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Development of the Static Granular Bed Reactor for full-scale application

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Development of the Static Granular Bed Reactor for full-scale application

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

Biological treatment systems have been found to be one of the most cost effective processes for treating agricultural, industrial, and food processing wastewaters. Biological processes convert organic wastes into useable end products: methane, carbon dioxide, and water. High-rate anaerobic treatment processes are particularly effective treating these types of wastewaters and have many advantages compared to other biological processes. Benefits include energy savings and low sludge production, compared to aerobic systems. High-rate anaerobic processes are capable of higher organic loads (OLR) and shorter hydraulic retention times (HRT) than low-rate systems. Due to shorter hydraulic retention times and higher OLR capacities, high-rate anaerobic systems are typically smaller in size than their low-rate counterparts.

A new anaerobic biological process has been developed by Ellis and Mach (US patent pending, Serial No. 60/302,504) in the biotechnology research and development group in the Civil, Construction, and Environmental Engineering Department at Iowa State University. This new process is called the "Static Granular Bed Reactor" (SGBR). The SGBR uses a downflow reactor filled with active, anaerobic granular biomass. Influent wastewater is distributed evenly across the reactor and passed downward through the granule bed. Several laboratory-scale studies have demonstrated the superior performance in terms of COD and TSS removal and effluent volatile fatty acids concentration of the SGBR treating both low and medium-strength wastewater.

Thesis Organization

The sections of this thesis are organized into chapters, covering two separate research projects using the SGBR. Chapter 2 details an on-site pilot demonstration of the SGBR treating slaughterhouse wastewater. Chapter 3 pertains to a laboratory study that investigated the effects of pentachlorophenol addition on the SGBR. Supplemental material from each research project is contained in the appendices at the end of the document. Appendices A and B pertain to the project discussed in Chapter 2. Appendix C pertains to the project discussed in Chapter 3. The overall objective of these research projects was to evaluate the performance of the SGBR at various conditions. Prior to this research, the SGBR had not been tested at pilot-scale or subjected to loadings with inhibitory compounds. The information gathered from these two studies will enhance the development of this new and novel biological treatment system.

CHAPTER 2. ON-SITE PILOT DEMONSTRATION OF THE STATIC GRANULAR BED REACTOR (SGBR)

Background

SGBR process

The static granular bed reactor (SGBR) consists of a dense bed of anaerobic granules operated in a downflow mode without flow recirculation (Mach and Ellis, 2000). The system does not require recirculation pumping, solids/liquid/gas separation devices, or complex underdrain or backwashing systems (Mach and Ellis, 2000). The SGBR is capable of maintaining high solids retention times (SRTs) despite changes in hydraulic retention times (HRTs) (Jung *et al.*, 2002). The dense granule arrangement inside the reactor increases the contact between microorganisms and the wastewater. The efficient granule structure also promotes syntrophic relationships between the various microorganisms involved in the breakdown of the wastewater being treated by the system.

The first research with the SGBR system compared two SGBRs with different height to diameter ratios. SGBR 1 had a diameter of 4 inches whereas SGBR 2 had a diameter of 2.5 inches, each with a one liter working volume (Mach and Ellis, 2000). These reactors were fed a synthetic substrate consisting of non-fat dry milk amended with sodium bicarbonate and micronutrients at a strength of 1 g COD/L (Mach and Ellis, 2000). The work of Mach and Ellis (2000) compared the performance of the two systems under various HRTs (36- to 6-hours) at ambient temperatures (22 ± 2°C). Total and soluble chemical oxygen demand (COD) removal was greater than 94% for both reactors throughout the research period (Mach and Ellis, 2000). Overall, Mach and Ellis (2000) concluded that the SGBR with a larger height to width ratio (SGBR 2) was more representative of a plug flow system, and resulted in better performance than the other SGBR with a smaller height to width ratio (SGBR 1).

Comparison study

The objective of this research was to study the high-rate anaerobic treatability of Hormel Foods wastewater and compare the performance of the Anaerobic Sequencing Batch Reactor (ASBR) and SGBR systems over a range of HRTs and organic loading rates (OLRs). Influent characteristics of the Hormel Foods wastewater used in the laboratory study were as follows: 1,912 ± 782 mg COD/L, 480 ± 340 mg VFA/L, 534 ± 184 mg SS/L, 800 ± 390 mg BOD_5/L, and pH 6.7 ± 0.4 (Jung *et al.*, 2002).

Both systems were operated over a range of HRTs from 48- to 8-hours. Over the length of the entire study, the SGBR outperformed the ASBR in terms of COD removal efficiency, BOD_5 removal efficiency, effluent volatile fatty acid (VFA) concentration, and

effluent suspended solids (SS) concentration. Better performance of the SGBR was especially noticeable at HRTs less than 24 hours. As a result of biomass washout during decanting, COD removal decreased and effluent SS concentration increased in the ASBR as HRT was lowered from 24- to 18-hours. Complete failure of the ASBR system occurred at an 8-hour HRT. Table 1 summarizes the results of the SGBR and ASBR systems from the laboratory comparison study.

Table 1. Summary of results for ASBR and SGBR over entire study (Jung *et al.*, 2002).

Characteristic	ASBR	SGBR
COD removal efficiency, (%)	75-93	89-96
BOD_5 removal efficiency, (%)	91.8 (average)	96.3 (average)
Effluent VFA, (mg/L as HAc)	65 ± 21	25 ± 24
Effluent SS, (mg/L)	143 ± 201	26 ± 16
Specific Methanogenic Activity (SMA), (g-CH_4/g-VSS/d)	0.751	1.018
Effluent pH	7.4 ± 0.4	7.6 ± 0.4

Objectives

Successful performance of the laboratory SGBR led to the development of an on-site pilot-scale system treating slaughterhouse wastewater from Hormel Foods Corporation. This system operated under various HRTs in order to demonstrate this technology on a large scale basis and to develop full-scale design parameters. Results from the pilot study were compared to other high-rate anaerobic digestion configurations treating slaughterhouse wastewater.

Literature Review

Anaerobic treatment of industrial wastewaters

Anaerobic treatment of industrial wastewaters has advanced in development due to the design of reactors which incorporate immobilized biomass. The advantage of using systems with immobilized biomass allows for high loading rates at short hydraulic retention times (Forster, 1994). Borja *et al.* (1995) reported the use of a hybrid reactor combining an anaerobic filter and a sludge blanket that was highly efficient in retaining biomass and led to biomass accumulation over time. A survey by Wheatley *et al.* (1994) reported European industrial anaerobic systems predominately treat food and fermentation wastes as well as wastewaters from the paper industry.

Pérez *et al.* (1997) reported the use of two high-rate anaerobic thermophilic treatment systems, an anaerobic filter (AF) and a fluidized bed, for the treatment of distillery wastewater. The fluidized bed was capable of achieving a maximum organic removal efficiency of 97% at an OLR of 32 kg COD/m^3·d compared to 75% organic removal efficiency for the AF at an OLR of 20 kg COD/m^3·d. Overall, the fluidized bed was capable of operating at higher OLRs and achieved higher COD removals than the AF (Pérez *et al.*, 1997).

Codigestion of olive oil mill effluents (OME) and swine manure was reported using an upflow anaerobic sludge blanket (UASB) reactor (Angelidaki *et al.*, 2002). At high COD concentrations anaerobic degradation of OME wastewaters is unstable due to the lack of ammonia, alkalinity, and the inhibitory effects of polyphenols (Angelidaki *et al.*, 2002). Batch experiments showed that digestion of OME or swine manure alone was inhibitory due to low nitrogen content and/or high aromatic compound concentrations and high ammonium concentrations in the wastes, respectively (Angelidaki *et al.*, 2002). Seventy-five percent COD reduction was achieved with a 50/50 mixture (by weight) of OME and swine manure (Angelidaki *et al.*, 2002).

The use of UASB reactors for high-rate anaerobic treatment of instant coffee wastewater was studied by Dinsdale *et al.* (1996). Both thermophilic and mesophilic treatment was investigated. Overall, the thermophilic UASB was able to achieve 70% COD removal at an OLR of 11.4 kg COD/m^3·d whereas the mesophilic UASB achieved 78% COD removal at an OLR of 10 kg COD/ m^3·d (Dinsdale *et al.*, 1996).

Benefits of anaerobic treatment

High and medium strength wastewaters with considerable nitrogen content can be treated by anaerobic processes (Ruiz *et al.*, 1997). The nitrogen content, along with other characteristics, of typical slaughterhouse wastewaters makes it a good candidate for treatment using anaerobic systems. These other favorable characteristics include: high concentration of biodegradable organics, sufficient alkalinity, adequate phosphorus, adequate micronutrients, low toxic compound concentrations, and temperatures between 20 and 30°C (Massé and Masse, 2000). Benefits include energy production in the form of methane, low sludge production, no aeration cost, and destruction of pathogens (Johns, 1995; Massé and Masse, 2000; Mateu *et al.*, 1992; Sayed *et al.*, 1988; Wheatley *et al.*, 1997). Anaerobic processes are also beneficial for small operations or slaughterhouses that operate on a limited basis because anaerobic bacteria can survive for long periods without being fed (Massé and Masse, 2000).

High-rate anaerobic processes are capable of operating at higher organic loads and shorter hydraulic retention times than low-rate systems (Johns, 1995). Due to shorter hydraulic retention times and higher OLR capacities, high-rate anaerobic systems are typically smaller in size than their low-rate counterparts.

Limitations in treating slaughterhouse wastewaters

High protein and lipid contents make degradation of slaughterhouse wastewaters by anaerobic systems difficult (Salminen and Rintala, 2002). Unionized ammonia from protein degradation and long-chain fatty acids (LCFA) from lipids can be inhibitory at high concentrations (Rinzema *et al.*, 1994; Salminen and Rintala, 2002).

Accumulation of coarse suspended solids, colloidal matter, or soluble substrate components from slaughterhouse wastewaters can negatively affect the performance and stability of anaerobic treatment processes (Ruiz *et al.*, 1997). Sayed *et al.* (1988) also experienced poor removal of coarse SS in a UASB treating slaughterhouse wastewater. Johns (1995) reported that the use of large-scale dissolved air flotation (DAF) units for pretreatment of slaughterhouse wastewaters to remove suspended solids, oils, fats, and greases were unreliable or required large quantities of chemical addition for successful pretreatment. Massé and Masse (2001) found longer reaction periods were needed with high SS concentrations at lower temperatures.

Influent wastewater temperatures, which typically range worldwide between 20-35°C, can also be an issue in high-rate anaerobic treatment (Johns, 1995). High temperatures coupled with the high fat content of most slaughterhouse wastewaters can lead to treatment difficulties due to fat emulsification (Johns, 1995).

Massé *et al.* (2001) found particulate hydrolysis and acidification to be limiting factors during the start-up of ASBRs treating slaughterhouse wastewaters at 20 and 25°C. Acidification of the soluble fraction of slaughterhouse wastewater was found to be the rate-limiting step in the conversion to methane-COD (Sayed *et al.*, 1988). Sayed *et al.* (1984) also found the low rate of hydrolysis to be the limiting factor in UASB treatment of slaughterhouse wastewater at low temperatures (20°C). Accumulation of soluble COD was found to occur in the UASB sludge bed at low temperatures (Sayed *et al.*, 1988). Massé *et al.* (2001) suggest increasing hydrolysis and acidification may be achieved by maintaining high biomass concentrations in the reactor.

Effective anaerobic treatment also requires control of parameters such as pH, alkalinity, and nutrients due to the sensitivity of anaerobic processes to environmental disturbances (Forster, 1994; Wheatley *et al.*, 1994). Accumulation of inhibitory compounds, such as potassium or ammonium ions, from either metabolic processes or man-made sources must also be controlled and/or prevented (Forster, 1994).

Anaerobic processes treating slaughterhouse wastewater

An UASB reactor treating slaughterhouse wastewater was capable of steady performance up to an OLR of 5 kg COD/m^3·d with COD removal efficiencies higher than 90% (Ruiz *et al.*, 1997). Higher OLR caused an increase in effluent solids and a decrease in COD removal to 59% at an OLR of 6.5 kg COD/m^3·d along with sludge flotation inside the reactor (Ruiz *et al.*, 1997). Hydraulic, organic, and temperature shocks caused a decrease in

COD removal ranging from 10-25% and a decrease in methane production by 20% (Ruiz *et al.*, 1997). The system recovered from each shock event within 24 hours (Ruiz *et al.*, 1997).

An AF reactor treating the same slaughterhouse wastewater showed COD removal efficiencies ranging from 63-84% at OLRs ranging from 0.5-6 kg COD/m^3·d (Ruiz *et al.*, 1997). Again, reactor performance decreased at OLR higher than 6 kg COD/m^3·d with sludge washout observed up to OLR of 11 kg COD/ m^3·d (Ruiz *et al.*, 1997). Similarly, the AF was also able to recover from hydraulic, organic, and thermal shocks within 24 hours (Ruiz *et al.*, 1997).

