Phase I Final Report
Anaerobic Pretreatment of Crab Processing Wastewater

Submitted by:

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INTRODUCTION

The crab processing industry in Virginia handles several million pounds of blue crabs per year, and is important to the Commonwealth from an economic and cultural perspective. The industry is mostly composed of small companies which buy crabs each day during the season from local crabbers, and to a lesser degree, out-of-state sources. These crabs must be immediately cooked, cleaned and packaged in order to maintain acceptable sanitary standards. The processors use one or more "cookers", in which they subject the live crabs to steam. As the steam passes over the crabs, it condenses, dissolving a significant load of organic and inorganic compounds, and mixes with brackish water draining from the live crabs. It is typical for a single cook to produce approximately one gallon of wastewater for every 30 pounds of live crabs cooked (Boardman *et al.*, 1993). This wastewater is high in biologically degradable matter, with a typical 5 day BOD (BOD_5) of 20,000 mg/L. By way of comparison, typical municipal sewage has a BOD_5 of 200 mg/L. Since most of the crab processors are small companies located on the waterfront, they usually discharge their wastewater directly into the adjacent body of water.

It is the objective of this project to design and test both laboratory and field high-rate anaerobic treatment systems capable of removing the high level of BOD and suspended solids in the crab processing wastewater. Also, it is our ambition to design a prototype system in such a way that it will be affordable to a small crab processing company, and will minimize the requirement for skilled on-site management.

This report presents results and a discussion of the lab-scale system. Thus far, the pilot-scale system has not been in operation long enough for any data to be collected. As this is Phase I of a two phased study, data for the pilot plant, and a more rigorous analysis of both systems will be presented in the final report for Phase II.

EXPERIMENTAL PLAN

The project consists of two main components: the design and evaluation of a bench-scale treatment system at Virginia Tech in Blacksburg, and the design and evaluation of a pilot-scale system located at Virginia Tech's Seafood Technology Center in Hampton, Virginia.

Bench-Scale Study

Work was begun on the bench-scale systems in September, 1993. Two systems with differing hydraulic flow designs were placed in operation (see Figures 1 and 2). Both systems consist of two anaerobic stages followed by an aerobic stage. The two systems have the same total working volumes (12 liters). System A would best be described as an upflow anaerobic sludge bed (4 liters) with anaerobic clarifier (4 liters), while system B is a two stage sequential upflow packed (urethane foam cubes) bed anaerobic filter (4 liters each). Each system has an aeration tank (4 liters) as the final stage. Both systems were maintained at 33° - 35°C, and fed by a common line delivering refrigerated feed wastewater.

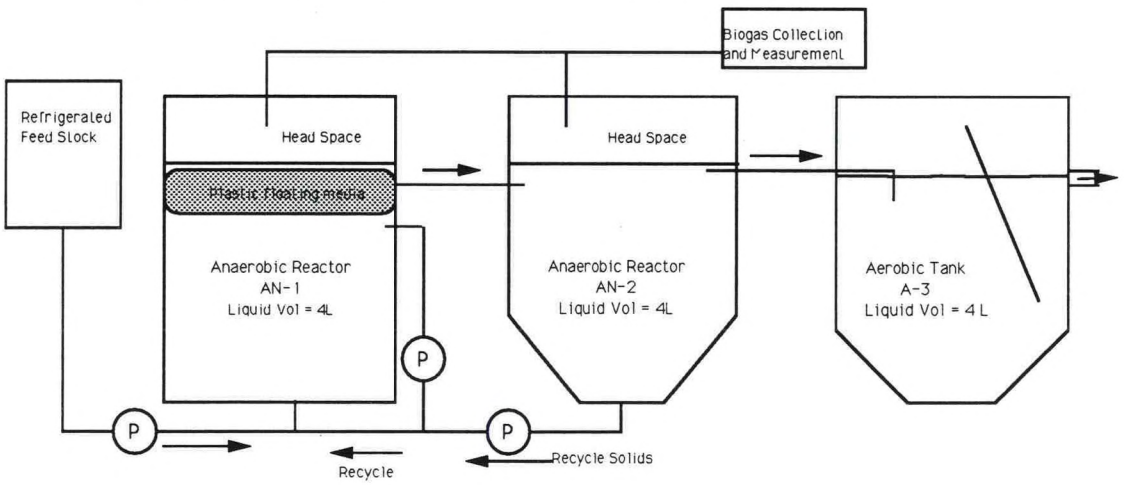

Figure 1 Bench-Scale System A schematic

Systems A and B were innoculated with anaerobic sludge from a local POTW on October 11, 1993 (Day 0), resulting in an initial mixed liquor suspended solids (MLSS) of approximately 5,000 mg/L.

