Waste Management and Byproducts Recovery for the Blue Crab (*Callinectes sapidus*) Industry

Part IV: Removal of Nutrients and Ammonia from Crab Processing Waters

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Chapter 1. Introduction

Seafood processing companies have come under significant pressure to upgrade the level of treatment of their wastewater discharges. Along with most other industries which discharge wastewater to a receiving water, seafood processors were impacted by the 1972 Federal Water Pollution Control Amendments, and subsequent federal legislation such as the 1977 Clean Water Act.

Initially, discharge requirements were based on best practical technology (BPT), a relatively lenient system of standards based on current industry practices. A more restrictive set of requirements proposed for implementation in 1984 were based on best available technology (BAT). For some industries, including blue crab processors, it was later deemed economically unfeasible by EPA (Brinsfield *et al.*, 1978) to impose BAT based standards at that time, and thus a more lenient set of requirements, essentially the same as BPT, were authorized.

These were referred to as best conventional pollutant control technology currently available (BCT). As shown in Table 1, the only parameters included in the federal guidelines for blue crab processors were five day biochemical oxygen demand (BOD_5), total suspended solids (TSS), oil and grease (O&G), and pH. Different limits were placed on new sources than on pre-existing plants.

The discharge of water to the environment is regulated by the United States Environmental Protection Agency (EPA) through the National Pollutant Discharge Elimination System (NPDES). All public and private dischargers of water to the environment are potentially subject to regulation through the NPDES permitting process. The permit specifies the maximum allowable limits for certain pollutants in the wastewater.

In most cases, the EPA has delegated this regulatory responsibility to the states. In Virginia, the Department of Environmental Quality (DEQ) has authorization from EPA to administer this program in Virginia through the issuance of Virginia Pollutant Discharge

Elimination System (VPDES) permits. EPA requires that DEQ be no less stringent in setting discharge limits than are allowed by EPA standards.

Industries may discharge their liquid wastes to a publicly owned treatment works (POTW). POTW's are generally empowered to set limits and fees on the discharge to their collection system of industrial wastewater which adversely affects the performance or efficiency of the POTW processes.

Martin (1992) discussed the impact of this regulatory structure on the seafood industry. and identified three areas of concern to regulators and the industry: "(1) effluent treatment and disposal, (2) solid waste disposal, and (3) by-product recovery." Wheaton (1984) argued that many blue crab processors in Maryland would not be able to meet treatment guidelines and remain in business. Stringent standards may very well spell doom for small locally owned and operated blue crab processing companies.

Scope of the Research

There are several identifiable waste streams generated in a typical crab processing company. Harrison *et al.* (1992) has presented an excellent description of many aspects of the blue crab processing industry in Virginia and Maryland, including a discussion of these waste streams.

This research study is concerned only with the wastewater generated by the pressure cooking of the live crabs (the retort water). In the cookers, the introduced steam condenses as it passes down through the charge of live crabs. The continued introduction of steam forces the condensate out the bottom of the cooker in a continuous fashion during the cook cycle. Since the steam pressure builds faster than the water can escape, the pressure in the cook pot rises to the necessary level during the cooking cycle. As the steam passes over the bodies of the crabs, it dissolves internal organs and fluids, and washes away sea water and debris previously retained in spaces in and around the shells of the crabs. As a result, the wastewater is high in organic content (COD of 20,000 mg/L) and in dissolved minerals. Although this wastewater stream is not the most voluminous in a processing plant, it is one of the most concentrated.

Research Goals

Previous work, which will be described in the following section on a review of the literature, has attempted to identify wastewater treatment techniques which are useful and practical for crab processor. Most of these studies have concluded that primary treatment such as screening and settling, and simple aeration of the wastewater will not result in a wastewater of acceptable quality (Brinsfield *et al.*,1978; Creter and Lewandowski,1975; Geiger *et al.*,1985; Wheaton *et al.*,1984). Other research studies have been conducted on anaerobic technologies to treat high strength wastewaters generated by seafood processors with a variety of results (Balslev-Olesen *et al.*, 1990, Battistoni *et al.*,1992, Harrison *et al.*,1992, Mendez ,1992, Soto *et al.*,1991, Wolf, 1993).

Limitations of space and funds for capital investment and operating costs, and lack of trained manpower for treatment plant operation and maintenance argue against a conventional, full-scale, activated sludge, wastewater treatment facility. Discharge to a sanitary sewer system is a potential alternative for those processors so located. However, the surcharges for high organic and nutrient content may be costly. A possible solution is an anaerobic system with an aerobic finishing step which would be relatively small and easy to operate. The effluent from this plant could then be discharged to a POTW for final treatment or directly to the environment depending on the individual circumstances of the processor company. As Anderson *et al.* (1982) pointed out, anaerobic treatment systems have several advantages over aerated systems: low sludge production; start/stop operation without prolonged lag time; and production of useful biogas as a fuel source.

It was the goal of this study to design and evaluate a system composed of multiple stages including anaerobic and aerobic processes. Specifically, this study had several primary objectives:

1. Determine the feasibility of using anaerobic pretreatment for the reduction of biochemical oxygen demand.
2. Determine the kinetic coefficients of the anaerobic stage.
3. Reduce the concentration of ammonia-nitrogen in the wastewater by means of nitrification.

Additional secondary objectives included an investigation of the effect of adding certain trace metals, and a brief look at ammonia toxicity.

Chapter 2. Literature Review

The following review presents the published literature on blue crab cooker wastewater studies and studies of other seafood processes which generate wastewater. An overview of anaerobic metabolism is presented as well as a review of various anaerobic treatment technologies. Also considered are studies on methanogenesis and toxic inhibition and trace metal limitation of anaerobic processes in biological reactors. Inhibition of nitrification by ammonia is also reviewed.

Federal Discharge Limitations

Different requirements have been imposed on existing facilities than on new sources of discharge, as is shown in Table 1.

Table 1. Federal effluent guidelines for the conventional blue crab processing category. 40 CFR 408 - Subpart B.(1)

	Existing Source			New Source		
	Direct Discharge		Indirect Discharge	Direct Discharge		Indirect Discharge
	Maximum(2)	Average(3)		Maximum(2)	Average(3)	CFR 403
BOD-5	no limit	no limit	no limit	0.30	0.15	(5)
TSS	2.2	0.74	no limit	0.90	0.45	(5)
O&G	0.60	0.20	no limit	0.13	0.065	(5)
pH	(4)	(4)	no limit	(4)	(4)	(5)

Note: Units are in lb/1000 lb raw seafood processed

(1) Applies to existing facilities manually processing more than 3000 lbs of raw seafood on any day during the calendar year and all new sources.

(2) Maximum for any one day

(3) Average of daily values for 30 consecutive days

(4) Within the range of 6.0-9.0

(5) Set by POTW with an approved pretreatment program

Virginia Discharge Limitations

As of July, 1994, the DEQ has proposed a new general permit (State Water Control Board, 1994) for seafood processors, including conventional (handpicked) and mechanized blue crab processors. The limitations are consistent with those shown above in Table 1.

Crab Processing Industry

The blue crab found in the Chesapeake Bay is identified by the scientific name *Callinectes sapidus*. These organisms are members of the phylum Arthropoda, class Crustacea. They are characterized by an exoskeleton composed of chitin and calcium carbonate, which must be periodically shed and replaced to allow growth in size. The organisms possess an open circulatory system, in which the arteries end in sinuses. The blood (more properly referred to as "hemolymph") bathes the tissues of the body and eventually is returned to the heart after passing over the internal surfaces of the gills, where it is reoxygenated (Campbell, 1990).

Blue crabs are typically caught in wire traps (pots) baited with dead fish. However, during the dredging season in mid-winter, the crabs are removed from the sand bottom by dredges pulled behind boats. Blue crab processing plants typically operate for 125 days out of the year (Chao *et al.*, 1983). The crabs must be cooked while still alive because of decomposition which occurs rapidly upon death. Thus, most crabs are transported immediately by boat or truck to a processor for cooking. The crabs are typically rinsed with fresh water to remove external sand and debris. The crabs are then placed in stainless steel perforated pans and loaded into a pressure cooker, which typically holds 1000-1500 lbs (454 -681 kg) of live crabs. The cooking cycle, which lasts 10 to 15 minutes, requires a pressure of 15 psi and a temperature of about 120°C. Approximately 30-50 gallons (114-189 L) of wastewater is generated per 1000 lb (454 kg) of live crabs (Harrison *et al.*, 1992). Once out of the cooker, the crabs have changed from a bluish gray color to bright red. After being allowed to cool, the meat can either be picked out of the body and claws by hand or, in larger processing plants, by machine. It is then typically sold as fresh, frozen, or canned meat.

Crab Cooker Wastewater Characteristics

Retort water is only one of several waste streams generated in blue crab processing plants. However, it is one of the most concentrated wastewater stream in terms of BOD, along with the wastewater stream from the "Quik Pik," and Harris claw system which produces a brine waste with very high BOD and solids concentrations (Harrison *et al.*,1992).

Hanover *et al.* (1975) tested the effluent from ten separate cooks at a crab processing plant and found that BOD_5 averaged 16,557 mg/L and chemical oxygen demand (COD) averaged 55,568 mg/L.

Wheaton *et al.* (1980) reported an average BOD_5 of 9,000 mg/L for six crab processing plants studied. The TSS averaged 1,500 mg/L at these plants.

Chao *et al.* (1983) found that the production of wastewater ranged from 25 to 50 gallons per 1000 pounds of live crabs. They measured BOD_5 in the range of 10,000 to 14,000 mg/L with the COD ranging from 20,000 to 25,000 mg/L. They measured TSS in cooker effluent at 700 to 1,000 mg/L and ammonia-nitrogen (NH_3-N) at 200 to 250 mg/L.

A summary of the values obtained by Chao *et al.* (1983), Wheaton *et al.* (1980), and Hanover *et al.* (1974) is presented in Table 2 which is taken from Harrison *et al.* (1992).

Table 2. Blue crab cooker effluent pollutant characterization found in published literature.[1]

Type	Chao *et al.* (1983) Range	Wheaton *et al.* (1980) Average	Hanover *et al.* (1974) Average
No. of samples	-	-	10
No. of plants	-	6	-
Flow (gal/ 100 lb.)	25-50	-	-
BOD5 (mg/L)	10,000-14,000	9,000	16,557
COD (mg/L)	20,000-25,000	-	55,568
TSS (mg/L)	700-1,000	1,500	-
NH3-N (mg/L)	200-250	-	-
pH	7.0-7.5	-	-

[1] adapted from Harrison *et al.*(1992).

Harrison *et al.* (1992) surveyed three blue crab processing plants in Virginia and measured various pollutant levels in several waste streams. Table 3 presents data obtained for the cooker (retort) wastewater. Samples were obtained and analyzed on two different visits to each plant.

Table 3. Concentrations of retort effluent from three blue crab processing plants.[1]

	mg/L								
Plant No.	COD	BOD-5	TSS	VSS[2]	Cl	O&G	TKN-N[3]	NH3-N	TP[4]
1	32,940	27,359	1,790	1,550	6,770	22	-	-	-
1	35,240	-	6,200	4,710	-	10	3,940	160	185
2	29,000	28,500	1,460	1,305	5,100	-	-	-	-
2	21,510	17,380	1,010	910	-	50	2,240	70	102
3	31,040	18,780	653	535	-	-	-	-	-
3	23,920	13,720	1,980	1,640	-	-	2,510	130	160

[1] adapted from Harrison *et al.* (1992)

[2] VSS = volatile suspended solids

[3] TKN-N = Total Kjeldahl Nitrogen

[4] TP = total phosphorus

Samuels, *et al.* (1992) in studying the feasibility of using crab solid waste as animal feed, determined that 44.1 % of the dry matter was protein, and upon drying, "a pungent ammonia odor was detected." While this present study is concerned with wastewater and not the crab solid waste, it is of interest that a high level of protein was detected in the crab shell waste material, and that ammonia was plainly evident.

Biological Treatment of Seafood Wastewaters

Recognizing the financial and space limitations at most seafood processing companies, Creter and Lewandowski (1975) installed a pilot-scale treatment system at a processing company in Maryland. The wastewater was a combined flow from blue crab and oyster processing, with a daily flow of about 2,000 gallons per day (7600 L/d), a BOD (assumed to be BOD_5 but not so stated) ranging from 400 to 1,200 mg/L, and suspended solids of approximately 250 mg/L.

Dissolved solids ranged from 1,000 to 8,252 mg/L. The treatment train included a screen, an aerated "roughing tank" with a 900 gallon (3411 L) capacity, followed by a 1250 gallon (4740 L) "batch processing tank" which was also aerated. The wastewater was then chlorinated and discharged to an estuary. The effluent was reported to have a BOD of 160 mg/L. Dissolved solids were reported to be higher in the effluent than in the influent, but no value was reported.

Wheaton *et al.* (1984) operated a pilot-scale system at a blue crab processing plant in Wingate, Maryland for a year. The raw wastewater included retort water as well as washing and cleaning waters. While a mean value for daily flow was not reported, a graph was presented described as "smoothed daily water use for a typical year for a blue crab processing plant" in which the values ranged from 4 to 12 m^3/day. The BOD_5 for the retort water had a mean value of 9,000 mg/L with a range of 4,000 to 24,000 m/L. The TSS was reported with a mean value of 1,500 mg/L. The combined crab processing water had a mean BOD_5 of 753 mg/L and a mean TSS of 577 mg/L. The treatment train included a screen (20 or 40 mesh followed by 60 mesh) for all water except the retort water. A 3790 L septic tank was used as a sump to collect the various streams. A pump transferred wastewater periodically to an above ground swimming pool (5.5 m diameter, 1.22 m deep) which was used as aeration tank for biological treatment. This was followed by a chlorine contact chamber, from which the effluent flowed into the adjacent estuary. A significant reduction ($p<0.001$) in BOD_5 was obtained to a mean value of 258 mg/L. However, effluent levels exceeded BAT discharge guidelines.

A year later, in a related lab-scale study (Geiger *et al.*, 1985), a sequencing batch reactor was constructed consisting of two 18 L tanks, the first a holding tank and the second an aerated reactor. To simulate consolidated effluent from a crab plant, retort water was diluted to 5% concentration with a reported COD of about 1,200 mg/L, a BOD_5 of approximately 1,000 mg/L and a pH of 7.8. Activated sludge from a local wastewater treatment plant was acclimated and used as seed for the system. The authors reported that "mixed liquor suspended solids (MLSS) were maintained at approximately 3000 to 7000 mg/L to be consistent with concentrations in an extended aeration system." The sequence began by adding 12 liters of 5% retort water to 6 liters of settled sludge. An aeration period followed. Aeration periods of 6, 12, 18, and 24 hours were evaluated. At the end of the aeration period, settling was allowed for 30 minutes, after which time, 12 liters of supernatant was removed. Results indicated that the 24 hour period was most effective in reducing organic content and TSS in the effluent. Effluent BOD_5 was 498 mg/L in the 6 hour cycle, and 114 mg/L in the 24 hour cycle. Effluent TSS was 390 mg/l after 6 hours, and 41 mg/L after 24 hours.

Anaerobic treatment of fish processing wastewater was studied by Balslev-Olesen *et al.* (1990). Two hydraulic configurations were compared, which the authors described as (1) anaerobic upflow fixed-bed filter (AF), and (2) anaerobic fluidized bed (AFB). The AF had an empty volume of 365 liters, and was filled with clam shells. The AFB had an empty volume of 359 liters with quartz sand used as a support medium. The AF included a recirculation pump to maintain an upflow velocity of between 0.1 to 1.2 m/h while the AFB maintained an upflow velocity of between 2 to 20 m/h. The hydraulic retention time of both systems was varied from 0.5 to 5 days.

Concentrated herring brine was diluted to simulate the whole plant fish canning wastewater. The COD was initially 10 g/L and later increased to 17.4 g/L. The mixture contained 1.2% NaCl initially and later 4% NaCl. Acidity was controlled through the addition of KOH to maintain a pH of about 6.8 and the reactors were operated at 35 °C. The loading rate to each system ranged from 3.3 kg COD/m^3·d to 10 kg COD/m^3·d. Effluent COD concentrations increased in the AF system from 1.77 g/L at the low loading to 4.59 g/L at the highest loading. In the AFB system, effluent COD was 1.75 g/L at the low loading, increasing to 2.4 g/L at the highest loading. The authors referred to these results as "steady state results" even though loadings were increased rapidly with less than a week at a given loading rate in some cases.

The authors stated that "increases in salt concentration to 4% during the laboratory experiments did not cause any inhibition." No data is provided regarding biomass concentration in the reactors. However, the authors did state that the "generation of the biomass in the reactors took about 6 months." Biogas production was estimated at approximately 0.50 m^3/kg COD removed. Gas composition was reported to be approximately 2/3 methane, 1/3 carbon dioxide, and 1.5% hydrogen sulfide. Also, recovery after periods of no feeding was studied. Both systems recovered quickly (as indicated by biogas production) after shutdowns as long as three months.

Various toxicity issues were studied by Soto *et al.* (1991) as they pertained to the anaerobic treatability of fish canning wastewater. Four 0.9 L reactors were used to study waste streams from mussel, fish meal, and octopus processing. Each of these wastewaters possessed a different set of characteristics. COD values ranged from 18.5 g/L to 55.2 g/L, TSS from 1.07 g/L to 16.56 g/L, and chloride levels as high as 15.82 g/L. The mussel waste contained a high concentration of sugars; the fish meal was high in suspended solids, protein and fats, while the octopus waste was high in protein and salts. Two of the reactors were operated in the mesophilic range (37°C), and two were operated in the thermophilic range (55°C). The VSS of biomass in the reactors ranged from 2.06 to 3.68 g/L. The pH was maintained in the range of 7.3 to 7.62. The inoculum biomass was obtained from an upflow anaerobic sludge blanket (UASB) reactor treating sugar mill waste, and was acclimated by feeding mussel waste in small doses over a three month period. The hydraulic regime is not clearly stated, but it is assumed that the reactors were operated in an upflow mode.

The reactors were fed each of the three wastes during sequential periods, and at HRT's of 9 to 33 days. With mussel waste, at an organic loading rate (OLR) of 1.0 kg COD/m^3·d and an HRT of 18 days, the COD removal percentage was 90% in the mesophilic reactor. Under the same conditions, the thermophilic reactor showed 92.9% removal. With fish meal waste, at an OLR of 1.8 kg COD/m^3·d and a 33 day HRT, COD removal was 87% at 37°C and 76% at 55°C. The octopus waste was applied at an OLR of 1.2 kg COD/m^3·d with HRT of 9 days and 18 days in the mesophilic and thermophilic reactors respectively. The removals were 84.3% in the mesophilic reactor and 82.5% in the thermophilic reactor.

The authors speculated that ammonia toxicity played a role in the lower removals at higher temperature when treating the two wastes which had a high protein content. They reported that total ammonia-nitrogen increased to 3-4 g/L, and rose more quickly in the higher temperature reactor. This appeared to be related to an inhibition of the methanogenic population.

An assay for ammonia toxicity was conducted using a synthetic medium with ammonium chloride and acetate as the carbon source. It was found that 50% inhibition occurred at 2.8 g/L NH_4-N at pH 7.4.

The authors stated that "the presence of sulfate in ratios COD/SO_4^{2-} close to 8, and its virtually total reduction to sulfide during the anaerobic process, caused toxic levels of H_2S inside the digester." They went on to say that a 5% concentration of H_2S in the biogas would indicate toxicity if the pH were below 7, and would result in instability.

Although the authors mentioned chloride and sodium as potential inhibitory factors, they implied that this did not prove to be the case by stating "reactors were acclimatized to salinity by a weekly feeding." Sodium levels are not reported; chloride concentration was as high as 15.82 g/L.

Another study of fish-canning wastewaters was conducted by Mendez *et al.* (1992). In this pilot-scale study a three stage anaerobic system was evaluated which consisted of a 7 m^3 pre-digester, a "15 m^3 Central Activity Digester" and a 3 m^3 clarifier. A heating line into the main digester is shown on a diagram, but we are not provided with information about the temperature of operation. Two waste streams were used as feed. Tuna wastewater exhibited a COD of 34.5 g/L, TSS of 4 g/L, chloride concentration of 14.0 g/L with a protein content of 77% of the organic matter present. The mussel wastewater had a COD of 18.5 g/L, TSS of 1.4 g/L, and chloride concentration of 13.0 g/L. Carbohydrate was the predominant constituent at 74% of the mussel organic matter, with protein at 22%.

The reactor was inoculated with biomass from digesters at a paper mill, and a municipal wastewater treatment plant. The startup involved feeding with slightly diluted tuna wastewater for 30 days. An HRT of 5.0 to 7.5 days was used throughout the study. The reactor was fed increasing loadings of tuna waste (diluted with sea water to a COD of 20-25 g/L) until day 200 when the feed was stopped for 47 days. Then, a combination of tuna and mussel wastewaters were fed with a gradual switch to all mussel waste. This last operational period lasted an additional 114 days. During the entire test period, chloride concentration was maintained at 14 g/L by using sea water to dilute wastewater. The authors stated that "no substantial changes were registered as salinity increased." The VSS concentration in the reactor had a mean value of 10 g/L.

The COD removals were greatest for the combination of tuna and mussel waste at an OLR of 3.2 to 3.8 kg COD/m^3·d with a 90-95% removal. HRT ranged from 5.6 to 7.5 days. Mussel waste alone at 4.2 kg COD/m^3·d and HRT of 5.0 days showed 75-85% COD removal, and tuna waste alone showed a similar removal of 80% at an OLR of 4.5 kg COD/m^3·d and HRT of 5.0 days.

The authors stated that ammonia (unstated concentration) "produced from degradation of the protein-rich effluents ... not only does not create inhibition, but the increased overall alkalinity makes the system more stable against organic overloads."

Crab retort and "combined plant effluent" were treated anaerobically by Harrison *et al.* (1992). Using wastewater from the same source, although at a prior time period, as the current study, a draw and fill procedure was used to feed several 2 L reactors which were mixed by magnetic stirrers. Certain reactors received full strength retort water, while others received retort water mixed with other wastewater streams, and in some cases, wastewater which had been coagulated by pH adjustment. Considering just the reactor receiving retort water only, a MLSS of 4,000 mg/L was maintained using an anaerobic sludge innoculum from a local POTW (the same souce as used in the current study). Once a day, a given amount of mixed liquor was removed from the reactor, and replaced by the same volume of feed. The initial food to microorganism ratio (F/M) was 0.3 for the first ten days, when "indications of reactor failure appeared." The F/M was decreased to 0.15 for the next 20 days. For the last 24 days of the study, the F/M was increase to 0.25. The authors report that the SRT for the reactor was 153 days until day 31 and then 136 days during the final 24 days. Hydrualic retention time was 18.2 days prior to day 31 and 12.5 days thereafter. Effluent soluble COD stablized after day 20, inspite of the doubling of F/M on day 31, at approximately 700 mg/L. Gas production did show a marked increase when F/M was increased on day 31, and averaged about 0.6 L per gram of COD removed. TKN-N in the feed averaged 2,000 mg/L, and was about 1,200 mg/L in the effluent at the end of the study. Approximately 90% of the TKN-N in the effluent was in the form of ammonia/ammonium-nitrogen. VFA's in the effluent stabilized by the end of the study at less than 10 mg/L each (acetic, propionic, iso-butyric, and n-butyric acids.

The apparatus used for the study by Harrison *et al.* (1992) was utilized by a subsequent investigator (Wolfe, 1993) without a break in operation using the same feed source. Operation of the reactor continued for an additional 216 days. During the first 40 days of this study, the F/M ratio was 0.40, and was then decreased to 0.35 for the balance of the study. During the period from day 48 to day 161, effluent COD was in the range of 1,500 to 2,500 mg/L (85 to 91% removal). Soluble BOD_5 averaged 1,400 mg/L. SRT varied from 96 to 248 days (MLVSS averaged 4,000 mg/L), while HRT was maintained at 12.5 days. After day 161, failure of the reactor began, as evidenced by a steadily rising effluent COD and decrease in daily gas production. By day 216, when the reactor was abandoned, the effluent COD had reached 9,300 mg/L.

In the Wolfe study, several constituents were considered as possible inhibitory factors. Ammonia toxicity to anaerobic cultures was discussed, and dismissed as a likely cause. The ammonia in the reactor peaked at about 1,800 mg/L at pH 7.5. As discussed later in this literature review, this is indeed below the threshold previously found to be toxic to anaerobic cultures. Sodium was the most abundant cation, measured at about 5,000 mg/L during the period of decline and failure. As discussed later in this review, this also is below the recommended (by Kugelman and McCarty, 1965) maximum operating level of 6,900 mg/L Na when other cations are also present. Wolfe concluded by speculating that the combination of high ammonia concentration combined with high cation concentrations may have exerted a synergistic effect and led to the decline of the culture. However, the data indicate that the decline in performance began rather suddenly on or about day 161, with rapidly rising COD concentrations. Unless a threshold effect occurred in which the combination of the toxic constituents reached an intolerable level, some other factor, unidentified by Wolfe, caused the death of the microorganisms. The increase in COD after day 161 is so steep and steady, that it is possible that little treatment occurred after that date, and the rising COD values were the result of simple dilution of feed by reactor contents.

The Mixed Culture Anaerobic Environment

A mixed culture of anaerobic bacteria is a complex community. Frequently the bacteria in such a culture are categorized based on their substrate requirements into four basic categories. Mosey (1983) described these groups as the fermentative bacteria (acidogens), the acetic acid formers (the acetogens), the acetate utilizing methane formers (the acetoclastic methanogens), and the hydrogen utilizing methanogens. Also generally present are sulfate reducing bacteria which can use a wide variety of compounds as electron donors, such as aromatic compounds, fatty acids, amino acids, and alcohols (Zinder, 1993). Iron reducers may also be present, which use a variety of organic compounds as electron donors and Fe^{3+} as a terminal electron acceptor (Lovely and Phillips, 1987).

The first step in the anaerobic breakdown of a complex substrate in the absence of an aerobic (molecular oxygen present) or anoxic (nitrite or nitrate present) environment is referred to as fermentation. Ferry (1993) states that the fermenters convert polymeric compounds to H_2, CO_2, formate, acetate, and higher volatile fatty acids." Since these compounds, with the exception of hydrogen, tend to react with water and dissociate liberating hydrogen ions, an acidic effect is exerted, and thus the term, "acidogens."

Hydrolysis of polymeric carbohydrates results in the liberation of various sugars, such as glucose. Typical reactions stated by Mosey (1983) for the fermentation of glucose are:

$C_6H_{12}O_6 + 2\,H_2O \rightarrow 2\,CH_3COOH + 2CO_2 + 4H_2$ (Acetic Acid) (1)

$C_6H_{12}O_6 + 2\,H_2 \rightarrow 2\,CH_3CH_2COOH + 4H_2O$ (Propionic Acid) (2)

$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 4H_2$ (Butyric Acid) (3)

Protein degradation by fermentation leads to similar products as shown above, but includes various nitrogenous and sulfur containing compounds such as methylated amines and methylated sulfides (Zinder, 1993). These amines and sulfides, particularly trimethylamine, give degraded crab wastewater its distinctively offensive aroma (Abazinge *et al.*, 1993).

Previously, some species of methanogens were believed to be able to use medium chain fatty acids as substrate. However, Bryant *et al.* (1967) discovered that what was once considered to be a pure culture of a methanogenic species was in fact a symbiotic culture of two species of obligate syntrophic organisms: a hydrogen producing acetogen and a hydrogen consuming methanogen.

The acetogenic bacteria are Gram positive eubacteria and utilize longer chain fatty acids to produce acetate, hydrogen, and carbon dioxide. However, the energetics of these reactions are such that they are not thermodynamically feasible unless the hydrogen concentration remains very low (Boone and Bryant,1980; Dwyer *et al.*, 1988; McInerney 1986; Zinder 1993).

The methanogens utilize primarily acetate or hydrogen as electron donors. However, researchers have found that methanogens can also use a number of other one and two carbon compounds as a substrate (Table 4 from Zinder, 1993). In every case, a carbon atom is reduced to a negative four valence state and combined with hydrogen to form methane. Depending on the starting substrate, this reduction yields varying amounts of energy.

