8	12.879
Jan31	19.234	6.145
Feb3	21.733	8.911
Feb5	18.476	-0.442
Feb7	22.785	15.078
Feb10	21.123	11.197
Feb12	16.308	9.292
Feb14	19.333	7.492
Feb17	19.642	15.398
Feb19	20.223	11.183

t-Test: Paired Two-Sample for Means	**Kaldnes**	**Ringlace**
Mean	19.97114	10.48941
Variance	10.03812	17.92647
Observations	22	22
Pearson Correlation	0.64839	
Pooled Variance	13.98229	
df	21.00000	
Hypothesized Mean Difference	0	
t	13.67993	
P(T<=t) two-tail	0.00000	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	4	
t	7.90886	
P(T<=t) two-tail	9.89103E-08	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	8	
t	2.13779	
P(T<=t) two-tail	0.04445	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	9	
t	0.69502	
P(T<=t) two-tail	0.49466	
t Critical two-tail	2.07961	

Table 3: Kaldnes Media SCVFA Production Relative to the Control, Run #1

Run #1 Data	Kaldnes total	Control total
Aug 16	9.8	-4.065
Aug 19	14.7	7.0766
Aug 21	3.4	-3.0105
Aug 23	2.7	-0.4671
Aug 26	3.5	2.3196
Sep 3	5.6	-5.3592
Sep 6	5.2	4.3331
Sep 9	10.4	8.8946
Sep 12a	3.7	-0.0035
Sep 12b	2.8	-2.1214
Sep 20	5.9	1.432
Sep 23	1.2	-0.8361
Sep 25	1.3	0.8434
Sep 27	3.6	2.9888
Oct 6	4.2	5.0826
Oct 7	8.1	9.4374
Oct 15	0.6	1.2

t-Test: Paired Two-Sample for Means	**Kaldnes**	**Control**
Mean	5.09931	1.63208
Variance	14.02844	18.48901
Observations	17	17
Pearson Correlation	0.44972	
Pooled Variance	16.25873	
df	16	
Hypothesized Mean Difference	0	
t	3.36655	
P(T<=t) two-tail	0.00393	
t Critical two-tail	2.11991	
Hypothesized Mean Difference	2	
t	1.42463	
P(T<=t) two-tail	0.17348	
t Critical two-tail	2.11991	
Hypothesized Mean Difference	3	
t	0.45366	
P(T<=t) two-tail	0.65616	
t Critical two-tail	2.11991	

Table 4: Kaldnes Media SCVFA Production Relative to the Control, Run #2

Run #2 Data	Kaldnes total	Control total
Jan5	18.2	6.22
Jan6	20.6	5.087
Jan7	17.5	0.172
Jan9	14.3	-2.062
Jan10	18.4	3.901
Jan14	14.6	2.815
Jan15	16.9	4.572
Jan17	22.6	2.454
Jan20	25.8	3.911
Jan22	20.7	0.2
Jan24	21.9	1.5
Jan27	27.1	10.706
Jan29	21.8	2.172
Jan31	19.2	-1.185
Feb3	21.7	3.615
Feb5	18.5	-7.619
Feb7	22.8	4.862
Feb10	21.1	4.648
Feb12	16.3	-0.038
Feb14	19.3	1.929
Feb17	19.6	2.448
Feb19	20.2	2.686

t-Test: Paired Two-Sample for Means	**Kaldnes**	**Control**
Mean	19.97114	2.40882
Variance	10.03812	12.51800
Observations	22	22
Pearson Correlation	0.49944	
Pooled Variance	11.27806	
df	21.00000	
Hypothesized Mean Difference	0	
t	24.44127	
P(T<=t) two-tail	0.00000	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	16	
t	2.17426	
P(T<=t) two-tail	0.04126	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	17	
t	0.78257	
P(T<=t) two-tail	0.44261	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	18	
t	-0.60912	
P(T<=t) two-tail	0.54898	
t Critical two-tail	2.07961	