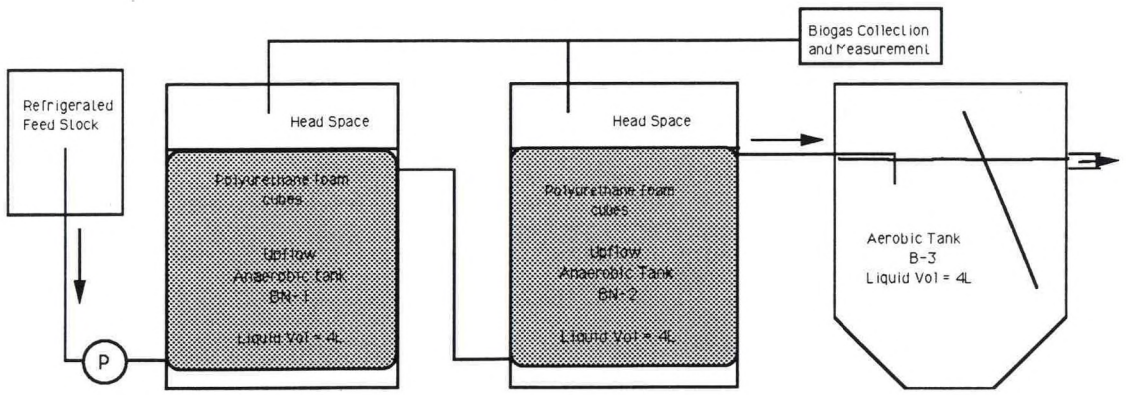

Figure 2 Bench-Scale System B schematic

Crab cooker wastewater was obtained from the Graham & Rollins Seafood Company in Hampton Virginia and stored at 4° C in Blacksburg. It was diluted with tap water to a concentration of approximately 1,000 mg/L chemical oxygen demand (COD) for the initial purpose of acclimation of the bacteria. In order to increase the organic loading rate while keeping the hydrualic flow rate constant, the dilution rate was decreased at approximately one month intervals once the effluent COD stabilized. By the end of December, each system was receiving approximately 12 grams of COD per day, which is equivalent to a volumetric loading rate of about 1 gram of COD per liter of each system.

Calcium carbonate was mixed with distilled water, neutralized to a pH of approximately 7.5 with HCl, and added as a slurry to each anaerobic reactor to provide alkalinity.

Pilot-Scale Experiment

Assembly of the pilot-scale system in Hampton was completed by the end of December and the system is now in operation. Crab cooker wastewater is pumped automatically from one of the two retort cookers in use at the Graham-Rollins Seafood Company, located approximately three hundred feet away from the Virginia Tech Seafood Technology Center. A 250 gallon holding tank in the

wastewater treatment building receives the wastewater. It is equipped with a self-regulating overflow system so that excess wastewater is discharged directly to the Hampton River, as is allowed by the VPDES permit held by Graham-Rollins. A schematic of the pilot-scale system is shown in Figure 3. Wastewater is metered out of the holding tank into the anaerobic reactor (labeled CN-1) which has a working liquid volume of 160 gallons (600 liters). Wastewater flows from CN-1 into the anaerobic clarifier (CN-2), also with a liquid volume of 160 gallons, where settled biomass is returned to the bottom of CN-1, and clear effluent flows to the aeration tank (C-3), which has a liquid volume of 120 gallons. This tank has an integral settling chamber to separate sludge from liquid. Final effluent is discharged to the Hampton River.

This system was inoculated with anaerobic sludge from the same source as the lab-scale systems in Blacksburg. The pilot-scale system is configured identically to lab-scale system A. Currently, 72 liters (approximately 20 gallons) per day of wastewater is being fed into the treatment system. Once test results show that the bacteria have become acclimated to the feed, the feed loading will be increased. The behavior of the lab-scale systems will be used as a guide for this start-up process.

The pilot scale system has a heating system with coils inside tanks CN-1 and CN-2 to maintain their temperature within the range of 30-35°C. A small electric water heater equipped with a continuously running circulator pump provides heated water. Provision is made for the collection of biogas from the anaerobic stages.