Table 4. Methanogenic reactions.[1]

Reactants	Products	Organisms
Hydrogen+Bicarbonate+H^+	Methane+water	most methanogens
Carbon monoxide+water	methane+bicarbonate+H^+ *Methanosarcina*	*Methanobacterium* and
Ethanol+bicarbonate	acetate+H^++methane+water	hydrogenotrophic methanogens
Acetate+water	methane+bicarbonate	*Methanothrix* and *Methanosarcina*
Methanol	methane+bicarbonate+ water+H^+	*Methanosarcina* and other methylotrophic methanogens
Methanol+hydrogen	methane+water other methylotrophic methanogens	*Methanosphaera stadtmanii*,
Trimethyl amine+water	methane+bicarbonate+ ammonium+H^+	*Methanosarcina* and other methylotrophic methanogens
Methyl mercaptan+water	methane+bicarbonate+ hydrogen sulfide+H^+	some methylotrophic methanogens

[1] from Zinder (1993).

Figure 1 is adapted from Costello *et al.* (1991a), who diagrammed how the consortium of bacteria functions to degrade complex substrates into methane, carbon dioxide, hydrogen, and water.

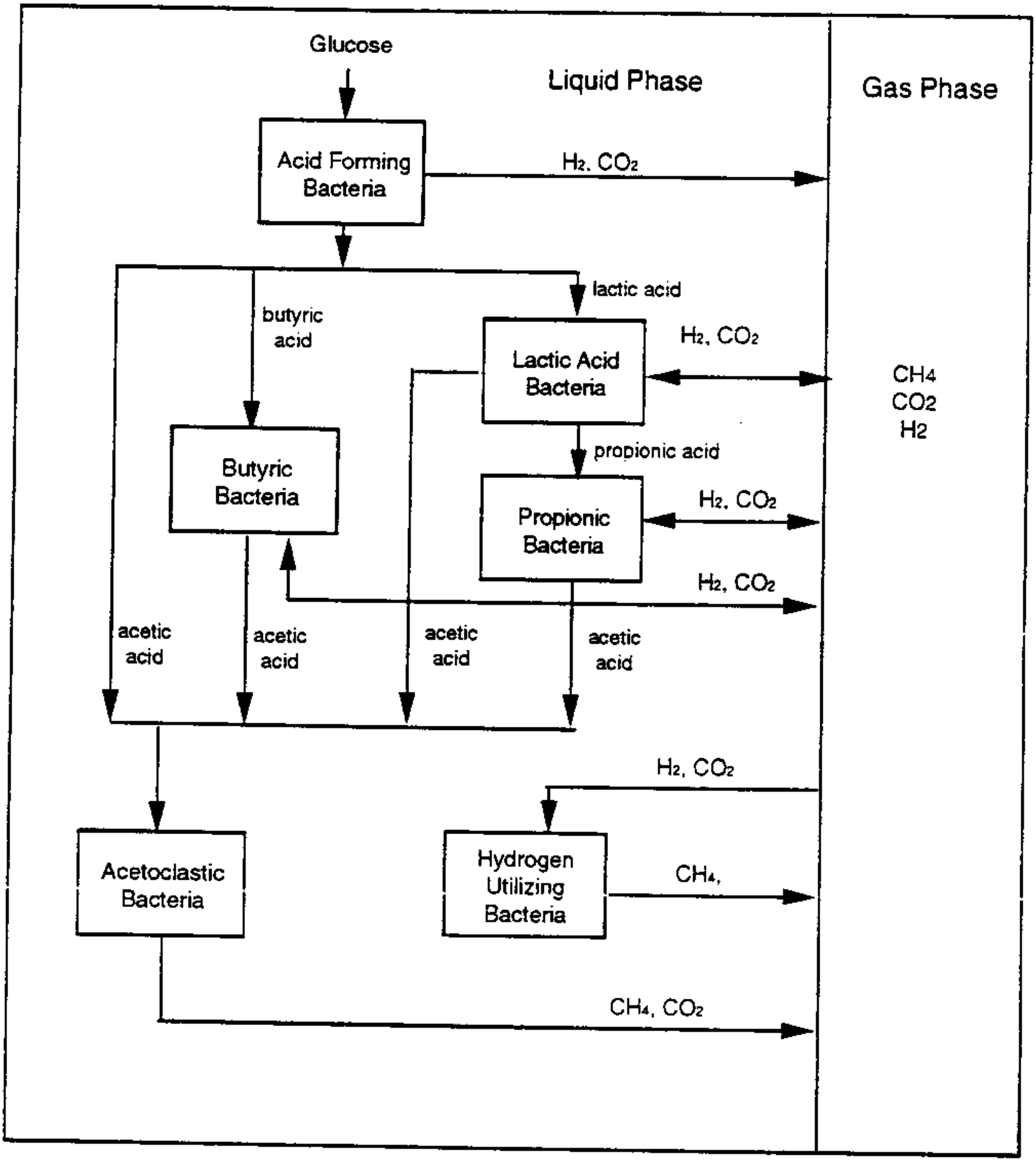

Figure 1. Relationships among populations in an anaerobic reactor ecosystem model; adapted from Costello *et al.* (1991a).

The optimum temperature for methanogenesis was found to be 35°C, by Lin *et al.* (1987). However, essentially equal substrate removal efficiencies were found to exist in the range of 25 to 35°C.

In addition to the fermentative/methanogenic consortium, competition for substrate takes place with the sulfate reducers and the iron reducers. As shown in Table 5 (from Zinder, 1993), there is a hierarchy of energy yield available to the various groups, and Zinder states that when organic substrate is limiting, iron reducers will out compete others if oxidized iron is available, followed by sulfate reducers, methanogens, and acetogens.

Table 5. Hydrogen and acetate utilization by iron reducing bacteria, sulfate reducing bacteria, methanogens, and acetogens.[1]

Reactants	Products	$\Delta G°$ (kJ/rxn)[2]
Hydrogen+ferric iron	Ferrous iron+H^+	-914
Hydrogen+sulfate+H^+	Bisulfide+water	-152
Hydrogen+bicarbonate+H^+	Methane+water	-135
Hydrogen+bicarbonate+H^+	Acetate+water	-105
Acetate+ferric iron+water	bicarbonate+ferrous iron+H^+	-809
Acetate+sulfate	bicarbonate+bisulfide	-47
Acetate+water	methane+bicarbonate	-31

[1] from Zinder (1993).
[2] $\Delta G°$ values from Thauer *et al.* (1977).

Acetogens are inhibited by an accumulation of acetic acid (Kaspar and Wuhrman, 1978) as well as by hydrogen as mentioned previously. Since certain methanogens consume acetate and others consume hydrogen, the methanogens can be viewed as the organisms which control culture conditions in terms of acidity (acetoclastic methanogens) and redox potential (hydrogen consuming methanogens) (Mosey, 1983).

Symbiotic pairs of organisms have been identified with a variety of substrate usage, free energy of reactions and doubling times (Zinder, 1993). The ethanol consuming pair has a doubling time of less than 24 hours, while the propionate and benzoate pairs have doubling times more on the order of 6 to 7 days. Mosey (1983) referred to the doubling times of fermentative bacteria as short as 30 minutes and suggested that hydrogen utilizing methanogens could double in 6 hours but acetate consumers and acetogens required several days. Based on these growth rates, it is clear why a rapid increase in loading to an anaerobic bioreactor would result in an accumulation of fatty acids and a drop in pH.

Thus, it has been demonstrated that mixed anaerobic cultures exhibit a consortia type environment, in which each member group depends on other member groups for physiological success. Acidogens break down complex molecules to simpler ones. Acetogens prevent the accumulation of the medium chain fatty acids by converting them to acetate and hydrogen. Methanogens consume acetate and hydrogen preventing an accumulation of hydrogen which would shut down the acetogenic oxidation of medium chain fatty acids.

Toxicity and Inhibition in Anaerobic Reactors

Based on work by previous researchers at Virginia Tech (Harrison *et al.*, 1993; Wolf, 1993), there was concern in this study that inhibition and/or toxicity would result in failure of biological treatment of crab cooker wastewater over time. This section reviews some of the pertinent work which has been done in the past dealing with toxicity and inhibition in anaerobic systems. There also was a concern relating to inhibition of nitrification in the aerated stage of the treatment system. That issue will be discussed in the section on nitrification.

Anderson *et al.* (1982) defined inhibition in anaerobic systems as pertaining to these parameters:

1. reduction in production of biogas
2. drop in pH accompanied by an increase in volatile fatty acid concentration
3. decrease in COD removal efficiency
4. lag in recovery from stop/start operation

5. overload instability

Of these, gas production and increase in VFA concentration are the most easily quantifiable for operational monitoring purposes. The authors state that methane production should be between 0.34 and 0.36 m^3 per kg of COD removed, if the BOD is at least 50% of the COD. They reported that this translates to a methane yield of 0.91 to 0.93 m^3 for every kilogram of organic carbon metabolized. The authors also stated that "volatile acid concentrations above 500 mg/L indicate either that the ratio of food to micro-organisms (or organic loading rate) is too high or that the system is inhibited," and that an increase in the concentration of propionic acid is an indicator of inhibition of the acetogenic bacteria.

Hydrogen Ion Concentration (pH)

Clark and Speece (1970) and found that no inhibition was detected in methanogenic cultures between pH 6.0 and 8.0 for packed bed reactors, with inhibition being evidenced at pH 5.5.

Keefer and Urtes (1962) found that the bacteria survived at pH levels below 5.5 for months, but that a lag period ensued upon returning the culture to a more neutral pH. On the other hand, cultures maintained at high pH, above 8.2, exhibited no lag period upon return to neutrality.

Alkaline and Alkali Earth Metals

The most common metal cations found in natural waters and wastewaters are sodium, potassium, magnesium, and calcium. Kugelman and McCarty (1965) studied the inhibitory effects of these cations singly and in combinations in anaerobic reactors. They used 8 L reactors, mixed by recirculation of biogas, which were fed a minimal medium with acetate as the only carbon source. The innoculum was digested sludge from a local POTW. The reactors were operated at a 15 day solids retention time (SRT), an organic loading of 0.5 g/day/L, and at 35°C. They found that there may exist antagonistic or synergistic effects when more than one cation is present. Antagonistic effects are those in which the inhibitory effect of one cation is mitigated by the presence of one or more other cations. Conversely, a synergistic effect is when the inhibitory effect of two or more cations is greater than the sum of their individual effects when each is present alone.

Kugelman and McCarty found that fifty percent inhibition, measured in terms of substrate utilization, when each metal was tested alone, occurred at the following concentrations: sodium (7.36 g/L), potassium (5.85 g/L), magnesium (1.94 g/L), and calcium (4.4 g/L). However, antagonistic effects were present, and the authors suggested that the upper limits for satisfactory digester performance would be: sodium (6.9 g/L), potassium (5.85 g/L), magnesium (3 g/L), and calcium (5 g/L). Optimum concentrations of these cations were estimated to be at 0.01 M each for the monovalent cations, and 0.025 M for the divalent ones.

Ammonia

Kugelman and McCarty (1965), in the same study as mentioned above, also studied the inhibition due to the ammonium ion. They maintained the pH of the reactor at 7.0 and observed 50% inhibition at 4.5 g/L when ammonium was the only cation present. However, antagonistic effects were significant when sodium (0.01 M), potassium (0.005 M), and calcium (0.005 M) were present, increasing the reaction rate to over 100 % of the control reaction rate. The authors labeled this effect "stimulation" because the otherwise inhibitory cation caused an increase in activity when certain other ions were present. Magnesium was found to have no additional effect when added to the above mentioned metals, but yielded approximately the same reaction rates when replacing calcium in the solution. The authors suggested that an ammonium ion concentration of 0.01 M (0.18 g/L) for optimum culture activity, but concluded that 4.5 g/L ammonium ion could be present if antagonists were present. The authors did not explore the impact of pH on the toxicity of ammonium.

Toxicity of ammonia is widely accepted to be pH dependent. The pKa for ammonium is 9.3, and at pH 7.3, only about 1% of the total is in the unionized toxic form. Sathananthan (1981) evaluated ammonia inhibition of methanogenesis and determined that a concentration above 80 mg/L NH_3-N would result in inhibition. This implies that at pH 7.3, the total concentration of NH_3/NH_4-N would have to be around 8,000 mg/L, and about 800 mg/L at pH 8.3 to become inhibitory to anaerobic cultures.

In studies done by Parkin *et al.* (1983) an acclimation to ammonia occurred at concentrations up to 7,500 mg/L NH_4-N and pH of 7.5. However, cultures loaded with 10,000 mg/L NH_4-N or more showed severe inhibition. In a test of reversibility with concentrations as high as 14,000 mg/L, cultures "recovered to full gas production rapidly" once the ammonium was removed from the feed.

Fatty Acid Toxicity

Anderson *et al.* (1982) studied the relationship between pH and inhibition of anaerobic processes at high volatile fatty acid (VFA) concentrations. The authors stated that their own experimental work, as well as reports in the literature, indicate that a free (unionized) concentration of 30 mg/L as acetic acid was the threshold value for inhibition. Based on the dissociation of the VFA's, all of which have pKa's of 4.75 to 4.87 (CRC Handbook of Chemistry and Physics, 1979) the concentration of VFA's at pH 7.0 would need to be above 1,500 mg/L, and at pH 7.8, the VFA concentration would need to be in excess of 8,000 mg/L to be associated with inhibition.

It is important to recognize that VFA's may be the result of, not the cause of, inhibition. Anderson *et al.* (1982) asserted that this is usually the case.

Sulfide Toxicity

Hydrogen sulfide is produced by sulfate reducing bacteria which use sulfate as a terminal electron acceptor in anaerobic environments. Similar to VFA's discussed previously, the presence of sulfide may be evidence of methanogenic inhibition as well as the cause of it. Methanogens may be out competed for substrate by sulfate reducers which derive more energy per mole of substrate than do methanogens (Anderson *et al.* 1982).

Lawrence and McCarty (1965) introduced various heavy metals (copper, zinc, nickel, and iron) into anaerobic reactors first as sulfate salts, and later as chloride salts. Two concentrations were used: 200 mg/L and 400 mg/L as sulfur. During the sulfate phase, gas production approximated that in the control reactor. When the switch to chloride salts was made, an immediate decline in gas production occurred in those reactors receiving nickel, copper, and zinc. No change was noted with iron. Sulfide measurements showed that essentially all of the sulfate was reduced to sulfide, and that as much as 400 mg/L sulfide was present without any negative impact on reactor performance. Although reference is made to the pH being maintained at "normal levels", the authors did not state what the pH was. They concluded that sulfide generation is beneficial for the prevention of heavy metal toxicity in the concentration ranges tested.

Parkin *et al.* (1983) exposed methanogenic cultures grown on acetic acid to sodium sulfide. They found that 50 mg/L S^{-2} caused some inhibition, and for a continuously fed anaerobic filter, 600 mg/L S^{-2} was the "maximum tolerable concentration."

Maillacheruvu *et al.* (1993) studied the toxicity of both hydrogen sulfide and dissolved sulfide (DS) to both methanogens and sulfate reducers in complete mix reactors and anaerobic filters. They found that filters with fixed film biomass were consistently more resistant to the effects of these toxicants than complete mix reactors. Depending on the substrate fed, in complete mix reactors, sulfide was inhibitory to methanogens at levels ranging from 60 to 150 mg/L S, and DS was inhibitory to sulfate reducers at concentrations ranging from 150 to 400 mg/L S. In anaerobic upflow filters, hydrogen sulfide was tolerated at levels above 150 to 200 mg/L S by methanogens. A DS level of 400 mg/L S was not inhibitory to sulfate reducers in systems fed acetate (1000 mg/L DS for propionate fed systems). The authors observed cyclic variations in reduced sulfur levels and volatile acid COD levels during their long term (two year) studies. They concluded that "process failure occurred when the amplitude of cyclic variation increased continuously in successive cycles."

Isa *et al.* (1986a) found that sulfate levels up to 5.000 mg/L S could be tolerated with little impact on methane production in acetate/ethanol fed fixed film reactors. The results of this study indicated that inhibition of methanogens occurred to a significant degree only at levels of free hydrogen sulfide approaching 1,000 mg/L S. They recommended that if a two stage reactor were to be used, the first stage should be managed to produce acetate rather than ethanol, which is a hydrogen precursor, as hydrogen leads to greater production of hydrogen sulfide. They suggest that this can be done by maintaining the pH of the fermentation step above 6.0.

Heavy Metal Toxicity

In the study discussed previously concerning the beneficial effects of sulfide on heavy metal toxicity, Lawrence and McCarty (1965) stated that zinc and copper toxicity affected both the acidogens as well as the methanogens, as evidenced by changes in VFA concentrations and methane production. They added that " microorganisms responsible for hydrolysis of complex organics to organic acids were as much or more seriously affected by heavy metals than the methane-forming bacteria." However, the authors also showed that heavy metals which formed insoluble sulfides would be rendered non-toxic if sufficient sulfate was present in the feed of an anaerobic digester. An exception would be chromium which does not form an insoluble sulfide salt.

Toxicity due to heavy metals is rare in anaerobic treatment systems according to Anderson *et al.* (1982). They did mention that certain industrial wastes, such as distillery and swine processing wastes, contain high concentrations (no value specified) of copper, and thus the sludge may present disposal problems.

The toxicity of nickel to methanogenic cultures was evaluated by Parkin *et al.* (1983). They found that gas production was negatively impacted as nickel concentration was increased from 50 to 500 mg/L. In acclimated cultures fed continuously with acetate as the sole carbon source, 250 mg/L nickel could be tolerated without a decrease in gas production. However, 350 mg/L of nickel resulted in a decrease in gas production. The effects of nickel were found to be reversible if the exposure was to less than 800 mg/L. Above that level, and with exposures longer than a day in duration, irreversible effects were seen.

Nutrient Limitation

Anaerobic systems are dependent on living organisms as are other biological treatment processes. Thus, the feed for these systems must include all necessary nutrients which cannot be synthesized by the organisms themselves. Since anaerobic systems are not autotrophic, the required nutrients include a carbon source and certain essential elements such as nitrogen, phosphorus, and sulfur which are found in the proteins and nucleic acids of all living things. Also necessary are certain other elements which may be necessary for the proper functioning of enzymes, coenzymes, and cofactors.

Nitrogen and Phosphorus

The anaerobic bacteria have a requirement for nitrogen and phosphorus in order to build biomass. Souza (1986) recommended a COD/N ratio below 70 in order to provide sufficient nitrogen for growth. The phosphorus requirement was stated as no more than a COD/P ratio of 350.

Goodwin *et al.* (1990) evaluated the requirement for phosphorus as well as several metals in a UASB employing sucrose as a feed. In those reactors fed a substrate with low phosphorus content (3.5 mg/L), both acidogens and methanogens were adversely impacted compared to a control (22 mg/L P) in terms of VFA formation and methane production.

Trace Heavy Metals

The growth of a particular methanogen (*Methanobacterium thermoautotrophicum*) was found to be dependent on certain trace metals by Schönheit *et al.* (1979). The authors supplemented a defined medium which included hydrogen and carbon dioxide as the sole energy and carbon sources with nickel, cobalt, molybdenum, and iron. It was determined that the production of one gram of cells (dry weight) required 150 nmol of nickel, 20 nmol of cobalt, 20 nmol of molybdenum, and 10 μmol of iron. The authors speculated that growth is possible only in the presence of these metals, and that they are usually present in even carefully formulated media due to the exposure of the apparatus to stainless steel fittings, syringe needles, etc.

Goodwin *et al.* (1990) tested the effect of a collection of trace metals (iron, nickel, manganese, zinc, boron, cobalt, copper, and molybdenum) on the performance of a UASB. They found that performance (in terms of acetic acid utilization and methane production) of the reactor slowly decreased after start up. Upon an introduction of the trace metal solution, acetate levels dropped and gas production increased. They did not attempt to identify which metals in the mixture were essential or at what concentration.

Streicher *et al.* (1990) examined the effect of certain supplements on the anaerobic treatment of diluted whey in a fluidized bed reactor. Meat extract, ammonium, and blood had no significant effect. The addition of a mixture of iron, nickel, cobalt, and yeast extract did result in an increase in COD removal efficiency and biogas production within several days. The authors do not report the exact concentrations or total loadings of these supplements.

Nutrient Removal

As discussed previously, the degradation of protein rich wastewater liberates ammonia from amino acids. The presence of ammonia in wastewater discharges is of concern due to its toxicity to fish and other aquatic life. The in-stream toxicity measured as LC_{50} (lethal concentration at which 50% of the test organisms die) depends on several factors such as pH, temperature, salinity and types of fish species present. LC_{50}'s below 1 mg/L NH_3-N have been reported by Colt and Tchobanoglous (1976) and Coche (1981). As a result, environmental regulators are interested in limiting the discharge of ammonia to the environment.

Ammonification

Much of the nitrogen contained in some wastewaters is found in organic compounds. Proteins are composed of amino acids, each of which contains an amino group. These amino groups are removed from amino acids during the degradation of the proteins releasing free ammonia. The ammonia will react with water to form the ammonium ion (NH_4^+)and depending on the pH of the solution, a certain fraction of the total concentration will remain as free NH_3. Figure 2, taken from Wong-Chong and Loehr (1976), illustrates the increase in ammonia as the concentration of organic nitrogen decreases. Many organisms are capable of ammonification.

Nitrification

Sharma and Ahlert (1977) present a comprehensive overview of nitrification. Certain bacteria are capable of converting ammonia to oxidized forms of nitrogen through a process

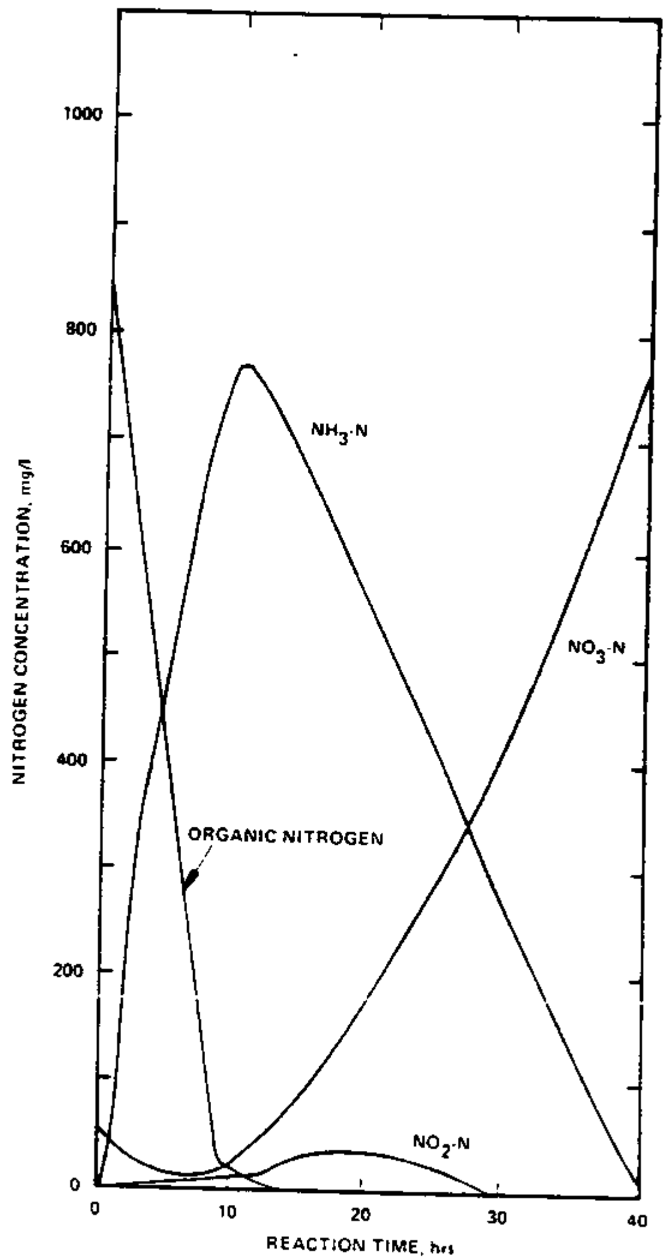

Figure 2. Nitrification kinetics of organic nitrogen; adapted from Wong-Chong and Loehr (1978)

known as nitrification. The most common have been identified as the genera *Nitrosomonas* and *Nitrobacter*. *Nitrosomonas* has been demonstrated to oxidize ammonia to nitrite while *Nitrobacter* completes the oxidation by converting nitrite to nitrate. Nitrifiers derive their energy from these reactions, and are thus called autotrophs. The conversions of ammonia to nitrite and nitrite to nitrate are oxygen demanding reactions and can occur only in an aerobic environment. This is often referred to as "nitrogenous oxygen demand (NOD)." For every mg of ammonia converted to nitrate, 4.57 mg of oxygen are required .

Nitrifiers grow more slowly than do heterotrophic bacteria. In an environment in which there is abundant BOD and limited ammonia, heterotrophs and nitrifiers compete for carbon, space and oxygen. As typical yield factors for heterotrophs are in the range of 0.4 to 0.6 (mg biomass produced per mg substrate utilized) (Metcalf and Eddy,1989), and yields for nitrifiers are typically in the range of 0.04 to 0.13 for *Nitrosomonas* and 0.02 to 0.07 for *Nitrobacter*, heterotrophs typically dominate (Sharma and Ahlert, 1977). Nitrification is temperature dependent (Metcalf and Eddy,1989) with little growth occurring as temperatures fall below 10°C. pH is also an important factor. It appears that an optimum pH for both oxidations would fall in the range of pH 7.3 to pH 7.6 (Eckenfelder, 1990).

Doubling times for *Nitrobacter* are as long as 5 days (Sharma and Ahlert. 1977), and therefore washout is of concern. Many design engineers choose to specify sludge ages (mean cell residence times) in the range of 15 to 20 days in order to protect biological treatment reactors from loss of nitrification as a result of these various factors (Metcalf and Eddy, 1989).

Inhibition of Nitrification by Ammonia

Researchers have discovered that ammonia can be inhibitory to nitrification. The ammonium ion, which predominates at pH values below the pKa of 9.3, is believed to be non-toxic. Thus, the toxicity of ammonia is dependent both upon the total NH_3/NH_4 concentration.

and the pH of the solution. Suthersan and Ganczarczyk (1988), citing Anthonisen (1974), reported that free ammonia inhibited *Nitrobacter* when present in the range of 0.1 to 1.0 mg/L NH_3-N. Inhibition was defined as an accumulation of nitrite. The oxidation of nitrite to nitrate is more rapid than the conversion of ammonia to nitrite. Therefore, an accumulation of nitrite is an indication that *Nitrobacter* is inhibited. Suthersan and Ganczarczyk stated that in their own study, they were able to acclimate a culture of *Nitrobacter* to levels of 2.5 mg/L NH_3-N (pH = 8.0) without inhibition of nitrification. They also reported that *Nitrosomonas* was inhibited at pH values of 8.8 and 9.2 in the presence of 60 mg/L total NH_4-N.

Wong-Chong and Loehr (1978) found that *Nitrobacter* was inhibited by free ammonia concentrations ranging from 3.5 mg/L to 50 mg/L based on the degree of acclimation.

Sulfur

Sulfur is typically present in wastewaters in either its reduced (sulfide) or oxidized (sulfate) inorganic forms, and as a constituent of proteins. A mass balance of sulfur should be theoretically possible for a treatment system, but appears to be difficult. Wable (1992) found it impossible to balance sulfur in his study of COD removals in the anaerobic stage of phosphorus removal systems. He remarked that "unusually high effluent sulfate concentrations ... were recorded when the influent contained a VFA" and went on to say that sulfate concentration increased in the clarifier (presumably the aerobic clarifier) with no obvious explanation.

Reactor Configurations

Feilden (1983) described the most commonly utilized anaerobic reactor configurations. Those discussed were the batch reactor, the constant volume stirred tank reactor (CSTR), the plug flow reactor, CSTR plus plug flow, CSTR's in series. In all of these, hydraulic retention time (HRT or θ) equals solids retention time (SRT or θ_c). The author stated that it is very difficult to separate solids from the reaction mixture without also retaining inert or non-degradable solids. As a result, much attention has been paid to configurations which would allow retention of active biomass while allowing low HRT's.

Anaerobic Upflow Filter

Young and McCarty (1967) studied anaerobic upflow filters, in which a support surface is provided for the attachment of biomass and the media remain submerged, as opposed to a trickling filter which only dampens the attached growth on the media. Chiang and Dague (1992) studied the effect of height to diameter ratio on the performance of such static reactors, and found that there was no significant difference in terms of COD removal or methane production among reactors with ratios ranging from 1.2 to 14.3. Based on tracer studies, they found that even the tall reactors could best be described as completely mixed. They recommended against very tall reactor design, seeing no benefits from such configurations.