Table 5: Ringlace Media SCVFA Production Relative to the Control, Run #1

Run #1 Data	Ringlace total	Control total
Aug 16	6.2	-4.065
Aug 19	11.2	7.0766
Aug 21	0.3	-3.0105
Aug 23	3.2	-0.4671
Aug 26	5.7	2.3196
Aug 30	1.7	-1.4
Sep 3	4.7	-5.3592
Sep 6	7.3	4.3331
Sep 9	10.2	8.8946
Sep 12a	2.9	-0.0035
Sep 12b	3.0	-2.1214
Sep 20	6.8	1.432
Sep 23	2.7	-0.8361
Sep 25	4.2	0.8434
Sep 27	8.2	2.9888
Oct 6	7.9	5.0826
Oct 7	12.4	9.4374
Oct 8	5.6	1.16
Oct 15	3.4	1.2

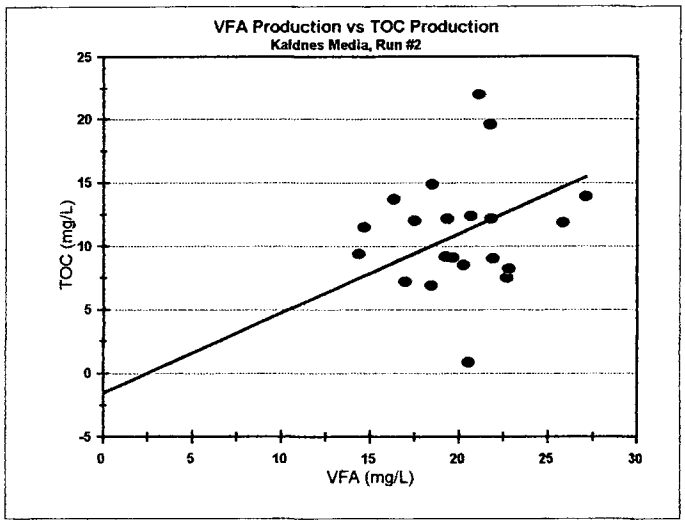
t-Test: Paired Two-Sample for Means	**Ringlace**	**Control**
Mean	5.66473	1.44765
Variance	10.80676	16.92190
Observations	19	19
Pearson Correlation	0.82474	
Pooled Variance	13.86433	
df	18	
Hypothesized Mean Difference	0	
t	7.89365	
P(T<=t) two-tail	0.00000	
t Critical two-tail	2.10092	
Hypothesized Mean Difference	3	
t	2.27817	
P(T<=t) two-tail	0.03514	
t Critical two-tail	2.10092	
Hypothesized Mean Difference	4	
t	0.40634	
P(T<=t) two-tail	0.68928	
t Critical two-tail	2.10092	

Table 6: Ringlace Media SCVFA Production Relative to the Control, Run #2

Run #2 Data	Ringlace total	Control total
Jan5	10.7	6.22
Jan6	11.7	5.087
Jan7	4.9	0.172
Jan9	8.1	-2.062
Jan10	13.1	3.901
Jan14	4.5	2.815
Jan15	9.0	4.572
Jan17	15.3	2.454
Jan20	17.1	3.911
Jan22	13.1	0.2
Jan24	11.7	1.5
Jan27	14.2	10.706
Jan29	12.9	2.172
Jan31	6.1	-1.185
Feb3	8.9	3.615
Feb5	-0.4	-7.619
Feb7	15.1	4.862
Feb10	11.2	4.648
Feb12	9.3	-0.038
Feb14	7.5	1.929
Feb17	15.4	2.448
Feb19	11.2	2.686

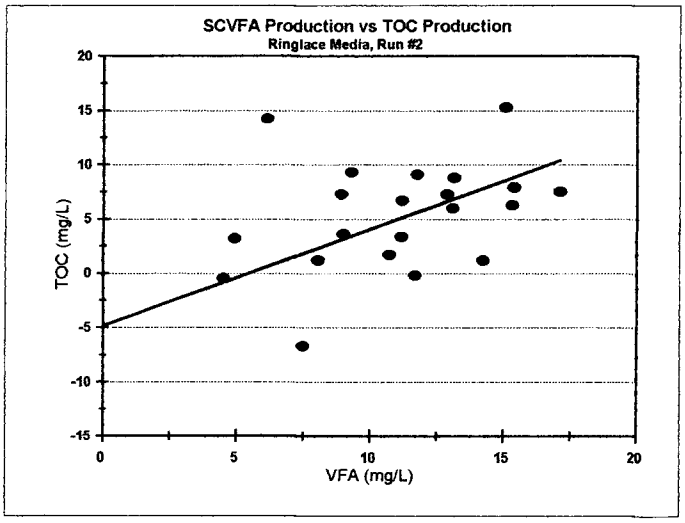
t-Test: Paired Two-Sample for Means	**Ringlace**	**Control**
Mean	10.48941	2.40882
Variance	17.92647	12.51800
Observations	22	22
Pearson Correlation	0.63780	
Pooled Variance	15.22224	
df	21	
Hypothesized Mean Difference	0	
t	11.25709	
P(T<=t) two-tail	0.00000	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	6	
t	2.89848	
P(T<=t) two-tail	0.00859	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	7	
t	1.50537	
P(T<=t) two-tail	0.14712	
t Critical two-tail	2.07961	
Hypothesized Mean Difference	8	
t	0.11227	
P(T<=t) two-tail	0.91167	
t Critical two-tail	2.07961	