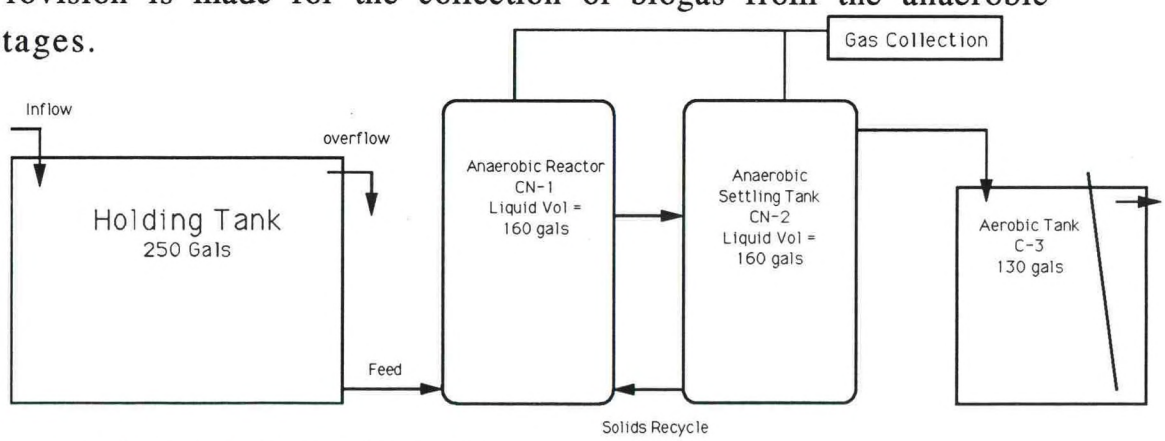

Figure 3 Pilot-Scale System C schematic

Test Methods

At appropriate intervals, the following parameters were measured: chemical oxygen demand (closed micro-reflux method, according to Standard Methods, 1992), five day biochemical oxygen demand (Standard Methods, 1992), total organic carbon (Model DC80 Carbon Analyzer, Rosemont Analytical Inc., Dohrman Division, Santa Clara, CA), ammonia (Orion selective electrode method), alkalinity and volatile fatty acids (two step titration as described by Anderson and Yang (1992), total suspended solids and volatile suspended solids (Standard Methods, 1992). Metals were determined by atomic absorption spectrophotometry (Perken-Elmer Model 703). Biogas was first collected by water displacement, but the high volumes produced per day quickly made that impractical. An alternative technique was adopted wherein biogas was collected daily in plastic bags which were later emptied using water displacement to measure gas volume. Carbon dioxide content and hydrogen sulfide content were analyzed using Sensidyne Analyzer Tubes (Gastec Corporation, Yokohoma, Japan).

RESULTS

The following results are for the bench-scale study. Results for the pilot-scale system will be presented during Phase II of the project.

COD And BOD_5 Removal

Figure 4 shows the soluble COD and soluble BOD_5 reductions in system A with an organic loading of approximately 7.6 grams of COD per day during the period from day 24 to day 55. This loading was accomplished by diluting the full strength wastewater with tap water so as to use a constant hydraulic flow throughout the experiment. Samples were taken periodically of the feed wastewater, and the effluent from each stage. Samples were filtered using 0.45 μm glass

fiber filters. Suspended solids were measured and the filtrate was analysed. COD and BOD were determined for the filtrate. Therefore, the values indicated in this report are for soluble COD (SCOD) and soluble BOD_5 ($SBOD_5$) in the effluents from each reactor.

In system A, SCOD was reduced from a feed concentration of 2,120 mg/L to 499 mg/L, which represents a total reduction of 76%. At the end of a 31 day period, the $SBOD_5$ of 1,425 mg/L in the feed was reduced by 98% to a value of 20 mg/L in the final effluent.

In the B system (Figure 4), the SCOD reduction was 86% to an SCOD of 280 mg/L. The $SBOD_5$ results for system B indicate a final aerobic effluent $SBOD_5$ of 18 mg/L, for a total $SBOD_5$ reduction of 99%.

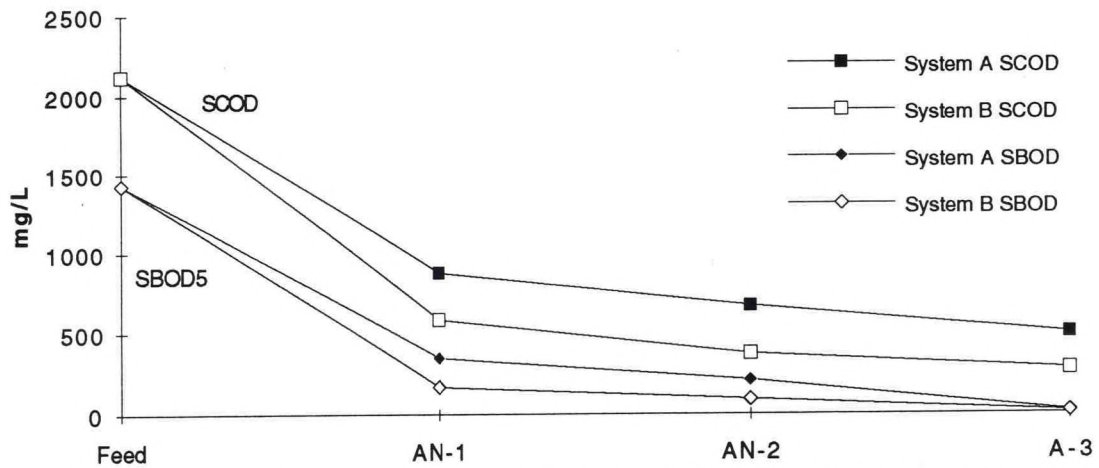

Figure 4 SCOD & SBOD5 at Various Points in Systems A & B. Organic Loading approx. 7.6 gm/day. (AN-1 and AN-2 represent first and second anaerobic stages; A-3 is the aerated third stage.)

The organic loading was step increased to approximately 12.3 grams of SCOD per day (3,429 mg/L) for days 56 through day 90 (Figure 5). The results for SCOD and $SBOD_5$ are shown in Figure 5. System A achieved a total SCOD reduction of 82% to 632 mg/L, and a total $SBOD_5$ reduction of 99% to a value of 6 mg/L. System B achieved an SCOD reduction of 83% to 587 mg/L, with an $SBOD_5$ reduction of 99% to $SBOD_5$ of less than 1 mg/L.