Anaerobic Fluidized Bed Reactor

Traditional fluidized bed reactors operate in an upflow mode with a vertical velocity sufficient to suspend the particles placed in the reactor. A biofilm develops on the particles, which may be sand, pumice, granular activated carbon or some other inert substance with appropriate density and surface area. Sreekrishnan *et al.* (1991) evaluated the effect of variations in dilution rate, COD loading, and amount of inoculum on the development of the biofilm in a fluidized bed reactor using sand (600 μm) as the fluidized surface. The authors concluded that high dilution rates and high inoculum rate increased biofilm formation. Additionally, they observed that inoculum with high methane production rates developed biofilm faster than inoculum with low methane production, and concluded that methanogens are more likely to adhere to surfaces than fermenters.

In a bioreactor utilizing a polyurethane matrix, Isa *et al.* (1986b) came to a similar conclusion, i.e., that methanogens colonize and adhere to such surfaces more so than do sulfate reducers. In fact, they observed that methanogens will displace sulfate reducers from surfaces even when sulfate reducers initially predominated.

Upflow Anaerobic Sludge Blanket (UASB)

Much research during the 1980's on reactor design and configuration was the result of the pioneering work by Lettinga *et al.* (1980) who described the phenomenon of granulation of biomass in certain upflow anaerobic bioreactors. They observed that under certain conditions of substrate characteristics and reactor design, bacteria which did not form large particles with high settling velocities would be washed out of the reactor. Those bacteria which tended to grow into larger particles would be retained in the reactor. Eventually, particles reaching several millimeters in diameter would grow and be retained in the reactor, reaching concentrations up to 45,000 mg/L VSS. In order to facilitate separation of gas from liquids and solids in the reactor, angled ledges and an inverted cone were placed in the reactor column, such that gas would be directed into a collection tube while effluent liquid would flow in a somewhat serpentine fashion to escape the reactor.

Upflow Blanket Filter (UBF)

Guiot and van den Berg (1984) described a variation on the UASB concept which they called an upflow blanket filter. It differed from the UASB in that the complex solids liquid gas separation apparatus was replaced by a layer of floating plastic rings. These rings occupied only the top one third of the reactor volume. A sludge blanket was allowed to develop in the bottom two thirds of the reactor. The feed was a synthetic substrate utilizing sucrose as the carbon source. The initial inoculum was 9.8 g/L VSS obtained from UASB's treating sugar and acetate.

Loadings up to 51 g COD/L/day were studied. Biomass accumulated in the reactor to a maximum value of 28.5 g VSS/L. The maximum COD removal rate demonstrated was 1.2 g COD/g VSS. The authors cited benefits of the combined sludge blanket plus floating filter design as being colonization of the filter by biomass, solids separation function of the filter, lower cost than a packed filter due to less packing, and avoidance of channeling which may occur in a packed bed filter. They also compared the UBF to a downflow packed bed filter fed the same waste, and observed that the maximum biomass retained in the downflow filter was 3.7 g VSS/L, with a removal capacity of 3 g COD/L/day.

Kinetic Models Developed For Anaerobic Systems

Most kinetic models developed for describing anaerobic systems are based on the Monod equation and some incorporate an inhibitory feature similar to the Haldane equation. Mosey (1986) developed a model which focused on the formation of VFA's from a simple sugar substrate. Dinopoulou *et al.* (1988) described the acidogenesis phase by considering several inhibition models. Costello *et al.* (1991a and 1991b) included factors in their model for physiochemical as well as biological and hydraulic considerations. Interactions with the gas phase as well as product inhibition and pH inhibition were incorporated. The interactions of sulfides and their metal salts were incorporated in the model presented by Gupta *et al.* (1994) who recognized that sulfate reducers play a major role in the dynamics of many anaerobic reactors.

A kinetic model by Guiot (1991) described the behavior of the reactor described by Guiot and van den Berg (1984). This configuration is very similar to that employed in this present study. Guiot asserted that it is not necessary to model each of the complex interactions by the various groups of microorganisms. He concluded that it is sufficient to simplify the system since the conversion of acetate to methane and carbon dioxide is the rate limiting step. Soluble COD is lost from the system by the generation of methane, and its escape in the biogas. The model assumes that biomass accumulation can be disregarded over the finite period of analysis, and that the reactor is a complete mix environment. Table 6 provides the nomenclature used by Guiot (1991) and taken from his paper.

The mass balance equations used by Guiot (1991) are:

$$\mu_0 X_D - DX_e = (\mu_0 - 1/\theta_X)X_D = (dX/dt)_D \tag{4}$$

$$DS_0 - DS_e - k_0 X_D = 0 \tag{5}$$

$$DS_0 - DS_e - \omega DX_e - \omega_{CH_4} Q_{CH_4} = \omega (dX/dt)_D \tag{6}$$

Based on the standard Haldane equation, Guiot incorporates inhibition due to unionized VFA's:

$$ko = \frac{k_{0max} S_e}{K_s + S_e + \pi S_e^2/K_i} \tag{7}$$

When the pH of the reactor is high, i.e., over pH 7, the fraction of unionized VFA's is small, and therefore π is small. Equation (7) becomes the standard Monod equation. Equations (8) and (9) are also taken from Guiot (1991) and predict removal efficiency and methane production.

$$E = 1 - \frac{[S_0 - K_s - k_{0max} X_D \theta_d + \{(S_0 - K_s - k_{0max} X_D \theta_d)^2 + 4K_s S_0\}^{1/2}]}{2S_0} \tag{8}$$

$$Q_{CH_4} = \frac{\omega}{\omega_{CH_4}} b_0 X_D + \frac{(1 - \omega Y)}{2\omega_{CH_4}\theta_d} [S_0 - K_s - k_{0max} X_D \theta_d - \{(S_0 - K_s - k_{0max} X_D \theta_d)^2 + 4K_s S_0\}^{1/2} \tag{9}$$

Table 6. Nomenclature for kinetic model[1].

b_o	= "non-growth" parameter (biomass basis) (d_{-1})
D	= dilution rate of reactor (d_{-1})
E	= soluble COD removal efficiency [E=1-Se/So]
k_o	= observed specific rate of substrate removal (g COD/g VSS/day)
k_{omax}	= maximum observed specific rate of substrate removal (g COD/g VSS/day)
Ki	= inhibition constant (g COD/L)
Ks	= half-saturation constant (g COD/L)
m_o	= "non-growth" parameter (substrate-COD basis) (g COD/g VSS/day)
μ_o	= observed specific growth rate (d_{-1})
μ_{omax}	= maximum observed specific growth rate (d_{-1})
π	= fraction of unionized VFA
Q_{CH4}	= volumetric flow rate of methane (STP) (vol/vol/day)
S_e	= soluble substrate concentration (g COD/L) in reactor and effluent
S_o	= feed soluble COD concentration (g COD/L)
θ_d	= hydraulic residence time (d)
θ_x	= solids residence time (d)
θ_{Xc}	= critical solids residence time (d)
X_D	= biomass concentration in the reactor (g VSS/L)
X_e	= solids concentration in effluent (g VSS/L)
Y	= true growth yield (g VSS/g COD)
ω	= biomass conversion factor into COD (g COD/g VSS)
ω_{CH4}	= conversion factor of methane volume(STP) into COD (g COD/L)

[1] adapted from Guiot (1991).

The minimal attainable substrate concentration is given by equation (10).

$$S_{min} = \frac{K_s b_o}{Y k_{omax} - b_o} \qquad (10)$$

Guiot observed that the performance of his reactor during a step-up in loading differed from its performance during a step-down, and thus labeled this effect, hysteresis. Consequently, two sets of kinetic coefficients were required to completely describe its behavior. This hysteresis was evidently due to the inhibitory effect of high concentrations of VFA's which accumulated when the system was overloaded.

Since biogas was not quantified in this study, the simpler Monod model was used for determining kinetic coefficients. By rearrangement (Metcalf and Eddy, 1989), the following linear relationships apply:

$$\frac{\theta X}{(So-S)} = \frac{Ks}{k}\frac{1}{S} + \frac{1}{k} \qquad (11)$$

$$\frac{1}{\theta c} = \frac{Y(So-S)}{X\theta} - Kd \qquad (12)$$

where k = specific substrate utilization (d^{-1})

Ks = half velocity constant (mg/L)

Kd = endogenous decay rate (d^{-1})

So = feed substrate conc. (mg/L)

S = substrate conc. in reactor (mg/L)

θ = hydraulic retention time (d)

θc = solids retention time (d)

X = biomass conc. (mg/L VSS)

Y = Yield (mg VSS/mg COD)

Chapter 3. Materials and Methods

This chapter will present the experimental apparatus used, the source of wastewater feed and biomass inoculum, and the sampling and analytical techniques used to obtain data for the study.

Experimental Apparatus

Three treatment systems were assembled for this research study: two lab-scale systems (A and B) at Virginia Tech in Blacksburg, and a pilot-scale system (C) located at the Virginia Tech Seafood Research and Extension Center in Hampton, Virginia.

Heat Cabinet for Lab-Scale Systems (A and B)

The anaerobic reactors (Aan1, Aan2, Ban1, Ban2) were maintained at 33 - 35°C in a thermostatically controlled, heated cabinet which continuously passed air through a plenum above the experimental chamber. Four hundred-watt electric light bulbs supplied heat as demanded by the thermostat. The heated air was then directed into the experimental chamber through small holes in the back wall of the chamber. A small fan (4" diameter) ran continuously to exhaust air from the chamber.

Wastewater Feed for Lab-Scale Systems A and B

Crab cooker wastewater was obtained periodically from Graham and Rollins, Inc., in Hampton, Virginia. When possible, it was collected directly out of the cooker in 5 gallon carboys and transported immediately to Blacksburg. On some occasions, cooker wastewater was collected by the staff at the crab company and placed overnight in their freezer room. On one occasion during the spring of 1994, the harvest of crabs was insufficient for the crab company to operate daily, and wastewater had to be obtained from the holding tank of the pilot plant. In every case, the wastewater was transported to Blacksburg and stored at 4^{o} C until fed into the systems. Since each batch of wastewater had slightly different characteristics, and since the wastewater was subject to change slightly during storage, weekly analyses were performed on the feed wastewater.

Feed Regime During the Acclimation Period

The initial time period during which diluted wastewater was used will be referred to as the "acclimation period." The feed pump was a peristaltic pump by Masterflex (Cole-Parmer, Inc., Chicago, ILL). Initially, gas accumulated in the tubing between the refrigerator and the feed pump interfering with the supply of wastewater to the systems. By a rearrangement of the feed tubing, with provision for release of the gas, the feed flow was eventually stabilized during the acclimation period. A common line delivered wastewater to the vicinity of the feed pump. At that point, a "y" fitting supplied wastewater to two pump heads which supplied systems A and B.

The wastewater was initially diluted with tap water to 5% for the purpose of acclimation of the biomass. The feed flow rate was set at approximately 3 L/day, but mechanical and plumbing difficulties caused considerable variations. The dilution rate was decreased at approximately one month intervals. The waste concentration was 10% from day 27 to day 55, 25% from day 56 to 91, and 50% from day 92 to day 133. After day 133, full strength wastewater was delivered to both systems A and B.

On two occasions, calcium carbonate (2 g/L of reactor vol.) was mixed with distilled water, adjusted to a pH of approximately 7.5 with HCl, and added as a slurry to each anaerobic reactor resulting in alkalinity which averaged 5,000 to 6,000 mg/L as $CaCO_3$ over the study period.

Feed Regime During the Study Period

Beginning on day 133, full strength wastewater was supplied to systems A and B. The feed pump was set to deliver approximately 2 L of waste per day to each system. Occasionally, obstructions in the liquid or gas tubing caused variations in liquid levels and effluent volumes. On day 168, the pumping rate was reduced to approximately 1 L/day. During the study period, the effluent flows from A3 and B3 were collected and measured daily. A record of these flows is included in Appendix A.

Biomass Inoculum for Lab-Scale Systems A and B

The anaerobic reactors of Systems A and B were inoculated with anaerobic sludge from the Peppers Ferry Wastewater Treatment Plant, Radford, VA, on October 11, 1993 (Day 0), resulting in an initial mixed liquor volatile suspended solids (MLVSS) of approximately 5,000 mg/L. The aerobic reactors of systems A and B were inoculated with mixed liquor (approximate MLVSS of 2,000 mg/L) obtained from an experimental "University of Cape Town" (UCT) style treatment system operated on the campus of Virginia Tech, which was treating municipal sewage. On day 134, approximately 440 mL of mixed liquor in each aerobic reactor was replaced with an equal volume of mixed liquor from the same UCT system as provided the original biomass. The MLVSS of the reactors was measured before and after the replacement. There was no significant change in concentration. On day 239, the entire contents of reactor B3 were removed and replaced with new mixed liquor from the UCT system which had a MLVSS of approximately 1360 mg/L.

System A: A Three Stage System Employing an Upflow Anaerobic Bed Filter (UBF)

System A consisted of three reactors in series: two anaerobic stages followed by an aerobic stage (Figure 3).

Reactor Aan1

The first stage (Aan1) was a 4 L polyethylene reactor 6 inches (in.) in diameter by 10 in. high (15 cm by 25 cm). Fittings in the walls and top of the reactor were polypropylene bulkhead fittings with a neoprene washer. Influent entered the bottom of the reactor. A length of vinyl tubing was attached to the bulkhead fitting on the interior of the reactor. It positioned a downward-facing elbow over the center of the bottom of the tank such that the flow was deflected against the bottom of the reactor and outward in a radial pattern. The reactor contained 60 polyurethane foam pieces forming a layer approximately 3 in. (7.5 cm) thick . Each piece was 1 in. (2.5 cm) square by 1/2 in. (1.25 cm) thick, but will be referred to as "cubes." The porosity of the foam was 20 holes per inch. The density of the foam cubes was such that they were buoyant even when covered with a biofilm. Effluent flowed up through this layer of floating cubes and

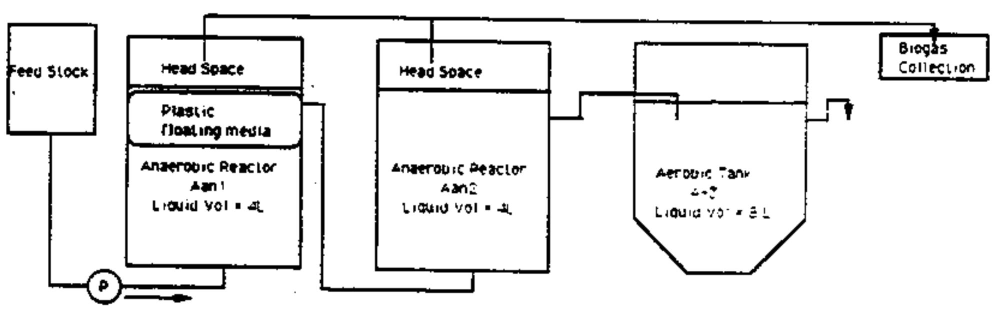

Figure 3. System A schematic; final configuration.

exited by gravity overflow through a vinyl tube which connected Aan1 to Aan2. Initially, a recirculation system returned liquid from near the top of Aan1 to merge with the feed line at the bottom of Aan1. This recirculation was discontinued on day 167. A fitting in the top of Aan1 was provided for gas collection. A vinyl tube attached to this fitting was connected to a gas collection bag which was replaced daily. An additional fitting was installed in the top of the reactor for sampling purposes.

Reactor Aan2

The second stage (Aan2) was a 4L anaerobic clarifier, identical in dimensions to reactor Aan1. Initially, flow from Aan1 entered Aan2 at its mid-height position. Sludge was pumped from the bottom of Aan2 to the bottom of Aan1. Effluent flowed out of Aan2 by gravity through an overflow standpipe to tank A3. On day 167, the recycle of sludge from Aan2 to Aan1 was discontinued and the connecting line from Aan1 to Aan2 was reconnected so that it entered Aan2 at its bottom through an elbow fitting as described above for reactor Aan1. The effluent arrangement was not altered.

Reactor A3

The third stage of treatment was an aeration tank (A3). Initially, A3 had a volume of 4 L with an integral partition to provide for some settling of biomass, and was located in the heat cabinet. The pH in A3 was monitored and found to stabilize at approximately 8.7. Beginning on day 145, hydrochloric acid was added on three successive days to reduce the pH below 7.3. The pH returned to 8.7 within hours after each acid addition. It was concluded that a continuous pH monitoring and control system would be required to maintain the pH in the 7.1-7.3 range. As this equipment was not available, pH adjustment with acid was discontinued. On day 175 , this tank was replaced by a tank with an 8 L volume, new aerobic biomass from an actively nitrifying treatment system was added and the reactor was removed from the heat cabinet and operated at room temperature, which was maintained between 20° and $25^{\circ}C$. There was no sludge recycle from that time on. However, the nature of the standpipe overflow resulted in some settling, and consequently the solids concentration in the effluent was lower than that of the mixed liquor.

System B: A Three Stage System Employing an Upflow Anaerobic Packed Filter (UPF)

The reactors in system B were identical in size and shape to the corresponding reactors in system A, but differed in flow pattern and packing (Figure 4).

Reactors Ban1 and Ban2

Reactors Ban1 and Ban2 were filled with the polyurethane foam cubes (9 in. layer). Reactors Ban1 and Ban2 each contained 180 foam cubes of the same size and type as described above for reactor Aan1. During the entire study period, the wastewater flowed upward through Ban1 and upward through Ban2. At no time was there any recycle of sludge or wastewater. Effluent from Ban2 flowed through a standpipe by gravity to the aerobic stage, reactor B3.

Reactor B3

The aerobic reactor B3 was identical to A3 in volume, shape, and configuration, and was initially housed in the heat cabinet with the anaerobic reactors. The pH of B3 stabilized at about 8.7. Beginning on day 145, hydrochloric acid was added on three successive days to reduce the pH below 7.3. The pH returned to the 8.7 level within hours after each acid addition. As with A3, it was concluded that a continuous pH monitoring and control system would be required to maintain the pH in the 7.1-7.3 range. As this equipment was not available, pH adjustment with acid was discontinued. The B3 reactor was changed to one with a volume of 8 L on day 175. Some of the same biomass as was added to Reactor A3 on this date was added to Reactor B3 to maintain the VSS at 2,000 mg/L, and the reactor was removed from the heat cabinet. On day 239, an integral funnel clarifier was inserted into reactor B3 while maintaining the total volume of the reactor-clarifier at 8 L. The temperature of the reactor was maintained between 20 and 25°C from day 176 to 280 .

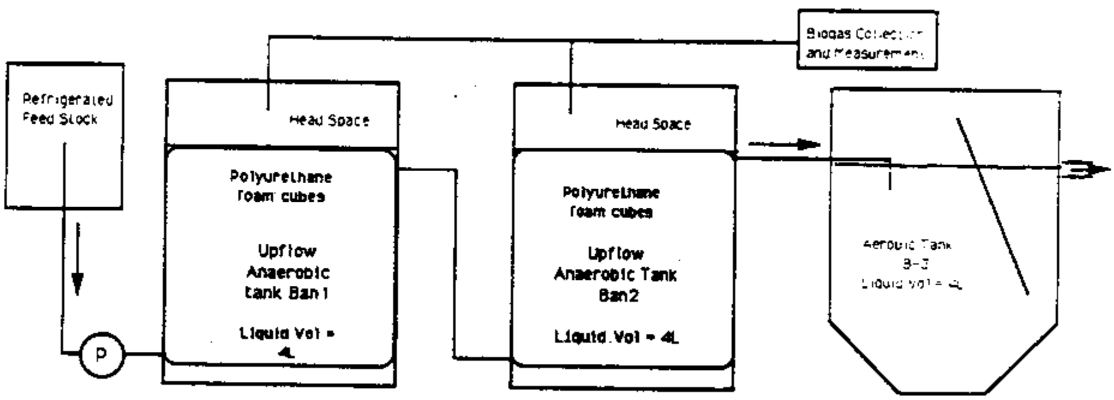

Figure 4. System B schematic.

System C: The Pilot Plant

The pilot-scale system in Hampton, VA, was completed by the end of December, 1993. Crab cooker wastewater was pumped automatically from one of the two retort cookers in use at a seafood processing company to a small concrete block building located on the grounds of the Virginia Tech Seafood Research and Extension Center, a distance of approximately 300 feet (92 meters).

Collection and Pumping System

A semi-flexible copper pipe was attached to the discharge of a crab cooker pot which held 1200 lb (545 kg) of live crabs when fully loaded. The pipe terminated above the open top of a collection drum with an approximate volume of 55 gallons (208 L). The drum was supported in the horizontal position by four metal legs approximately three feet above the ground. A boiler drain was installed for direct collection of samples. A vertical galvanized iron pipe equipped with a strainer and foot valve rose approximately four feet to a centrifugal pump, which was controlled by a float switch in the collection drum. When sufficient cooker water collected in the drum, the float switch activated the pump until only about 2 gallons (7.5 liters) of wastewater remained in the drum. The pump forced the wastewater through a 3/4 in. PVC pipe which was fixed along the wharf at the low tide level. Operation of the pump was automatic and required no intervention by the crab plant personnel, except when danger of freezing necessitated the draining of the water in the pump and standpipe. A fitting was installed to facilitate the priming of the pump by a garden hose upon return to service.

Pilot-Scale Reactor Sizes

The 250 gallon (946 liter) holding tank was 44 in. in diameter and 48 in. tall. Reactors C1 and C2 held 160 gallons (600 liters) and were cylindrical, 34 in. in diameter (0.87 m) and 66 in. tall (1.69 m) and equipped with air-tight lids. Reactor C3 was a 55 gallon drum, and reactor C4 was rectangular, 30 in. deep by 36 in. wide by 30 in. tall, holding 120 gallons (454 L). All of the reactors were polyethylene tanks.

Anaerobic/Aerobic Five Stage Pilot Plant

A schematic of the pilot-scale system is shown in Figure 5. The holding tank in the wastewater treatment building received the wastewater. It was equipped with an overflow so that excess untreated wastewater was discharged directly to the Hampton River, as was allowed by the VPDES permit held by the crab plant. Wastewater was pumped out of the holding tank by a peristaltic pump (Masterflex by Cole-Parmer, Inc., Chicago, IL) into the bottom of the anaerobic upflow reactor (C1). The feed pump was controlled by a float switch in the holding tank so that it would not operate if the liquid level dropped below a pre-determined level. This prevented the feed pump from pumping air into Reactor C1 if the feed wastewater was used up during unattended operations. Reactor C1 contained a 12 in. (0.31 m) layer of polyurethane foam cubes of the same type as used in systems A and B. The wastewater then flowed from near the top of C1 by gravity into the mid-height point of the anaerobic clarifier (C2). Settled biomass was returned to the bottom of C1 by a centrifugal pump which operated intermittently, controlled by a timer. Fittings were installed in the tops of C1 and C2 for the release of biogas, which was exhausted outside the building. The clarified effluent flowed from near the top of C2 to the first aeration tank (C3), which had a liquid volume of 50 gallons. Reactor C3 was operated as a CSTR without recycle. The overflow from C3 flowed by gravity into the final aeration reactor C4, which had an integral settling chamber to separate sludge from liquid. Final effluent was discharged to the Hampton River.

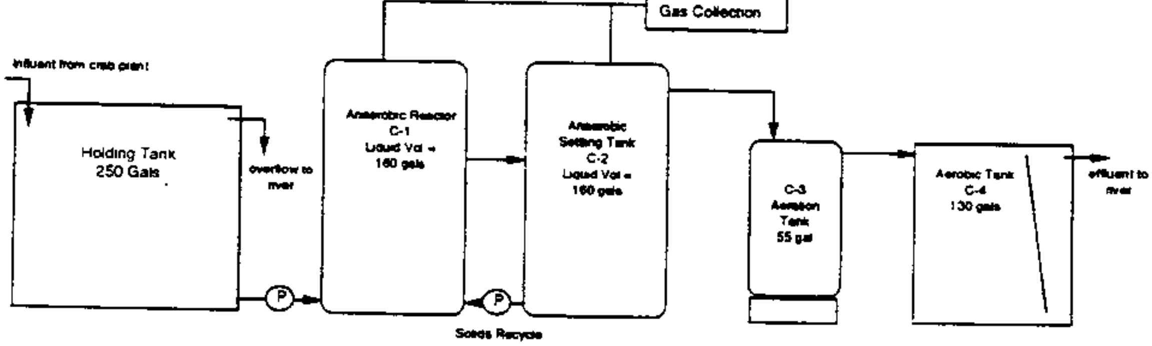

Figure 5. Pilot-Scale System C schematic.

Heating System

Because it was impractical to heat the entire building housing the pilot plant, an integral heating system was installed in reactors C1 and C2. A coil (25 feet [7.7 m]) of 1/2" (1.27 cm) soft copper tubing was installed in the inside of each reactor. An in-line pump continuously circulated water through the tubing. The water was heated by a 6 gallon capacity electric hot water heater. The thermostat of the water heater was adjusted to maintain the temperature of C1 at 35°C. Because the warm water passed through C1 first, and then C2 before returning to the heater, the temperature of C2 was lower than C1. This was deemed acceptable in light of the role of reactor C2 as a clarifier only. The holding tank, and reactors C3 and C4 were operated at ambient temperature.

Inoculation and Acclimation

The system was inoculated with anaerobic sludge from the same source as the lab-scale systems in Blacksburg. Aerobic sludge was obtained from a local POTW for reactors C3 and C4. The first introduction of biomass and wastewater into the pilot plant was in January, 1994.

Biogas Collection and Measurement

A sister study by another researcher was conducted to develop and evaluate an economical system from the utilization of biogas from crab cooker wastewater. Details of the collection, measurement, and analysis of the gas can be found in Rodenhizer (1994).

Apparatus for the Study of Nitrification

It was of interest to investigate the potential inhibition of nitrification due to free ammonia toxicity, and due to competition with heterotrophs in a high BOD environment.

BOD bottles were used to study nitrification at four different pH levels: 6.8, 7.3, 7.8, and 8.3, with two replicates at each pH level. Also, a set of BOD bottles at the same pH values was used to compare a high BOD environment to a low BOD environment. Biomass was obtained from the previously mentioned UCT experimental system in which active nitrification was known to be occurring. The sludge was centrifuged at 1,000 rpm for 20 minutes. Approximately equal portions were placed in each bottle, and the volume brought up to 200 mL with either Aan2 effluent (BOD_5 approximately 3,500 mg/L) or B3 effluent (BOD_5 approximately 100 mg/L). The initial VSS concentrations generally ranged from 1,500 to 2,000 mg/L. Aan2 effluent was bubble stripped with air to reduce the ammonium concentration to the range of 800 to1000 mg/L N. Also, a bottle was set up at each pH value containing ammonium chloride in distilled water at an initial concentration of 800 mg/L nitrogen to serve as a control, and to demonstrate the effect of bubble stripping at each pH level. Each bottle was equipped with a diffuser stone aerator and aerated continuously for 21 days. Due to difficulty in maintaining the pH at the desired level during the first week, additional biomass was added on day 8. Readings of pH were taken at least every other day, and acidified phosphate buffer or sodium hydroxide was added to bring the pH to the desired level. Distilled water was added to replace water lost to evaporation.

Apparatus for the Determination of Kinetic Coefficients

Because of the difficulties in maintaining steady conditions for each system, a separate experiment was set up for the determination of kinetic coefficients for the anaerobic stage. No attempt was made to determine kinetics for the aerobic processes.

The apparatus was similar to that used by Lawrence and McCarty (1965) in their study of sulfide and heavy metal toxicity in anaerobic digesters, except that the reactors were 125 mL Erlenmeyer flasks containing 100 mL of mixed liquor (Figure 6). Five reactors were used. Equal aliquots from the bottoms of reactors Aan1 and Ban1 were obtained on day 221, which became day 0 for the kinetic study. A dilution water was prepared using the same inorganic ionic concentrations as the contents of Aan1 and Ban1. The sludge was diluted to an approximate VSS concentration of 4,000 mg/L. The five HRT's investigated were 10 , 12.5 , 16.7 , 25 , and 50 days. Since the flasks were CSTR's without solids recycle, HRT was equal to SRT. Oxygen was purged from all containers with nitrogen gas. Oxygen was stripped from the feed storage containers with nitrogen, and the feed containers were subsequently maintained tightly closed with an atmosphere of nitrogen above the feed wastewater. Care was taken when feeding each reactor not to introduce air.