Figure 1: Kaldnes Media Correlation Analysis, SCVFA Production vs TOC Production



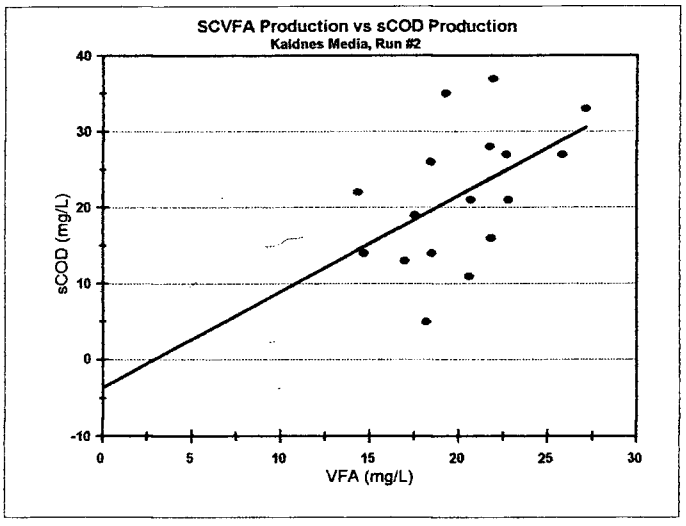
Slope: 0.627, Int.: -1.53, Pearson Coef.: 0.4505

Figure 2: Ringlace Media Correlation Analysis, SCVFA Production vs TOC Production



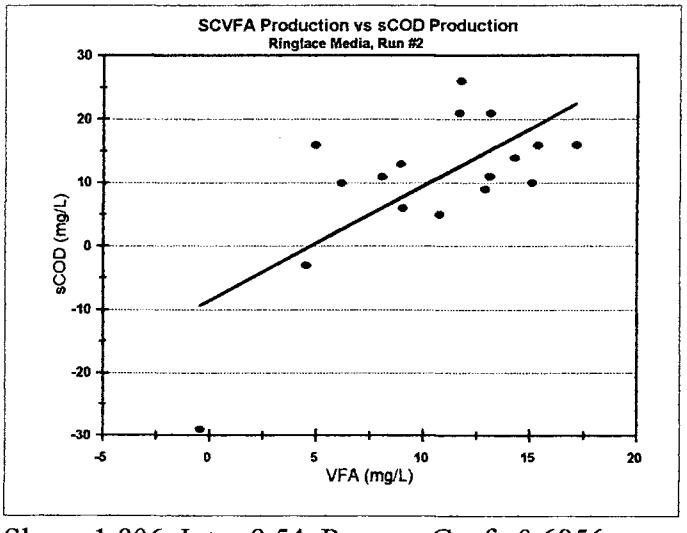
Slope: 0.889, Int.: -4.84, Pearson Coef.: 0.5840

Figure 3: Kaldnes Media Correlation Analysis, SCVFA Production vs sCOD Production