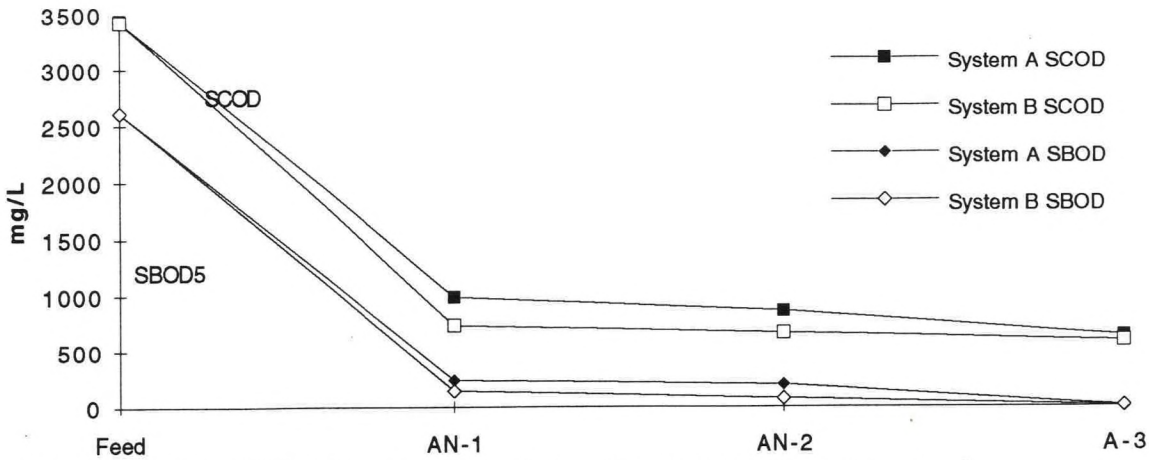

Figure 5 SCOD & SBOD5 at Various Points in Systems A & B. Organic Loading approx. 12.3 gm/day. (AN-1 and AN-2 represent first and second anaerobic stages; A-3 is the aerated third stage.)

Biogas Production

Figures 6 and 7 indicate biogas production for systems A and B, respectively. Between day 40 and day 55, while the organic loading averaged 7.6 grams COD per day, the gas production in system A averaged 2,800 mL per day, with a high value of 3,000 mL and a low of 1,400 mL. During this same period, system B averaged 4,000 mL per day, with a high of 5,800 mL and a low of 2,500 mL.

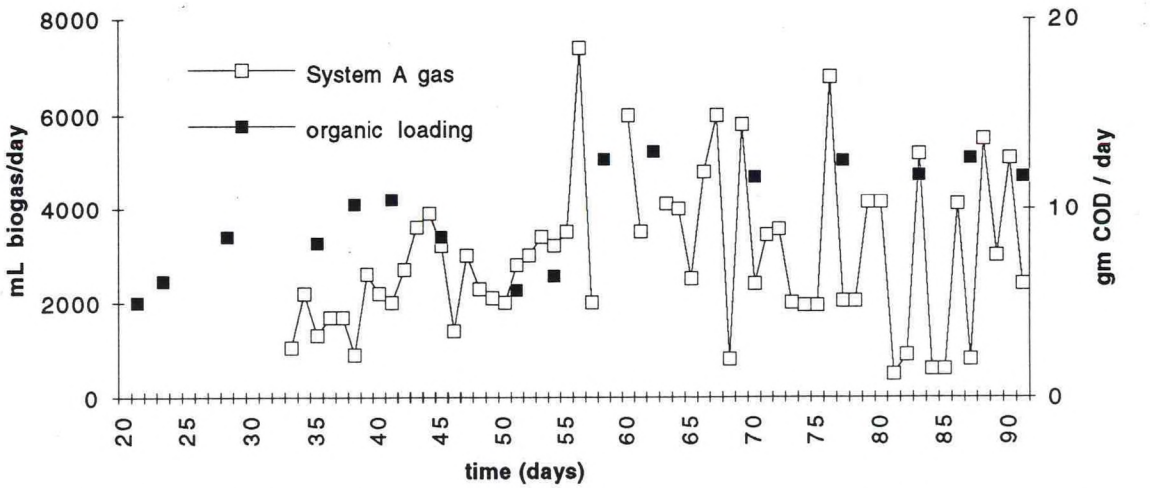

Figure 6 System A Gas Production; time is shown in days from initial innoculation with biomass.