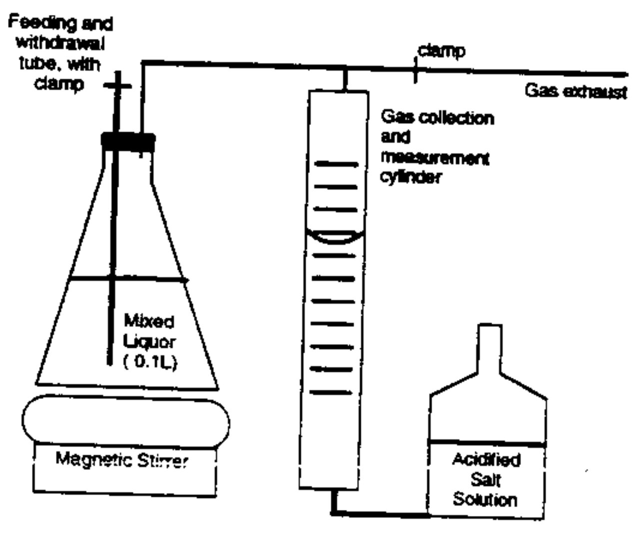

Figure 6. Apparatus for kinetic study; adapted from Lawrence and McCarty (1965).

Investigation of Trace Metal Deficiency

An identical set of five flasks were set up as described above for the kinetic study. The feed wastewater for this set of reactors was spiked with the following metals: iron (as ferrous chloride), nickel (chloride), cobalt (chloride), and molybdenum (sodium molybdate), such that the metals concentrations would be: iron, 10 mg/L, and nickel, cobalt, molybdenum at a one micro-molar concentration. This was done by preparing a 1 mM solution of the trace metals which was saturated with ferrous chloride. One mL of that solution was then added to each liter of wastewater. In all other respects, both sets of reactors were treated in an identical fashion.

Collection and Handling of Samples

Systems A and B

Feed and effluent samples were collected weekly from systems A and B. Samples were obtained by using a syringe to withdraw approximately 30 mL through a sampling port installed in the tubing of each system. Occasionally, samples of the aerobic mixed liquor were taken directly from the aeration tanks after the contents had been thoroughly mixed and stirred. All samples were stored at 4°C if the appropriate analysis was not to be performed immediately upon return to the laboratory.

System C

Samples were obtained on several occasions by draining liquid (approximately 200 mL) from the various sampling ports indicated on the schematic (Figure 5). The samples were placed in a styrofoam lined box along with a frozen gel-pack for transportation back to

Blacksburg for analysis. Samples were refrigerated once received in Blacksburg at 4°C until analysis could be performed, which was typically within 24 hours for COD and suspended solids.

Wet Chemistry

All tests were run in accordance with Standard Methods for the Analysis of Water and Wastewater (1992), when there was an appropriate procedure.

Chemical Oxygen Demand

Chemical oxygen demand (COD) was determined by use of the 5220 C: Closed Reflux Titrimetric Method (Standard Methods, 1992) using 20 x 150 mm culture tubes with screw caps. The titrant used was 0.05 N ferrous ammonium sulfate (FAS). Both cold and hot blanks were included in every trial. Due to limitations of the range of the reagents, samples were diluted with distilled water. Typically, feed was diluted 100:1; all others were diluted 25:1. All samples were filtered through Whatman 934-AH filters prior to testing (the exception to this was the feed, which was not filtered).

Biochemical Oxygen Demand

The five day biochemical oxygen demand (BOD_5) was determined using Method 5210 B (Standard Methods, 1992). No seed was added to the bottles as it was assumed that the sample aliquot contained sufficient bacteria. Dissolved oxygen levels were determined using an oxygen probe. Appropriate dilutions were made based on anticipated oxygen demand. All samples were filtered through Whatman 934-AH filters except the feed.

Titration for Alkalinity and Volatile Fatty Acids

A two step titration with 0.1 N HCl was used to determine alkalinity and volatile fatty acids (Anderson and Yang,1992). The mid-point was pH 5.1 and the end point was pH 3.5. The technique was based on the assumption that essentially the only ions in wastewater from anaerobic reactors which affect pH are the carbonate system ions and the dissociated fatty acid ions. While the originator of this technique verified its accuracy, this investigator came to believe that the technique was useful primarily as a qualitative indicator of the status of the reactors and not as a data collection technique. Thus, later in the study, fatty acids were also measured using gas chromatography as described below.

Suspended Solids in Effluents and Aerobic Mixed Liquor

Total and volatile suspended solids were measured using Methods 2540 D and E, respectively (Standard Methods, 1992).

Mixed Liquor Suspended Solids in Anaerobic Reactors

On two occasions, the main anaerobic reactors were opened briefly for solids sampling. Four foam cubes were removed from Aan1 and Ban1 (2 from near the top and 2 from the bottom). These cubes were dried at 105 C for 24 hours before weighing. The average weight of the cubes added to the reactors was deducted to obtain the solids adhering to foam cubes. This value per cube was multiplied times the number of cubes originally added to that reactor. Biomass was squeezed out of two other cubes into distilled water. The cubes were then returned to the reactor. The biomass squeezed out was analyzed for the VSS/TSS ratio, which was then applied to the value obtained from weighing the dried intact cubes. Also, liquid samples were withdrawn from the reactor through a nylon tube with a syringe, with equal portions removed every two inches (2.5 cm) vertically. This composite sample was then analyzed for TSS and VSS. The result was combined with the volatile solids found on foam cubes to arrive at the total volatile attached and suspended solids mass in the reactor. Therefore, the term "VSS" will be used to refer to the sum total of volatile solids both suspended and attached to the foam cubes.

Total Organic Carbon Analyzer

Samples were analyzed over a six week period during the acclimation phase using a Dohrman Total Organic Carbon Analyzer. Feed samples were diluted, but not filtered to remove suspended solids. Reactor effluents were diluted and filtered through Whatman 934-AH filters to remove suspended solids. Samples were oxidized in the furnace of the analyzer so as to completely oxidize particulate matter. Prior to injection, samples were acidified with phosphoric acid and bubbled with oxygen gas to remove carbon dioxide. Standard solutions were analyzed with every set of samples tested.

Chromatography and Spectrophotometry.

A Dionex Ion Chromatograph was employed according to Method 4110 B (4) for the measurement of certain cations (Na, NH_4, K, Mg, Ca) and anions (Cl, NO_2, NO_3, PO_4, SO_4). Samples were diluted and filtered through a 0.45 μm filter. The anion system specifications were: eluent was 1.80 mM Na_2CO_3, flow rate of 2.0 mL/min. with a pressure of approximately 1200 psi, regenerant was 0.05 H_2SO_4, with a sample volume of 50 μL. Similar conditions were used with cations except the eluent was 0.1mM methanesulfonic acid at 1.0 mL/min. with an SRS controller instead of regenerant.

Trace metals (Fe, Ni, Co, Mo) were measured in a graphite furnace using a Perken-Elmer 5100C atomic absorption spectrophotometer, according to <u>EPA Methods for Chemical Analysis of Water and Wastes</u>, EPA-600/4-79-020 Revised March, 1983. Method 219.2 was used for Cobalt; Method 236.1 was used for iron; Method 246.2 was used for molybdenum; and Method 249.2 was used for nickel.

Gas chromatography was used to measure volatile fatty acids at the end of the study. Acetic, propionic, n-butyric and iso-butyric acids were measured using a Tracor 560 gas chromatograph with a flame ionization detector. The column used was 60/80 Carbopack C/ 0.3% Carbowax 20M/ 0.1% H_3PO_4 in a 30" x 4 mm ID glass column. The oven was at 120°C, the inlet and detector were at 200°C, with a run time of 10 minutes. Carrier gas was N_2 at 4 mL/min., with H_2, at a flow of 30 mL/min., burned in air, at a flow of 300mL/min., in the FID. Samples were acidified with either 1% acetic-free formic acid or 0.5% phosphoric acid.

Chapter 4. Results and Discussion

The general characteristics of the crab cooker wastewater used for this study are presented in this chapter, as well as data which indicates changes which occurred during its storage.

Results from the experimental treatment systems referred to as Systems A and B are included in this chapter. The data for organic loading, effluent COD values and COD removals are presented, in addition to a summary of the various ions present in the treated effluents at various stages in each treatment system. Alkalinity, pH, and volatile fatty acids were monitored and are summarized. Also presented are the results of a nitrification study. A draw-and-fill study to determine kinetic coefficients for the anaerobic stage of treatment was conducted and results are presented along with the effect of addition of trace metals to the raw wastewater.

Results for the pilot plant are not presented. Mechanical difficulties and extended power outages due to thunderstorm activity plagued the pilot plant during the course of this research. The power outages resulted in loss of air flow to the aeration tanks, interruption of the heating system, and the operation of the feed pump. Thus, the results are not quantitatively reliable. Work on the pilot plant is continuing under the efforts of additional researchers.

Biogas data is not presented since the biogas generated by A, B and C reactors is the subject of a sister study by a fellow researcher (Rodenhizer, 1994).

Wastewater Characteristics

Samples were collected over an eleven month period from September, 1993 to July, 1994. Presented in Table 7 are a summary of the characteristics of the wastewater used during the study period.

Table 7. Characteristics of Crab Cooker Wastewater

Parameter	Unit	Mean	Min.-Max.
COD(1)	mg/L	18,900	9,300-33,700
BOD_5(1)	mg/L	14,100	12,200-15,500
TSS	mg/L	1,430	530-4,000
VSS	mg/L	1,150	250-2,200
pH	std. unit	7.1	6.8-7.4
NH_3/NH_4-N	mg/L-N	1060	470-1,770
VFA	mg/L-HAc	6,370	3,400-8,900
Alkalinity	mg/L-$CaCO_3$	780	60-2,000
<u>Metals</u>:			
Sodium	mg/L	1,770	890-2,570
Potassium	mg/L	600	340-870
Magnesium	mg/L	230	140-380
Calcium	mg/L	330	200-530
Iron	mg/L	5.6	2.5-8.9
Nickel	µg/L	95	26-150
Cobalt	µg/L	12	1-24
Molybdenum	µg/L	4	3-7
<u>Anions:</u>			
Chloride	mg/L	8,300	3,000-20,000
Nitrite	mg/L-N	12	nd(2)-30
Nitrate	mg/L-N	4	nd-19
Phosphate	mg/L-P	70	14-160
Sulfate	mg/L-S	250	30-460

(1) COD and BOD_5 values were not necessarily obtained for every sample. Therefore, comparison of minimum, maximum, and mean values for these two parameters is not appropriate.
(2) nd = not detected

The values indicated above are in general agreement with data collected by previous researchers as cited in the literature review. Harrison *et al.* (1992) obtained BOD_5 values considerably higher on several occasions than were measured during this study. They found TKN as high as 3,940 mg/L-N. TKN was measured on only one occasion in this study, at which time the value was 2,300 mg/L-N. However, Harrison *et al.* (1992) did not find ammonia-nitrogen to be as high as was measured here. This may be due to the timing and source of samples for analyses. The tests performed for this study were conducted on wastewater which had been transported and stored before the analyses were conducted, whereas Harrison et al. (1992) attempted to preserve sample specimens so as to establish the nature of the wastewater fresh out of the retort.

Changes in Wastewater During Storage

There was concern that the wastewater would change significantly over time while it was in storage. Because the source of the raw wastewater was located approximately 300 miles from the site of the experimental setup, it was impractical and costly to make frequent journeys to collect wastewater. Therefore, measurements were routinely made on each batch of wastewater used. In the case of the feed used for the kinetic study, repeated measurements of COD were performed on a single batch of wastewater which was refrigerated at 4°C. Figure 7 shows the COD measurements made over a 75 day period. The COD of the waste began and ended with values of approximately 17,000 mg/L. Since these measurements were made on unfiltered samples, the inclusion of varying amounts of suspended material is believed to have contributed some variability to the test results.

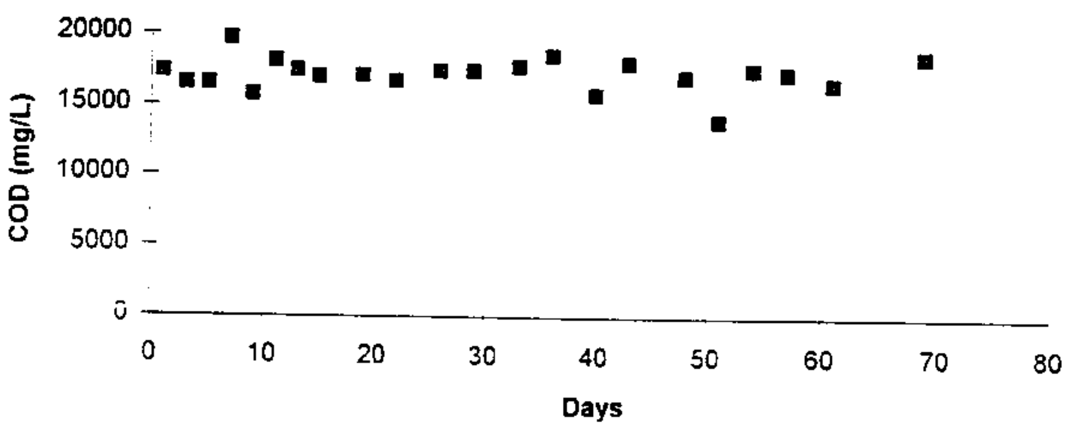

Figure 7. COD of feed stored over a 75 day period.

Volatile fatty acids (VFA) were measured in that same batch of wastewater over a two week time span. There was an increase in VFA's from 1,660 mg/L to 6,700 mg/L. This was assumed to be the result of fermentation which occurred in spite of the refrigeration. While fermentation alters the chemical composition of the feed, it does not generally reduce the COD. Since all scenarios for the full scale treatment of this wastewater involve provision of a holding tank, and since methanogenic activity may actually benefit from a prior fermentation step, this change in the makeup of the feed while in storage was deemed to be acceptable for the continuance of the research.

VSS in Anaerobic Reactors

The volatile suspended solids in the main anaerobic reactors (Aan1 and Ban1) were measured on just three occasions: at day 0, near the beginning of the study period on day 160 , and at the end of the study (day 280). Because of the presence of foam cubes in the reactors, it was necessary to open the reactors for the removal of cubes for testing. Since this exposed the contents to oxygen, it was done only twice during the study. Since anaerobic bacteria grow slowly, short-term variations in loading were not expected to result in great variations in solids production. It was therefore assumed that volatile solids accumulated in a linear fashion over time (Figure 8).

The primary anaerobic reactors, Aan1 and Ban1, were inoculated on day 0 with 5,500 mg/L VSS. The solids concentration increased by day 160 to 7,925 mg/L VSS in Aan1, and 21,700 mg/L VSS in reactor Ban1. By the end of the study, the VSS concentration of Aan1 had increased to 14,075 mg/L and Ban1 contained 27,760 mg/L. Because these reactors were not complete mix tanks, it is interesting to know how the solids were distributed. Table 8 presents the VSS found in the various regions of the reactors.

Table 8. Volatile Suspended Solids in Reactors Aan1 and Ban1.

	Suspended mg	On Cubes mg	Total Solids mg	VSS Concentration mg/L
Reactor Aan1				
Day 0	22,000	0	22,000	5,500
Day 160	8,600	23,100	31,700	7,900
Day 280	33,300	23,000	56,300	14,100
Reactor Ban1				
Day 0	22,000	0	22,000	5,500
Day 160	9,900	76,900	86,800	21,700
Day 280	27,300	83,700	111,000	27,800

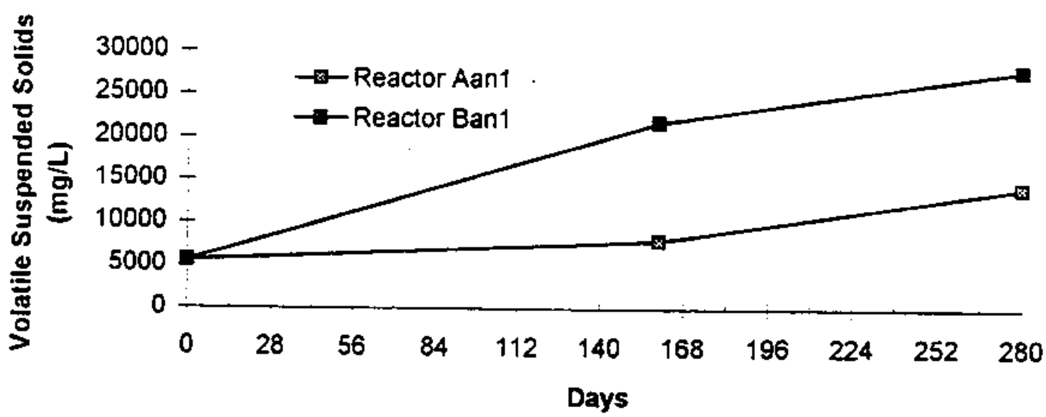

Figure 8. VSS in Reactors Aan1 and Ban1 over the course of the study.

COD Loading and Effluent Concentrations

Organic loading is the total mass of organic carbon compounds introduced into the system per day. In this study, we have used COD to measure loading, recognizing that other reduced species will be included in the measurement. Effluent values are expressed in terms of concentration (mg/L). Except where otherwise stated, values are for total COD for the feed (samples were not filtered) and are for soluble COD for effluents (suspended solids were removed prior to the test).

System A

The loading to System A and the effluent COD concentrations for each of the four stages in System A during the period from day 133 through 280 are shown in Figure 9 and summarized in Table 9. The loading to the system during Phase 1 (day 133-166) was erratic, ranging from 21,700 mg/d to 38,500 mg/d. This resulted from the variation in COD content of the feed obtained during March, 1994. The feed wastewater had a COD concentration ranging from 9,300 mg/l on March 4, 1994, to a high of 16,500 mg/L on March 26th. The initial flow was 2.33 L/d. The effluent from Aan1 gradually increased from a COD of 2,400 mg/L to 5,500 mg/L. The Aan2 anaerobic clarifier's effluent increased from 2,100 mg/L to 4,100 mg/L during this period. At times, the COD of the Aan2 clarifier was essentially the same as the COD of Aan1. The effluent from the aerobic stage, A3, ranged in COD from 1,600 mg/L to 3,400 mg/L during this period.

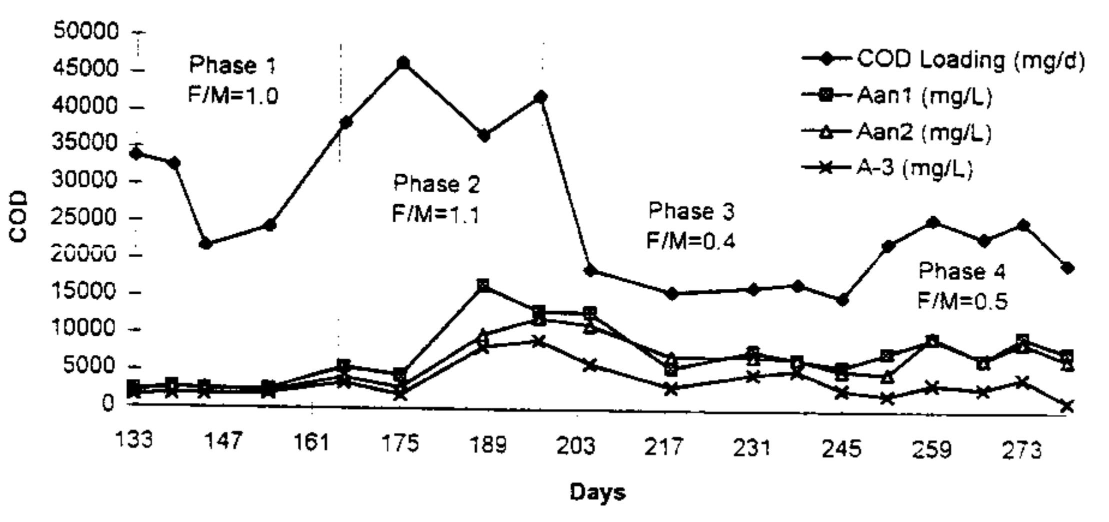

Figure 9. COD Loading and effluent COD in System A over time. F/M ratios are the average for the period.

The flow was reduced at the beginning of Phase 2 (day 167) to 1.38 L/d. However, the high strength of the feed resulted in COD loadings of 37,000 to 46,500 mg/d during this phase. The effluent COD of Aan1 increased as a result of the continued high loading, reaching a peak value of 16,600 mg/L on day 188. The COD of the Aan2 effluent peaked at 12,200 mg/L on day 197. At the beginning of this phase, the recycle from Aan2 to Aan1 was discontinued. The aerobic stage effluent, A-3, had a COD peak value of 9,200 mg/L on day 197.

Table 9. COD Loading and COD effluent concentrations in System A during four phases of the study period.

(mg/L) Phase	Loading (mg/d) Mean	min.-max.	Aan1 Effluent (mg/L) Mean	min.-max.	Aan2 Effluent (mg/L) Mean	min.-max.	A-3 Effluent Mean	min.-max.
1. Day 133-166	30,100	21,700-38,400	3,100	2,400- 5500	2,800	2,100- 4,100	2,100	1,550-3,400
2. Day 167-197	41,900	36,800-46,500	11,400	4,400-16600	8,300	2,800-12,200	6,500	1,900-9,200
3. Day 198-245	16,700	15,300-18,800	6,500	5,600- 7900	7,800	5,300-11,200	4,400	2,800-6,100
4. Day 246-280	23,500	2,0000-25,900	8,500	7,700-10000	7,600	4,980-10,000	2,900	1,400-4,400

During Phase 3 (day 198 - day 245), the feed rate was again reduced, to just under 1 L/d, because of a concern for overloading the reactors, and possible failure of the treatment systems. Coincidentally, the feed obtained at the beginning of May and continuing throughout the study, had a lower COD, ranging from 16,000 to 21,000 mg/L. Thus, the loading to the system stabilized at an average of 16,700 mg/d COD, with a range of 15,300 mg/d to 18,800 mg/d during this period. During Phase 3, the Aan1 effluent COD averaged 6,500 mg/L, while ranging from a low value of 5,600 mg/L to a high of 7,900 mg/L. The Aan2 effluent COD trended lower from 12,200 mg/L to 5,300 mg/L on day 197. A3 effluent COD ranged from 6,100 mg/L to 2,800 mg/L on day 245. A note of caution is inserted here regarding the performance of reactors A-3 as well as the aerobic reactor of system B, B-3. Because of the absence of nitrification in reactors A-3 and B-3, several manipulations of these reactors, and their contents took place over the course of the study. The details are discussed in the section on nitrification.

The final phase of the study period occurred from day 246 to day 280 when the feed rate to System A was increased approximately 25% to 1.21 L/d. This resulted in an increase in COD loading to an average of 23,500 mg/d (range 20,000 to 25,900 mg/d). During this period, the Aan1 effluent averaged 8,500 mg/L COD, ranging from 7,000 to 10,000 mg/L. The Aan2 effluent averaged 7,600 mg/L. The A-3 effluent ranged from 1,400 to 4,400 mg/L COD with an average value of 2,900 mg/L.

System B

The pattern of loading and effluent concentrations in System B is similar to that of System A. Since the feed was supplied continuously to both systems from a common line out of the refrigerated feed reservoir, the only variation in loading between the two systems occurred when the feed pump to System B delivered a slightly different amount of liquid per day than did the System A pump, as evidenced by the daily final effluent collection data. As a result, System B received a slightly higher loading during Phase 2 than did A, but slightly lower loadings during the balance of the study. Figure 10 shows a history of the loading and COD effluents concentrations for System B.

As shown in Table 10, the effluent COD from reactor Ban1 averaged 2,400 mg/L during Phase 1. Ban2 had a slightly lower effluent COD with an average of 2,250 mg/L. The aerobic effluent from B-3 averaged just under 1,800 mg/L COD. There was an upward trend in the effluent COD concentrations of all three stages during Phase 1 in response to the increased loading during this initial period.

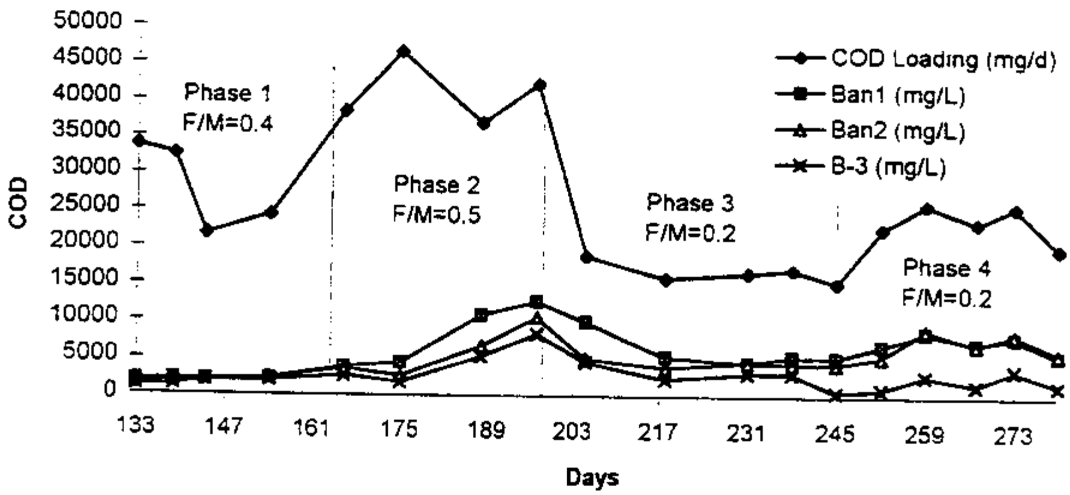

Figure 10. COD Loading and effluent COD in System B over time. F/M ratios are the average for the period.

Phase 2 showed a marked increase in effluent COD concentrations for all stages. Ban1 effluent increased from 4,300 mg/L to 12,600 mg/L. Ban2 increased from 2,600 mg/L to 10,600 mg/L, and B-3's effluent increased in COD concentration from 1,700 mg/L to 8,300 mg/L. The peak COD values for all three stages in System B occurred at the end of Phase 2.

Phase 3 was a period in which the effluent COD values for all three stages decreased for two weeks and then remained stable. The Ban1 effluent stabilized at approximately 5,000 mg/L from day 218 to day 245. Ban2 effluent averaged 4,300 mg/L during the same period. The aerobic stage effluent, B-3, averaged 2,700 mg/L for the entire period. but averaged 2,300 mg/L after the first 14 days of Phase 3.

Table 10. COD Loading and COD effluent concentrations in System B during four phases of the study period.

Phase	Loading (mg/d)		Ban1 Effluent (mg/L)		Ban2 Effluent (mg/L)		B-3 Effluent (mg/L)	
	Mean	min.-max.	Mean	min.-max.	Mean	min.-max.	Mean	min.-max.
1. Day 133-166	30100	21700-38400	2400	850- 3800	2250	1500- 3500	1800	1300-2600
2. Day 167-197	48500	42700-54000	9200	4300-12600	6600	2600-10600	5100	1700-8300
3. Day 198-245	15800	14500-17800	6000	4600- 9900	4400	3900- 5000	2700	570-4600
4. Day 246-280	22900	19500-25300	7200	5500- 8500	7100	5300- 9000	2100	1000-3600

The System B effluents mirrored the COD loading during Phase 4 of the study period, when the loading increased 45% from Phase 3. There was essentially no difference between the effluent concentrations of Ban1 and Ban2 during Phase 4, averaging 7,200 and 7,100 mg/L COD respectively. The aerobic effluent COD averaged slightly less during Phase 4 (2,100 mg/L instead of 2,700 mg/L) than in Phase 3 despite an increased loading.

Variation in Loading and Effluent COD

The sequence of loadings to Systems A and B does not constitute a series of steady state conditions with transitions. The response of anaerobic cultures with a complex substrate is slow, and true steady state may take a very long period of time to establish. Rather, the history of Systems A and B portray a real world condition in which steady state would never be attained. The wastewater under study here is produced by an intermittent and sometimes unpredictable process which is dependent upon a variety of factors such as weather, season of the year, economic conditions, and the adundance of the harvest of blue crabs. Even with the inclusion of an equalization basin, the flow and strength of wastewater will be highly variable, and the treatment scheme would need to be adaptable to such variation.

Viewed in this context, the behavior of these treatment reactors is quite informative. The variation in input to both systems was greater than that of the effluent. While the effluent COD concentration did rise in response to the marked increase in loading, the increase was moderated to some degree. The loading increased by 25,000 mg/day in System A and by 32,000 mg/day in System B from the low point during phase 1 to the high point during phase 2, while the final effluent concentration increased only about 8,000 mg/L in System A and about 7,000 mg/L in System B. When the loading was reduced at the end of phase 2, the final effluent concentration decreased within two weeks to a relatively stable level of 2,000 to 4,000 mg/L.