Another UASB, seeded with digested sewage sludge, was able to achieve satisfactory treatment of slaughterhouse wastewater with OLR up to 3.5 kg COD/m^3·d at a HRT of 7 h at temperatures as low as 20°C (Sayed *et al.*, 1984). This research also showed that the system was capable of recovering from and maintaining biogas production after unfed periods of time due to the presence of accumulated insoluble substrates in the reactor (Sayed *et al.*, 1984). Sayed *et al.* (1984) found that treatment efficiency of soluble ingredients, conversion of total COD to methane at OLRs greater than 1.5 kg COD/m^3·d, and treatment of higher loads were all more favorable at operating temperatures of 30 than at 20°C. Sludge wash-out from the UASB was not significantly different between the two operating temperatures (Sayed *et al.*, 1984).

The use of a DAF-UASB system to treat wastewater from a small slaughterhouse operation was investigated by Manjunath *et al.* (1999). The DAF was found to reduce influent waste strength by approximately 50% and increase the treatability compared to the raw wastewater (Manjunath *et al.*, 1999). UASB reactors treating DAF-pretreated wastewater and raw wastewater were compared. The system treating DAF-pretreated wastewater was capable of obtaining 90% COD removal at an OLR of 4.0 kg COD/m^3·d compared to 70% COD removal at an OLR of 3.5 kg COD/m^3·d from the system treating raw slaughterhouse wastewater (Manjunath *et al.*, 1999).

Start-up of ASBRs treating pork slaughterhouse wastewater at 20 and 25°C were investigated by Massé *et al.* (2001). The ASBRs operating at 20 and 25°C were able to achieve a total COD removal ranging from 47-94% and 72-95%, respectively, during the experiment (Massé *et al.*, 2001). Start-up time required for the 20°C-ASBR was 168 days and 136 days was required for the 25°C-ASBR (Massé *et al.*, 2001). Biomass activity and volatile solids (VS) concentrations were lower at 20°C, however methane content in the biogas was higher at 20°C than 25°C (Massé *et al.*, 2001). Sayed *et al.* (1984) also found organics conversion to methane was less complete at higher temperatures, especially at OLRs below 1.5 kg COD/m^3·d.

Two ASBRs operating at 30°C were able to achieve total COD reductions of 90-96% at OLRs of 2.07-4.93 kg/m^3·d (Massé and Masse, 2000). Seed sludge for these two ASBRs consisted of anaerobic granules from a milk processing plant and municipal anaerobic non-granular biological sludge. Both reactors experienced SS washout during start-up and SS

accumulation (biomass yield and undegraded SS) at rates of 0.068 g VSS/g COD removed (Massé and Masse, 2000).

Effective treatment of solid poultry slaughterhouse waste was achieved at loadings up to 0.8 kg VS/m^3·d with a HRT between 50-100 d (Salminen and Rintala, 2002). Accumulation of soluble COD, ammonia, VFA, decrease in specific methane yield and pH occurred at OLR of 2.1 kg VS/m^3·d (Salminen and Rintala, 2002). Inhibition was most likely caused by high ammonia concentrations, resulting from a buildup of LCFA and VFA and pH drop in the reactor.

Borja *et al.* (1994) investigated treatment of slaughterhouse wastewater using an anaerobic downflow filter reactor at 35°C. Rapid startup (35 d) was achieved with methanol addition to the influent resulting in 94.5% COD removal at an OLR of 10.1 kg COD/m^3·d (Borja *et al.*, 1994). Methanol addition to enhance the growth of methanogens was not required during the start-up of ASBRs operating at 20 and 25°C (Massé *et al.*, 2001). This system was also subjected to temperature, pH, influent flowrate, and influent COD shocks for periods of 5 and 10 h. These shocks resulted in effluent quality deterioration and decreased gas production. No long term effects from these shock conditions were noted and effluent quality and biogas production returned to normal within 15 h of returning the system to normal operating conditions (Borja *et al.*, 1994).

A combination AF/sludge blanket reactor treating slaughterhouse wastewater was capable of achieving COD removal ranging from 69-98% at OLR from 5-45 g COD/L·d (Borja *et al.*, 1995). At OLR of up to 25 g COD/L·d the system achieved 96% COD removal, however experienced rapid decreases in performance at higher loading rates (Borja *et al.*, 1995). Borja *et al.* (1995) concluded that the configuration of the reactor did not impact COD removal, but did enhance the retention of active biomass.

Núñez and Martínez (1999) utilized an expanded granular sludge (EGSB) reactor to treat slaughterhouse wastewater with OLRs up to 15 kg COD/m^3·d. During the study, COD removal efficiency averaged from 65-80%, and was found to be dependent on HRT (Núñez and Martínez, 1999). Effective removal of fats occurred, however, particulate matter did not undergo degradation in the EGSB (Núñez and Martínez, 1999). Observed biomass yield in the EGSB was 0.257 g VSS/g COD removed (Núñez and Martínez, 1999).

Table 2. Summary of anaerobic processes treating slaughterhouse wastewater.

Source	Influent COD, (mg/L)	Reactor type	OLR, (kg COD/m^3·d)	COD removal efficiency, (%)
Ruiz *et al.*, 1997	8,000 (average)	UASB AF	1.03-6.58 0.88-11.21	59.0-91.3 28.4-82.7
Manjunath *et al.*, 1999	1,100-7,250	DAF-UASB UASB	4.0 3.5	90.0 70.0
Massé *et al.*, 2001	7,501±771- 12,590±1,222	ASBR, 20°C ASBR, 25°C	0.46-2.44[a] 0.46-3.07[a]	47-94 72-95
Massé and Masse, 2000	6,908-11,500	ASBR	2.07-4.93[a]	90-96
Salminen and Rintala, 2002	1,000-14,900[b]	STR	0.5-2.1[c]	NA
Borja *et al.*, 1994	5,050 (average)	AF	10.1	94.5
Borja *et al.*, 1995	2,450 (average)	(AF/sludge blanket)	5-45	69-98
Sayed *et al.*, 1984	1,500-2,200	UASB	0.5-3	70
Núñez and Martínez , 1999	1,440-4,200	EGSB	2.8-10.2 (average)	65-80
Jung *et al.*, 2002	1,912±782	ASBR SGBR	0.44-7.23	75.9-92.8 88.7-96.1
Hormel On-site Pilot-scale system	2,179-4,391	SGBR	1.09-4.55	91.8-96.1

[a] based on sludge volume
[b] soluble COD
[c] kg VS/m^3·d

Materials and Methods

Overview of the on-site pilot-scale SGBR system

The on-site pilot-scale SGBR system consisted of a 1,000-gallon reactor vessel (700-gallon operating volume), 200-gallon fiberglass influent wastewater tank used to simulate DAF, 1,650-gallon polypropylene influent wastewater storage tank, 3/4-inch PVC piping and

fittings, a ChronTrol four-channel controller/timer (Cole-Parmer, Vernon Hills, IL), and four Masterflex peristaltic pumps (Models L/S 77521-40, I/P 07593-00, I/P 07591-00, Cole-Parmer, Vernon Hills, IL). Detailed drawings of each reactor vessel and system configuration used during the project period are included in Appendix A.

Over the course of the project, three 1,000-gallon reactor vessels were utilized. A 1,000-gallon high density polyethylene (HDPE) reactor vessel (referred hereafter as Reactor #1, Plastic Supply Inc., Brandon, MS) was originally purchased for use in the pilot-scale system. Reactor #1 incorporated a segmented underdrain system consisting of 3/4-inch perforated PVC pipe placed in the middle of approximately six inches of 3/8-inch pea gravel. Reactor #1 operated from March 27, 2002 until May 15, 2002.

Failure of Reactor #1 on May 15, 2002 resulted in the replacement of the reactor vessel with a 1,000-gallon polypropylene liquid storage tank (Reactor #2). Modifications to the 1,000-gallon liquid storage tank were made in order to use the tank as a replacement reactor vessel. A combination perforated PVC pipe/pea gravel underdrain system was utilized in Reactor #2. Unlike Reactor #1, this underdrain system was not segmented. Reactor #2 operated from July 30, 2002 to December 29, 2002.

A 1,000-gallon polypropylene reactor vessel (Reactor #3, Douglas Manufacturing, Burnsville, MN) was designed and fabricated to replace Reactor #2. Reactor #3 was similar in design to Reactor #1, and incorporated a segmented underdrain system consisting of approximately two inches of 3/8-inch pea gravel on top of approximately seven inches of 2-inch river rock. Three quarter-inch perforated PVC pipe was placed in the middle of the graded gravel bed for effluent discharge and backwashing. Reactor #3 operated from January 6, 2003 to May 31, 2003.

Biogas produced by the system was passed through a steel wool scrubber to remove hydrogen sulfide (H_2S) and measured using a Schlumberger gas meter. A representative schematic of the pilot-scale SGBR system is shown in Figure 1.

The system was operated on a continuous basis from a range of 48 to 16- hour HRT conditions. Table 3 shows the HRT and OLR schedule as well as the influent wastewater characteristics used in the pilot-scale system study. The liquid level inside Reactor #3 was visible via a built-in sight tube and was maintained at 700 gallons using an adjustable effluent overflow pipe. Influent wastewater was distributed above the granule bed via a perforated distribution pipe located in the headspace of the reactor. Backwashing of the system was accomplished using the effluent underdrain system and a Masterflex peristaltic pump with collected effluent. A biogas recirculation system (not shown) was later added as an additional backwashing option.

Seed sludge

Anaerobic granular seed sludge used in the SGBR system was obtained from City Brew Brewery, La Crosse, Wisconsin. Approximately 650 gallons of anaerobic granules

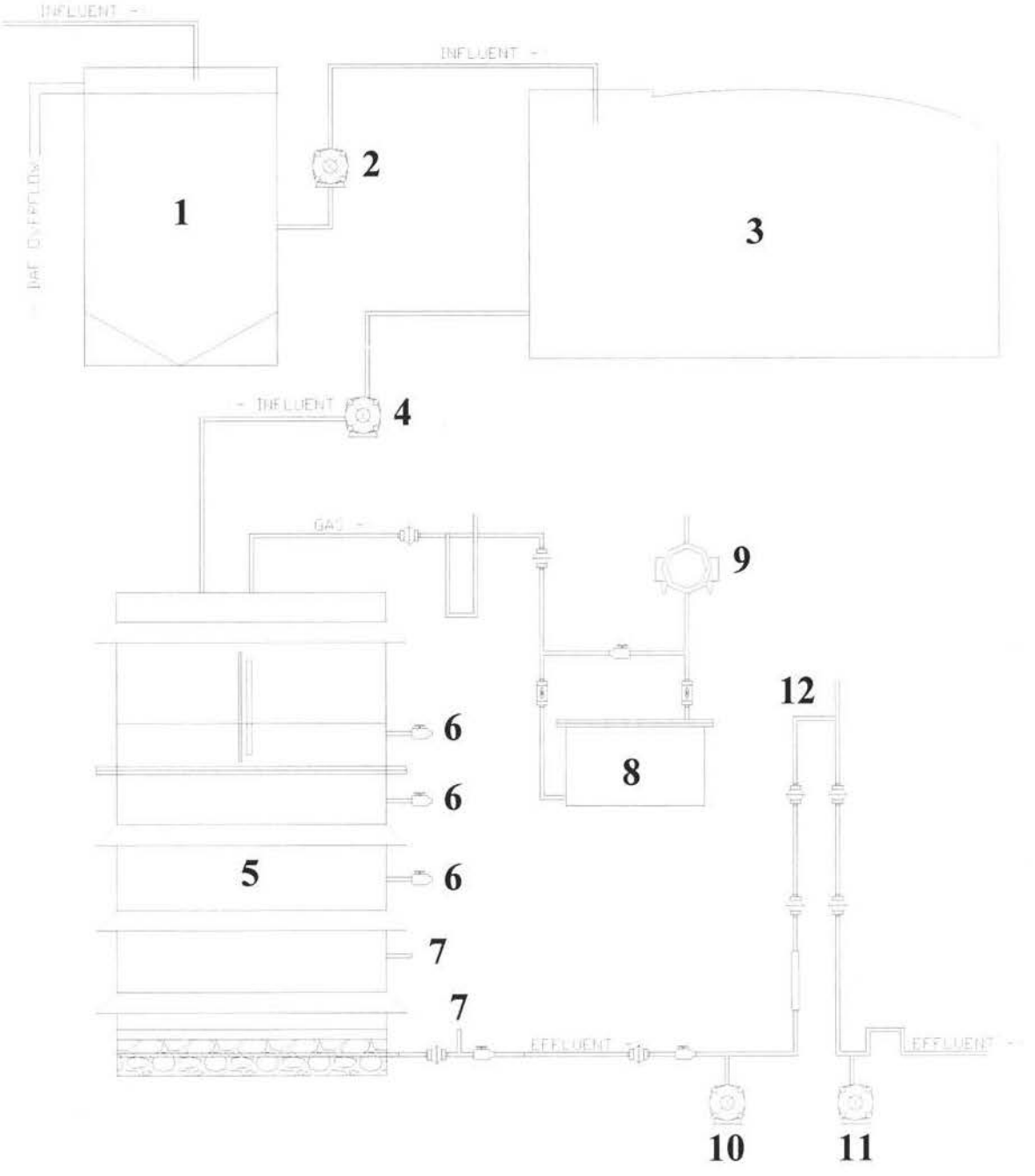

Figure 1. Representative schematic of the pilot-scale SGBR system. 1, DAF tank; 2, transfer pump; 3, influent wastewater storage tank; 4, feed pump; 5, SGBR; 6, sampling port; 7, temperature probe; 8, hydrogen sulfide scrubber; 9, gas meter; 10, backwash pump; 11, effluent sample pump; 12, effluent overflow pipe

Table 3. HRT and OLR schedule and influent wastewater characteristics[a].