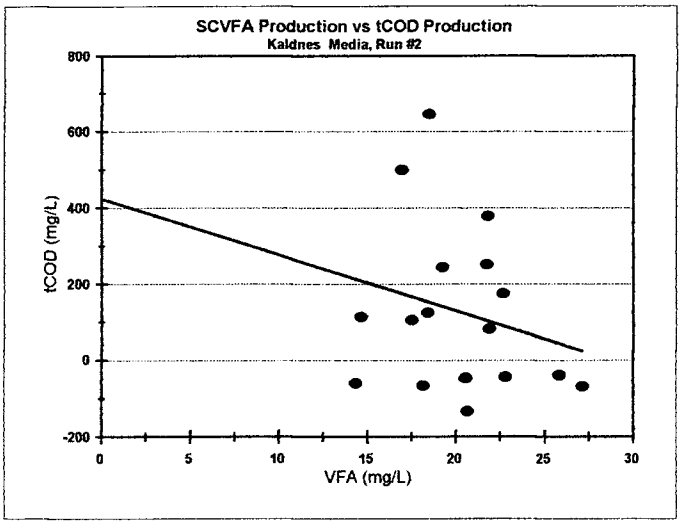
Slope: 1.257, Int.: -3.64, Pearson Coef.: 0.4902

Figure 4: Ringlace Media Correlation Analysis, SCVFA Production vs sCOD Production



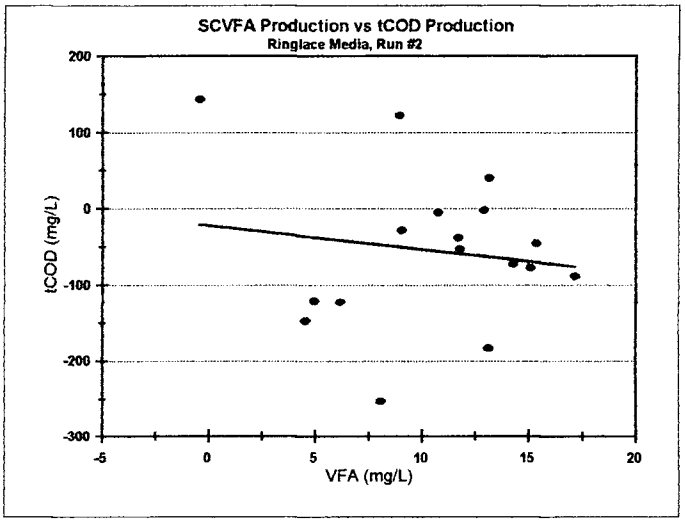
Slope: 1.806, Int.: -8.54, Pearson Coef.: 0.6856

Figure 5: Kaldnes Media Correlation Analysis, SCVFA Production vs tCOD Production



Slope: -14.70, Int.: 423.9, Pearson Coef.: 0.2335

Figure 6: Ringlace Media Correlation Analysis, SCVFA Production vs tCOD Production