Substrate Removal in Systems A and B

As discussed in the previous section on fixed and suspended volatile solids in the anaerobic reactors, an assumption of linearity was made regarding the accumulation of biomass in the Aan1 and Ban1 reactors. By interpolating between the initial VSS loaded into the reactors, and the mass measured on two occasions during the study, grams of COD removed per gram of VSS per day (specific removal) were calculated.

Reactors Aan1 and Ban1

Figure 11 illustrates the history of COD removal in reactors Aan1 and Ban1 in terms of grams of COD removed per gram of VSS. The chart shows that in both reactors, with the exception of the sudden increase in loading around day 166, specific removals decrease steadily over time until around day 217. At that time, removals in both reactors leveled off. Over the period of day 217 to day 280, removal in reactor Aan1 averaged 0.23 g COD/g VSS, while reactor Ban1 averaged 0.12 g COD/g VSS.

The main difference between Aan1 and Ban1 was that Ban1 had three times as many foam cubes which became filled and covered with biomass. Two consequences of this difference could explain the differing removal performance. First, the foam cubes occupied three times as much volume in Ban1 as they did in Aan1. If the flow of liquid through the reactor was excluded from this volume in both reactors, the HRT in Ban1 would be less than in Aan1. Secondly, the diffusion of substrate into the interior of the foam cubes may have resulted in a lower metabolic rate for those microorganisms so located. These factors would tend to decrease the removal efficiency of reactor Ban1 on a per gram VSS basis. The much higher VSS content of Ban1 mitigated this lower efficiency resulting in overall higher COD removals per day per reactor.

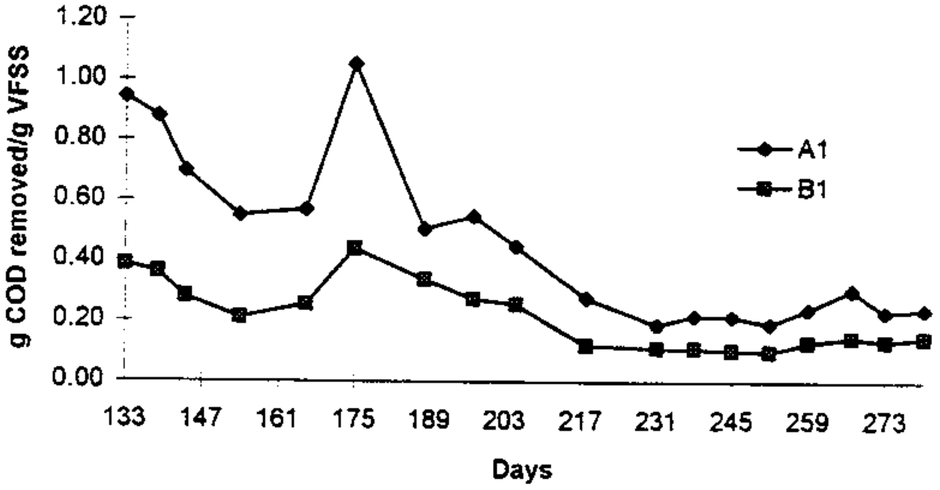

Figure 11. COD removed per gram of VSS in reactors Aan1 and Ban1.

The trend in removal efficiency may have been the result of a shift in population balance as fermenters outgrew acetogens and methanogens when loading was increased at the beginning of the study period. Fermentation alone does not result in COD reduction, since it is a conversion of organic carbon from one form to another. Fermentation outpacing methanogenesis is confirmed by the acclumulation of VFA's, which are substrate for acetogens and methanogens (Costello *et al.*, 1991a). It would be expected that over time, as the various populations stabilize, the VFA concentration would decrease, and COD removal per gram VSS would increase.

Reactors Aan2 and Ban2

The COD reduction in the Aan2 reactor was less than in Aan1, averaging 860 mg/L over the entire study period. If just the last 30 days were considered, the COD reduction in Aan2 was 670 mg/L.

Reactor Ban2 reduced the COD of the wastewater by an average of 930 mg/L. However, the period between day 197 and day 231, when the effluent from Ban1 was above 9,000 mg/L, reactor Ban2 exhibited reductions of about 5,000 mg/L. When the Ban1 effluent declined in strength to the range of 5,000 to 8,000 mg/L, from day 231 to day 280, the COD reductions in Ban2 averaged only 265 mg/L.

Reactors A3 and B3

The COD reduction in reactor A-3 averaged 2,800 mg/L over the course of the study period. When considering just the last 75 days of the study, the reduction was 4,200 mg/L COD.

The COD reductions in the aerobic reactor B-3 averaged 2,300 mg/L for the entire study period. If only the period subsequent to the change to an 8 L volume is considered (after day 176), the COD reduction is 3,000 mg/L. When the period during which the integral clarifier was in place is considered (after day 239), the COD reduction averaged 4,600 mg/L. A cautionary note is offered that the contents of B-3 were replaced with new mixed liquor on day 240, as described below.

BOD_5 of Feed and Effluents

A limited number of BOD_5 tests were conducted at the end of the study period between day 259 and day 280. While the relationship between anaerobic degradability and BOD_5 is vague (the BOD test is, after all, an aerobic test, and measures oxygen depletion), the results of these tests are presented in Table 11. Since BOD represents the oxygen demand of the effluent when it enters the environment, the conversion of COD to BOD_5 is of great interest.

Table 11. Average BOD_5 of wastewater feed and effluents for Systems A and B with corresponding values for average COD for days 259-280.

Parameter	Feed	Aan1	Aan2	A3	Ban1	Ban2	B3
COD (mg/L)	19,600	8,700	8,300	3,100	7,300	7,600	2,400
BOD_5(mg/L)	14,000	4,300	4,100	340	4,100	3,900	110
ratio COD/BOD_5	1.4	2.0	2.0	9.1	1.8	2.0	20

The spread between COD and BOD_5 declines from 5,600 mg/L in the feed to less than 3,000 mg/L in the final effluent. It is reasonable to suggest that some of the relatively non-biodegradable constituents of the feed are altered in the anaerobic stage, and rendered biodegradable in the final aerobic reactor. Additionally, some of the reduced species in the anaerobic effluent which are oxidized by the COD test, but not the BOD test, are inorganic compounds and elements (iron, sulfide, etc.) which may be oxidized chemically in the aerobic reactor. The presence of these reduced species in the anaerobic effluent tends to add to the non-degradable fraction and exaggerate the difference between COD and BOD_5. In a later section (on kinetic coefficient determination) it was calculated that the non-degradable portion of the wastewater used for that particular experiment was 2,900 mg/L.

Organic Carbon vs COD

On nine occasions over a six week period, organic carbon was measured so that a relationship could be established between TOC and COD (suspended solids were removed from reactor effluents but not from the feed for both TOC and COD). The data was separated into three groups: untreated wastewater, anaerobic effluent, and final effluent (Figures 12, 13, and 14). The ratio of COD to TOC was calculated with the following results:

Untreated Crab Cooker Wastewater:	COD/TOC = 2.52
Anaerobic Effluent:	COD/TOC = 2.54
Final Effluent:	COD/TOC = 3.6

The theoretical ratio for the oxidation of glucose yields an oxygen to carbon ratio of 2.667. Eckenfelder (1991) reported that the COD/TOC relationship for various industrial wastewaters ranged from 1.75 to 6.65, and that the ratio tended to decrease with biological

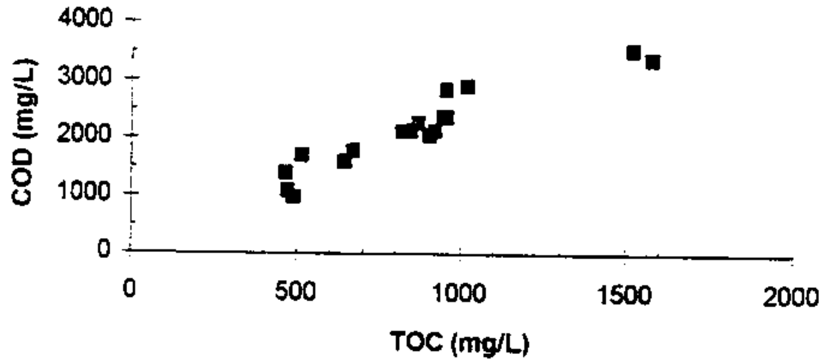

Figure 12. Relationship of TOC to COD for untreated crab cooker wastewater.

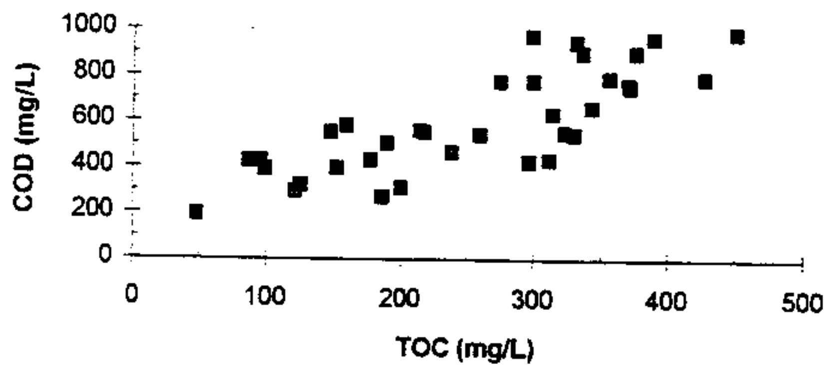

Figure 13. Relationship of TOC to COD for anaerobic treated crab cooker wastewater.

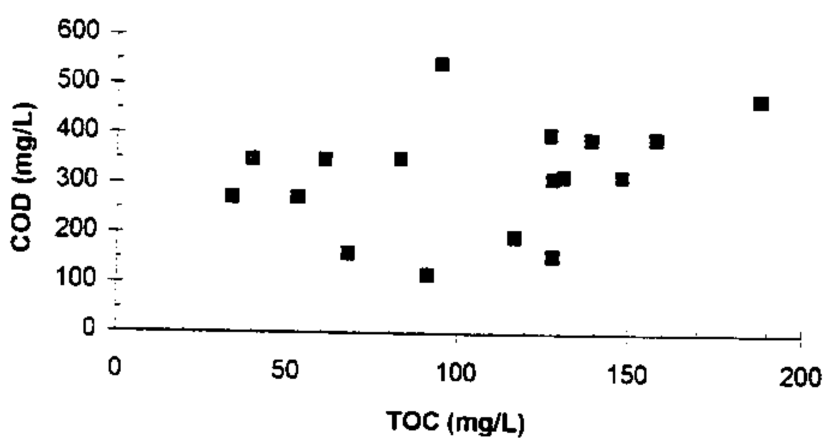

Figure 14. Relationship of TOC to COD for anaerobic and aerobic treated crab cooker wastewater.

treatment. He reported that the ratio of COD/TOC declined from 4.15 to 2.2 for domestic sewage during treatment.

The values for raw feed and anaerobic effluent agree well with these values. However, the higher value for the final effluent COD/TOC indicates that there remain in the aerobic stage effluent various reduced compounds which are oxidized in the COD test, but are not organic carbon compounds. Clearly, most of the organics have been removed by metabolic processes. Reduced metals and bisulfide ion would be among the likely possibilities.

Alkalinity and pH

Alkalinity of the feed averaged 980 mg/L-$CaCO_3$ (as calcium carbonate), with a rather wide variation from a low of 330 mg/L-$CaCO_3$ to a high of 2,100 mg/L-$CaCO_3$.

Powdered calcium carbonate (2,000 mg/L) neutralized with hydrochloric acid was added on two occasions as a slurry to the anaerobic reactors during the acclimation period to establish alkalinity. Thereafter, the alkalinity of the anaerobic reactors averaged between 5,000 and 6,000 mg/L-$CaCO_3$. Both aerobic reactors averaged about 4,400 mg/L-$CaCO_3$ throughout the study. Some variation occurred due to replacement and additions to mixed liquor, as mentioned previously.

The pH of the feed was typically close to neutrality, averaging 7.1 over the course of the study, but ranging from 6.8 to 7.4. VFA production in the feed would lower pH, but the relatively high alkalinity naturally present in the feed resisted a pH drop.

The pH of reactor Aan1 averaged 7.9 with very little variation, while reactor Aan2 had a slightly higher average pH at 8.0. The aerobic reactor, A3, operated generally at a pH of 8.7.

System B behaved in a very similar manner to A. Reactor Ban1 maintained its pH in the range of 7.7 to 8.4, with an average of 8.0. The pH increased in reactor Ban2 to an average of

8.2 with occasional periods of pH as high as 9.0. The pH in the B3 reactor was essentially the same as in A3, averaging 8.7 with a maximum recorded value of 9.0 and a minimum of 8.2.

Volatile Fatty Acids in Systems A and B

Over most of the study period, VFA's were estimated using a two step titration technique, as developed by Anderson and Yang (1992) and described in the previous chapter. At the end of the study, VFA's were analyzed using gas chromatography (GC), which yielded information on the concentrations of acetic, propionic, iso-butyric, and n-butyric acids.

While VFA's in the feed averaged about 4,000 mg/L-HAc (as acetic acid) during Phase 1, VFA's were detected in effluents from Aan1 and Aan2 during this phase, but not in the effluent from A3 or any System B reactors. However, when VFA's were measured on day 197 during Phase 2 with its high COD loadings, the feed contained 8,900 mg/L-HAc, and all the effluents contained several thousand mg/L-HAc.

During Phase 3 when loadings were reduced, VFA's diminished gradually in all reactors, but becoming non-detectable only in reactor Ban2 on one occasion, and in B3 on three out of four occasions. When the loading was increased again in Phase 4, VFA's reappeared in the effluent from all reactors. Table 12 summarizes these findings.

Analysis by GC at the end of the study revealed that most of the VFA's were in the form of acetic acid, followed by propionic acid. No iso-butyric acid was detected, and n-butyric acid was detected only in the feed wastewater.

Table 12. Mean Volatile Fatty Acids (VFA's) in reactor effluents during the four phases of the study period; values expressed in mg/L as acetic acid.

Phase	Day	Feed	Aan1	Aan2	A3	Ban1	Ban2	B3
1	133-166	4,100	100	60	nd	nd	nd	nd
2	197	8,900	8,100	4,500	4,100	6,300	3,400	2,100
3	205-251	7,200	2,900	2,700	270	2,000	600	10
4	252-280	6,800	3,000	2,400	240	3,000	2,300	200

nd = not detected.

The presence of VFA's in high concentrations in the anaerobic effluents indicated that there was an imbalance between fermentative, acetogenic and methanogenic activity. As noted in the literature, fermenters grow much faster than methanogens and acetogens. It appears from the data that the methanogenic/acetogenic populations did not catch up with the growth of fermenters during Phase 2 with its high loadings. The decline in VFA's from phase 2 levels to phases 3 and 4 indicate some recovery, but not to the phase 1 condition in which almost all of the VFA's were consumed.

A full-scale treatment system would quite likely be exposed to the conditions described here: a varied flow and feed strength, resulting in a varied strength and type of effluent. If such variations were short lived and not too extreme, and if the reactor culture was sufficiently diverse, the effluent quality might not be impacted to the point of unacceptable performance.

Cations and Anions

Over the course of the study, sodium and ammonium were the cations present in the highest concentrations, followed by potassium, calcium and magnesium. Chloride was by far the most abundant anion, followed by sulfate and phosphate. Table 13 presents values for these ions over the course of the study.

Sodium

As was noted in the literature review, high levels of sodium can be toxic to microorgansms. Kugelman and McCarty (1965) found that an upper limit for sodium, when in the presence of other common cations, for satisfactory anaerobic reactor performance would be about 6,900 mg/L. As noted in Table 13, sodium levels remained well below this level, and thus were not expected to cause anaerobic reactor failure.

Table 13. Mean Concentrations for certain cations and anions in Systems A and B. expressed in mg/L.

	Reactor					
	Aan1	Aan2	A3	Ban1	Ban2	B3
CATIONS:						
Ammonium-N	1050	1100	810	1280	1100	880
Calcium	260	260	190	260	210	160
Magnesium	130	140	160	130	140	160
Potassium	570	600	660	610	680	580
Sodium	1060	1000	1060	1000	1040	1010
ANIONS:						
Chloride	6900	6900	6700	7400	6700	6200
Nitrate-N	1	1	1	2	1	20
Nitrite-N	4	3	3	3	3	11
Phosphate-P	34	23	18	17	12	16
Sulfate-S	84	69	250	82	113	290

Ammonia

Ammonia-nitrogen was measured in the ionized form with ion chromatography by adjusting the pH of the sample to below pH 7.3 so that less than 1% would be unionized. Since the fraction which is ionized is a function of pH, and since the pH of the reactors varied from one another, the amount of free ammonia (FA) varied as well. For convenience, the sum of the ionized and unionized forms of ammonia-nitrogen will be referred to as Total Ammonia (TA).

TA concentration of the feed, as shown in Table 13, varied over the study period from a low of 470 mg/L to a high of 1,700 mg/L. The high values corresponded with the period of high COD wastewater. The TA concentration of anaerobic stages of System A was essentially the same as that of the feed, while it increased slightly in the anaerobic stages of System B. In the aerobic stages of both Systems A and B, the TA concentration was lower than the anaerobic stages.

Nitrite and Nitrate

Nitrite, while present in the raw wastewater, tended to become undetectable in the effluent of all stages during Phase 1, except in B3 when up to 72 mg/L NO_2-N were detected in B3 on day 154. During later phases, nitrite was not detected except on day 245 when 29 mg/L NO_2-N was measured in B3 as a result of manipulations which will be discussed in the section on nitrification.

Nitrate was generally present at very low levels or undetectable except for several isolated occasions. Exceptions to this occurred on day 245 and day 252 when substantial amounts of nitrate (as much as 217 mg/L NO_3-N) were measured in B3 as a result of manipulations to B3 which will be discussed in the section on nitrification.

Phosphate

Phosphate tended to decrease in concentration as the wastewater moved through each treatment system, decreasing from an average of 72 mg/L PO_4-P in the feed to a low of 18 mg/L PO_4-P in A3 and 16 mg/L PO_4-P in B3. This was probably the result of uptake by bacteria and chemical precipitation, most probably in the form of calcium phosphate.

Sulfate

The average concentration of sulfate in the feed was 250 mg/L SO_4-S (sulfate as sulfur). The concentration consistently decreased in the anaerobic stages of both systems to a mean of 84 mg/L SO_4-S in Aan1 and 82 mg/L SO_4-S in Ban1, probably due to the action of sulfate reducing bacteria accompanied by a production of H_2S and HS^-. Sulfate concentration increased in the aerobic stages of both systems. In reactor A3, the sulfate level returned to about the same as that in the feed, but in B3, the mean concentration of sulfate was substantially higher than in the feed (292 mg/L compared to 247 mg/L). An increase in sulfate concentration in the aerobic stage was also noted by Wable (1992) in his study of anaerobic/aerobic treatment for the removal of phosphorus. Since the feed is assumed to be high in protein (a TKN ranging from 2,000 to 4,000 mg/L-N according to Harrison *et al.*, 1992), the source of sulfur in addition to the original sulfate would be that liberated from the degradation of protein. Some sulfur is lost by the formation of hydrogen sulfide which was readily detectable, but not quantified. A sulfur balance was not attempted.

The concentration of sulfate and sulfide are important considerations in the operation of an anaerobic reactor, not only due to the unpleasant effects of odor, but also in light of the possibility of sulfide toxicity. As was noted in the literature review, several researchers have studied this issue. For example, Lawrence and McCarty (1965) found that 400 mg/L sulfide (at what was reported as simply "normal pH") could be tolerated without an observed negative impact, and Isa *et al.* (1986a) found that up to 5,000 mg/L-S sulfate could be tolerated (above pH of 7.0) with little impact on methane production. Since the concentration of sulfur present in these reactors, even considering a contribution from protein, is well below these levels, the likelihood of sulfide toxicity is low.

Nitrification in Reactors A3 and B3

Because the removal of nitrogen, particularly in the form of ammonia, is of great benefit to the protection of the Chesapeake Bay and the environment in general, the absence of nitrification in the aerobic stages of these experimental treatment systems was of concern. Without nitrification, biological denitrification is not possible.

It was assumed that ammonia toxicity was the cause of the lack of nitrification. Ammonia toxicity to nitrification is well studied as discussed in the literature review. Efforts as described in the Methods and Materials chapter to control pH were undertaken to reduce the fraction of TA which was in the toxic, unionized form. Several additional steps were undertaken as described here.

On day 206, pH adjustment with CO_2 gas was explored. CO_2 gas was bubbled into reactors A3 and B3 at a rate such that the pH was maintained between 7.0 and 7.5. This succeeded in maintaining the pH at the desired level for five days until the gas supply became exhausted. The day after the CO_2 bubbling ended, the pH of both reactors increased to 8.1, and within three more days, the pH was 8.7 in both reactors.

On day 239, the entire contents of reactor B3 were replaced (A3 was not altered). Mixed liquor from an experimental University of Cape Town (UCT) system operated on the campus of Virginia Tech in Blacksburg, Virginia, was obtained and settled. The supernatant of this settled UCT sludge was analyzed and found to contain 11 mg/L of ammonium-N, 5 mg/L of nitrate-N, and no nitrite. The initial MLVSS concentration added to B3 was 1,360 mg/L. A funnel was inserted into B3 to act as an integral clarifier. Solids were wasted to achieve a target sludge age of 20 days. After 6 days, on day 245, the pH of B3 was 8.15, the nitrite-N concentration of B3 effluent was 29 mg/L, and the nitrate-N concentration was 217 mg/L. On this same testing date, there was no nitrite or nitrate detected in reactor A3.

On days 247 and 249, the dissolved oxygen (DO) concentration of both A3 and B3 were checked. It was found to be 6.0 mg/L in A3 and 5.7 mg/L in B3 on day 247. On day 249, the DO in A3 was 6.2 mg/L and in B3 was 5.2 mg/L. Since nitrification is a strictly aerobic process, a minimal level of DO is essential. The measured levels of DO in both reactors indicate that the cultures were not oxygen limited. There is no significance implied in the difference between the DO measure in B3 compared to A3, even though nitrification is an oxygen demanding process. It is more likely a result of the aeration equipment present in the two reactors.

Anions were measured again on day 152. Nitate was down to 63 mg/L in B3 and no nitrate was detected. A3 continued to have neither nitrite or nitrate in detectable levels. Subsequently, both nitrite and nitrate disappeared from the B3 effluent.

It seems reasonable to conclude that the introduction of new mixed liquor into B3, with an intially low TA concentration, permitted the biomass to nitrify a significant fraction of the incoming ammonia, as evidenced by the high levels of nitrite and nitrate after a week. However, the incoming rate of TA was apparently more than the biomass could process. Consequently, pH increased rather than decreased (nitrification generates hydrogen ions), TA concentration became inhibitory, and nitrification activity ceased about two weeks after the mixed liquor was introduced.

Batch Study of Nitrification

Based on the speculation that the lack of nitrification in the aerobic stages of the two experimental systems was due to ammonia toxicity, a batch study was undertaken to see if nitrification would occur under a variety of pH and COD/BOD concentrations, and after extended aeration.

The beginning TA in the controls was 800 mg/L N. At the end of the study (21 days), the TA concentration in the controls varied inversely with increasing pH as shown in Table 14.

Table 14. Total ammonia/ammonium-nitrogen concentration in controls after 21 days of bubble air stripping at different pH levels; expressed in mg/L-N.

	pH			
	6.8	7.3	7.8	8.3
Day 0	800	800	800	800
Day 21	590	540	410	320
Theoretical Unionized Ammonia -Day 21	1.8	5.3	12.5	29.1

The TA concentrations in the "high strength" batch reactors (using anaerobic effluent: COD of 8,000 mg/L ; BOD_5 of 3,900 mg/L) was initially approximately 1,200 mg/L-N. The low strength batch reactors (using aerobic effluent: COD of 2,700 mg/L; BOD_5 of 155 mg/L.) had TA concentrations of about 800 mg/L-N.

The TA concentrations in all experimental batch reactors showed great variability over the course of the study. The high strength reactors (e.g. 2/pH level, 4 pH levels) had ending concentrations ranging from 600 mg/L TA to 1600 mg/L TA, with the lowest value recorded in one of the reactors at pH 8.3. The low strength group had ending TA concentrations ranging from less than 100 mg/L to 1000 mg/L, with the lowest concentrations occurring in one of the reactors at pH 8.3 and one at pH 7.3.

When tests were performed after one week, no nitrite was detected in any of the reactors, and only very low concentrations of nitrate were measured (less than 5 mg/L). On day 16, no nitrite was detected in any of the high strength group, but nitrite was found in three of the low strength reactors (140 mg/L at pH 6.8; 210 mg/L at pH 7.3; 160 mg/L at pH 8.3). Nitrate was not found at concentrations above 10 mg/L in any of the reactors on day 16.

At the conclusion (day 21) of the batch test, no nitrite was detected in any of the reactors in either group. Nitrate was found in only one of the high strength reactors (15 mg/L in one of the reactors at pH 8.3). However, substantial concentrations of nitrate were found in all of the low strength reactors (Figure 15). The pH 6.3 reactors contained 320 and 450 mg/L NO_3-N. The pH 7.3 reactors contained 97 and 18 mg/L NO_3-N. The pH 7.8 reactors contained 160 and 80 mg/L NO_3-N. The pH 8.3 reactors had 280 and 100 mg/L NO_3-N.

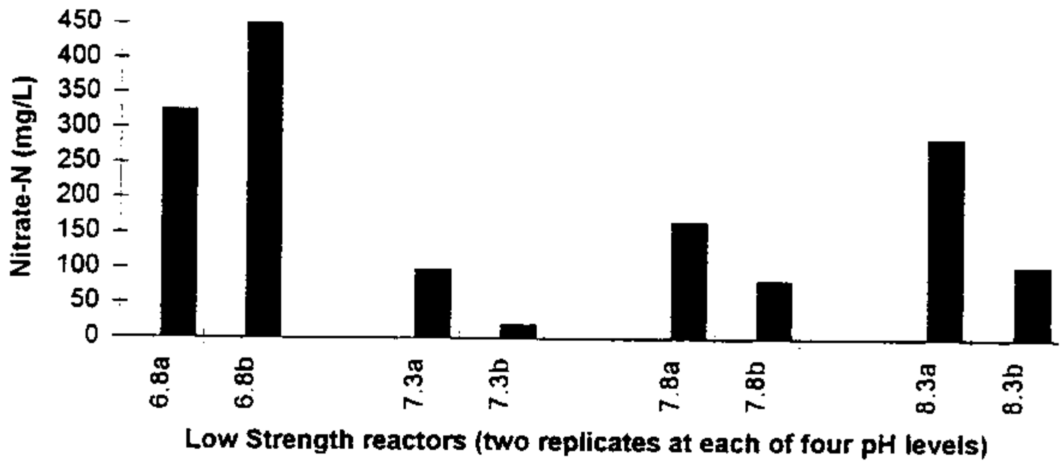

Figure 15. Nitrate in Low strength (initial BOD5 of 155 mg/L, TA concentration of 800 mg/L-N) reactors after 21 days; "a" and "b" refer to replicate reactors.

These results show that nitrification did occur given proper conditions. *Nitrosomonas* and *Nitrobacter* survived at least 8 and 13 days of inhibition, respectively (between day 8 when additional biomass was added and day 16 when nitrite was detected, day 21 when nitrate was detected).

Initial TA concentrations in the low strength reactors probably prevented the autotrophic nitrifying bacteria from growing during the inital phase of the experiment, but the low initial BOD_5 content of the wastewater (155 mg/L) also prevented extensive growth of the heterotrophs. It appeared that once TA levels became non-inhibitory due to air-stripping, the nitrifiers were able to grow, converting TA to nitrite and nitrate. Although it is difficult to know from this data exactly what combination of TA concentration and pH constitute a non-inhibitory condition, these results are consistent with the literature which suggests a level of free ammonia of 0.1 to 50 mg/L-N, depending on acclimation, as being the maximum non-inhibitory level for *Nitrobacter*, and the production of nitrate. For example, at pH 7.3, a TA concentration of 500 mg/L-N includes free ammonia at about 5 mg/L-N, which may be tolerated by acclimated organisms.