Parameter (mg/L except pH & as noted)	Days of SGBR operation[b]					
	1-32	128-135	136-170	171-191	192-224	227-255
HRT, h	48	48	40	36	32	28
OLR, g COD/L·d	2.20	1.09	1.52	2.15	2.80	2.91
Total COD	4,391 ± 774	2,179 ± 94	2,533 ± 450	3,225 ± 456	3,728 ± 517	3,395 ± 590
Soluble COD	2,242 ± 662	1,370 ± 45	1,547 ± 171	1,619 ± 153	1,828 ± 155	1,960 ± 277
BOD	NM[c]	NM[c]	1,555 ± 272	1,570 ± 78	1,694 ± 140	1,352 ± 156
TSS	1,682 ± 473	755 ± 544	586 ± 294	837 ± 177	945 ± 371	812 ± 309
VSS	1,430 ± 448	NM[b]	470 ± 287	651 ± 175	713 ± 296	609 ± 246
VFA (as HAc)	242 ± 100	477 ± 147	568 ± 116	526 ± 105	505 ± 162	491 ± 160
pH	7.48 ± 0.65	7.14 ± 0.13	7.18 ± 0.36	6.99 ± 0.31	6.77 ± 0.28	6.98 ± 0.19
Alkalinity (as $CaCO_3$)	517 ± 53	674 ± 123	722 ± 79	608 ± 97	561 ± 109	659 ± 62
Wastewater Temperature, °C	NM[c]	29 ± 0.8	30 ± 1.4	24 ± 0.9	23 ± 1.3	22 ± 1.5

[a] Format = average ± standard deviation

[b] Day 1 = April 2, 2002; Day 255 = December 12, 2002; Day 308 = February 3, 2003; Day 425 = May 31, 2003

[c] NM = Not measured

[d] Day 421-425 average HRT was 14.1 hours

Table 3. (continued)

Parameter (mg/L except pH & as noted)	Days of SGBR operation[b]						
	308-315	316-329	330-336	337-363	364-398	399-425	Entire Study
HRT, h	48	40	32	24	20	16[d]	
OLR, g COD/L·d	1.37	1.33	1.84	3.01	3.25	4.55	
Total COD	2,750 ± 1,094	2,213 ± 218	2,448 ± 242	3,009 ± 467	2,710 ± 352	3,031 ± 355	3,137 ± 814
Soluble COD	1,545 ± 111	1,541 ± 178	1,536 ± 184	1,807 ± 231	1,582 ± 159	1,650 ± 155	1,749 ± 368
BOD	1,393	1,641 ± 39	1,790	1,547 ± 49	1,355 ± 127	1,648 ± 200	1,543 ± 202
TSS	924 ± 828	385 ± 133	586 ± 155	748 ± 376	553 ± 176	731 ± 186	840 ± 491
VSS	758 ± 760	334 ± 117	522 ± 127	651 ± 366	494 ± 152	639 ± 173	704 ± 431
VFA (as HAc)	448 ± 111	522 ± 47	499 ± 161	544 ± 141	533 ± 108	503 ± 144	486 ± 159
pH	6.97 ± 0.33	6.75 ± 0.16	6.77 ± 0.42	6.65 ± 0.36	6.58 ± 0.29	6.77 ± 0.35	6.90 ± 0.44
Alkalinity (as $CaCO_3$)	573 ± 117	643 ± 34	627 ± 46	683 ± 90	660 ± 96	659 ± 110	630 ± 107
Wastewater Temperature, °C	22 ± 1.2	22 ± 1.0	23 ± 1.2	23 ± 1.3	25 ± 1.5	25 ± 1.5	24 ± 3.1

were used in the pilot-scale SGBR system. The anaerobic granules were transferred from 55-gallon drums by manually pouring them into the reactor and using a peristaltic or diaphragm pump. Excessive pumping of the granules was found to cause disintegration of the granule structure. Massé and Masse (2000) also reported slow disintegration of granular seed sludge due to mixing in ASBRs.

Analytical testing

Analytical testing of the SGBR system performance began on April 2, 2002 (Day 1). An influent grab sample collected after the influent wastewater storage tank and a 24-hour composite effluent sample were used for analysis. The 24-hour composite sample was obtained using a peristaltic pump on a programmable timer and was collected from the sample reservoir on the effluent discharge piping. The effluent sample was stored in a refrigerator at 4°C prior to analysis.

Analysis included total and soluble COD, VFA, pH, alkalinity, and total and volatile suspended solids measurements and were performed as described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). CODs, VFAs, and alkalinity were determined by the closed-reflux method, titration method, and titration method (pH endpoint of 4.50), respectively. An Orion Portable pH meter (Model 210A) with a combination electrode was used to measure pH on-site. Effluent flowrate measurements were also recorded and used to adjust the influent pumping rate.

Influent and effluent wastewater temperature monitoring began after the addition of the storage tank. Temperature monitoring of the interior of the SGBR was added on February 3, 2003 (Day 308). Interior reactor and effluent temperatures were monitored using a Fisherbrand dual-channel thermocouple with Type K beaded probes (Fisher Scientific, Cat. No. 15-078-39). Influent wastewater temperature was measured using a digital thermometer.

Biochemical oxygen demand (BOD_5) measurement was added to the analytical testing routine on August 17, 2002 (Day 138). Biogas analysis and H_2S measurement in the biogas began on September 6, 2002 (Day 158). Biogas composition analysis was performed in the Hormel Foods R&D Control Laboratories using a gas chromatograph. Hydrogen sulfide measurement was performed on-site using a Draeger accuro Bellows Pump with H_2S detector tubes. Biochemical oxygen demand and gas analysis occurred weekly, and H_2S measurement occurred on a biweekly basis since initiation.

Analysis of COD, VFA, alkalinity, and total and volatile suspended solids measurements were conducted five days per week during the 48- through 16-hour HRT conditions. Only temperature, flowrate, pH, and gas production measurements occurred during the remaining two days of the week. A summary of the analytical testing results for the influent and effluent wastewater throughout the study are shown in Tables 3 and 4, respectively.

COD balance and yield calculation

COD balance

The COD removed from the system was assumed to be: 1) effluent COD (soluble); 2) COD converted to methane gas; 3) biomass COD in the effluent; 4) COD utilized for biomass synthesis. COD converted to methane gas in the aqueous phase and COD converted to CO_2 were assumed to be negligible compared to the previous four components used in the COD balance calculations.

The COD of gaseous methane was determined as indicated below:

$$CH_4(gas) - COD \quad (g/day) = CH_4 \ generation \ rate \quad (L/day) * 2.86 \quad (g/L) \qquad (3.1)$$

The COD equivalent of biomass in the effluent was determined by the following equation:

$$VSS_{effl} - COD \quad (g/day) = VSS_{effl} \quad (g/L) * Influent \ flow \ rate \quad (L/day) * 1.42 \qquad (3.2)$$

Accumulated biomass inside the SGBR was calculated as follows:

$$Biomass \quad (g/day) = \left(TCOD_{\inf l} - SCOD_{effl}\right) * Q - \left(CH_4(gas) - COD + VSS_{effl} - COD\right) \quad (3.3)$$

Yield calculation

Biomass yield calculations were computed indirectly by performing a COD balance on the SGBR system. The difference between the influent COD and the COD recovered was assumed to be converted to biomass inside the reactor. Influent suspended solids were assumed to be completely destructed and no accumulation of substrate inside the SGBR was also assumed during the yield calculations. Therefore, any appearance of biomass in the effluent was the result of biomass growth. The biomass yield observed was estimated using the following expression:

$$Yield \quad \left(gVSS/gCOD_{removed}\right) = \frac{\left(Biomass - COD + VSS_{effl} - COD\right)/1.42 \quad (gVSS)}{COD \ removed \quad (g)} \qquad (3.4)$$

Results and Discussion

The results from system operation and analytical testing are presented here in subsections Day 1-32 (April 2-May 3, 2002), Day 128-255 (August 7-December 12, 2002), and Day 308-425 (February 3-May 31, 2003), corresponding to system operation with Reactors #1, #2, and #3, respectively. The SGBR system operated for several days between these time periods, but without analytical testing of system performance at these times.

System operation

Extenuating circumstances at the pretreatment building and with the Hormel Foods operating schedule caused brief periods of system shutdown lasting no more than a few days in each of the time periods presented. Such occurrences were beyond the control of the investigators and Hormel Foods and were somewhat anticipated at the beginning of the project. There were no apparent detrimental effects on the SGBR system as a result of these shutdowns.

Day 1-32

From initial startup through May 15, 2002 (Day 44), a few problems were encountered with the system. Some of the problems that were encountered included: lack of influent wastewater over the weekends when production was stopped at the Hormel Foods processing facility and clogging of the valve coming from the influent tank. Both of these problems were resolved with the addition of the influent storage tank and addressed in a timely manner without impact to the system's performance. However, on May 15, 2002 (Day 44), Reactor #1 developed a crack in the bottom plate of the HDPE tank. The system was subsequently shut down and Reactor #1 was returned to the manufacturer for repair. During the week of June 17, 2002, Reactor #1 was returned to the Austin site from the manufacturer after repairs were made. On June 27, 2002 (Day 87), the SGBR system was reassembled. Transfer of the granules back into Reactor #1 was completed over the following weekend and the system was put back on-line on July 2, 2002 (Day 92).

After reassembly and operating for less than 24 hours, a second failure of Reactor #1 occurred on July 3, 2002 (Day 93). Further investigation of the reactor vessel revealed that the tank had failed in three locations. Based on the assessment of the specialty contractor contacted to repair the SGBR tank on-site, Reactor #1 was abandoned and a 1,000-gallon liquid storage tank (Reactor #2) was purchased to act as a temporary replacement reactor.

Day 128-255

Reactor #2 was assembled on July 23, 2002 (Day 113). The system was again operational on July 30, 2002 (Day 120). After three weeks of successful operation at start-up conditions, the HRT was reduced from 48- to 40-hours on August 13, 2002 (Day 134). Further reductions in HRT to 36-hours, 32-hours, and 28-hours occurred on September 18 (Day 170), October 9 (Day 191), and November 13, 2002 (Day 226), respectively. Each reduction in HRT occurred without incident. This system configuration experienced several problems during its operation.

During the 32 and 28-hour HRT conditions several system shutdowns were caused by a clogged influent pipe feeding the influent tank. Each time the system was restarted without incident. Such occurrences would not be expected in a full-scale system due to larger pipe diameters and the fact that the wastewater from Hormel Foods would be pretreated with a DAF.

Several other problems were encountered with the mechanical operation/system configuration during the 28-hour HRT condition. On December 11, 2002 (Day 254), a gas leak was discovered in the access "porthole" located on the top of Reactor #2. Attempts to seal the leak proved unsuccessful.

A buildup of foam and scum was also found inside the headspace and on top of the granular biomass of Reactor #2 on December 13, 2002 (Day 256). Such an occurrence would be noticed on the top of the granule bed, as in this case, due to the downflow design of the SGBR system. Approximately 25 gallons of foam/scum was removed from the headspace of Reactor #2 and the system was restarted December 17, 2002 (Day 260). The cause of the foam/scum occurrence inside Reactor #2 was unclear. Possible explanations for this problem include the presence of a surfactant or cleaning agent in the influent wastewater stream being fed to the reactor from the Hormel Foods processing facility during this time. An increase in grease concentration in the influent wastewater, combined with the biogas production from the granules, may have resulted in a foam/scum layer on top of the granule bed inside the reactor. Temperature effects inside the reactor from the influent wastewater may have been a contributing factor. From a biological standpoint, filamentous bacteria that have been found to be present in anaerobic granules could have caused production of foam inside the reactor. Minimal operational difficulties were associated with this occurrence, and system performance did not deteriorate during this event.

On December 20, 2002 (Day 263), the effluent flowrate began to slowly decrease and the liquid level inside the reactor began to rise, indicating a problem with the reactor draining properly. The liquid level ultimately backed up into the influent distribution pipe inside the reactor and approximately a total of 1.5 gallons of granular biomass was lost out of the reactor influent feed pipe. Over the following week the same problem with decreasing effluent flowrate reoccurred, and Reactor #2 was shut down on December 29, 2002 (Day 272).