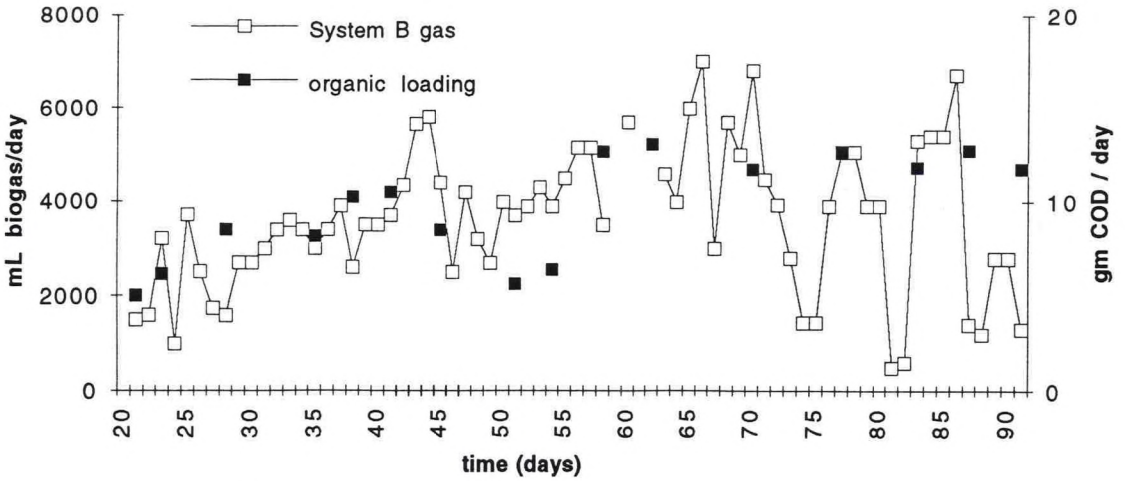

Figure 7 System B Gas Production; time is shown in days from initial innoculation with biomass.

During the period from day 56 through day 90, gas production variability increased. System A produced an average of 3,336 mL of biogas per day, while system B averaged just under 4,000 mL per day. The high production for system A was 7,400 mL while system B's high day was 7,000 mL. Lowest daily production was 500 mL in both systems A and B, co-incidentally on the same day.

The biogas contains approximately 14% CO_2 and less than 1% H_2S by volume. It is assumed that the balance of the gas is methane, although this has not yet been confirmed analytically.

Ammonia

From day 20 to day 55, ammonia-nitrogen (NH_3-N) was measured in the feed at approximately 65 mg/L NH_3-N (Figure 8) . The level of ammonia-nitrogen increased in reactor AN-1 and AN-2 in both systems to about 85 mg/L, and then decreased in the aerobic stages to an average of 30 mg/L.

From day 56 to day 90, the ammonia-nitrogen concentration in the feed averaged 143 mg/L NH_3-N (Figure 7), and this value increased to slightly over 180 mg/L in the anaerobic stages, before decreasing to an average of 94 mg/L in system A's aerated reactor and 111 mg/L in system B's aerated tank.

Data for other forms of nitrogen, along with that for sulfate and phosphate was incomplete during the study period. It is anticipated that a comprehensive picture of the fate of nitrogen and important anions will be elucidated during Phase II of the project.

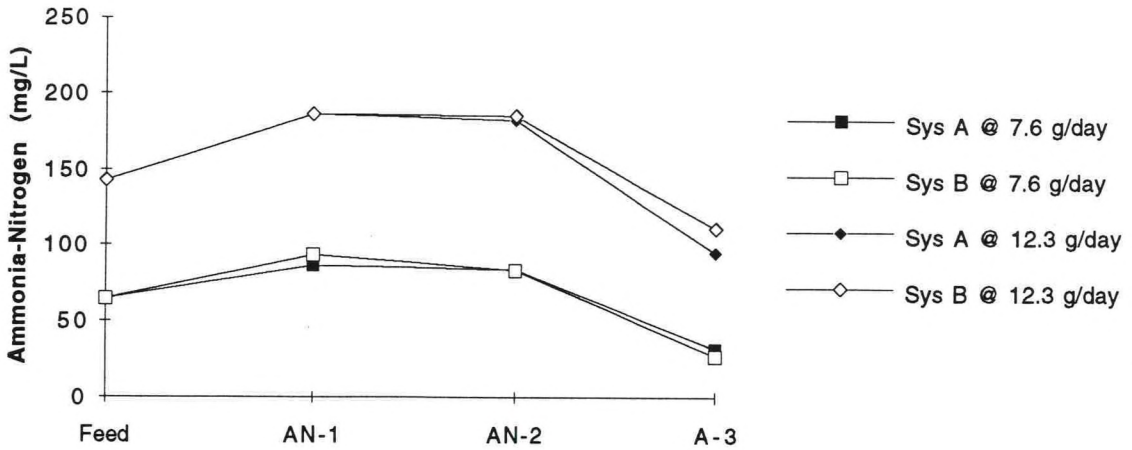

Figure 8 Ammonia-Nitrogen at various COD Loadings in Systems A & B. (AN-1 and AN-2 are the first and second anaerobic stages; A-3 is the aerated third stage.)

Alkalinity, Volatile Fatty Acids and pH

Alkalinity in the wastewater was measured at appoximately 400 mg/L as calcium carbonate (see Table 1). This value increased to as high as 2,348 mg/L as calcium carbonate in the anaerobic reactors due to the added calcium carbonate. The acidity of the feed (pH) was close to neutrality in the feed. The mixed liquor in the anaerobic reactors was slightly basic at pH around 7.8. In the earlier period, at loading of approximately 7.6 g COD/day, the pH in the aerated tanks dropped to about 7.4. However, when the loading was increased to 12.3 g COD/day, the pH of the aerated tanks rose to 8.2 and 8.4 respectively in systems A & B.