The high strength reactors in this batch study, using effluent from the anaerobic stage rather than from the aerobic stage, had both higher levels of TA (around 1,200 mg/L-N) and BOD_5 (3,900 mg/L). One would expect a longer time for the TA level to decline due to air-stripping when starting at a higher initial value. Metabolism of any protein remaining in the feed would result in the liberation of ammonia increasing the TA concentration. High BOD content would allow heterotrophs to grow while nitrifiers would not grow due to ammonia inhibition. The combination of these factors suggests that a longer lag time would be required for nitrification to occur in the high strength reactors, and this is exactly what was observed.

Kinetic Study Results

Figure 16 presents the VSS concentrations in the kinetic study, semi-continuous, batch-type, reactors which were maintained at the following HRT's (also equal to sludge age): 10 , 12.5, 16.7, 25 , and 50 days. The initial VSS concentrations of about 4,000 mg/L decreased in all reactors over time until about day 50. After day 50, the VSS concentrations appeared to stabilized at 570 mg/L in the 10 day reactor, ranging up to 1120 mg/L in the 50 day reactor, as presented in Table 15. The reactors were maintained at 35 ± 4°C.

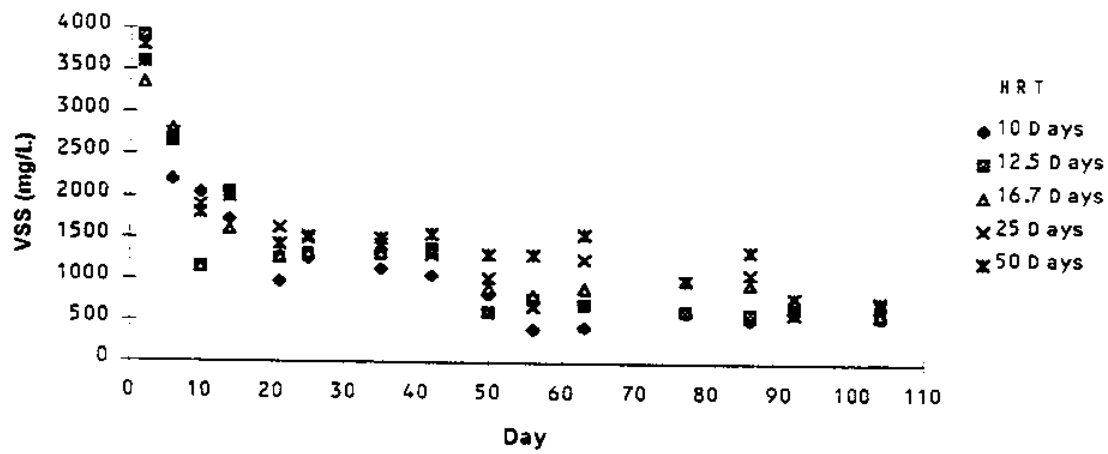

Figure 16. VSS in kinetic reactors.

Table 15. Mean values of VSS in kinetic study reactors during pseudo-steady-state period, expressed in mg/L.

	HRT, days				
	10	12.5	16.7	25	50
VSS	570	640	850	930	1120

The COD removals appeared to stabilize for all reactors by the 60th day of the study. The pseudo-steady state values for effluent COD ranged from 14,800 mg/L in the 10 day reactor to 4,800 mg/L in the 50 day reactor, as shown in Table 16. The effluent COD generally declined as HRT increased, except for the 12.5 day reactor, which exhibited the highest effluent values.

COD removals are listed in Table 16 and charted in Figures 17 and 18. COD removals averaged 3,200 mg/L for the 10 day reactor, 2,600 for the 12.5 day reactor, 7,400 mg/L in the 16.7 day reactor, 8,300 mg/L in the 25 day reactor, and 13,200 mg/L in the 50 day reactor. As expected, the COD removals increased as HRT increased, except for the 12.5 day reactor.

Table 16. Mean values of effluent COD and COD removal in kinetic study reactors during pseudo-steady-state period, expressed in mg/L.

	HRT, days				
	10	12.5	16.7	25	50
effluent COD	14800	15400	10700	9700	4800
COD reduction	3200	2600	7400	8300	13200

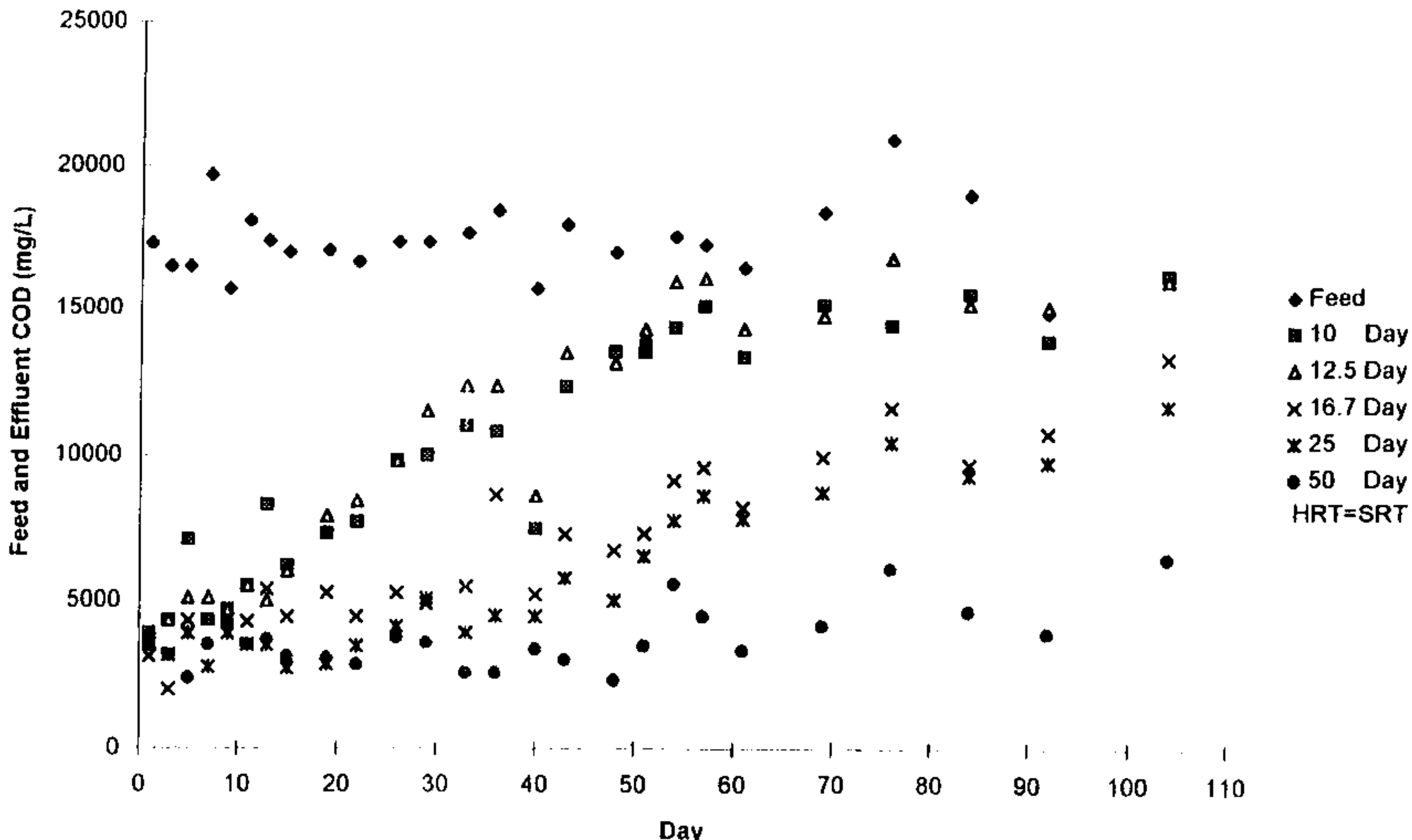

Figure 17. COD in kinetic study reactors over time.

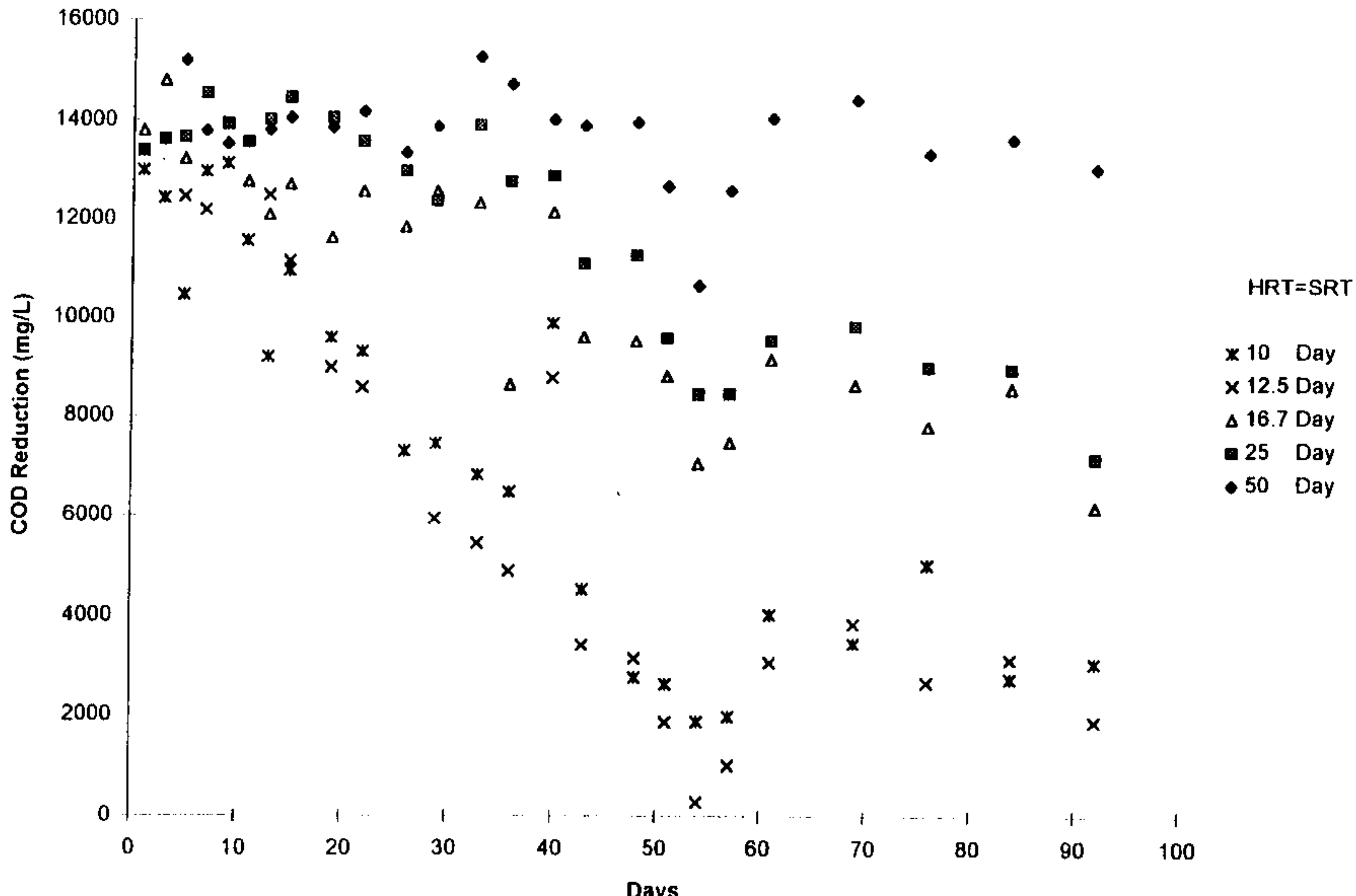

Figure 18. COD removals in kinetic study reactors.

Volatile fatty acids in the kinetic study

Mean values for VFA's in the kinetic study reactors over the period from day 57 to day 99 are presented in Table 17. Acetic acid was present in all reactors, but found in decreasing concentration as the HRT was lengthened, ranging from about 4,400 mg/L in the 12 day and 10 day HRT reactors to 900 mg/L in the 50 day HRT. Propionic acid was present and decreased in concentration from 1,200 mg/L to 280 mg/L as HRT increased. Except for the 12.5 day reactor, the concentration of iso-butyric acid generally decreased as HRT increased, becoming non-detectable in the 50 day HRT reactor.

These results cannot be easily compared to the four phases of the main study with systems A and B. In the kinetic study, a consistant and constant feeding regime was followed over the course of the study. With systems A and B, the loading was variable and biomass increased over time. Thus, population imbalances would be more likely to develop and be maintained in A and B than in the kinetic reactors, with corresponding differences in the production and consumption of VFA's.

Table 17. Mean values of volatile fatty acids in kinetic study reactors at various hydraulic retention times over the period from day 57 to day 99, expressed in mg/L.

		HRT, days				
	Feed	10	12.5	16.7	25	50
Acetic acid	3840	4360	4440	3280	3130	940
Propionic acid	1040	1190	1160	1070	1050	280
iso-Butyric acid	270	540	580	240	180	nd
n-Butyric acid	480	600	1060	220	110	nd

nd = not detected

In the 12.5 day HRT reactor, the n-butyric acid concentration averaged over 1,000 mg/L while the 10 day HRT reactor had an n-butyric acid concentration of 600 mg/L. This corresponds to the higher effluent COD in the 12.5 day reactor as compared to the 10 day reactor.

Unplanned Heat Excursion

It was discovered on day 56 that the temperature of the 12.5 day reactor was approximately 45°C, apparently due to heat from the magnetic stir plate. This time corresponded to a low point in COD removal for most of the reactors. Insulation was inserted under all of the flasks, and COD removal performance improved rapidly, as seen in Figure 18. While the temperature of every reactor was not measured because of a reluctance to admit air into the flasks, the relatively poor performance of the 12.5 day reactor suggests that it suffered relatively greater than did the other reactors. Specifically, there appeared to be inhibition of the butyric acid consuming bacteria in the 12.5 day HRT reactor, resulting in an accumulation of n-butyrate and high effluent COD's. Due to this anomolous behavior, the performance of the 12.5 day HRT reactor was considered to be inconsistent with the behavior of the other reactors in the study, and was omitted from the kinetic calculations.

Specific Substrate Utilization and F/M Ratios

Specific substrate utilization (mg COD removal per mg VSS per day) for the 10 and 16.7 day HRT reactors were 0.56 and 0.52, respectively. This value dropped to 0.36 for the 25 day HRT reactor and to 0.24 for the 50 day reactor, as shown in Figure 19. The value for the 12.5 day HRT reactor was inconsistent with the pattern of the other reactors, measuring 0.33. The low values for the longer HRT reactors are probably more a consequence of the light loading than removal capability.

The F/M ratio for the reactors decreased in a linear fashion with increasing HRT, from 2.7 for the 10 day reactor to 0.3 for the 50 day reactor. At lower F/M ratios, the longer HRT reactors were more efficient (as high as 73% - Figure 20), but less mass of substrate was removed per unit mass of VSS.

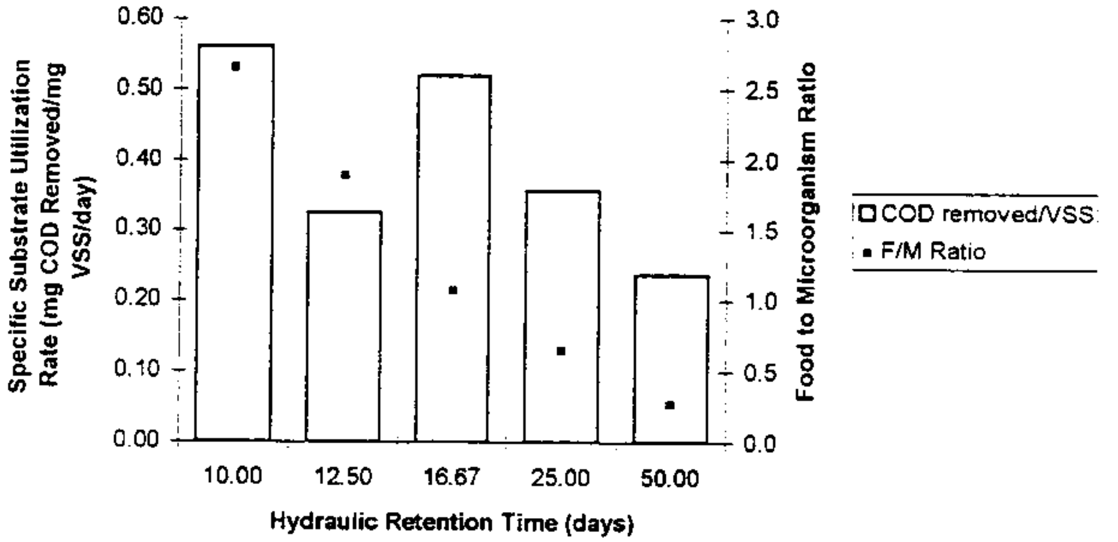

Figure 19. Specific substrate utilization rate and F/M ratio in anaerobic reactors at various HRT's.

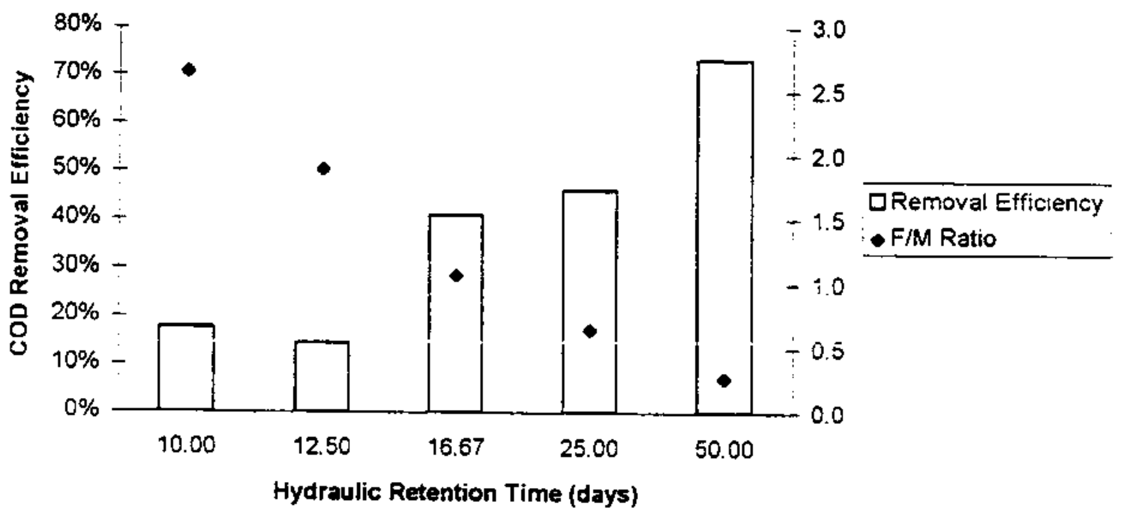

Figure 20. COD removal efficiency and F/M ratio in anaerobic reactors at various HRT's.

In the previous work done with this wastewater by Harrison *et al.* (1992) and Wolfe (1993), kinetic coefficients were not calculated. However, it was found by Harrison *et al.* that at an F/M ratio of 0.25, effluent soluble COD was about 700 mg/L, and VFA's were quite low (averaging less than 40 mg/L). In the work done by Wolfe, a reactor was operated initally at an F/M or 0.4 for 40 days, and then altered to an F/M of 0.35 for another 120 days. During that latter period, effluent soluble COD averaged 2,100 mg/L with a corresponding BOD_5 of 1,400 mg/L, for a removal efficiency of 85-90%.

Kinetic Coefficients

The Monod model was used for the calculation of kinetic coefficients for the anaerobic reactor study. While this model was developed with substrate concentration expressed in terms of BOD_5, it can be applied to COD data if the non-degradable portion is subtracted from the measured values. The non-degradable portion is the COD which would remain if the HRT (and SRT) were infinitely long, allowing all biodegrable material to be removed. Since it is not practical to conduct such an exercise, the non-degradable portion can be estimated by plotting substrate concentration in COD versus 1/HRT. This plot, as shown in Figure 21, yielded a value of 2,900 mg/L as the non-degradable portion. It is interesting to note that while this value appears to be high, it is in general agreement with the measured values for the final (aerobic stage) effluents during the final study phase of Systems A and B. In those cases, A3 effluent had a COD of 3,100 mg/L and a BOD_5 of 340 mg/L (difference of 2,760 mg/L) and B3 had a COD of 2,400 mg/L and a BOD_5 of 110 mg/L (difference of 2,290 mg/L). Stripping and oxidation of H_2S could account for some of the loss in non-degradable COD during treatment in the aerobic stage.

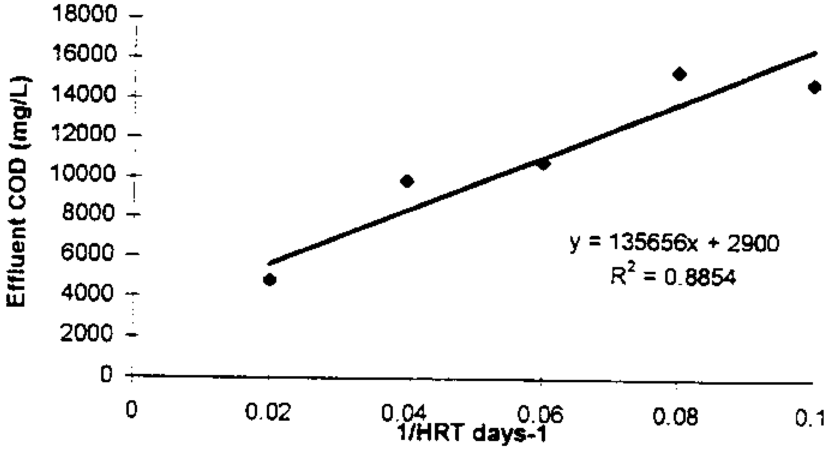

Figure 21. Effluent COD versus 1/HRT for anaerobic reactors at five HRTs.

If the Monod model is a valid representation of this anaerobic system, specific substrate utilization (mg COD/mg VSS/day) approaches a maximum value as the degradable substrate concentration increases. This theoretical relationship is presented in Figure 22 with measured data points indicated. Omitting the point corresponding to the 12.5 day reactor, there appears to be a reasonable agreement with the theoretical curve.

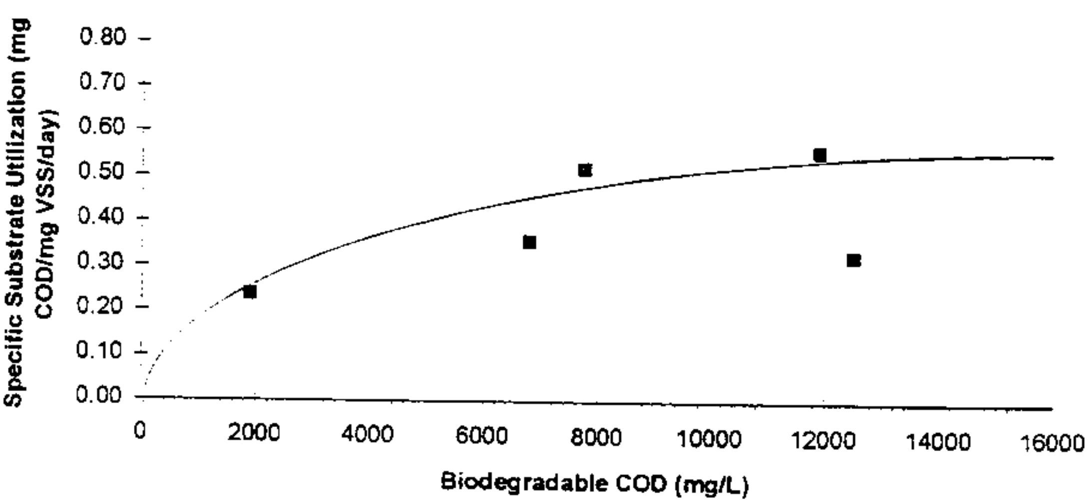

Figure 22. Specific Substrate Utilization Rate versus Biodegradable Effluent Substrate Concentration (mg/L COD).

Solving for the kinetic coefficients after deducting for non-degradable COD:

Substrate Utilization (k) = 0.68 day^{-1}
Half Velocity constant (Ks) = 3,500 mg/L (degradable COD)
Yield (Y) = 0.19 mg VSS/ mg COD
Decay rate (Kd) = 0.028 day^{-1}

Figures 23 and 24 are plots of the linear relationships which allow the determination of Y, Kd, k, and Ks from the data points. As was mentioned above, the data for the reactor at 12.5 day HRT reactor were omitted from these calculations.

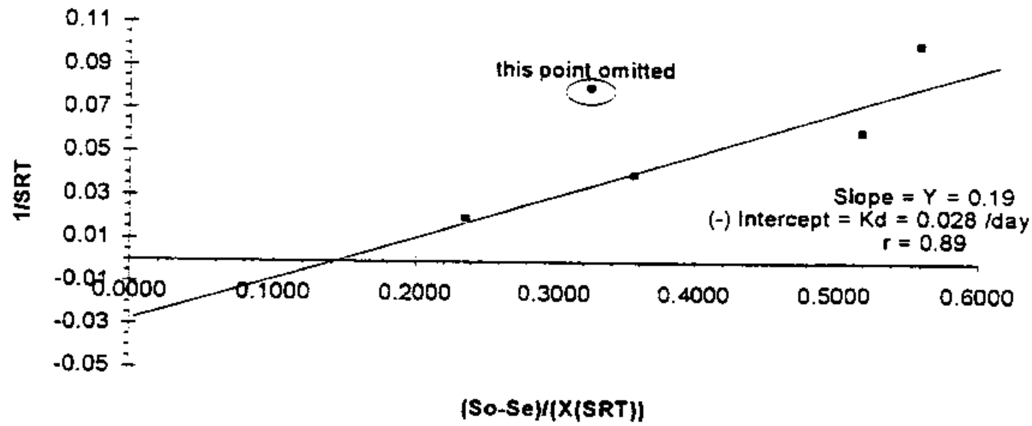

Figure 23. Linear plot for derivation of Yield and Kd in anaerobic reactors at various HRT's.

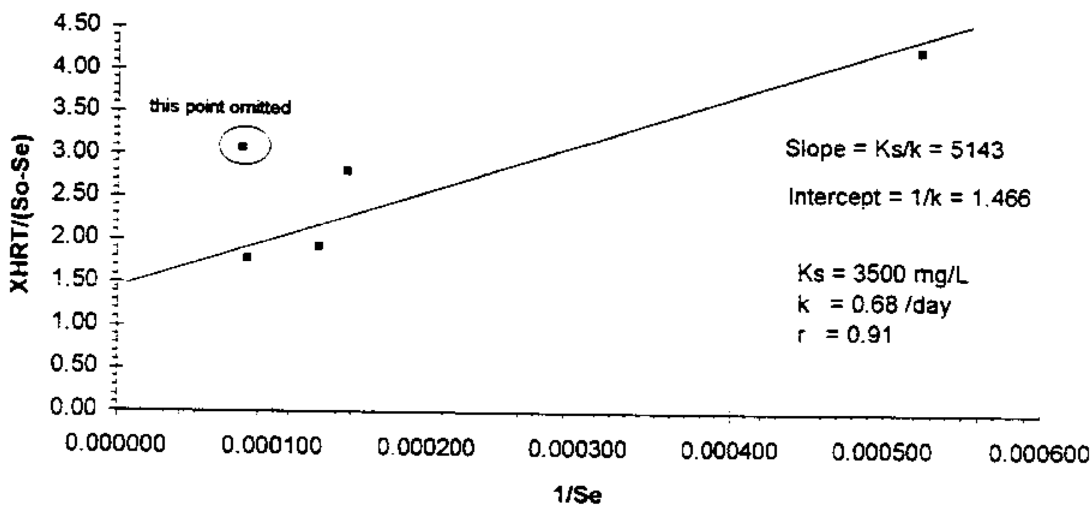

Figure 24. Linear plot for derivation of k and Ks in anaerobic reactors at various HRT's.

Effect of Trace Metal Addition to Feed

It was speculated that sulfide precipitation along with relatively low natural initial concentrations of certain trace metals might be limiting the growth of the methanogenic population. As noted in the literature review, certain metals, including nickel, cobalt, and molybdenum, have been found to be essential for the growth of methanogens. This study was intended to investigate the impact of the addition of the trace metals nickel, cobalt, and molybdenum on the COD removal performance of the anaerobic culture. Iron was added to precipitate sulfide in hopes of retaining the trace metals in solution. As discussed in the Methods and Materials chapter, 1 μmole of each of the three trace metals was added to 1 L of feed.