The decision to shut down Reactor #2 was two-fold; the inability to isolate sections of the underdrain for backwashing and the completion and availability of Reactor #3 from the manufacturer. Unlike Reactor #1, Reactor #2 did not have a segmented underdrain system that allowed different section to be isolated during backwashing. During each procedure the entire reactor was subject to backwashing, and was ineffective at delivering an equal flow of backwash water to the entire granule bed. Despite backwashing of Reactor #2 the decreasing effluent flowrate problem returned after a short time following each backwashing event. The uneven distribution of flow during backwashing was thought to have contributed to the decreasing effluent flowrate problem from Reactor #2. Reactor #3 was designed with a segmented underdrain system, similar to Reactor #1, and its installation allowed for greater control and distribution of backwash flow to the granule bed during backwashing procedures. The design of Reactor #3 was similar to Reactor #1, but with improvements that included the use of stronger and thinner polypropylene instead of HDPE, sampling ports along the granule bed, a built-in liquid level sight tube, increased reactor headspace, and steel reinforced gussets around the exterior of the reactor.

Day 308-425

Startup of Reactor #3 began on January 6, 2003 (Day 280). During the transfer of granules and throughout the following three weeks until January 26, 2003 (Day 300), the same problem with poor drainage of effluent from the reactor occurred. On January 21, 2003 (Day 295), investigation of the granular biomass from Reactor #3 revealed a large amount of "fines" present (small pieces of biomass), a decrease in size of the granules, and a significant loss of granular structure since initial seeding.

A biogas backwash/recirculation system was added to the underdrain of Reactor #3 in attempt to solve the effluent drainage problem. This system used produced biogas and recirculated it back into the reactor via the underdrain piping. Biogas backwashing/recirculation was performed using two underdrain segments while concurrently using the remaining two underdrain segments to drain effluent from the reactor. This process was attempted, with limited success, until January 26, 2003 (Day 300).

An on-site investigation of the system on January 21, 2003 (Day 295), revealed the performance of an improper backwashing procedure by the system operator. The operator had the backwash pump operating in a reverse flow mode, and was withdrawing liquid from the reactor instead of adding liquid via the underdrain in an upflow manner. It was believed that this may have compounded the effluent drainage problem in Reactor #3.

At this time, three samples were taken from the system; one at the 525-gallon level (Sample #1), one at the 350-gallon level (Sample #2), and one from the effluent discharge pipe at the bottom of the reactor (Sample #3). Sample #1 contained almost no granular biomass. Sample #2 contained a higher quantity of biomass than Sample #1 and also a large amount of "fines." The granules that were present in Sample #2 were relatively smaller in

size when compared to stored granules from the initial seeding. This comparison was made in the Iowa State University Biotechnology Laboratory upon return from the Hormel Foods site. Sample #3 also contained a high quantity of "fines." There were no full-size granules present in this sample.

An experiment was setup in the Iowa State University Biotechnology Laboratory in an attempt to find the probable cause of the decrease in size of the granular biomass and presence of "fines." This experiment was to simulate the transfer of granules using a peristaltic pump. Granules used in this experiment were from the same source as the granules used in the pilot-scale system at Hormel Foods, and were stored at 4 ^{o}C from the time of acquisition. Examination of granules transferred through the peristaltic pump resulted in decreased size and an increase in "fines" when compared to granules that had not been transferred through the peristaltic pump. The granules from the laboratory experiment were almost identical to the samples taken from the pilot-scale SGBR system at Hormel Foods.

Therefore, alternative means of transferring granules were investigated including the use of a progressive cavity pump, air-operated diaphragm pump, and manual loading of the new granular biomass into Reactor #3. In order to minimize the stress imposed on the granules during transfer the decision was made to manually load the majority of the new granular biomass into Reactor #3. A discussion with the operator of the City Brew Company UASB reactor resulted in the decision to use an air-operated diaphragm pump to transfer the remaining biomass (approximately 50-100 gallons). The air-operated diaphragm pump was also chosen over the progressive cavity pump due to availability and cost.

New granules were obtained from the City Brew Company, La Crosse, Wisconsin. No problems occurred while reseeding and restarting the system on February 3, 2003 (Day 308). Operation of Reactor #3 began at a 48-hour HRT and was rapidly reduced to 40, 32, and 24-hours on February 10 (Day 315), February 24 (Day 329), and March 3, 2003 (Day 336), respectively. Each reduction in HRT occurred without incident and this rapid reduction in HRT and increase in OLR provide valuable insight into the capability of the SGBR. Subsequent reductions in HRT to 20 and 16-hours occurred on March 31, 2003 (Day 364) and May 5, 2003 (Day 399), respectively.

Maintaining the 700-gallon operating volume inside the reactor became problematic throughout the 20-hour HRT condition. Beginning on April 12, 2003 (Day 376) the liquid level inside the reactor had risen over 770 gallons and persisted through May 16, 2003 (Day 410). During this time, operation of the system was stopped and subsequently restarted once when the liquid level was below 770 gallons. Typically, the system was stopped and restarted during the same day. On April 18, 2003 (Day 382) multiple attempts at backwashing with biogas from the system were not beneficial in solving this problem. Despite the rise in liquid level, a decrease in effluent flowrate from the system was not observed. Based on this information, increased headloss through the granule bed inside the

reactor was thought to have contributed to the problem. Therefore on April 22, 2003 (Day 386) the effluent overflow pipe was lowered by removing the middle sections of the pipe. The overflow pipe was then adjusted accordingly to maintain the 700-gallon operating volume inside the reactor. This action did provide temporary relief; however, the operating volume remained above 700 gallons.

On May 7, 2003 (Day 401) fifty (50) gallons of biomass was wasted from the SGBR. Over the course of its operation, biomass growth and accumulation was expected to occur in the SGBR. Biomass growth and accumulation likely decreased the void space and increased the headloss through the granule bed, resulting in problems with maintaining the 700-gallon operating level. Using an assumed yield of 0.1 g VSS/g $COD_{removed}$ and the concentration of VSS inside the reactor, it was calculated that a total of 200 gallons of biomass had accumulated since the startup of Reactor #3 on February 3, 2003 (Day 308).

Unfortunately, between May 8, 2003 (Day 402) and May 9, 2003 (Day 403) influent wastewater accumulated inside the reactor headspace and biogas piping. The pressure inside the reactor subsequently increased due to the continued production of biogas inside the reactor. The high pressure split the welds below the bolted flange on the bottom section of the reactor and deformed the top section of the reactor. The pressure was only able to increase to a certain level before the manometer on the gas piping acted as a pressure relief valve to equilibrate the pressure inside the reactor. An unknown quantity of granules was washed out of the reactor through the biogas collection system. The system was stopped on May 9, 2003 (Day 403).

The damage sustained to the reactor on May 9, 2003 (Day 403) was neither extensive nor detrimental to the operation of the system, and the decision was made to continue to operate the system without repair. On May 12, 2003 (Day 406) the remaining 150 gallons of biomass was wasted from the SGBR. The system was restarted the same day. Over the next day, the liquid level inside the reactor steadily rose, but was still below the 700-gallons operating level. Again, between May 13, 2003 (Day 407) and May 14, 2003 (Day 408), accumulation of influent in the headspace and gas piping occurred. No further damage to the reactor occurred, and a small, unknown quantity of granules was lost through the biogas collection system.

After the second influent wastewater backup on May 13 the effluent overflow pipe no longer provided effective hydraulic control inside the reactor. Therefore, a pump was attached to the effluent piping to maintain the operating volume. The effluent pump was adjusted to match the flowrate of the influent pump, and thus keeping the liquid level inside the reactor constant. This setup was operational on May 16, 2003 (Day 410). This arrangement was utilized through the end of the study on May 31, 2003 (Day 425). Also, on May 16, 2003 (Day 410), the operating volume was lowered to approximately 600-gallons. This provided more headspace in case the influent wastewater accumulated inside the reactor while the system was unattended. No additional operational problems were experienced after

this time, and operation of the pilot-scale SGBR system was terminated on May 31, 2003 as scheduled.

Organics removal

Day 1-32

Effluent TCOD decreased below 200 mg/L after initial start-up of the system (except Day 19). Effluent SCOD followed the same trend as TCOD and TCOD removal during this period averaged 96.1%. Effluent BOD was not measured during this time period.

Day 123-255

Both effluent TCOD and SCOD gradually increased during this time period, although remaining below 300 mg/L. OLR during this time period also increased from 1.09 g COD/L·d to 2.91 g COD/L·d. Average TCOD removal during this time ranged from 93.4-94.9% and initially increased as OLR increased. The decline in TCOD removal for the 32 and 28-hour HRT conditions during this time period may be a result of accumulated influent solids or biomass washout from the system. Effluent BOD values remained below 65 mg/L.

Day 308-425

Again, both effluent TCOD and SCOD gradually increased during this time period. OLR during this time period also ranged from 1.33 g COD/L·d – 4.55 g COD/L·d. TCOD removal ranged from 91.8-94.2% during the same time. Thus, as OLR more than tripled during this time, TCOD removal remained fairly constant. Average effluent BOD values remained below 75 mg/L throughout this period.

Suspended solids concentrations

Day 1-32

Effluent TSS and VSS were below 30 mg/L during this time period. Washout of biomass and/or accumulated solids inside the reactor may explain the increase in effluent suspended solids concentrations over this time period. Fluctuations in SS concentrations may have been caused by shutting down the system over the weekends during this time. Additional influent storage for continuous operation of the system was added after this time period.

Day 128-255

Fluctuations in effluent SS concentrations during this time period are representative of influent SS concentration fluctuations. A large spike in TSS and VSS on Day 252 was the

result of a combination of high influent SS concentration and backwashing the reactor due to high liquid level inside the SGBR.

Day 308-425

Increasing effluent TSS and VSS concentrations were most likely due to increasing OLR during this time period. Average effluent TSS and VSS concentrations during the 24-, 20-, and 16-hour HRT conditions were almost double the effluent concentrations during the 48-, 40-, and 32-hour HRT conditions.

From a sample taken on April 1, 2003 (Day 365) the total and volatile solids concentrations in the SGBR were 87.8 g/L and 75.2 g/L, respectively. Using the average daily effluent VSS concentration for this period and assuming that the total biomass in the SGBR remained constant throughout the study, an estimate of the solids retention time (SRT) in the SGBR was 2,910 days. This SRT would be an overestimation for a full-scale system due to the necessity for regular biomass wasting to accommodate biomass accumulation inside the SGBR. The long SRT found in the pilot-scale system was similar to the long SRTs reported with the SGBR used in the comparison study by Jung *et al.* (2002). SRTs for the lab-scale SGBR ranged from 600 to over 1000 days. Mach and Ellis (2000) also reported an SRT of approximately 500 days for an SGBR at a 6-hour HRT.

pH, VFA, and alkalinity

Effluent pH, VFA, and alkalinity averaged 7.38, 21 mg/L as HAc, and 1,232 mg/L as $CaCO_3$, respectively, from Day 1-32. From Day 128-255 these same parameters ranged from 7.31-7.78, 20-22 mg/L as HAc, and 1,084-1,233 mg/L as $CaCO_3$. Similar results occurred for Day 308-425. pH, VFA, and alkalinity ranged from 7.04-7.14, 16-25 mg/L as HAc, and 1,084-1,200 mg/L as $CaCO_3$, respectively. Massé and Masse (2000) experienced similar increases in effluent pH and alkalinity while treating slaughterhouse wastewaters in ASBRs. Due to the generation of bicarbonate from the conversion of protein to ammonia, addition of alkalinity to the feed wastewater was not required at any point in time. The increase in alkalinity provided buffer capacity to the system to handle changes in influent wastewater pH.

Table 4 summarizes the results of analytical testing for the effluent wastewater from the SGBR system. Results shown are from Day 1-32, 128-255, and 308-425, which represent April 2-May 3, 2002, August 7-December 12, 2002, and February 3-May 31, 2003, respectively. Graphical timelines depicting the trends in influent and effluent wastewater parameters from the pilot-scale SGBR system are found in Appendix B.

Biogas production

Cumulative methane production, shown in Appendix B, was calculated using the measured methane content of the biogas as shown in Table 5. Methane production was

corrected to STP conditions using recorded temperature and atmospheric pressure readings (when available).

The step-wise nature of the cumulative methane production for Day 1-32, shown in Appendix B, Figure B21, was the result of shutting the system off over the weekend during this time. Excellent results for cumulative methane production were experienced for Day 128-255, as the actual methane production was close to 100% of the theoretical value at the end of this period. Methane production results for Day 308-425 were not as useful as the previous period. Around the beginning of the 32-hour HRT condition (Day 336) the cumulative actual methane production exceed the cumulative theoretical methane production. This condition continued until the end of the project. Although the exact cause of this occurrence could not be determined, it was suspected that malfunction of the gas meter was to blame.

Table 4. Summary of effluent wastewater characteristics[a].