At the end of the study period, volatile fatty acid (VFA) concentration decreased in the first anaerobic reactor of each system to essentially zero. In the A system, VFA's reappeared in the aerated reactor but not in the B system.

Table 1. pH, Alkalinity (as Calcium Carbonate) and Volatile Fatty Acid Concentration (as Acetic acid) in Systems A & B at two COD Loadings.

	System A				System B		
	Feed	AN-1	AN-2	A-3	B-1	B-2	B-3
COD Loading = 7.6 g/day							
pH	7.19	7.65	7.75	7.4	7.84	7.81	7.35
Alkalinity (mg/L as CaCO3)	393	1190	1030	143	1325	1253	35
VFA (mg/L as HAc)	570	39	162	135	<6	<6	132
COD Loading = 12.3 g/day							
pH	7.0	7.8	7.8	8.2	7.9	7.9	8.4
Alkalinity (mg/L as CaCO3)	441	1851	1906	686	2348	2148	1257
VFA (mg/L as HAc)	1284	<6	<6	84	<6	<6	<6

Metals

Metals were analyzed in the feed solution using atomic absorption spectrophotometry. Table 2 shows the levels of those metals.

Table 2. Metal concentrations in Crab Processing Wastewater.

Common Metals			Trace Metals		
Calcium	274	mg/L	Cadmium	1	µg/L
Magnesium	260	mg/L	Chromium	3	µg/L
Sodium	3325	mg/L	Cobalt	1	µg/L
Potassium	646	mg/L	Molybdenum	3	µg/L
			Nickel	26	µg/L

DISCUSSION

COD and BOD_5 Removal

Organic removal performance differs slightly between systems A and B, with system B showing slightly better performance in terms of soluble COD. When considering soluble BOD_5, there does not appear to be an important difference in the two designs. Most of the

removal occurs in the first anaerobic reactor, with less removal in the second anaerobic reactor and the aerated tank.

Biogas Production

Biogas production generally tracks COD loading, but with increasing variability in its daily production. As the loading is increased, there appears to be a cyclic pattern of gas production. System A tends toward a cycle with peaks of production roughly every 4 days. System B tended to exhibit a peak every 5 days. No statistical analysis has been performed to determine if the difference in the behavior of the two systems is significant.

Ammonia

Ammonia-nitrogen, which is present in the full strength wastewater at a concentration of several hundred milligrams per liter, increases in concentration in the anaerobic reactors due to the hydrolysis of proteins and the deamination of the resulting amino acids. Some of this ammonia is converted to nitrite and nitrate in the aeration stage. However, much remains as ammonia. It is believed that improved performance from the aeration stage can be achieved, resulting in the increased conversion of ammonia to nitrite and nitrate. Work during Phase II of this study will focus on biological nitrogen removal by denitification, and also air-stripping of the remaining ammonia.

Volatile Fatty Acids and pH

Volatile fatty acids (VFA) are generated by the group of microorganisms generally referred to as "acidogens". According to Ferry (1993) they utilize organic macromolecules such as proteins, lipids and carbohydrates as electron donors generating short chain fatty acids, such as acetic, propionic, and butyric acids. By-products are hydrogen gas and carbon dioxide.

Another group, which are known as the "acetogens", utilize some of these shorter molecules and produce acetate. They are extremely sensitive to the partial pressure of hydrogen, which is inhibitory. Methanogens utilize hydrogen and acetate to reduce carbon dioxide to methane releasing energy in the process and maintaining a low partial pressure of hydrogen in the process, allowing the acetogens to produce more acetate. A build-up of short chain fatty acids is therefore indicative of an inhibition of the methanogens, which in turn leads to inhibition of the acetogens. Thus, methane production is closely tied to fatty acid concentration.

At the loadings tested thus far, our data does not indicate an accumulation of VFA. However, our sampling frequency may miss short term build-ups corresponding with methane production curtailment. Future studies will be designed to elucidate this.

Toxicity Concerns

There was initial concern regarding toxicity as a result of a previous study (Boardman *et al.*, 1993). Sodium levels were suspected of being a problem in the earlier study. However, toxicity has not yet been detected in this work. Soto *et al.* (1993 reported that sodium did not exert a inhibitory effect on the biological treatment of a similar wastewater (fish canning waste) until it reached a level of about 6,000 mg/L. As noted in Table 2, sodium concentrations in the feed to the reactors are well below 6,000 mg/L. It is hoped that as the strength of feed in this study is slowly increased, the bacteria will have an opportunity to become acclimated to the total dissolved solids concentration of the wastewater. Nonetheless, it is important to note that toxicity is a complex issue, and there may be additive or synergistic effects due to the presence of numerous cations in solution. This concern is addressed at some length in the study of blue crab waste treatability referenced earlier (Boardman *et al.*, 1993).

Another possible toxicity problem relates to the ammonia concentration. Previous studies have reported no inhibitory effect on methanogens until ammonia reached a concentration in the range of 3,000 to 4,000 mg/L at the pH values typically attained in these reactors (Soto *et al.*, 1991). Since ammonia levels are not anticipated to approach such values in this study, it is not anticipated that ammonia will become inhibitory. However, there is the possibility, again, that some additive or synergistic effects may occur resulting in inhibition being seen at lower levels of ammonia.