The COD of the feed for this study was in the range of 16,000 to 19,000 mg/L over the 76 day period of the study. A feed sample was obtained for metals analysis on day 6 of the study. Iron was present in the feed at 10.8 mg/L (0.2mM), nickel at 148 μg/L (2.5 μM), cobalt at 68 μg/L (1.2 μM), and molybdenum at 22 μg/L (0.23 μM).

All reactors showed a steady decline in VSS concentration and COD reduction performance. The effluent COD of all reactors increased steadily over the course of the study regardless of sludge age/hydraulic retention time. COD reductions were consistently inferior to those with corresponding HRT's (HRT=SRT) in the kinetic study group without trace metal additions. Table 18 presents COD removal efficiency for the group of reactors with trace metal additions compared to those without metals added.

Table18. COD removal efficiency of reactors with three trace metals added compared to reactors without metal addition.

	HRT, days				
	10	12.5	16.7	25	50
% removal with metals	14	13	18	25	50
% removal without metals	18	12	44	50	72

Figure 25 presents the effluent COD at all HRT's over the course of the experiment. Volatile fatty acids present in the reactors at the end of the experiment (day 76) are presented in Table 19.

Table 19. VFA's in reactors with trace metal addition after 76 days; expressed in mg/L.

	HRT, days				
	10	12.5	16.7	25	50
Acetic acid	4700	4200	4700	4600	3700
Propionic acid	1400	1200	1300	1100	920
iso-Butyric acid	550	760	850	600	180
n-Butyric acid	1300	1300	1300	1030	240

It is tempting to state that the addition of trace metals resulted in inhibition. In fact, molydate is known to be inhibitory to sulfate reducing bacteria (Widdel, 1988). However, it is prudent to simply state that the addition of these metals at these concentrations did not improve the performance of the reactors in terms of COD removals.

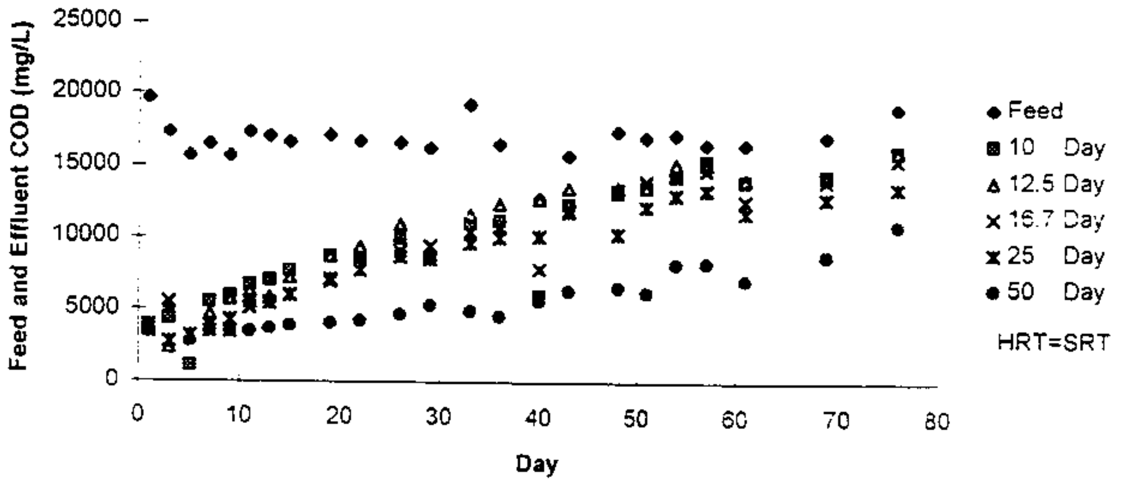

Figure 25. COD in reactors with trace metals.

Chapter 5. Summary, Conclusions, and Recommendations

This chapter will draw together observations made throughout the Results and Discussion Chapter, and offer some recommendations for application of what has been learned in this study. Also, areas for further research will be identified.

Summary

As detailed in the literature review and reinforced by the results of this study, the wastewater from crab processing facilities is highly varied over the course of the year. This variability, combined with variations in flow during different seasons and even during the work week, present serious difficulties for treatment plant design. The design must be capable of responding to rapid increases in loading, and the biomass must survive periods of starvation.

COD removal in Reactors and Anaerobic Reactor Design

This study has established that an upflow anaerobic reactor treating crab cooker wastewater can operate over an extended period of time without signs of failure. Accumulation of inactive or under performing biomass did appear to occur over time. In spite of this, COD reductions in the range of 84-88% did occur, reducing the COD concentration from around 20,000 mg/L in the feed to about 7,000 mg/L in the anaerobic effluent, and 2,400 to 3,100 mg/L in the final (aerobic) effluent. A single upflow reactor with an HRT of around 3.2 days and sufficient retention of biomass appears to be as effective as two reactors in series. Colonization sites (such as on and in the pores of the foam cubes) appear to accelerate the accumulation of biomass, although much of it may become inactive. It does not appear necessary to provide solids settling and recycle if sufficient packing is provided.

Implications of VFA Accumulation

Because the mixed anaerobic culture is composed of a consortium of substrate specific groups, the accumulation of specific fatty acids provides insight as to the balance of these groups in the reactor. A ramp up in loading occurred early in the study period and was accompanied by an accumulation of fatty acids. This indicated that the fermenters in the culture were out producing the acetogens and methanogens. It is unclear as to whether inhibition of the acetogens and/or methanogens was occurring, or whether, given sufficient time, those groups would have grown in numbers sufficient to keep pace with the fermenters. High loading resulted in an accumulation of butyric acids, as in the 10, 12.5, 16.7 and 25 day reactors, whereas butyric acid did not accumulate in the 50 day HRT reactor.

Toxicity of Cations and Anions to the Anaerobic Stage

It is unclear as to whether there was inhibition due to cations or anions in the anaerobic stages. Levels of cations or anions which, according to the literature, would have been toxic to the anaerobic bacteria were not detected in this study. Sodium and chloride were both well below the reported levels for inhibition. Hydrogen sulfide has been reported to be toxic, and is generated by sulfate-reducing bacteria. Based on studies in the literature and the sulfate levels in this wastewater, it is doubtful that sulfide toxicity to the anaerobic reactors occurred. The possibility of synergistic toxicity among the various cations and anions present was not specifically investigated, but is a possibility which may warrant study.

Nutrient Limitation

Nitrogen was in abundance in this wastewater, and ortho-phosphate was measured in the effluent at several milligrams per liter. Therefore, it does not appear that these nutrients would production of essential enzymes, but the addition of iron, nickel, cobalt, and molybdenum at 1 μmole/L each did not result in an increase in growth or COD removals.

Nitrification and Ammonia Toxicity

It is clear that high levels of ammonium/ammonia were inhibitory to nitrifying bacteria in the aerobic stages of these experimental systems. Efforts to induce nitrification failed, and only after air stripping of ammonia, and BOD depletion, was nitrification observed to occur in a batch study. Physical and/or chemical removal of ammonia, possibly as a pretreatment step to biological nitrogen removal, will be required in a full scale application of anaerobic treatment of this wastewater.

Conclusions

Specifically, the following conclusions were drawn from this research study:

- The overall treatment performance of an upflow anaerobic packed filter, Ban1, was superior to an upflow anaerobic bed filter, reactor Aan1, due to higher biomass retention. Also, there was less variation in the effluent COD concentration of the three stage system incorporating Ban1 as compared to a similar system incorporating Aan1, in spite of almost identical variation in loadings.
- Specific substrate removal was higher in reactor Aan1 than in Ban1, apparently due to a higher fraction of active biomass. Lower actual HRT in Ban1 compared to Aan1 also may have been an important factor. Diffusion into the central core of the biomass filled foam cubes, which were more abundant in Ban1, also may have been a limiting factor.
- The Monod model kinetic coefficients for the anaerobic stage were determined to be: $k=0.68\ day^{-1}$, $Ks=3{,}500\ mg/L$, $Y=0.19$, and $Kd=0.028\ day^{-1}$.
- While VFA's accumulated in the reactors under periods of high loading, none of the species measured were in the reported range of toxicity to anaerobic processes. There appeared to be sufficient nitrogen and phosphorus available to sustain growth.

- Nitrification did not occur in the aerobic stage of the continuous flow studies, apparently due to ammonia toxicity, and competition with heterotrophs. Nitrification occurred in a batch study, apparently due to a decrease in the TA (total ammonia/ammonium) concentration to non-inhibitory levels, and depletion of BOD.

Recommendations

Based on the results of this study, a design capable of reducing COD/BOD and ammonia would consist of a five stage system: Stage 1 would be a single upflow anaerobic reactor; Stage 2, an anoxic tank for denitrification, receiving recycle from Stage 5; Stage 3 would be the initial aerobic treatment for BOD reduction with an integral clarifier; Stage 4 would consist of an air stripping tower to lower the TA concentration to approximately 500 mg/L; Stage 5 would be a second aeration tank for nitrification followed by a clarifier. This arrangement would hopefully result in influent to the second aeration reactor containing a relatively low BOD content, and an acceptably low TA concentration such that nitrifying bacteria would not be inhibited. A pH controller for this final stage would be essential to maintain pH in the range of 7.1 - 7.3; i.e., low enough so that free ammonia would be less than 1% of the TA, but not so low as to be unsuitable for nitrifier growth. Supernatant from Stage 5 would be recycled to Stage 2 for denitrification.

It is premature to recommend implementation of this design in full scale applications. The issue of nitrification and denitrification must be resolved before final design parameters can be determined. Important considerations in the final design of a treatment facility would necessarily be based on the discharge circumstances (i.e. direct or sewer discharge, particular limits, etc.) of the processing company. A discharge permit for a new direct discharge to a receiving water would typically impose BOD_5, TSS, oil and grease, and pH limitations. Limits on ammonia, total nitrogen, total phosphorus, and toxicity might also be imposed. An indirect discharger who is discharging to a public sewer system would be motivated to avoid paying surcharges imposed by the local wastewater treatment authority, and would thus evaluate the cost/benefit of an on-site treatment facility designed to minimize those surcharges. It is beyond the scope of this project to evaluate these many factors. However, it is hoped that the results of this study will aid those with the responsibility of designing treatment facilities for the crab processing industry.

Areas for Further Study

The following areas warrant further study:

1. Why did activity of the VSS decrease over time in the anaerobic reactors?
2. If diffusion becomes a limiting factor in the anaerobic reactors, would the selection of a different packing improve performance?
3. Would high velocity recirculation in the anaerobic reactors lead to formation of granular sludge, or would the biomass simply wash out if there were not settling and recycle?
4. How long does it take for the methanogenic/acetogenic populations to "catch up" with the fermenters after a ramp up in loading?
5. How long a period of starvation can be sustained by the anaerobic stage, and by the aerobic stage, with a reasonably quick recovery to activity?
6. Are there nutrients which are lacking in the crab cooker wastewater which could enhance the performance of the treatment system if added?
7. Can nitrification be established in a continuous flow system incorporating air stripping? If so, can a subsequent denitrification stage be successful?
8. Are there toxic or inhibitory substances, or combinations of them, limiting the anaerobic treatability of this wastewater? Are there inhibitory factors in addition to ammonia affecting nitrification?

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Appendix

The appendix contains tabulated raw data collected over the period of this study.

		<--------TSS	------------------------>								<----VSS-	-------------------------->							
		Feed	A-1	A-2	A-3	B-1	B-2	B-3	-MLS	B-MLSS	Feed	A-1	A-2	A-3	B-1	B-2	B-3	A-MLVSS	B-MLVSS
11-Oct	0																		
19-Feb	131	650	1530	860	790	1170	770	350											
27-Feb	139	1380	1140	950	640	1340	990	560			1330	850	660	450	750	480	420		
4-Mar	144	940	930	720	540	680	940	470			760	730	480	380	460	380	240		
14-Mar	154	530	1800	540	470	730	490	230			250	940	270	200	430	110	20		
26-Mar	166	1153	1180	3960	4240	4200	507	307			967	720	3440	2640	2840	373	213		
averages		931	1316	1406	1336	1624	739	383			827	810	1213	918	1120	336	223		
4-Apr	175	2260	2280	1160	770	2410	850	650			2200	1390	660	520	1670	450	340		
17-Apr	188	1100	1500	1100	720	2190	5380	700	12000	13460	1040	1170	800	560	1420	2110	560	3990	5180
26-Apr	197	1830	2370	2380	840	1810	3090	1100			1740	1880	1840	700	1390	2170	940		
averages		1730	2050	1547	777	2137	3107	817	12000	13460	1660	1480	1100	593	1493	1577	613	3990	5180
4-May	205	4040	2050	1650	1100	8410	1320	970			2110	560	710	690	3200	730	540		
30-May	231	1580	1740	1740	2960	2320	1380	5300	2960	2320	1200	900	720	1600	1200	400	2320	1600	2320
6-Jun	238	1110	1030	430	6150	840	460	3390	6150	1690	890	570	270	2020	620	300	1690	2020	1690
13-Jun	245	720	680	530	500	2590	380	100	660	1590	630	440	290	330	1490	270	60	470	1250
averages		1863	1375	1088	2678	3565	885	2440	3257	1867	1208	618	498	1160	1628	425	1153	1363	1753
20-Jun	252	1640	1160	940	1330	2500	820	1060	6480	2960	910	550	400	550	1520	350	640	1800	2140
27-Jun	259	1020	530	530	1100	1270	500	320		2350	1020	270	320	750	870	210	160		1850
18-Jul	280	1460	920	970	1420	760	650	650	7410	2370	1010	560	550	960	470	360	270	3000	1470
averages		1373	870	813	1263	1510	657	677	6945	2560	980	460	423	753	953	307	357	2400	1820

Measured Solids in Reactors A1 and B1

		A1 conc	A1 mass	B1 conc	B1 mass
11-Oct	0	5500	22000	5500	22000
20-Mar	160	7882	31528	21680	86720
18-Jul	280	14075	56300	27760	111040

Est VSS		Conc	Mass	Conc	Mass	A1 Avg Conc	B1	A1 Avg Mass	B1	
11-Oct	0	5500	22000	5500	22000					
21-Feb	133	7480	29920	18950	75799					
27-Feb	139	7569	30277	19556	78226					
4-Mar	144	7644	30575	20062	80248			1.0	0.4	F/M
14-Mar	154	7793	31171	21073	84293	[illegible],437	18401	29746	73603	
20-Mar	160	7882	31528	21680	86720					
26-Mar	168	8192	32767	21984	87936					
4-Apr	175	8656	34625	22440	89760			1.1	0.5	F/M
17-Apr	188	9327	37308	23099	92395	9,258	23031	37033	92124	
26-Apr	197	9792	39166	23555	94219					
4-May	205	10204	40818	23960	95840					
17-May	218	10875	43501	24619	98475					
30-May	231	11546	46185	25277	101109			0.4	0.2	F/M
6-Jun	238	11907	47630	25632	102528	11,360	25095	45442	100380	
13-Jun	245	12269	49075	25987	103947					
20-Jun	252	12630	50520	26341	105365					
27-Jun	259	12991	51965	26696	106784			0.5	0.2	F/M
5-Jul	267	13404	53616	27101	108405	12,601	26313	50405	105253	
11-Jul	273	13714	54855	27405	109621					
18-Jul	280	14075	56300	27760	111040					

			<--------System A-------->					<--------System B-------->					<--------BOD5 (mg/L)-------------->						
Date	Day	Feed	Q	COD Ldg	A-1	A-2	A-3	Q	COD Ldg	B-1	B-2	B-3	Feed	A-1	A-2	A-3	B-1	B-2	B-3
11-Oct	0																		
21-Feb	133	14474	2.33	33724	2363	2105	1550	2.33	33724	1846	1514	1255							
27-Feb	139	13935	2.33	32469	2787	2710	1780	2.33	32469	2090	1858	1316							
4-Mar	144	9302	2.33	21674	2456	2605	1786	2.33	21674	1935	2084	1860							
14-Mar	154	10450	2.33	24349	2520	2320	1940	2.33	24349	2220	2250	1940							
26-Mar	166	16500	2.33	38445	5500	4125	3375	2.33	38445	3825	3525	2625							
4-Apr	175	33700	1.38	46506	4400	2800	1900	1.60	53920	4300	2600	1700							
17-Apr	188	26700	1.38	36846	16600	9900	8300	1.60	42720	10800	6600	5200							
26-Apr	197	30600	1.38	42228	13200	12200	9200	1.60	48960	12600	10600	8300							
4-May	205	19800	0.95	18810	12950	11240	6100	0.90	17820	9900	4950	4570							
17-May	218	16650	0.95	15818	5600	7200	3100	0.90	14985	5200	3900	2300							
30-May	231	17400	0.95	16530	7900	7200	4800	0.90	15660	4600	4600	3100							
6-Jun	238	17900	0.95	17005	6800	6900	5400	0.90	16110	5300	4300	3100							
13-Jun	245	16060	0.95	15257	5860	5290	2830	0.90	14454	5100	4250	570							
20-Jun	252	18700	1.21	22627	7730	4980	2140	1.18	22066	6810	5290	1020							
27-Jun	259	21400	1.21	25894	9800	10000	3600	1.18	25252	8500	9000	2800	15531	4718	4557	283	5100	4830	290
5-Jul	267	19355	1.21	23420	6970	6970	3100	1.18	22839	7160	6970	1550	12230	3450	3540	298	3780	3540	92
11-Jul	273	21200	1.21	25652	10000	9200	4400	1.18	25016	8000	8400	3600	14100	3225	3562	420	3581	3450	27
18-Jul	280	16520	1.21	19989	7870	6880	1380	1.18	19494	5510	5900	1570	14550	5625	4538	373	3788	3788	20
Average		18925	1.48	26513	7295	6368	3705	1.49	27220	5872	4922	2688	14103	4255	4049	344	4062	3902	107
Avg day 133-166		12932	2.33	30132	3125	2773	2086	2.33	30132	2383	2246	1799							
Avg day 167-197		30333	1.38	41860	11400	8300	6467	1.60	48533	9233	6600	5067							
Avg day 198-245		17562	0.95	16684	7822	7566	4446	0.90	15806	6020	4400	2728							
Avg day 246-280		19435	1.21	23516	8474	7606	2924	1.18	22933	7196	7112	2108							

COD REMOVAL (from prior stage)

		A1 VSS	A-1	A-2	A-3	B1 VSS	B-1	B-2	B-3
11-Oct									
4-Mar	144	30575	9163	16.5	871.5	80248	9684	-71.5	111
14-Mar	154	31171	7356	168	522.5	84293	7656	-173	227
26-Mar	166	31528	7975	-115	-153	86720	9650	-503	262.5
4-Apr	175	32767	12100	2150	1563	87936	20800	1463	1363
17-Apr	188	34625	13600	600	-1950	89760	19400	950	-600
26-Apr	197	37308	15450	2700	1850	92395	16050	1100	300
4-May	205	39166	12250	1835	5620	94219	15300	6300	3205
17-May	218	40818	12625	2075	6120	95840	13025	3650	2125
30-May	231	46185	9125	-450	2400	101109	12425	300	1150
6-Jun	238	47630	10850	450	1650	102528	12350	650	1350
13-Jun	245	49075	11120	1040	3265	103947	11880	950	3705
20-Jun	252	50520	9650	1815	2995	105365	10570	665	3750
27-Jun	259	51965	10250	-1235	3890	106784	11550	-1345	4345
5-Jul	267		13408	1415	5385		13218	860	6435
11-Jul	273		10278	-715	3685		12278	-820	4085
18-Jul	280	56300	10990	2055	6660	111040	13350	855	5580
			11012	863	2773				

COD Removed/G VSS

day	A1	B1
144	300	121
154	236	91
166	253	111
175	369	237
188	393	216
197	414	174
205	313	162
218	309	136
231	198	123
238	228	120
245	227	114
252	191	100
259	197	108
280	195	120

Ammonia and Anions in Effluents

Date	Day	<---------NH4/NH3-N-------->							<----NO2-N------------------->							<----NO3-N----------------->						
11-Oct	0	Feed	A-1	A-2	A-3	B-1	B-2	B-3	Feed	A-1	A-2	A-3	B-1	B-2	B-3	Feed	A-1	A-2	A-3	B-1	B-2	B-3
27-Feb	139	918	691	439	300	448	729	518	15	0	0	0	0	0	14	5	1	0	1	1	0	1
4-Mar	144	520	674	640	609	625	625	575	3	0	0	0	0	0	0	4	1	1	1	1	1	1
14-Mar	154	474	340	598	258	679	590	250	1	0	0	0	0	0	72	3	1	1	7	19	1	1
26-Mar	166	939	907	825	606	921	935	687	3	0	0	0	0	0	29	3	0	0	0	0	0	2
4-Apr	175	505	517	456	442	1094	566	261	27	0	0	0	0	0	0	0	0	0	0	0	0	0
17-Apr	188								10	2	3	15	3	10	10	0	0	0	0	0	0	0
26-Apr	197	1700	2300	2450	2360	3380	3318	3366	0	4	5	0	4	7	8	0	0	0	0	0	0	0
4-May	205	1770	1580	1840	960	1560	1510	1020	0	0	8	11	0	0	0	8	6	4	5	3	7	3
17-May	218								8	10	0	0	0	0	0	19	0	0	0	0	1	4
30-May	231	950	1030	1080	1433	900	951	1410	7	8	9	0	0	0	0	10	0	0	0	0	0	0
6-Jun	238	1114	1078	1040	897	1372	880	847	26	0	0	16	6	0	0	0	0	0	0	0	0	0
13-Jun	245	907	1055		1018	1517		355	30	7	8	0	8	7	29	0	2	0	0	0	0	217
20-Jun	252	1160	1065	1097	437	1148	1064	638	25	10	9	0	12	9	0	0	0	0	0	0	0	63
27-Jun	259	1455	1176	1694	645	1172	1161	726	0	8	5	2	5	6	0	0	0	0	0	0	0	0
18-Jul	280	1385	1188	1182	618	1811	1168	757	19	9	0	0	9	0	0	11	3	7	2	2	0	2
average		1061	1046	1112	814	1279	1125	878	12	4	3	3	3	3	11	4	1	1	1	2	1	20

Date	Day	<----PO4-P------------------->							<----SO4-S------------------->						
11-Oct	0	Feed	A-1	A-2	A-3	B-1	B-2	B-3	Feed	A-1	A-2	A-3	B-1	B-2	B-3
27-Feb	139	42	17	7	4	5	5	1	302	26	20	348	20	14	265
4-Mar	144	32	7	5	6	4	5	2	415	58	44	369	63	25	348
14-Mar	154	43	25	23	4	20	21	2	378	54	21	405	24	57	422
26-Mar	166	14	1	3	1	2	2	2	250	34	90	427	130	50	267
4-Apr	175	44	25	5	50	25	5	1	192	28	18	257	29	23	191
17-Apr	188	103	38	5	4	14	6	3	309	60	190	196	144	290	370
26-Apr	197	50	31	3	6	3	4	19	210	24	16	16	23	100	152
4-May	205	95	74	30	8	43	20	3	177	89	86	110	58	410	390
17-May	218	34	38	10	5	6	0	1	29	71	2,074	112	102	236	324
30-May	231	163	37	74	150	16	11	84	240	57	23	90	124	82	216
6-Jun	238	87	62	14	5	5	3	1	92	214	21	38	62	97	178
13-Jun	245	96	67	30	9	29	22	33	77	121	54	230	72	60	123
20-Jun	252	87	27	13	8	31	25	83	292	170	38	235	116	60	210
27-Jun	259	88	26	29	6	32	47	5	455	171	94	414	103	83	381
18-Jul	280	100	30	87	2	23	292	2	290	82	255	540	166	106	542
average		72	34	23	18	17	31	16	247	84	203	252	82	113	292

NOTE: A "0" means "not detected."

		<---------pH -------------------->							<----Alkalinity (as CaCO3)----------->						
11-Oct		Feed	A-1	A-2	A-3	B-1	B-2	B-3	Feed	A-1	A-2	A-3	B-1	B-2	B-3
19-Feb	131	7.0	7.8	7.8	8.5	7.9	7.8	8.4	334	2899	3126	2462	3712	3343	2396
27-Feb	139	7.1	8.1	8.1	8.7	8.1	8.1	8.7	690	5130	5400	3000	5700	5500	3500
4-Mar	144	7.0	8.1	8.1	8.7	8.1	8.1	8.7	557	4415	4430	3374	4323	4714	3968
14-Mar	154	7.3	8.1	8.0	8.8	8.0	8.1	8.9	695	3900	4040	2900	4400	4300	2700
26-Mar	166	6.8	8.0	8.1	8.7	8.1	8.1	8.9							
26-Apr	197	7.2	7.8	8.5	8.4	8.0	8.4	8.3	1220	4200	5375	5420	5250	6660	7450
4-May	205	7.4	7.5	7.9	8.9	7.7	9.0	9.0	1120	2330	4130	6720	2760	7300	7300
30-May	231	7.3	7.9	7.9	8.6	8.4	8.4	8.7	850	5860	5360	5770	6080	6100	6500
6-Jun	238	7.3	7.9	8.1	8.7	8.4	8.7	8.9	1200	6400	6500	8280	6280	6060	6100
13-Jun	245	7.1	7.9	7.9	8.7	7.9	8.0	8.2	480	5960	6340	4000	5800	5880	700
20-Jun	252	7.3	8.0	8.1	8.8	8.0	8.1	8.8	1540	7020	7310	2820	6630	6660	3820
18-Jul	280	7.1	7.8	7.9	8.9	7.9	7.9	8.8	2050	5940	6760	3900	7150	7095	4800
average		7.1	7.9	8.0	8.7	8.0	8.2	8.7	976	4914	5343	4422	5280	5783	4476
minimum		6.8	7.5	7.8	8.4	7.7	7.8	8.2	334	2330	3126	2462	2760	3343	700
maximum		7.4	8.1	8.5	8.9	8.4	9.0	9.0	2050	7020	7310	8280	7150	7300	7450

Volatile Fatty Acids in Systems A and B

		<----VFA (as Acetic acid)-------->						
11-Oct		Feed	A-1	A-2	A-3	B-1	B-2	B-3
27-Feb	139	5500	66	0	0	0	0	0
4-Mar	144	3397	0	71	0	0	0	0
14-Mar	154	3470	229	121	0	0	0	0
		4122	98	64				
26-Apr	197	8944	8110	4533	4090	6300	3400	2050
4-May	205	6670	5360	5750	215	3900	0	0
30-May	231	7150	2170	1970	600	650	560	0
6-Jun	238	7300	1920	1640	270	975	38	32
13-Jun	245	7640	1980	1490	0	2440	1810	0
		7190	2858	2713	271	1991	602	8
20-Jun	252	4960	930	1050	113	2400	1170	77
18-Jul	280	7700	4800	3600	350	3350	3380	320
		6330	2865	2325	232	2875	2275	199
average		6170	2377	1917	537	2001	996	226

Values for Day 252 and 280 are detailed below:

		Feed	A-1	A-2	A-3	B-1	B-2	B-3
		<----Acetic acid by GC------------->						
20-Jun	252	4030	402	848	113	2138	992	77
18-Jul	280	5450	3835	2940	288	3002	3035	283
		<----Propionic acid by GC------------>						
20-Jun	252	1144	648	249	0	332	225	0
18-Jul	280	1416	1200	816	75	430	362	46
		<----iso-butyric acid by GC------------>						
18-Jul	280	0	0	0	0	0	0	0
		<----n-butyric acid by GC------------>						
18-Jul	280	1590	0	0	0	0	0	0