Parameter (mg/L except pH & as noted)	Days of SGBR operation[b]					
	1-32	128-135	136-170	171-191	192-224	227-255
HRT, h	48	48	40	36	32	28
Total COD	167 ± 50	141 ± 12	145 ± 18	149 ± 15	202 ± 27	231 ± 33
Soluble COD	146 ± 44	121 ± 16	119 ± 11	106 ± 9	142 ± 18	140 ± 23
TCOD Removal, %	96.1 ± 1.3	93.4 ± 0.3	94.0 ± 0.8	94.9 ± 0.9	94.4 ± 0.8	93.5 ± 1.2
BOD	NM[c]	NM[c]	41 ± 11	35 ± 5	45 ± 7	49 ± 14
TSS	11±5	10± 2	14± 8	12± 3	25± 8	38± 16
VSS	5 ± 4	NM[b]	9 ± 8	5 ± 3	14 ± 9	25 ± 16
VFA (as HAc)	21 ± 4	21 ± 4	20 ± 5	22 ± 5	20 ± 2	21 ± 6
pH	7.38 ± 0.33	7.59 ± 0.15	7.78 ± 0.29	7.49 ± 0.25	7.31 ± 0.14	7.32 ± 0.19
Alkalinity (as $CaCO_3$)	1,232± 157	1,084± 116	1,156± 120	1,114± 265	1,139 ± 59	1,233 ± 74
Wastewater Temperature, °C	NM[c]	25 ± 1.1	25 ± 1.6	21 ± 1.3	20 ± 1.8	19 ± 2.3

[a] Format = average ± standard deviation

[b] Day 1 = April 2, 2002; Day 255 = December 12, 2002; Day 308 = February 3, 2003; Day 425= May 31, 2003

[c] NM = Not measured

[d] Day 421-425 average HRT was 14.1 hours

Table 4. (continued)

Parameter (mg/L except pH & as noted)	Days of SGBR operation[b]						
	308-315	316-329	330-336	337-363	364-398	399-425	Entire Study
HRT, h	48	40	32	24	20	16[c]	
Total COD	147 ± 14	179 ± 17	175 ± 14	214 ± 17	228 ± 29	249 ± 35	193 ± 46
Soluble COD	101 ± 16	126 ± 10	129 ± 5	149 ± 9	148 ± 16	168 ± 19	137 ± 27
TCOD Removal, %	94.2 ± 1.2	92.3 ± 1.3	92.8 ± 1.0	92.8 ± 0.9	91.8 ± 1.3	92.1 ± 1.1	93.6 ± 1.5
BOD	38	43 ± 2	50	51 ± 10	73 ± 15	68 ± 3	51 ± 16
TSS	13± 6	18± 3	17± 3	33± 7	31± 7	36 ± 11	23 ± 13
VSS	9 ± 3	17 ± 3	16 ± 3	30 ± 7	28 ± 6	33 ± 10	18 ± 13
VFA (as HAc)	16 ± 2	20 ± 2	20 ± 1	22 ± 3	25 ± 7	22 ± 3	22 ± 5
pH	7.08 ± 0.25	7.04 ± 0.05	7.09 ± 0.05	7.08 ± 0.05	7.09 ± 0.07	7.14 ± 0.06	7.30 ± 0.29
Alkalinity (as $CaCO_3$)	1,084 ± 89	1,121 ± 46	1,128 ± 50	1,200 ± 76	1,162 ± 78	1,162 ± 99	1,169 ± 105
Wastewater Temperature, °C	20 ± 0.6	20 ± 1.0	21 ± 1.4	25 ± 3.6	26 ± 1.9	26 ± 2.0	23 ± 3.4

Table 5. Summary of biogas methane content[a].

	Days of SGBR operation[b]					
	1-32	128-135	136-170	171-191	192-224	227-255
HRT, h	48	48	40	36	32	28
Daily Biogas Production @ STP, L	893 ± 558	NM[d]	1,697 ± 611	1,923 ± 408	2,390 ± 567	3,168 ± 1,614
Biogas Methane Content, %	90[c]	NM[d]	80.5 ± 2.2	78.1 ± 8.3	89.6 ± 3.3	88.9± 5.2
Daily Methane Production @ STP, L	833 ± 520	NM[d]	1,404 ± 505	1,637 ± 315	2,140 ± 521	2,839 ± 1,490

[a] Format = average ± standard deviation

[b] Day 1 = April 2, 2002; Day 255 =December 12, 2002; Day 308 = February 3, 2003; Day 425 = May 31, 2003

[c] Assumed value

[d] NM = Not measured

[e] Day 421-425 average HRT was 14.1 hours

Table 5. (continued)

	Days of SGBR operation[b]						
	308-315	316-329	330-336	337-363	364-398	399-425	Entire Study
HRT, h	48	40	32	24	20	16[e]	
Daily Biogas Production @ STP, L	1,011 ± 74	1,294 ± 141	2,050 ± 450	3,636 ± 737	3,371 ± 766	3,425 ± 1,527	2,407 ± 1,301
Biogas Methane Content, %	97.5	90.6 ± 1.1	91.4	90.8 ± 3.3	88.6 ± 4.3	92.0 ± 1.4	88.4 ± 6.1
Daily Methane Production @ STP, L	952 ± 72	1,177 ± 133	1,883 ± 420	3,258 ± 730	2,996 ± 716	3,151 ± 1,438	2,147 ± 1,191

COD balance and yield calculation

COD balance

Daily influent total COD was balanced against: 1) residual effluent COD (soluble); 2) COD converted to methane gas; 3) biomass COD in the effluent; 4) COD utilized for biomass synthesis. The biomass COD in the effluent was determined based on the daily VSS measurement of the effluent sample. The COD utilized for biomass synthesis could not be regularly determined, therefore the COD not recovered in the COD balance was assumed to be biomass accumulated inside the SGBR. Table 6 shows the results of the COD balance for the SGBR system throughout the study.

Table 6. Summary of equivalent COD for each time period

Day	HRT (h)	OLR (g COD/L·d)	Infl. COD (g/day)	Effl. COD (g/day)	CH_4-COD (g/day)	VSS_{effl}-COD (g/day)	Recovery (g/day)
1-32	48	2.20	5,809 ± 1,030	193 ± 57	2,535 ± 1,552	9.33 ± 7.41	2,545 ± 1,556
128-135	48	1.09	NM[a]	NM[a]	NM[a]	NM[a]	NM[a]
136-170	40	1.52	4,484 ± 785	191 ± 18	3,770 ±816	23.00 ± 17.47	3,793 ± 814
171-191	36	2.15	5,754 ± 817	184 ± 18	4,395 ± 761	13.71 ± 8.69	4,409 ± 763
192-224	32	2.80	7,650 ± 973	281 ± 43	5,734 ± 1,271	41.86 ± 27.39	5,776 ± 1,276
227-255	28	2.91	8,311 ± 977	302 ± 50	5,762 ± 1,508	65.03 ± 32.82	5,827 ± 1,516
308-315	48	1.37	4,043 ± 1,472	145 ± 11	2,720 ± 107	16.14 ± 7.01	2,736 ± 107
316-329	40	1.33	3,699 ± 420	209 ± 20	3,330 ± 409	36.81 ± 6.66	3,366 ± 412
330-336	32	1.84	4,689 ± 226	252 ± 16	3,874 ± 591	50.00 ± 1.80	3,924 ± 593
337-363	24	3.01	8,905 ± 1,322	400 ± 28	7,673 ± 1,316	111.78 ± 25.75	7,785 ± 1,329
364-398	20	3.25	9,305 ± 1,590	479 ± 68	7,686 ± 1,646	132.98 ± 26.22	7,819 ± 1,659
399-425	16[b]	4.55	12,254 ± 820	646 ± 112	7,616 ± 3,099	180.56 ± 75.42	7,797 ± 3,096

[a] NM = Not measured; [b] Day 421-425 average HRT was 14.1 hours

Yield calculation

Results of the biomass yield calculations during each HRT condition, as described in section 3.4.2, are presented in Table 7. The average overall yield for Reactor #1 was much higher than Reactors #2 and #3 because the methane produced over the weekends while the system was shut down was not recorded. Thus, the recovered COD value during this time was lower than the actual value resulting in a higher calculated yield using the assumptions stated in section 4.6.1. Núñez and Martínez (1999), Massé and Masse (2001), and Sayed *et al.* (1984) reported biomass yields from systems treating slaughterhouse wastewater of 0.257, 0.065-0.112, and 0.15-0.50, respectively. These systems were an EGSB, three different UASBs operating between 20-30°C, and a UASB, respectively.

Table 7. Biomass yields at different HRT conditions.

Day	Reactor	HRT (h)	OLR (g COD/L·d)	Average Yield per condition ($gVSS/gCOD_{removed}$)	Average Overall Yield ($gVSS/gCOD_{removed}$)
1-32	1	48	2.20	0.389	0.389
128-135	2	48	1.09	NM[a]	0.161
136-170		40	1.52	0.093	
171-191		36	2.15	0.141	
192-224		32	2.80	0.184	
227-255		28	2.91	0.221	
308-315	3	48	1.37	0.204	0.145
316-329		40	1.33	0.070	
330-336		32	1.84	0.093	
337-363		24	3.01	0.092	
364-398		20	3.25	0.114	
399-425		16[b]	4.55	0.288	

[a] NM = Not measured

[b] Day 421-425 average HRT was 14.1 hours

Comparison of pilot-scale SGBR performance

As one of the objectives of the study, the performance of the pilot-scale SGBR system treating Hormel Foods slaughterhouse wastewater was compared to other high rate anaerobic processes treating slaughterhouse wastewaters. Table 2 in the literature review section summarizes the results of various anaerobic configurations treating slaughterhouse wastewaters, and can be compared with the results from the pilot-scale SGBR in Table 4.

Figure 2 represents the COD removal efficiency at different OLR conditions of the various anaerobic digestion systems, including the pilot-scale SGBR. Only the results from the systems in Table 2 that included COD removal efficiency at different OLR conditions were plotted against the pilot-scale SGBR in Figure 2. As Figure 2 shows, the pilot-scale SGBR achieved greater COD removal efficiency than all the other anaerobic configurations at each OLR condition. Only the 25°C ASBR (Massé and Masse, 2001) achieved a greater COD removal efficiency than the pilot-scale SGBR at an OLR of approximately 1.9 kg COD/m^3·d.

Figure 3 is a comparison plot between the pilot-scale SGBR, laboratory-scale SGBR, and laboratory-scale ASBR treating Hormel Foods slaughterhouse wastewater. Both the SGBRs had higher COD removal efficiencies at all OLR conditions compared to the laboratory-scale ASBR. COD removal efficiency was nearly identical for the pilot- and lab-scale SGBRs, with the SGBR from the laboratory study having slightly higher COD removal efficiencies at the higher OLR conditions.

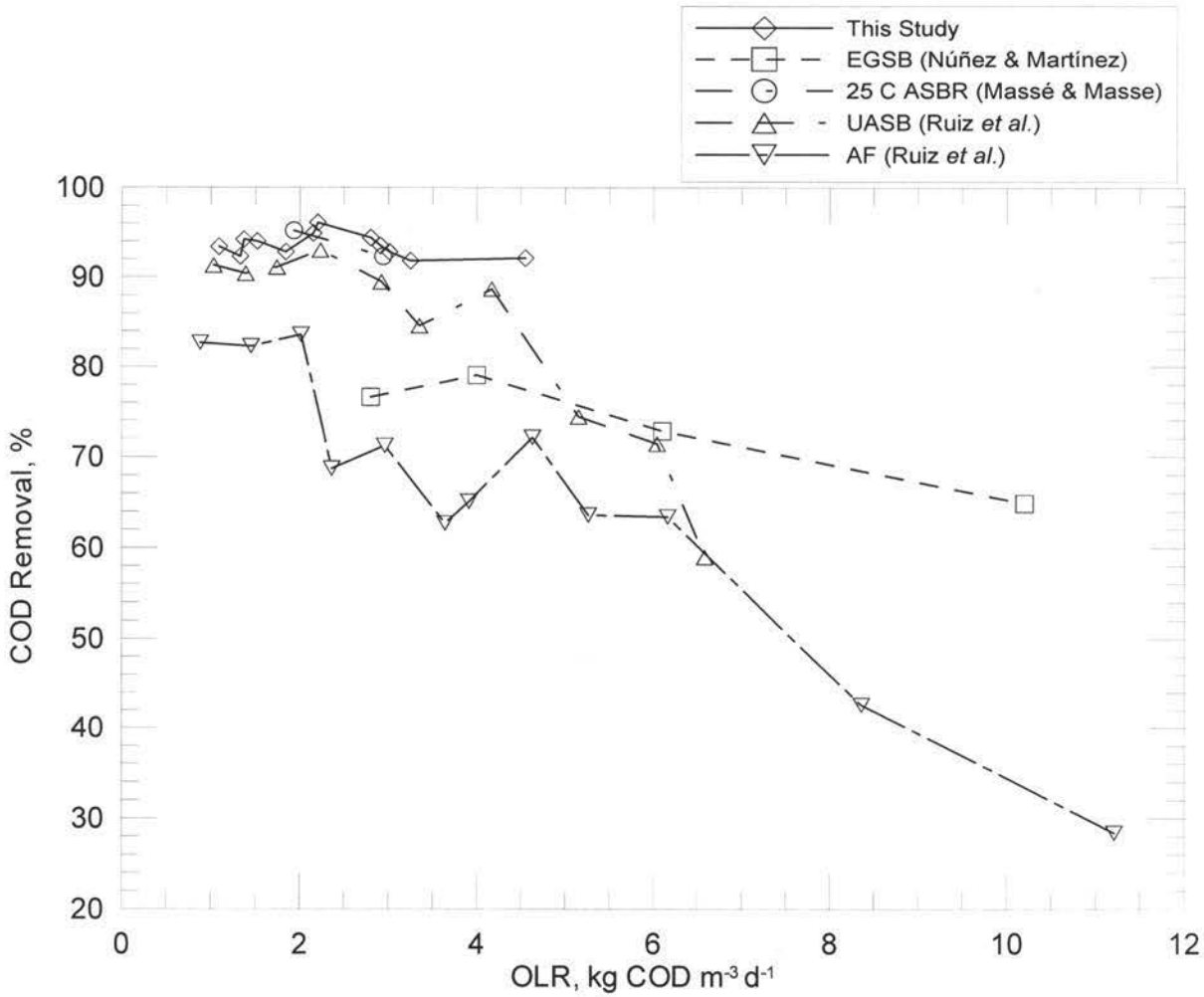

Figure 2. Comparison of COD removal efficiency between the pilot-scale SGBR and different anaerobic systems treating slaughterhouse wastewater at various OLR conditions.