Slope: -3.122, Int.: -22.1, Pearson Coef.: 0.1436

APPENDIX C: PHOTOS

Figure 1: BF-System Influent Processing Reactors, Runs #1 and 2

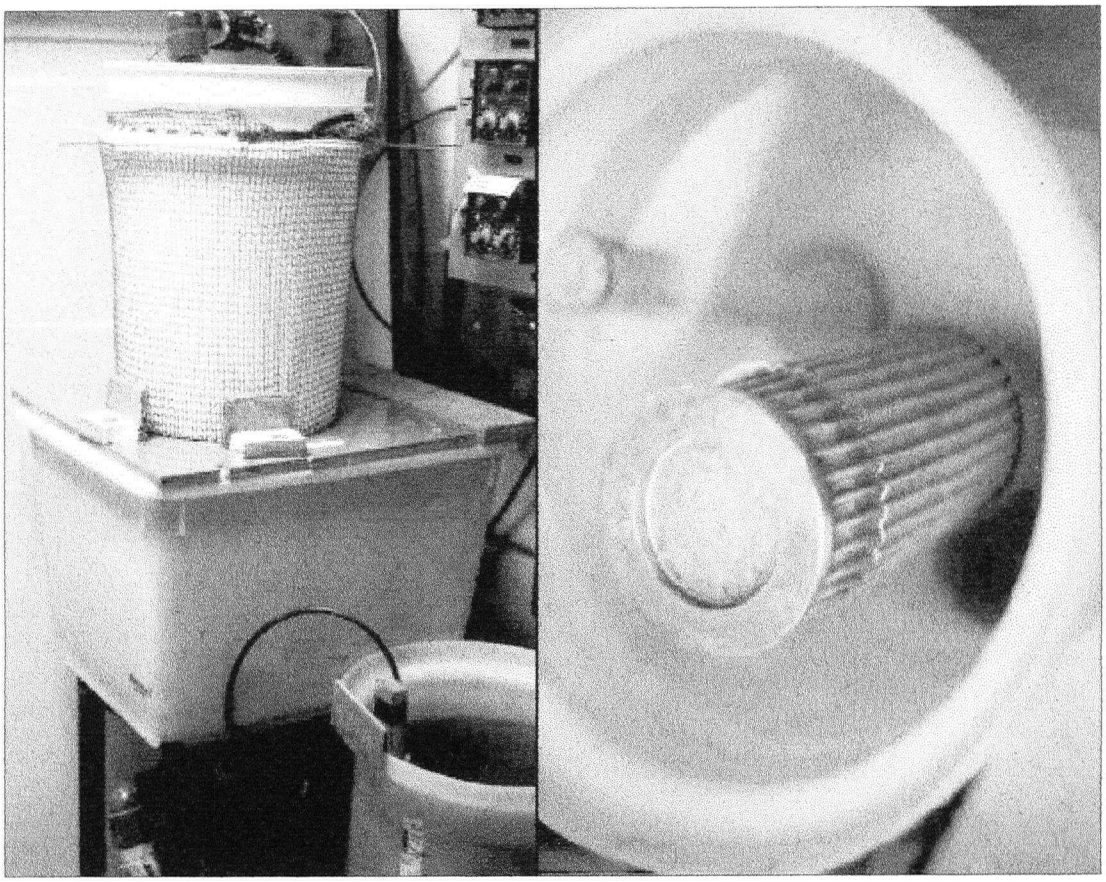