Date	Day	[COD]	<--System A---> gas (L)	Q (L)	<--System B---> gas (L)	Q(L)	A1	A2	A3	B1	B2	B3
11-Oct	Day 0											
21-Feb	133	14474	11.80	2.40	8.65	2.80						
22-Feb	134		12.50	1.85	14.30	1.40						
23-Feb	135		10.10	2.40	4.50	3.90						
24-Feb	136		13.60	4.00	13.40	3.55						
25-Feb	137		19.50	2.00	25.00	0.60						
26-Feb	138		17.10	1.80	20.20	2.00						
27-Feb	139	13935	17.60	1.75	19.70	1.75	2787	2710	1780	2090	1858	1316
28-Feb	140		16.10	2.70	25.50	3.75						
1-Mar	141		19.50	2.10	14.00	1.45						
2-Mar	142		16.30	2.60	17.70	2.80						
3-Mar	143		20.10	2.00	22.50	2.00						
4-Mar	144	9302	4.60	2.05	5.10	2.15	2456	2605	1786	1935	2084	1860
5-Mar	145		12.60	2.00	13.50	2.20						
6-Mar	146		12.90	2.20	14.10	2.35						
7-Mar	147		5.90	3.40	15.00	2.55						
8-Mar	148		15.00	2.00	12.30	3.90						
9-Mar	149		10.10	2.10	12.20	1.00						
10-Mar	150		11.40	2.50	13.00	2.50						
11-Mar	151		15.10	2.50	16.60	2.80						
12-Mar	152		13.40	2.50	14.80	2.40						
13-Mar	153		18.00	2.70	15.40	2.80						
14-Mar	154	10450	10.60	1.90	13.20	1.60	2520	2320	1940	2220	2250	1940
15-Mar	155		17.00	2.40	14.50	3.00						
16-Mar	156		14.80	2.00	14.20	2.70						
17-Mar	157		16.00	2.30	18.60	1.50						
18-Mar	158		13.70	2.50	16.00	2.40						
19-Mar	159		19.90	2.50	23.80	3.10						
20-Mar	160		24.10	2.30	27.80	2.80						
21-Mar	161		13.70	2.10	22.80	1.35						
22-Mar	162		22.40	2.40	23.70	2.95						
23-Mar	163		23.80	2.30	19.70	2.70						
24-Mar	164		23.30	2.70	24.70	2.40						
25-Mar	165		20.10	1.85	16.00	2.30						
26-Mar	166	16500	19.40	2.40	15.90	2.20	5500	4125	3375	3825	3525	2625
27-Mar	167		6.80	1.50	26.10	2.90						
28-Mar	168		17.90	0.90	15.90							
29-Mar	169		14.80	0.95	16.10	0.80						
30-Mar	170		10.60	0.70	12.00	1.60						
31-Mar	171		10.10	0.90	13.00	0.80						
1-Apr	172		8.50	1.00	12.00	1.20						
2-Apr	173		15.00	1.40	15.50	1.40						
3-Apr	174		10.95	1.20	18.63	0.70						
4-Apr	175	33700	16.95	0.60	21.60	0.50	4400	2800	1900	4300	2600	1700
5-Apr	176		19.80	1.80	23.25	2.10						
6-Apr	177		21.55	1.50	28.30	1.70						
7-Apr	178		16.70	1.20	20.20	1.45						
8-Apr	179		16.40	1.10	16.30	0.80						
9-Apr	180		15.50	1.30	23.50	0.80						
10-Apr	181		17.30	1.80	16.50	2.90						
11-Apr	182		16.05	1.70								
12-Apr	183		16.60	1.90	21.25	1.80						
13-Apr	184		19.30	1.30	25.20	1.90						
14-Apr	185		19.50		23.50	2.50						
15-Apr	186				22.40	1.80						
16-Apr	187		15.40	1.90	22.70	1.90						
17-Apr	188	26700	12.70	1.30	20.00	2.50	16600	9900	8300	10800	6600	5200
18-Apr	189		17.40	1.70	22.80	1.70						
19-Apr	190		17.05	1.50	22.80	1.90						

	day	[COD]	gas (L)	Q (L)	gas (L)	Q(L)	A1	A2	A3	B1	B2	B3
20-Apr	191		16.90	1.50	23.10	1.80						
21-Apr	192		17.30	1.75	23.00	1.95						
22-Apr	193		18.90	1.90	25.00	2.10						
23-Apr	194		16.70	1.75	25.20	2.00						
24-Apr	195		18.40	1.90	24.60	1.90						
25-Apr	196		12.10	1.35	23.20	1.90						
26-Apr	197	30600	21.50	1.90	26.00	2.10	13200	12200	9200	12600	10600	8300
27-Apr	198		14.90	1.85	17.10	1.75						
28-Apr	199		5.30	1.80	19.50	2.00						
29-Apr	200		10.70	0.85	13.10	0.60						
30-Apr	201		7.20	1.10	7.30	1.10						
1-May	202			1.05	4.20	0.98						
2-May	203		7.20	1.05	4.20	0.98						
3-May	204		3.00	0.80	3.00	0.95						
4-May	205	19800	2.50	0.90	3.50	0.90	12950	11240	6100	9900	4950	4570
5-May	206		3.25	1.00	4.85	0.90						
6-May	207		3.25	0.90	4.85	0.90						
7-May	208		3.10	0.70	5.00	0.80						
8-May	209		3.80	1.00	6.90	0.70						
9-May	210			0.90	6.40	0.90						
10-May	211		4.00	1.90	7.10	0.90						
11-May	212		5.40		7.10	0.90						
12-May	213		4.90	0.80	8.30	0.90						
13-May	214		6.20	0.90	8.40	1.00						
14-May	215		5.00	0.60	6.40	0.80						
15-May	216		6.50	0.80	13.00	0.75						
16-May	217		6.40	0.80	7.90	0.75						
17-May	218	16650	5.00	0.80	5.80	1.10	5600	7200	3100	5200	3900	2300
18-May	219		4.35	0.75	6.60	0.90						
19-May	220		4.35	1.70	6.60	0.70						
20-May	221		2.80		2.90	1.00						
21-May	222		2.00	0.80	7.50	0.10						
22-May	223		6.60	0.95	7.50	1.05						
23-May	224		7.40	0.80	8.50	0.80						
24-May	225		7.90	0.90	8.10	0.90						
25-May	226		11.50	1.60	11.90	0.70						
26-May	227		7.90		1.80	1.00						
27-May	228		8.30	1.00	8.50	1.00						
28-May	229		2.70	0.90	8.10	0.90						
29-May	230		7.90	0.90	6.50	1.40						
30-May	231	17400	8.20	1.00	1.00	0.80	7900	7200	4800	4600	4600	3100
31-May	232		7.80	1.10	8.60	1.10						
1-Jun	233		9.00	0.60	9.10	1.20						
2-Jun	234		3.40	0.95	8.50	0.90						
3-Jun	235		9.10	1.05	7.40	1.40						
4-Jun	236		8.80	1.00	9.20	0.30						
5-Jun	237		8.20	1.20	8.80	1.40						
6-Jun	238	17900	8.70	0.70	8.50	1.00	6800	6900	5400	5300	4300	3100
7-Jun	239		1.30	1.00	8.80	0.80						
8-Jun	240		8.60	1.00	8.10	1.00						
9-Jun	241		8.30	1.00	8.20	1.00						
10-Jun	242		8.50	0.60	8.20	0.95						
11-Jun	243		8.70	1.00	8.60	0.90						
12-Jun	244		7.70	0.50	8.40	0.95						
13-Jun	245	16060	9.80	1.60	9.60	0.90	5860	5290	2830	5100	4250	570
14-Jun	246		9.20	0.80	8.90	0.95						
15-Jun	247		8.20	1.00	9.00	0.95						
16-Jun	248		9.10	0.70	9.60	0.90						
17-Jun	249		7.00	1.05	9.40	0.95						
18-Jun	250		1.20	0.55	6.90	0.70						

	day	[COD]	gas (L)	Q (L)	gas (L)	Q(L)	A1	A2	A3	B1	B2	B3
19-Jun	251		5.80		9.80	0.70						
20-Jun	252	18700	9.70	1.05	11.80	1.35	7730	4980	2140	6810	5290	1020
21-Jun	253		8.50	1.00	11.00	1.35						
22-Jun	254		9.80	1.60	11.20	1.35						
23-Jun	255		9.20	1.15	9.30	1.15						
24-Jun	256		8.90	1.30	9.80	1.15						
25-Jun	257		8.80	1.40	4.40	1.15						
26-Jun	258		9.70	1.05	10.50	1.15						
27-Jun	259	21400		1.30	10.10	1.45	9800	10000	3600	8500	9000	2800
28-Jun	260			0.90	12.10	1.10						
29-Jun	261		9.80	1.30	10.40	1.20						
30-Jun	262		10.30	1.90	10.40	1.20						
1-Jul	263		6.30	1.30	10.90	1.20						
2-Jul	264		10.40	1.20	10.50	1.20						
3-Jul	265		10.30	0.95	10.00	1.15						
4-Jul	266		10.40	1.30	10.80	1.20						
5-Jul	267	19355	6.10	0.85	9.70	1.10	6970	6970	3100	7160	6970	1550
6-Jul	268		9.80	1.40	9.90	1.20						
7-Jul	269		6.70	1.15	10.50	1.15						
8-Jul	270		11.40	1.40	11.20	1.20						
9-Jul	271		11.30	1.00	9.60	1.80						
10-Jul	272		2.00	1.20	11.20	0.90						
11-Jul	273	21200	12.40	1.60	11.50	1.40	10000	9200	4400	8000	8400	3600
12-Jul	274		10.50	0.80	10.60	1.20						
13-Jul	275		6.50	1.20	8.90	1.00						
14-Jul	276		9.20	1.20	10.90	1.10						
15-Jul	277		11.00	1.00	10.60	1.20						
16-Jul	278		12.70	1.50	11.60	1.25						
17-Jul	279		2.80	0.80	7.70	1.10						
18-Jul	280	16520	10.00	1.30	10.80	0.40	7870	6880	1380	5510	5900	1570
				1.20		1.10						

Date	Day	<---------Sodium------------>							<----Potassium-------------------->						
11-Oct	0	Feed	A-1	A-2	A-3	B-1	B-2	B-3	Feed	A-1	A-2	A-3	B-1	B-2	B-3
27-Feb	139	1844	655	780	751	763	657	640	567	391	414	400	411	410	359
4-Mar	144	891	279	274	271	273	270	268	382	309	310	329	305	316	334
14-Mar	154	1930	890	753	910	803	769	895	344	320	307	369	341	343	351
26-Mar	166	2174	795	788	832	795	809	801	594	499	469	649	501	500	487
4-Apr	175	1482	848	810	707	825	974	739	465	528	417	511	726	584	392
26-Apr	197	1200	936	780	1065	826	855	884	500	630	680	890	725	776	869
4-May	205	2570	1700	1400	1270	1600	1200	1500	666	570	700	900	530	920	800
30-May	231	1800	1300	1400	1430	1470	1570	1400	680	660	666	785	790	774	800
6-Jun	238	1635	1143	1216	1376	1048	1339	1374	635	656	636	702	670	700	733
13-Jun	245	1700	1200		1034	946		798	560	617		645	602		291
20-Jun	252	1520	1030	1040	1135	1071	1047	1001	815	669	746	703	722	631	543
27-Jun	259	1400	1128	918	1093	1070	1105	1032	828	792	1,009	771	801	766	704
18-Jul	280	2845	1932	1886	1862	1518	1851	1842	872	796	855	967	804	1,385	892
average		1769	1064	1004	1057	1001	1037	1013	608	572	601	663	610	675	581

Date	Day	<----Magnesium-------------------->							<----Calcium--------------------->						
11-Oct	0	Feed	A-1	A-2	A-3	B-1	B-2	B-3	Feed	A-1	A-2	A-3	B-1	B-2	B-3
27-Feb	139	162	78	86	93	82	83	89	271	162	154	116	142	146	91
4-Mar	144	244	131	129	124	131	132	121	332	190	132	108	149	121	88
14-Mar	154	209	160	156	178	166	146	163	270	188	185	94	196	185	77
26-Mar	166	205	90	103	120	90	92	109	392	187	129	75	157	106	66
4-Apr	175	138	64	49	54	57	55	42	255	189	77	58	136	55	50
26-Apr	197	175	140	164	185	148	172	181	300	345	199	245	389	233	277
4-May	205	224	160	130	200	120	175	190	530	400	460	270	370	330	320
30-May	231	380	180	180	288	175	162	403	230	280	620	375	290	202	128
6-Jun	238	248	143	138	179	151	177	194	345	301	383	223	303	229	184
13-Jun	245	236	144		190	147		91	302	293		340	329		229
20-Jun	252	253	158	157	182	187	158	160	408	313	321	287	373	331	275
27-Jun	259	291	177	252	212	194	174	189	500	353	365	277	363	367	236
18-Jul	280	172	101	92	85	91	97	77	207	165	155	60	178	169	51
average		226	133	136	161	134	135	155	334	259	265	194	260	206	159

Trace Metals in Feed (by AA)

	mg/L	ug/L	ug/L	ug/L	ug/L	ug/L
	Fe	Ni	Co	Mo	Cr	Cd
25-Jan		26	1.0	3.0	3.0	1.3
24-May	8.86	153	24	7		
19-Jul	2.48	105	10	3		

Correlation of COD with TOC

Raw Wastewater

TOC	COD
490	968
466	1394
473	1084
515	1703
922	2125
960	2360
821	2107
871	2263
848	2120
957	2840
908	2045
1021	2911
905	2045
948	2360
645	1587
668	1780
1580	3368
1522	3522

r= 0.942
slope= 2.1
Y intercept= 329

Anaerobic Effluents

TOC	COD
239	465
297	425
343	658
200	310
260	542
330	542
300	774
186	271
450	984
427	787
372	748
312	433
376	898
336	898
177	429
121	292
389	960
371	760
214	560
125	320
356	787
314	630
218	550
147	550
331	944
323	550
152	393
159	580
96	426
87	426
48	194
299	968
275	774
189	503
99	387

r= 0.80
slope= 1.67
Y intercept= 166

Aerobic Effluents

TOC	COD
128	155
95	542
128	310
117	194
148	315
91	118
139	390
83	351
127	400
68	160
188	472
131	315
158	393
40	348
34	271
61	348
53	271

r= 0.295
slope= 0.758
Y intercept= 235

Ammonia Toxicity Study

pH before adjustment

Date	a	temp	Anaerobic Effluent as Feed 6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	Aerobic Effluent as Feed 6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	Control 6.8	7.3	7.8	8.3
29-Jun	0		6.80	6.80	7.30	7.30	7.80	7.80	8.30	8.30	6.80	6.80	7.30	7.30	7.80	7.80	8.30	8.30	6.8	7.30	7.80	8.30
6-Jul	7		6.77	6.79	7.28	7.35	7.69	7.73	7.94	8.36	6.92	6.85	7.30	7.34	7.65	7.71	8.04	7.93	6.71	7.10	7.37	8.09
7-Jul	8	23.7	6.81	6.84	7.33	7.41	7.75	7.78	8.08	8.40	6.88	6.86	7.34	7.38	7.83	7.84	8.22	8.03	6.76	7.23	7.61	8.25
8-Jul	9	21.4	6.73	6.77	7.27	7.28	7.62	7.78	8.09	8.36	6.82	6.75	7.29	7.30	7.80	7.82	8.19	8.00	6.72	7.17	7.52	8.10
9-Jul	10		6.78	6.80	7.26	7.29	7.50	7.76	8.03	8.35	6.87	6.74	7.31	7.23	7.68	7.80	8.14	7.68	6.71	7.06	6.92	8.07
11-Jul	12	21.0	6.79	6.84	7.26	7.33	7.50	7.73	7.99	8.31	6.87	6.59	7.21	7.34	7.60	7.74	7.86	6.62	6.66	6.97	6.63	8.05
14-Jul	15	23.4	6.76	6.84	7.21	7.31	7.43	7.70	7.91	8.14	6.50	6.16	6.56	7.32	7.48	7.62	7.18	6.05	6.70	6.88	6.90	6.88
15-Jul	16	23.4	6.77	6.84	7.24	7.30	7.61	7.78	8.07	8.18	6.38	6.26	6.40	7.23	7.46	7.62	7.38	6.75	6.68	7.12	7.14	7.49
17-Jul	18	23.8	6.75	6.81	7.21	7.28	7.53	7.76	8.07	8.28	6.16	5.97	6.02	6.96	6.70	7.13	6.79	6.09	6.72	7.04	7.11	7.27
18-Jul	19	24.8	6.70	6.77	7.25	7.21	7.55	7.68	8.02	8.11	6.04	6.12	6.29	6.82	6.41	6.76	6.57	6.50	6.81	7.09	7.17	7.61
20-Jul	21	25.0	6.84	6.79	7.24	7.31	7.58	7.82	7.51	8.22	5.86	5.92	6.79	6.64	6.12	6.17	6.26	7.37	6.92	7.18	7.30	7.82
22-Jul	23	22.2	6.76	6.74	7.16	7.25	7.30	7.74	6.17	8.14	5.86	5.90	7.30	6.25	6.10	6.07	6.23	7.52	6.69	7.01	7.08	7.58

Total NH3/NH4 -N conc.

Date	ay	Anaerobic Effluent as Feed 6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	Aerobic Effluent as Feed 6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	Control 6.8	7.3	7.8	8.3
29-Jun	0	1,142		1,176		1,274		1,231		774		757		805		875		800	800	800	800
6-Jul	7	1,234	858	839	834	595	760	433	1,276	586	598	614	647	912	663	572	420	600	581	538	553
12-Jul	13	1,251		1,449		782		582		979		1,174		849		744		656			
15-Jul	16	1,026	1,070	1,060	940	556	956	441	974	766	590	553	746	628	700	547	336	452	370	318	275
20-Jul	21	1,227	891	1,592	1,311	846	1,433	626	1,400	930	843	<100	911	764	1,000	563	17	586	538	406	316

VOLATILE SUSPENDED SOLIDS

Date	ay	Anaerobic Effluent as Feed 6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	Aerobic Effluent as Feed 6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b
29-Jun	0	1675	1875	1875	1650	1275	1800	2025	1875	2350	2250	1325	1575	1675	1575	1625	1675
23-Jul	24	1100	790	920	1040	700	1360	580	1130	1570	1767	1400	1650	1550	1700	1500	1375

NITRITE CONCENTRATION

Date	ay	6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b
29-Jun	0																
6-Jul	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15-Jul	16	0		0		0		0			140	212		0			162
20-Jul	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

note zero means "not detected"

NITRATE CONCENTTRATION

Date	ay	6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b
29-Jun	0																
6-Jul	7	5	2	2	3	1	2	2	3	4	1	2	3	2	2	1	2
15-Jul	16	2	0	0	0	0	0	0	0	0	10	6	0	1	0	0	8
20-Jul	21	2.5	1.3	0.3	0	2.1	0	15.2	2.5	324	448	97	18	165	81	285	102

note zero means "not detected"

COD

Date	ay	6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b
29-Jun	0	8000	8000	8000	8000	8000	8000	8000	8000	2667	2667	2667	2667	2667	2667	2667	2667
6-Jul	7	3970		2975		3770		2380		2180		3370		4960		2180	
9-Jul	10	4960		3770		3370		2580		2180		2975		2580		2180	
25-Jul	26	3680	4480	3520	4480	2560	4000	1920	4000	3040	3040	2560	3040	2880	3040	2560	2080

BOD5

Date	ay	6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b	6.8a	6.8b	7.3a	7.3b	7.8a	7.8b	8.3a	8.3b
29-Jun	0	3930	3930	3930	3930	3930	3930	3930	3930	155	155	155	155	155	155	155	155
25-Jul	26	<10	<10	<10	27	21	45	<10	57	22	34	26	62	23	22	5	8

Volatile Suspended Solids Values

		Trace Metals Effect Study					Kinetic Study				
HRT:		50 D	25 Da	16 Da	12 Da	10 Da	10 Da	12 Da	16 Da	25 Da	50 Days
		VSS	VSS	VSS	VSS	VSS	VSS	VSS	VSS	VSS	VSS
18-May	0										
20-May	2	6100	5050	5050	3550	4150	3600	3900	3350	3600	3800
24-May	6	3250	3150	3150	3200	2650	2200	2650	2800	2750	2700
28-May	10	1900	2200	1950	1550	1550	2050	1150	1150	1900	1800
1-Jun	14		1675	1600	1550	1525	1725	2050	1600	2000	2000
8-Jun	21	1675	1350	1200	1200	1275	975	1250	1275	1625	1425
12-Jun	25	2250	1650	1350	1125	1200	1250	1300	1325	1500	1525
22-Jun	35	1750	1400	1300	1200	1050	1125	1300	1325	1425	1500
29-Jun	42	1425	1275	1200	775	1275	1050	1375	1350	1300	1550
7-Jul	50	1050	1075	775	850	1050	825	600	900	1025	1300
13-Jul	56	1025	1375	633	450	550	400	762	817	675	1300
20-Jul	63	1150	650	683	588	600	430	700	900	1250	1550
3-Aug	77	1300	925	750	838	700	600	625	1000	1000	1000
12-Aug	86						520	588	950	1075	1350
18-Aug	92						620	662	783	800	600
30-Aug	104						580	560	600	710	750
Average							568	642	850	934	1121
Std Dev.							141	71	133	211	347

Kinetic Study - COD Values

Feed without added Metals

		10 Days		12 Days		16 Days		25 Days		50 Days		Feed
		Se	So-Se	Se	So-Se	Se	So-Se	Se	So-Se	Se	So-Se	Conc.
18-May	0											
19-May	1	3900	13000	3500	13400	3100	13800	3500	13400	3500	13400	17300
21-May	3	4330	12437	4330	12437	1970	14797	3150	13617	3150	13617	16500
23-May	5	7100	10467	5100	12467	4330	13237	3900	13667	2360	15207	16500
25-May	7	4330	12970	5100	12200	2750	14550	2750	14550	3500	13800	19700
27-May	9	4700	13133	4700	13133	3900	13933	3900	13933	4300	13533	15700
29-May	11	5500	11567	5500	11567	4300	12767	3500	13567	3500	13567	18100
31-May	13	8300	9200	5000	12500	5400	12100	3480	14020	3680	13820	17400
2-Jun	15	6200	10960	6000	11160	4450	12710	2700	14460	3100	14060	17000
6-Jun	19	7320	9600	7930	8990	5290	11630	2850	14070	3050	13870	17080
9-Jun	22	7730	9318	8450	8598	4470	12578	3460	13588	2850	14198	16680
13-Jun	26	9830	7320	9830	7320	5290	11860	4160	12990	3780	13370	17385
16-Jun	29	10015	7475	11530	5960	4910	12580	5100	12390	3590	13900	17385
20-Jun	33	11020	6838	12390	5468	5510	12348	3930	13928	2560	15298	17700
23-Jun	36	10820	6493	12390	4923	8660	8653	4525	12788	2560	14753	18490
27-Jun	40	7500	9913	8625	8788	5250	12163	4500	12913	3375	14038	15750
30-Jun	43	12375	4552	13500	3427	7310	9617	5810	11117	3000	13927	18000
5-Jul	48	13548	2772	13160	3160	6774	9546	5032	11288	2322	13998	17030
8-Jul	51	13550	2637	14320	1867	7350	8837	6580	9607	3480	12707	13930
11-Jul	54	14400	1880	16000	280	9200	7080	7800	8480	5600	10680	17600
14-Jul	57	15150	1993	16130	1013	9640	7503	8660	8483	4520	12623	17310
18-Jul	61	13380	4057	14360	3077	8260	9177	7870	9567	3340	14097	16520
26-Jul	69	15200	3473	14800	3873	10000	8673	8800	9873	4200	14473	18480
2-Aug	76	14480	5060	16860	2680	11700	7840	10510	9030	6150	13390	21020
10-Aug	84	15610	2753	15220	3143	9760	8603	9365	8998	4680	13683	19120
18-Aug	92	13970	3065	15147	1888	10820	6215	9840	7195	3930	13105	14950
30-Aug	104	16300		16100		13400		11800		6540		
54-76 eff			18%		12%		44%		50%		72%	18186
Averages		14823	3195	15415	2604	10657	7361	9698	8321	4807	13211	18018
std dev		938	1132	828	1280	1604	1032	1361	877	1121	1250	1940

SUMMARY OF DATA AND CALCULATED VALUES

θ=θc	X	Se-Snd	So-Se	Effic	Rem/V	F/M
10	570	11923	3194.7	18%	0.56	2.7
12.5	640	12515	2604	14%	0.33	1.9
16.667	850	7757	7361.3	41%	0.52	1.1
25	934	6798	8320.5	46%	0.36	0.6
50	1120	1907	13211	73%	0.24	0.3

(So-Se)/Xθ	1/θc	1/(Se-Snd)	Xθ/(So-Se)
0.5605	0.10	0.000084	1.78
0.3255	0.08	0.000080	3.07
0.5196	0.06	0.000129	1.92
0.3563	0.04	0.000147	2.81
0.2359	0.02	0.000524	4.24

r=	0.89	r=	0.91
Y=	0.19	Ks=	3500
Kd=	0.028	k=	0.68

1/θc	Se
0.1	14823
0.08	15415
0.06	10820
0.04	9840
0.02	4807

Snd = 2900
(non-degradable COD)

Volatile Fatty Acids by GC — Daily Data Log

	Feed WITH TRACE METALS											
	2ml 50 D	4ml 25 D	6ml 16 D	8ml 12 D	10ml 10 D	Feed w/ Metals	10 ml 10 D	8ml 12 D	6ml 16 D	4ml 25 D	2ml 50 D	Feed w/ O metals
Date: 7/14												
Acetic Acid	1803	2418	2638	2651	2543	905	2650	3521	1477	1533	554	1010
Propionic	546	600	689	693	682	260	964	1050	538	470	28	379
iso-Butyric	0	0	381	63	510	0	304	230	0	0	0	0
n-Butyric	369 ?		1061	995	767	286	944	?	0	0	0	277
Date: 8/2												
Acetic Acid	3663	4248	4716	4634	4665	3243	4700	4698	3162	3149	556	4412
Propionic	925	1129	1262	1252	1414	910	1236	1266	1155	1056	73	1228
iso-Butyric	175	603	850	756	546	194	542	719	317	168	-	293
n-Butyric	235	1033	1256	1309	1337	703	1138	1109	278	264	-	800
Date: 8/10												
Acetic Acid							3005	2855	1601	1796	299	3355
Propionic							865	845	900	772	-	865
iso-Butyric							300	408	145	151	-	329
n-Butyric							922	567	-	75	-	546
Date:												
Acetic Acid												
Propionic												
iso-Butyric												
n-Butyric												

Kinetic Study - COD Values

Feed with Trace Metals

		50 Days		25 Days		16 Days		12 Days		10 Days		Feed
	Da	Se	So-Se	Se	So-Se	Se	So-Se	Se	So-Se	Se	So-Se	Feed Conc.
18-May	0											
19-May	1	3900	15800	3500	16200	3900	15800	3900	15800	3500	16200	19700
21-May	3	5100	12200	2750	14550	5500	11800	2360	14940	4330	12970	17300
23-May	5	2750	12950	3150	12550	3150	12550			1100	14600	15700
25-May	7	3500	13000	3900	12600	3500	13000	4700	11800	5500	11000	16500
27-May	9	3500	12200	4300	11400	3500	12200	5700	10000	5900	9800	15700
29-May	11	3500	13800	5500	11800	5100	12200	6300	11000	6700	10600	17300
31-May	13	3700	13300	5400	11600	5400	11600	5800	11200	7000	10000	17000
2-Jun	15	3900	12700	6000	10600	6000	10600	7200	9400	7700	8900	16600
6-Jun	19	4070	13010	7120	9960	6915	10165	8750	8330	8750	8330	17080
9-Jun	22	4270	12410	7730	8950	8540	8140	9360	7320	8750	7930	16680
13-Jun	26	4720	11910	8690	7940	9070	7560	10960	5670	10015	6615	16630
16-Jun	29	5290	10960	8500	7750	9450	6800	8880	7370	8690	7560	16250
20-Jun	33	4920	14358	9640	9638	10430	8848	11610	7668	11020	8258	19278
23-Jun	36	4525	11999	10030	6494	10820	5704	12390	4134	11210	5314	16524
27-Jun	40	5625	7125	10125	2625	7875	4875	12750	0	6000	6750	12750
30-Jun	43	6375	9375	11812	3938	12000	3750	13500	2250	12375	3375	15750
5-Jul	48	6580	10840	10345	7075	13354	4066	13548	3872	13160	4260	17420
8-Jul	51	6190	10840	12190	4840	13930	3100	13550	3480	13550	3480	17030
11-Jul	54	8200	9000	13000	4200	14400	2800	15200	2000	14400	2800	17200
14-Jul	57	8260	8260	13380	3140	14750	1770	15150	1370	15340	1180	16520
18-Jul	61	7080	9440	11800	4720	12590	3930	14160	2360	13970	2550	16520
26-Jul	69	8800	8240	12800	4240	14000	3040	14400	2640	14400	2640	17040
2-Aug	76	10900	8140	13490	5550	15470	3570	16070	2970	16070	2970	19040
Mean Value		8648	8616	12894	4370	14242	3022	14996	2268	14836	2428	17264
Removal Efficiency		50%		25%		18%		13%		14%		