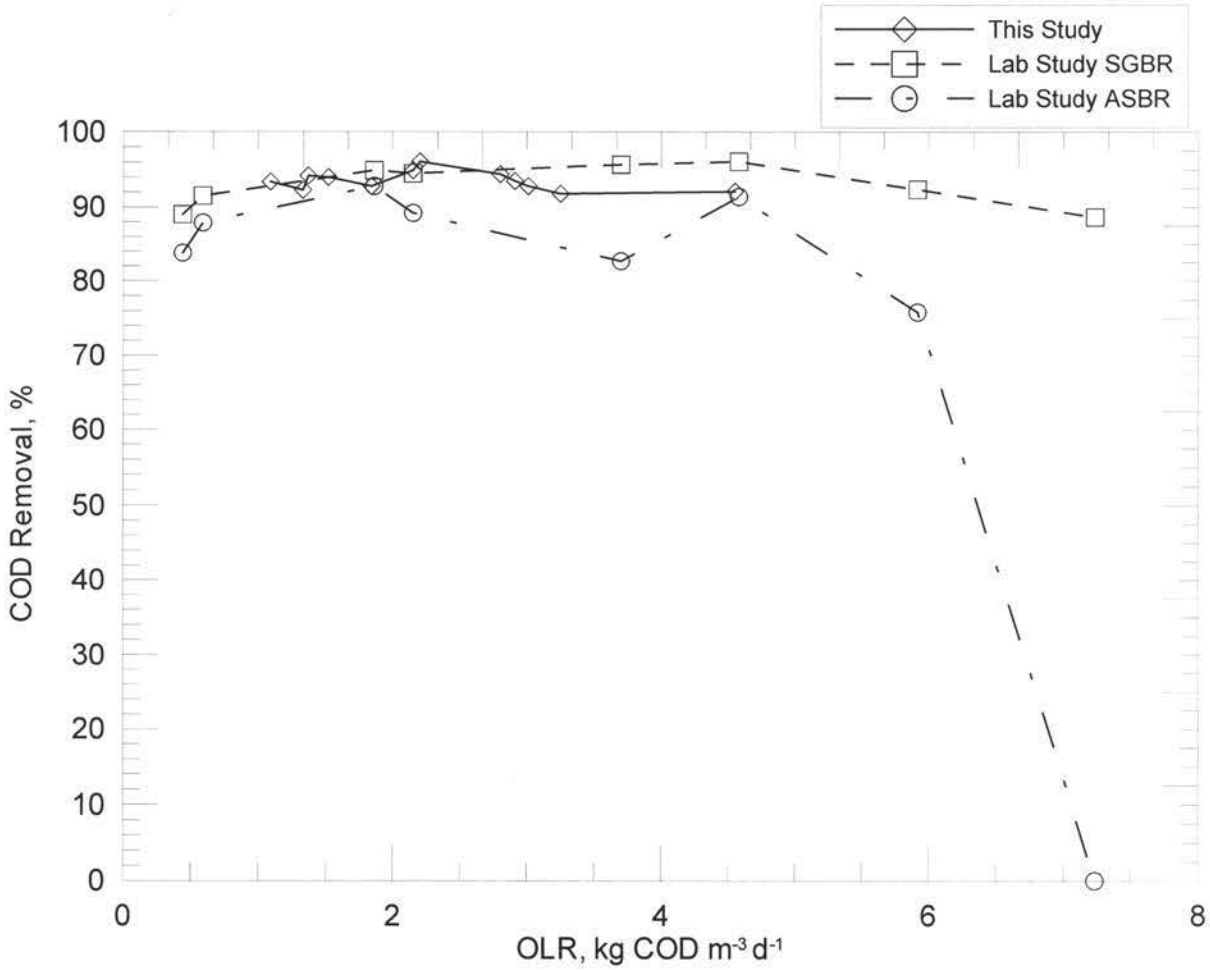

Figure 3. Comparison of COD removal efficiency between the pilot-scale SGBR, lab-scale SGBR, and lab-scale ASBR treating Hormel Foods slaughterhouse wastewater at various OLR conditions.

Granule size analysis

Size analysis was completed during the study to examine any changes in size of the granular biomass. A sample taken from the SGBR on April 1, 2003 (Day 365) was compared to seed granules from the reseeding of Reactor #3 on February 3, 2003 (Day 308). Size analysis was performed in the Materials Analysis and Research Laboratory of the Civil, Construction and Environmental Engineering Department at Iowa State University using a Pixera Pro CCD camera mounted on a light microscope combined with Noesis Vision's Visilog image processing and analysis software.

Results, shown below in Figure 4, revealed that the size distribution of the sample taken 57 days after startup from the SGBR was nearly identical to the size distribution of the

stored seed granules. There was a slight variation in the mean diameters of the granules between the two samples examined. The mean diameter of the granules taken from the pilot-scale SGBR and stored seed granules were 0.95 mm and 0.90 mm, respectively. Although the difference between the mean diameters of the two samples was small, the increase in granule diameter may have been a result of bacterial growth.

The image and size analysis equipment and software used in the granule size analysis had limitations that may have inaccurately portrayed the true size distribution of the samples examined. The major limitation with the analysis software was that the software first calculates the area of each object in the image, and then back-calculates the object's diameter assuming the object is spherical in shape. However, many of the granules are not spherical as shown in Figure 5, and this limitation could not be removed from the size analysis of the two samples. Other limitations included the inability of the software program to consistently distinguish between contrasts in the color of the biomass. Large granules were denser, and thus darker, than small pieces of biomass making them easier to quantify and measure. The analysis software also cropped any granules around the frame of the image not representing the true size of these granules. Manual editing of the images was used to minimize these two limitations of the software during the analysis of the two samples.

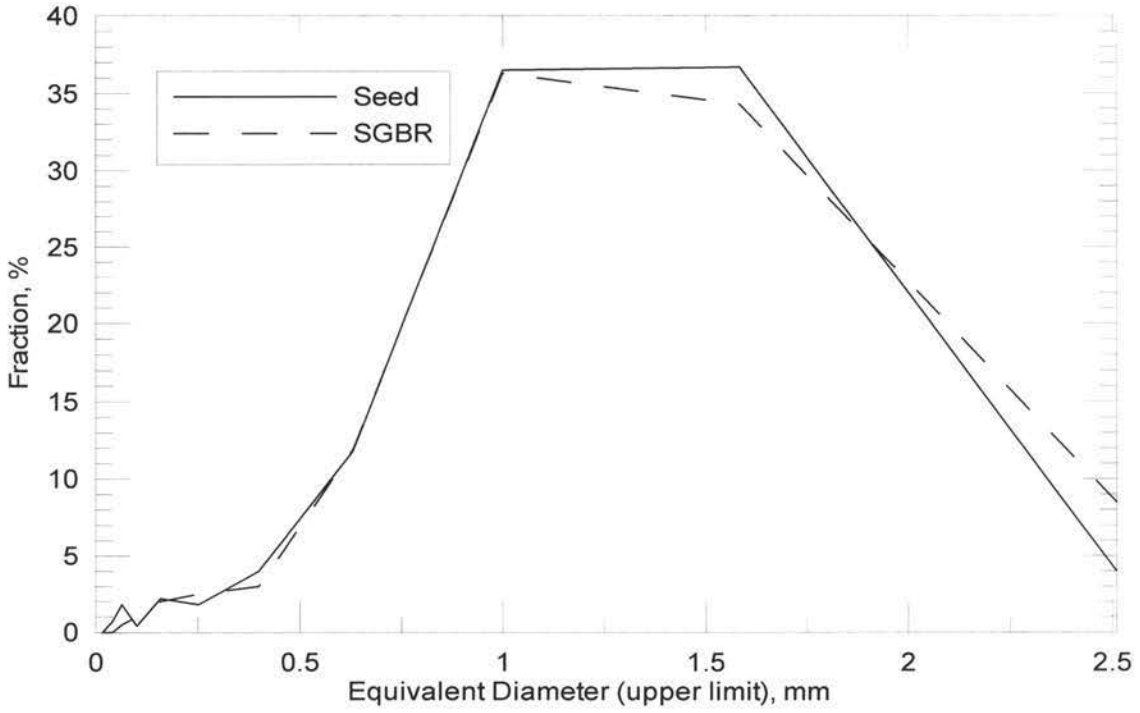

Figure 4. Size distribution of anaerobic granules from the pilot-scale SGBR and stored seed granules.

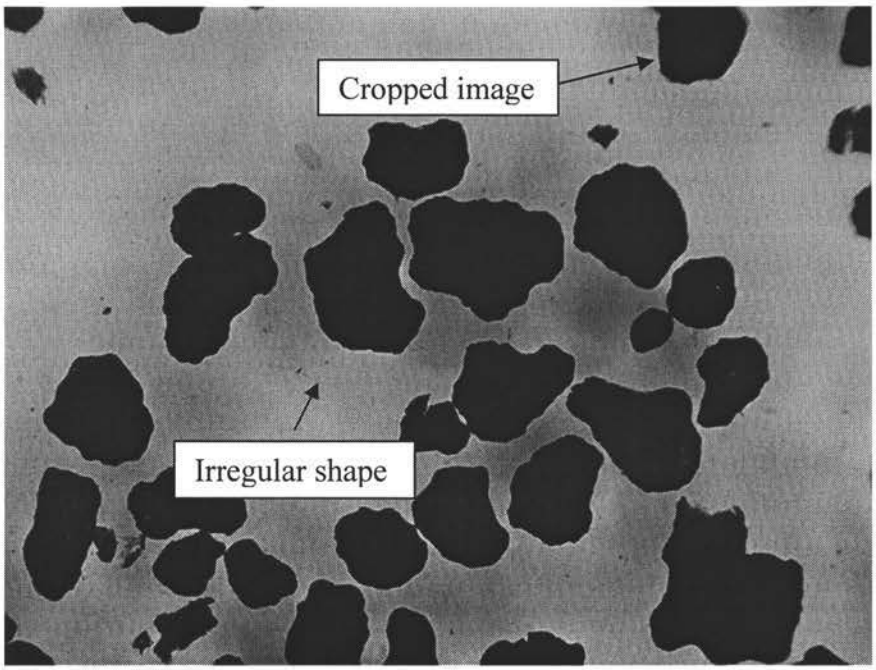

Figure 5. Image of stored seed granules sample used for size analysis showing the irregular shape and cropped images of the granules.

System hydraulics

Headloss

The SGBR utilizes a downflow design. Consequently, headloss in the system is attributed to losses through piping, fittings, underdrain, and the granule bed. From the results of the pilot-scale system, headloss was low for the majority of the operation. Two instances occurred that caused headloss to increase, and these increases appeared to be due to accumulation of solids (either biomass or influent solids) within the granule bed. If the increase in headloss was due to clogging of the underdrain, periodic backwashing would have alleviated the problem. New flocculent cell growth that occurred separate from the granules could have occupied the void spaces between existing granules. Therefore, as the system operated over time and biological growth and synthesis of new cells occurred, the volume of void space in the granule bed decreased while simultaneously increasing the headloss through the system. Ideally, this accumulated biomass (and/or influent solids) should be wasted out of the system periodically (e.g., during backwashing). As an indication of headloss over time, Figure 6 shows the height of the liquid level inside the reactor, as well as the height of the effluent overflow pipe. Hydraulic control of the system became

problematic around April 12, 2003 (Day 376), shown by the drastic rise in liquid level at that time. Shutdown of the system to drain the reactor until the liquid level was back around 700 gallons did not work, as the liquid level rose once the system was turned back on. Lowering (removal of the middle sections of pipe) the effluent overflow pipe to 37" on April 22, 2003 (Day 386) was a temporary solution until April 28, 2003 (Day 392) when the headloss through the granule bed exceeded the difference between the height of the overflow pipe and the 700-gallon liquid level (approximately 33"). Finally, on May 16, 2003 (Day 410) because the headloss through the granule bed was greater than 33" the effluent overflow pipe was replaced with a pump to control the liquid level inside the reactor. The operating volume of the SGBR was also lowered to 600 gallons to provide more headspace as a safeguard against future increases in liquid level inside the reactor above the desired operating volume.