CONCLUDING REMARKS

The results of Phase I as they relate to COD and BOD_5 removal are very encouraging. As more data on biogas production is collected, a better understanding of the relationship between organic loading and gas production will be developed. We would also like to better understand the gas production cycle as it relates to the metabolism of the various populations within the micro-flora.

The removal of nitrogen presents a significant challenge. The anaerobic environment generates ammonia from protein catabolism. The ammonia must be removed before discharge. This will be accomplished through a combination of biological denitrification and air-stripping.

Assembly of the pilot-scale plant was hampered by equipment and facility delays and bad weather. Additionally, a continuous supply of wastewater was not available once the crab potting season ended in late fall. Only those crabs obtained through dredging were available to process. However, since the pilot-scale system is now in full operation, there will be time in Phase II to satisfactorily evaluate the effectiveness of the pilot-plant.

Several photographs are included showing the assembly of the pilot plant in Hampton, as well as the bench-scale sytems in

Blacksburg. Also included is a photograph of the collection basin and transfer pump located on-site at the crab processing plant in Hampton.

ACKNOWLEDGEMENTS

We wish to thank the DEQ and NOAA for the funding which has made possible this study of the anaerobic pretreatment of crab processing wastewater. We also wish to express our appreciation to Mr. John Graham, president of Graham-Rollins,Inc., for his cooperation and assistance in this effort. Also, the staff at the Virginia Tech Seafood Technology Center, particularly Mr. Tom Rippen and Mr. Robert Lane, have been instrumental in the successful initiation of this project.

REFERENCES

Anderson, G.K., and G. Yang. 1992. Determination of Bicarbonate and Total Volatile Acid Concentration in Anaerobic Digesters Using a Simple Titration. Water Environment Res. Vol. 64, no 1. pg. 53-59.

Boardman, G.D. , G.J. Flick, T. Harrison, and C. Wolfe. 1993. Management and Use of Solid and Liquid Wastes from the Blue Crab (*Callinectes sapidus*) Industry, Part I: Waste Treatability Studies. National Sea Grant Office (in press).

Ferry, James G., ed. 1993. Methanogenesis. Chapman & Hall. New York.

Soto, M., R. Mendez and J.M. Lema. 1991. Biodegradability and Toxicity in the Anaerobic Treatment of Fish Canning Wastewaters. Environmental Technology. Vol 12. pg 669-677.

Soto, M., R. Mendez, and J. Lema. 1993. Sodium Inhibition and Sulphate Reduction in the Anaerobic Treatment of Mussel Processing Wastewaters. J. Chem.Tech. Biotechnol. Vol 58. pg1-7.

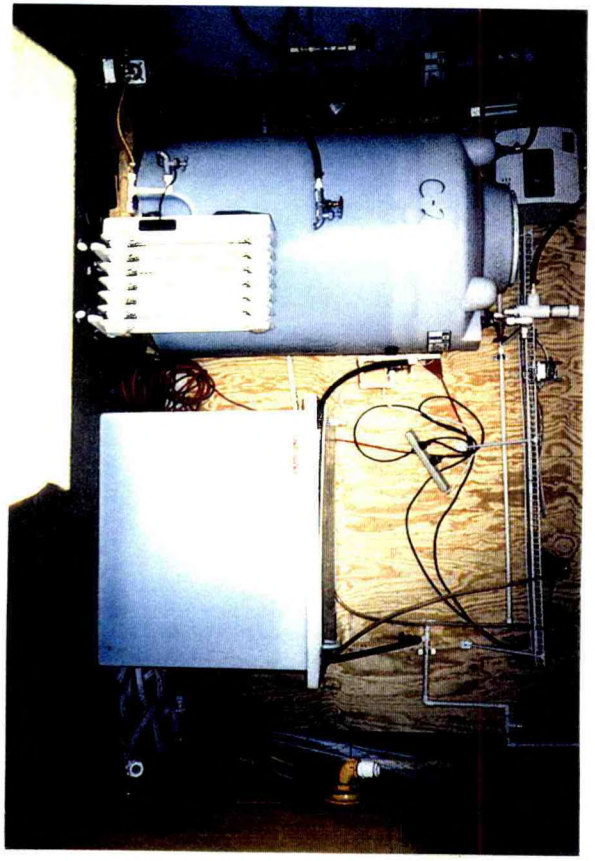

Photo 3 Inside pilot plant building in Hampton, VA, showing reactors CN-1, CN-2, C-3 and a partial view of the primary gas storage tank.