Figure 2: Ringlace Media Biolog GN Plate, Run #2

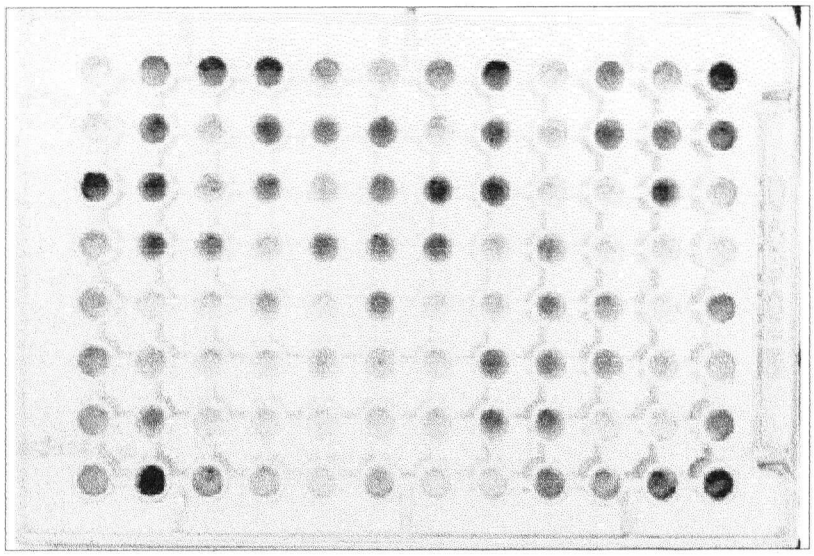


Figure 3: BF-System Reactors

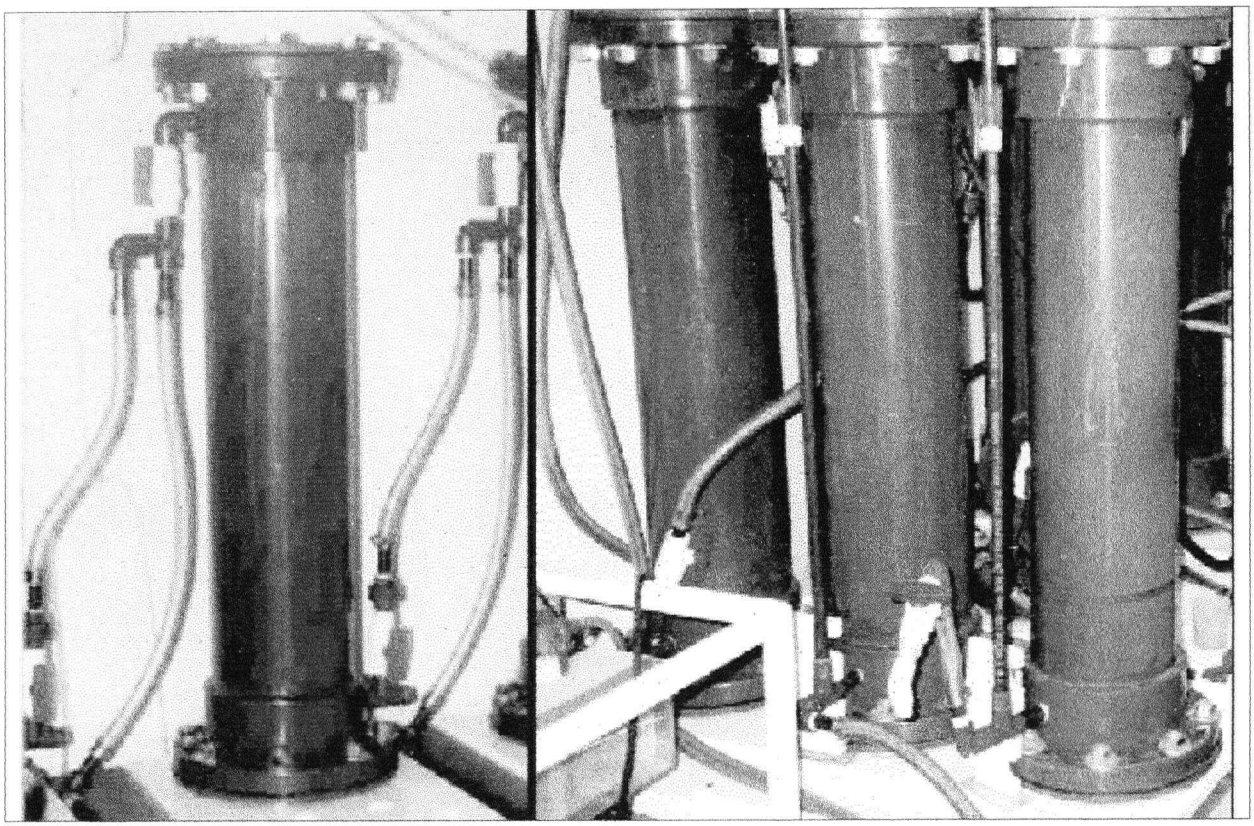


Figure 4: BF-system Influent Pumps