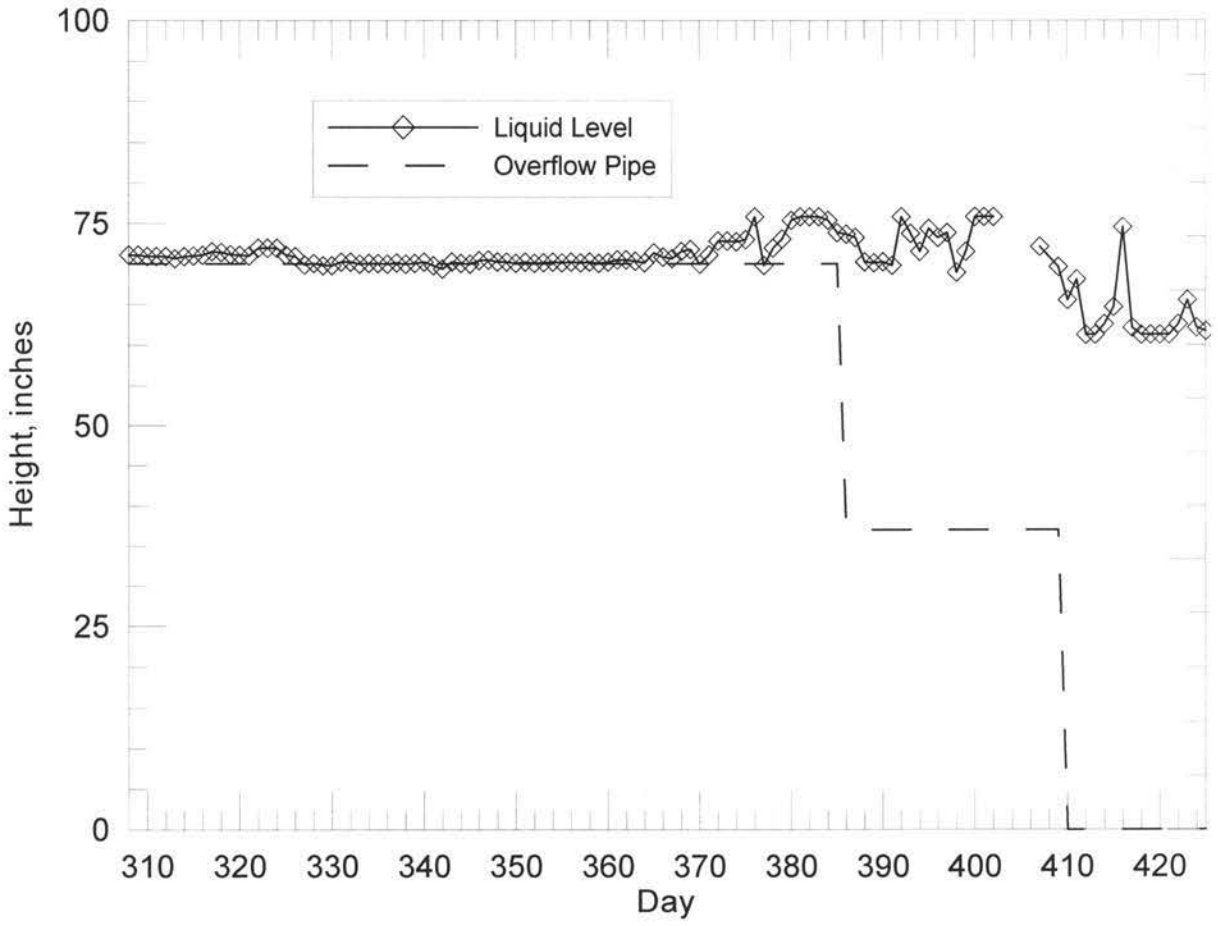

Figure 6. Liquid level inside the SGBR and height of the effluent overflow pipe for Day 308-425 (February 3 – May 31, 2003).

Hydraulic control

Hydraulic control of the SGBR is paramount for successful operation of the system. Therefore, it is recommended that a system and procedure be in place for maintaining and adjusting the fluctuations in liquid level in a full-scale system. Hydraulic control could easily be incorporated into the setup of the SGBR and the layout of the City of Austin wastewater treatment facility.

Increased headloss through the granule bed resulting from growth of the anaerobic granules and synthesis of the new bacterial cells must be controlled by routinely wasting biomass solids by backwashing the granule bed to remove accumulated biomass solids. For example, in a full-scale system at the City of Austin wastewater treatment facility, a backwash pump would fluidize the granule bed inside the SGBR (which has been taken off line) similar to the procedure used to backwash a sand filter at a water treatment plant. Using clarified effluent from one of the existing secondary clarifiers, the accumulated flocculent solids and fines would be washed out of the system via a backwash trough located above the granule bed. The backwash water would then be circulated to the clarifier (or an alternate clarifier) where any solids that were washed out of the system would be separated. This procedure is a possible solution for removing the fines that accumulate in the SGBR over time, and may also be an efficient procedure for solids wasting. Settled solids from the bottom of the clarifier could then be pumped back into the SGBR if needed. The backwash trough could be the same structure used to distribute the influent wastewater inside the SGBR headspace during normal operating conditions.

Conclusions

SGBR biological process

Results from both the previous laboratory comparison study and the current pilot-scale study demonstrate effective biological treatment of Hormel Foods slaughterhouse wastewater was possible using anaerobic granules. Analytical testing characterized the Hormel Foods influent wastewater stream prior to pretreatment and showed excellent results for treatment of this wastestream using the SGBR. Not only did the results show consistent performance of the system at different HRT and OLR conditions, but the SGBR's ability to withstand daily changes in influent wastewater characteristics was also demonstrated. Another benefit of the process was its ability to recover from long periods without sustained feeding (Day 33-127). Treatment efficiency of the system after restart with Reactor #2 (Day 128) was nearly identical to the period before shutdown with Reactor #1 (Day 1-32).

The necessity to reseed the system due to the destruction of the granular biomass also showed rapid startup of the SGBR was possible. Reactor #3 was started at a 48-hour HRT

and stepped down to a 24-hour HRT in 8-hour increments over a 29-day period. During this time OLR more than doubled from 1.37 g COD/L·d to 3.01 g COD/L·d, with a reduction in TCOD removal efficiency of only 1.4% (94.2-92.8%). Borja *et al.* (1994) was able to achieve rapid startup (35 days) of an anaerobic downflow filter treating slaughterhouse wastewater with the addition of methanol to enhance growth of methanogens. The addition of supplemental nutrients was not required during the rapid startup of the SGBR system with Reactor #3.

Throughout the entire study, the OLR ranged from 1.09-4.55 g COD/L·d and average total COD removal efficiency was greater than 90%. As expected from the laboratory comparison study, increased OLRs coupled with reduced HRTs only slightly affected performance of the SGBR. When compared to other high-rate anaerobic systems treating slaughterhouse wastewater in Table 2, the total COD removal efficiency of the SGBR system was more consistent and better than all of these systems. Unlike the use of ASBRs treating pork slaughterhouse wastewater investigated by Massé *et al.* (2001), startup of the SGBR system was rapid and high treatment efficiency was achieved within the first days of operation.

Limitations typically found in treating slaughterhouse wastewater were not experienced during this study using the SGBR system. High COD removal efficiencies, methane content (78.1-97.5%) in the biogas, and low effluent volatile fatty acids (16-25 mg/L as HAc) were evidence that no effects of inhibition from lipids and proteins occurred throughout the study. Accumulation of solid fractions of the influent wastewater was not apparent in the SGBR. Any accumulation that may have occurred did not negatively affect the performance and stability of the anaerobic process as suggested by Ruiz *et al.* (1997). Suspended solids removal was excellent throughout the entire study.

System operation

Biomass handling

Destruction of the original granular biomass could have resulted from a number of different events, either mechanical or biological. Based on the observations of the biomass samples taken on January 21, 2003 (Day 295), and the laboratory pump experiment, it is clear that multiple transfers of granules using the peristaltic pump was a major influence, if not the cause, of the destruction of the granular biomass. Overall, the majority of the granular biomass had been transferred six times between the three different reactors. A small quantity had been transferred a total of seven times, when including initial biomass seeding of Reactor #1. Complete loss of structure was experienced when the granular biomass was subjected to excessive shear forces, compressive forces, or mechanical agitation. In order to maintain the integrity of the granular biomass for use in the SGBR system, it is imperative to transfer the granules by means that minimize these risks. Full-scale applications will require

the use of pumps capable of handling fragile materials, such as a progressive cavity or diaphragm pump.

System hydraulics

Hydraulic control of the SGBR is vital to the successful operation of the system. From operation of the pilot-scale SGBR a mechanical system and procedure, as outlined in section 4.9.2, is recommended for maintaining and adjusting the fluctuations in the liquid level inside the SGBR. Increased headloss through the granule bed resulting from growth of the anaerobic granules and synthesis of the new bacterial cells must be controlled by routinely wasting biomass solids or backwashing the granule bed to remove accumulated biomass solids. Biomass yields experienced during the operation of Reactors #1, #2, and #3 were 0.389, 0.161, and 0.145 g VSS/g $COD_{removed}$, respectively.

The effluent overflow pipe used to maintain the liquid level inside the reactor worked well for the major portion of the project, but its function became unreliable at the higher flowrates experienced during the 20- and 16-hour HRT conditions. Under these conditions, adjustments made to the height of the effluent overflow pipe, coupled with the minimum available freeboard inside the reactor, were not sensitive enough to account for rapid changes in the liquid level due to increased headloss through the granule bed. Consequently, it was difficult at times to consistently maintain the 700-gallon operating volume.

Utilization of either an effluent overflow structure or pump arrangement is a possibility on a full-scale system. The pump arrangement could incorporate bypass piping, allowing the pump to be used for effluent discharge and backwashing purposes on strictly an as-needed basis. The overflow structure would thus be used for normal operation of the system.

Although the effluent pump installed on May 16, 2003 (Day 410) was successful at maintaining the desired operating volume inside the reactor, this arrangement complicates the daily operation of the system. However, incorporation of a liquid level sensor inside the reactor coupled with computer-controlled operation of the influent and effluent pumps would make this arrangement a feasible option on a full-scale system. Additional freeboard inside the reactor above the granule bed is also recommended in a full-scale system to accommodate fluctuations in the operating level.

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CHAPTER 3. EFFECT OF PENTACHLOROPHENOL (PCP) ADDITION TO THE STATIC GRANULAR BED REACTOR (SGBR)

Introduction

Numerous researchers have investigated the affect of toxic pollutants to anaerobic biomass found in wastewater streams, including the affects of pentachlorophenol (PCP) (Bhattacharya *et al.*, 1996; Montenegro *et al.*, 2001; Piringer and Bhattacharya, 1999; Tsuno *et al.*, 1996; and Wu *et al.*, 1993). PCP is an acutely toxic, chlorinated organic biocide used as an insecticide, fungicide, herbicide, and disinfectant (Bhattacharya *et al.*, 1996). PCP is commonly used in wood preservation and is an EPA priority pollutant (Bhattacharya *et al.*, 1996; Montenegro *et al.*, 2001; Piringer and Bhattacharya, 1999; Tsuno *et al.*, 1996; and Wu *et al.*, 1993). Due to its industrial application, PCP contamination from industrial wastewaters is highly possible and could be detrimental to the performance of wastewater treatment systems.

Anaerobic systems have been widely applied for the treatment of various industrial wastewaters. Anaerobic systems that utilize biomass granules, such as the upflow anaerobic sludge blanket (UASB) reactor, show promise for degrading a variety of simple and complex substrates. Another anaerobic system, known as the Static Granular Bed Reactor (SGBR), has also achieved high substrate removal efficiencies with medium and low strength wastewater. The SGBR consists of a dense bed of anaerobic granules operated in a downflow mode without flow recirculation. It does not require recirculation pumping, solids/liquid/gas separation devices, complex underdrains, or backwashing systems (Mach and Ellis, 2000). An added benefit is that the SGBR is capable of maintaining high solids retention times (SRTs) despite changes in hydraulic retention times (HRTs) (Jung *et al.*, 2002).

Objective

The objective of this research was to evaluate the effect of PCP addition to the SGBR system. Prior to this research, there has not been an investigation on the effect of toxic compounds to this new system configuration. PCP effects were examined in both batch and continuous reactor experiments.