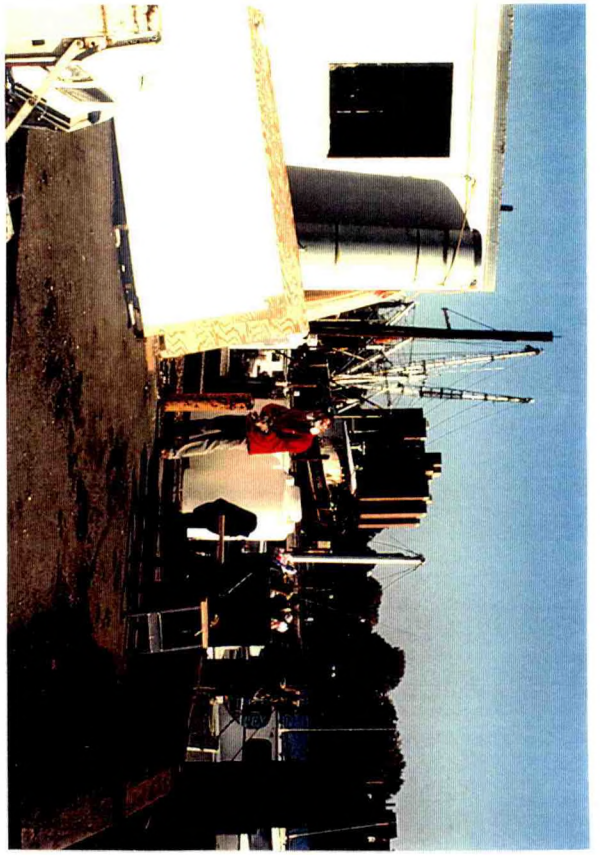

Photo 2 View of pilot plant building in Hampton, VA, showing building renovation and plant assembly in progress.

Photo 1 Pilot plant assembly in progress at the Virginia Tech Seafood Technology Center in Hampton, VA.

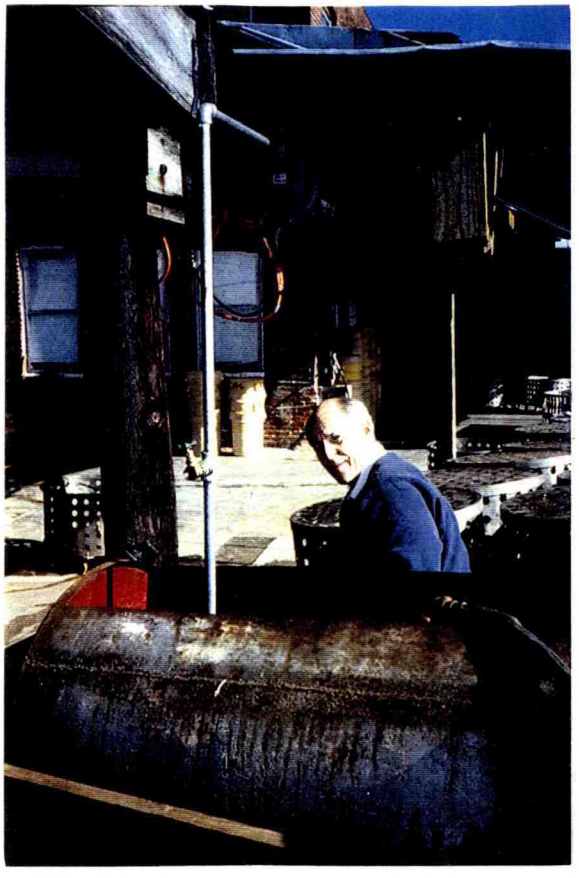

Photo 4 View of wastewater collection basin with transfer pump (overhead) located on site at Graham-Rollins, Inc. in Hampton, VA, approximately 300 feet from the Seafood Technology Center.

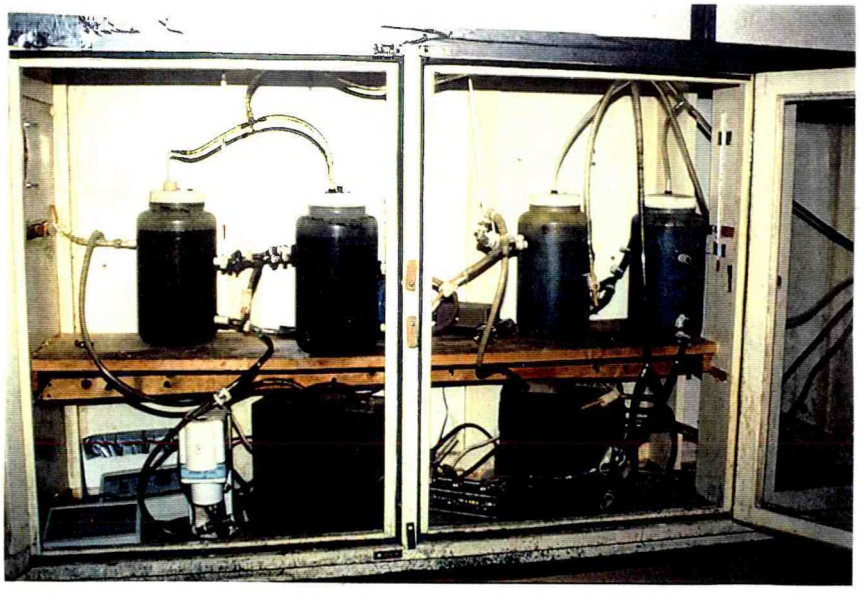

Photo 5 Bench-scale anaerobic/aerobic treatment system A (in the left half of the temperature control cabinet) and system B (in the right half of the cabinet) located in Blacksburg, VA.