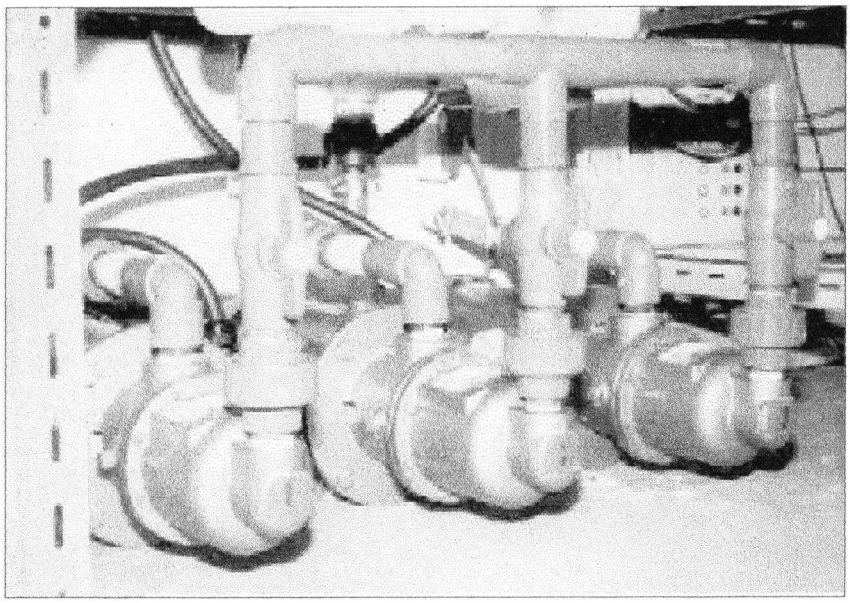


Figure 5: Reactor Mixing Chambers

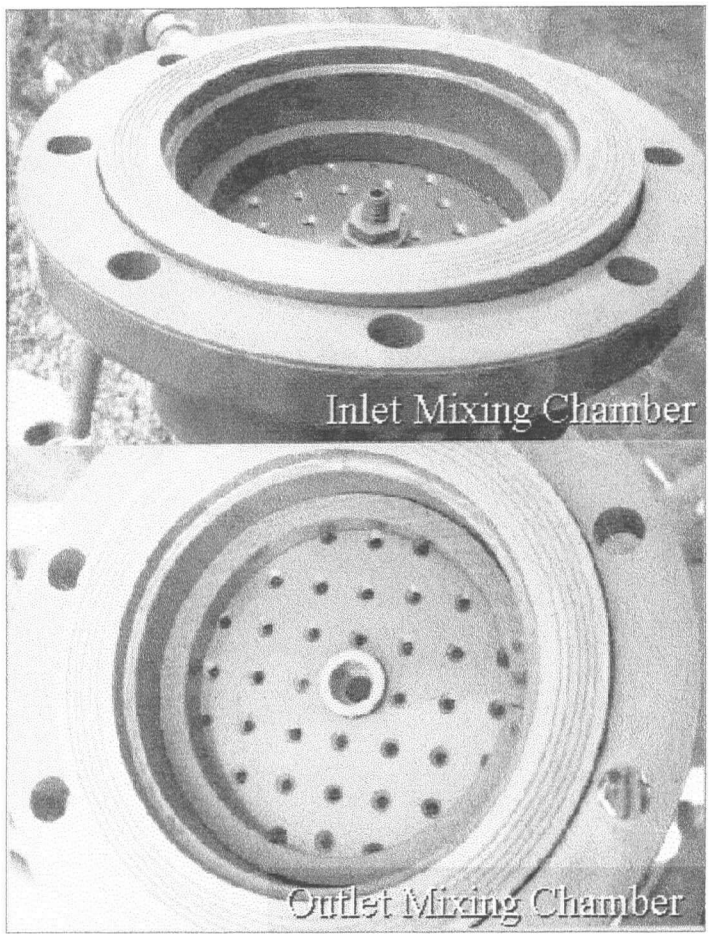


Figure 6: Ringlace Reactor, Outlet Mixing Chamber

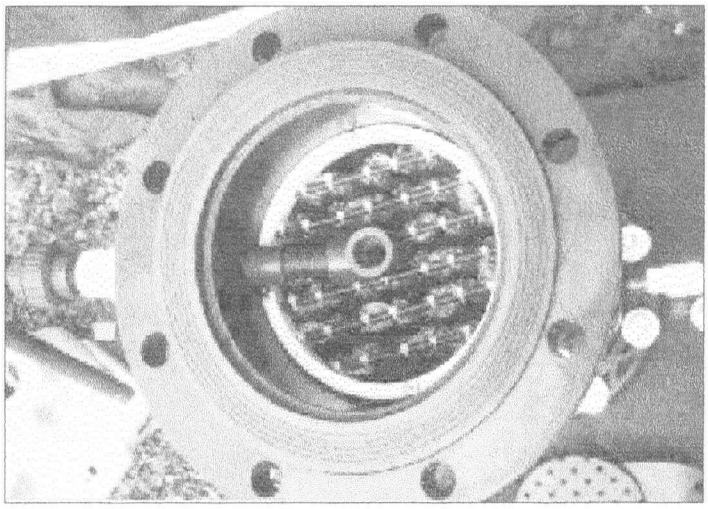


Figure 7: Ringlace cage, clean

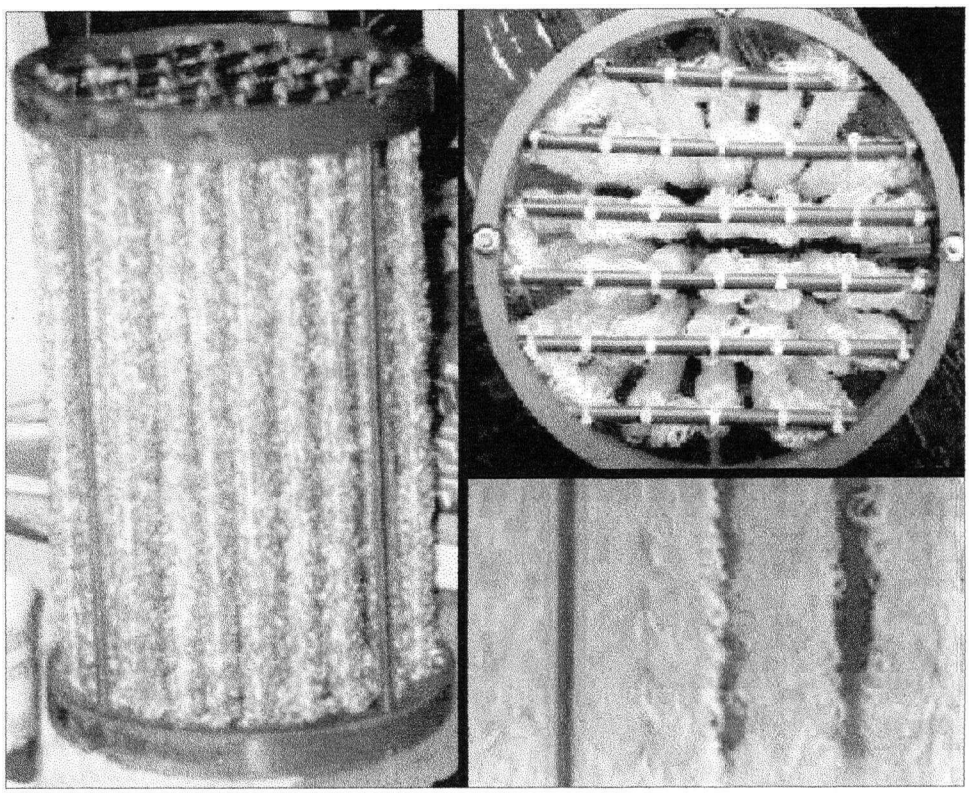


Figure 8: Ringlace cage, carrying solids

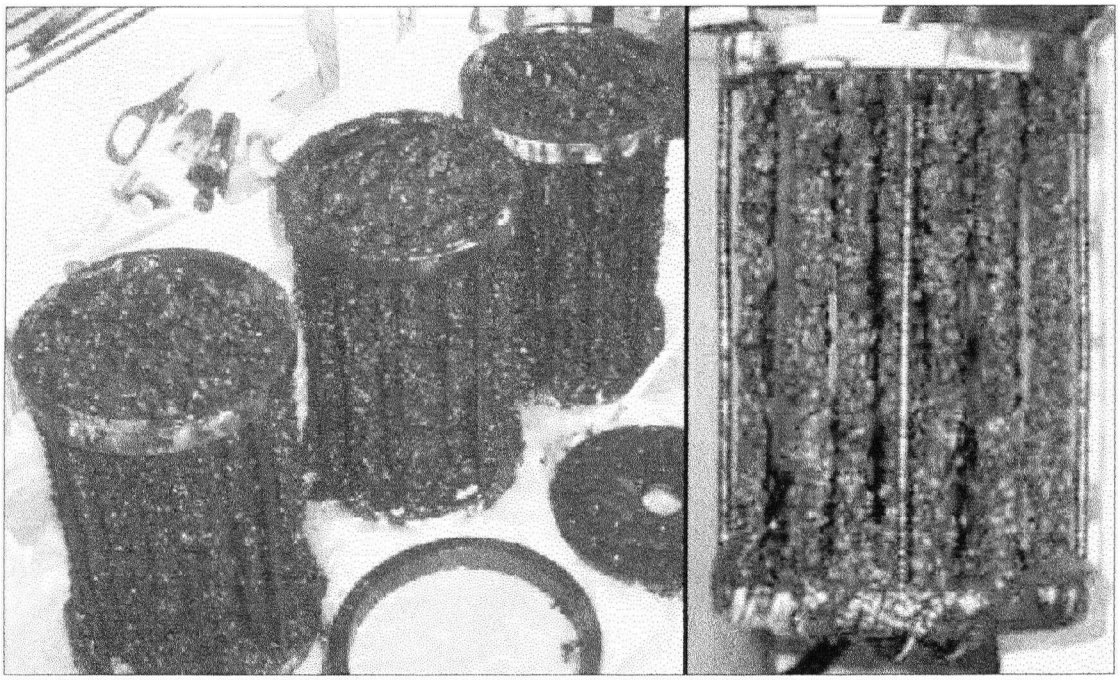


Figure 9: Kaldnes media bag