Literature Review

Anaerobic granules

Anaerobic granular sludge is, in reality, dense microbial communities that typically are comprised of millions of self-immobilized bacterial cells per gram biomass (Liu, Xu *et al.,* 2002). Granules are formed by the agglutination of suspended biomass in a process known as granulation (Yu *et al.,* 2001). A number of physicochemical and biological parameters and interactions are involved during the complex granulation process (Liu, Xu *et al.,* 2002, and Yu *et al.,* 2001).

Granular sludge is desirable in biological wastewater treatment for many reasons. Because of their dense microbial structure, large size, and relatively high density, granules are easily separated from purified effluent making it possible to maintain high numbers of microorganisms in the reactor. This allows for rapid contaminant transformation, high loading rates of waste, and relatively small space requirements (Liu, Xu *et al.,* 2002).

Considerable effort has gone into understanding and investigating theories, models, and factors influencing the granulation process. Currently, one set of conditions or parameters that can be applied universally to anaerobic sludge to promote the granulation process has not been found. Rather, the distinct differences found in granules seem to be dependent on the nature of substrate, pH, hydraulic retention time (HRT), and temperature (Quarmby and Forster, 1995). A variety of other factors including cation addition, polymer addition, organic loading rates (OLRs), presence/absence of hydrodynamic shear force, and seed sludge characteristics also influence the granulation process. Research suggests that the microbial and physical structures of granules are a result of selection pressure during the granulation process (Liu, Xu *et al.*, 2002, and Liu *et al.*, 2002).

Numerous research works detailing the microbial populations and microstructure of anaerobic granules found phylogenetic affiliation and localization of microbial populations including *Methanobacteriales, Methanosaeta, Methanomicrobiales, Methanococcales,* and *Methanothrix* species (Alibhai and Forster, 1986; Fang *et al.*, 1995; Liu *et al.*, 2002; MacLeod *et al.*, 1990; and Ouarmby and Forster, 1995). Stability and performance of anaerobic reactors is related to the complex interaction of microbial populations (Pereira *et al.*, 2002). Effective degradation of substrates requires a good association between the microorganisms involved in the anaerobic process (Thaveesri *et al.*, 1995).

Use of specific methanogenic activity (SMA) testing for inhibition

Research has found measuring the methane production per unit biomass versus time or the methane production per unit reactor volume versus time can be a good indicator of metabolic activity of anaerobic granules (Liu, Xu *et al.,* 2002). This accepted form of analysis is known as specific methanogenic activity (SMA) testing. SMA tests can also be

useful to indicate the presence of a toxic or inhibitory compound or intermediates to the anaerobic degradation process.

SMA tests comparing granular versus suspended sludge treating oleic acid showed the granular sludge exhibited significantly higher methanogenic activity for hydrogenotrophic methanogens than suspended sludge (Pereira *et al.,* 2002). This study also found that the accumulation of adsorbed, non-degraded substrate onto the biomass inhibited the initial methane production, reduced the methane production rate, and hindered the transfer of substrate and products (Pereira *et al.,* 2002).

Campos and Chernicharo (1991) developed a new method for measuring SMA to detect inhibition of anaerobic sludge by compounds usually considered to be non-toxic at low concentrations. The method employed the use of capillary manometers on a modified Warburg respirometer under mesophilic conditions to detect inhibition by lithium chloride. The repeatability, ease-of-use, and sensitivity made this method ideal for forecasting biological loading rates during start-up of anaerobic reactors and calculating inhibitory concentrations of toxic pollutants for systems already under steady-state conditions (Campos and Chernicharo, 1991). Acclimation of the biomass under anaerobic conditions to substrate (sodium acetate) and environmental conditions (35 °C, pH 6.8) preceded the addition of the inhibitory compound (lithium chloride) to ensure the sludge was biologically active (Campos and Chernicharo, 1991). This new method was used to determine the SMA of anaerobic sludge that was under both continuous and temporary exposure to the inhibitory compound at varying concentrations. This type of SMA test used a small amount of anaerobic biomass, and therefore could be utilized for a wide range of reactor sizes (Campos and Chernicharo, 1991). Mechanical agitation of the flask was employed, not the mixing of the biomass itself, during the SMA test. Therefore, the sludge structure was not damaged during the test and did not affect the SMA results (Campos and Chernicharo, 1991).

Colleran *et al.* (1992) described a variety of methods to determine the SMA of anaerobic biomass, including gas chromatographic analysis to determine methane content and electronic pressure transducer measurements to monitor pressure increases in the headspace of sealed test vials from the release of biogas produced in the anaerobic degradation process. These techniques were utilized in characterizing anaerobic sludge cultures, anaerobic biodegradability screening, and determination of toxicity/inhibition to individual anaerobic subpopulations. Colleran *et al.* (1992) noted that due to the complex syntrophic relationships of the individual subpopulations involved in the anaerobic degradation process, disruption at any one of the individual stages by toxic/inhibitory compounds will affect the overall process. The use of SMA testing procedures to determine the effect of such compounds can therefore determine the toxicity thresholds and the ability of anaerobic subpopulations to acclimate to various toxicants (Colleran *et al.*, 1992).

Rinzema *et al.* (1988) investigated the inhibitory effects of sodium on acetoclastic methanogens in granular sludge using SMA tests. Fang *et al.* (1997) used SMA tests to

examine the effect of nine aromatic pollutants on the bioactivity of anaerobic granules from a UASB reactor. These tests measured the rate of substrate (starch) conversion to methane in the presence of an individual aromatic pollutant. Fang *et al.* (1997) found that not only was the SMA in each sample dependent on the concentration of the pollutant, but that the chemical nature of the pollutant's functional group impacted the pollutant's toxicity. The more hydrophobic the functional group, the more toxic the pollutant was to the granule (Fang *et al.*, 1997). The sensitive methanogens, located in the interior of the granules, were protected by the layered structure of the granules and the measured inhibition to the toxic pollutants was less dramatic than expected (Fang *et al.*, 1997).

Fang and Chan (1997) showed phenol toxicity was not cumulative, permanent, nor progressive to anaerobic granules treating acetate-, benzoate-, and propionate-wastewaters. SMA batch tests were used to determine the concentration of phenol that caused 50% inhibition (IC_{50}) compared to control samples without phenol. Phenol added to upflow reactors with each of the three granule types below the IC_{50} values did not inhibit methanogenic activity. Phenol concentrations above the IC_{50} inhibited methane production and substrate degradation until phenol addition was eliminated and influent substrate concentration was reduced for a period of time (Fang and Chan, 1997). Fang and Chan concluded that each granule type exhibited a toxicity threshold for phenol and was dependent on the morphology and population dynamics of the granules themselves.

Effects of pentachlorophenol (PCP) on anaerobic systems

Both batch and continuous system tests have shown the effects of PCP on anaerobic biomass. Bhattacharya *et al.* (1996) were able to achieve 93% removal of 15 mg PCP/L from an upflow anaerobic column with proper acclimation of the biomass to the PCP. The biomass used in both the upflow anaerobic column and batch tests was anaerobically digested sludge from a municipal wastewater treatment plant. Batch tests by Bhattacharya *et al.* (1996) found that PCP inhibition was dependent on biomass concentration. Higher resistance to PCP was seen at higher biomass concentrations (measured as VSS) (Bhattacharya *et al.*, 1996). This differs from the conclusions of Fang and Chan (1997) who found no correlation between biomass concentration and toxicity/inhibition of anaerobic biomass from phenol. Acclimation to PCP was achieved by gradually increasing the PCP loading to the biomass; however, the mechanism for PCP removal from the system was unclear (Bhattacharya *et al.*, 1996).

Montenegro *et al.* (2001) achieved a removal rate of 1.07 mg PCP/g VS per day at a PCP concentration of 21 mg/L. COD removal efficiency, methane content of the biogas, and the methanogenic morphology of the granular biomass utilized were not significantly affected. Again, adaptation and biomass concentration most likely limited biomass toxicity to PCP since influent PCP concentrations were gradually increased from 2 to 21 mg/L over the research period (Montenegro *et al.*, 2001). Wu *et al.* (1993) also found that unadapted

granules exhibited greater inhibition at low PCP concentrations than granules that were adapted to PCP. Methanogenesis was inhibited in volatile fatty acid (VFA)-degrading granules at PCP concentrations greater than 1 mg/L and was more prevalent as the PCP concentration increased (Wu *et al.*, 1993). Wu *et al.* (1993) found butyrate degraders were the least sensitive consortia to PCP inhibition, similar to the findings of Montenegro *et al.* (2001).

Ninety-nine percent (99%) removal of 40 to 60 mg/L of influent PCP was achieved with PCP-acclimated anaerobic granules from a lab-scale UASB through dechlorination and mineralization of PCP (Wu *et al.*, 1993). Results showed that PCP was mineralized rather than adsorbed up to a maximum specific removal rate of 18 mg of PCP/g of VSS per day, with the suggestion that greater volumetric loading removal could be achieved with increased biomass concentration (Wu *et al.*, 1993). Dechlorination of PCP to intermediate chlorophenols was measurable only at PCP concentrations that resulted in inhibition of PCP degradation, methanogenesis, and VFA degradation (Wu *et al.*, 1993).

The fate of PCP in a laboratory-scale anaerobic digester was studied by Chen *et al.* (2000). PCP-acclimated digested sludge fed with raw sludge from a municipal wastewater treatment plant was subjected to a PCP loading rate of 7.5 mg/L·d. PCP was biologically transformed to intermediate chlorophenols in the solid and aqueous phases. An average of 99.64% of the intermediate chlorophenols was recovered in the solid phase (as effluent sludge) and overall more than 97% of the PCP remained in the digester (Chen *et al.*, 2000). Methane production and VS reduction of the PCP-acclimated digester was nearly identical to the control digester during the study, indicating that the daily PCP addition was not inhibitory to the performance of the system (Chen *et al.*, 2000).

In contrast, Piringer and Bhattacharya (1999) found adsorption to be the major mechanism of PCP removal in anaerobic acidogenic systems. Neither biodegradation nor intermediates of PCP were measured in both acclimated and unacclimated cultures with PCP concentrations ranging from 6 to 35 mg/L (Piringer and Bhattacharya, 1999). Delayed degradation of substrate was noted with the presence of PCP, indicating inhibitory effects to acidogens in the anaerobic sludge (Piringer and Bhattacharya, 1999). Tham and Kennedy (1994) also investigated the sorption of PCP by granular and dispersed anaerobic sludge. Granular sludge was obtained from five different industrial sources and processed in a blender to create dispersed sludge samples. No significant difference was found in adsorption of PCP between granular and dispersed sludge at a PCP concentration of 1 mg/L (Tham and Kennedy, 1994). Similar mechanisms for PCP adsorption was experienced by both sludge types for all five of the sludge sources, as indicated by the similar values calculated for the Freundlich adsorption isotherm of each sludge (Tham and Kennedy, 1994). Biodegradation of PCP was not detected with any of the sludge samples during the adsorption tests (Tham and Kennedy, 1994). Microbial composition and not spatial

arrangement of the granules was assumed to be the key factor in biosorption capacity differences between various sludge types (Tham and Kennedy, 1994).

Tsuno *et al.* (1996) used an expanded-bed, biological granular activated carbon (GAC) reactor to study the effect of PCP under anaerobic conditions. Biodegradation, dechlorination, and adsorption were all mechanisms for PCP removal with concentrations up to 400 mg/L. The GAC physically adsorbed aqueous-phase PCP before microbial biodegradation began in the reactor (Tsuno *et al.*, 1996). Removal by dechlorination was also indicated by the presence of intermediate chlorophenols in the effluent and extracted from the GAC. Tsuno *et al.* (1996) also found PCP removal was more pronounced in the presence of a cosubstrate, acetate. Montenegro *et al.* (2001) also supplemented fatty acids and methanol during degradation of PCP under anaerobic conditions. Other research has shown that PCP cannot be used as a sole carbon source in the anaerobic degradation process (Wu *et al.*, 1993).

Overall, a number of factors affect removal of PCP in anaerobic systems. Unacclimated biomass tends to exhibit an inhibitory response when exposed to PCP, whereas acclimated biomass has been shown to remove PCP by adsorption, dechlorination, and biodegradation. A wide range of PCP concentrations was found to cause varying levels of inhibition. Most research noted anaerobic biomass experienced a greater degree of inhibition as influent PCP concentrations increased (Wu *et al.*, 1993). The opposite seems to be true as increased biomass concentration exhibits more resistance to PCP inhibition (Bhattacharya *et al.*, 1996; Wu *et al.*, 1993). The work of Bhattacharya *et al.* (1996), Montenegro *et al.* (2001), and Wu *et al.* (1993) showed that anaerobic biomass acclimation to PCP is possible, given a gradual increase in PCP loading.

Table 8. Summary of the effect of PCP on anaerobic systems

Source	Reactor Type	Sludge Type	Influent PCP conc.	Removal
Bhattacharya *et al.*, 1996	Upflow Anaerobic Column	Municipal WWTP digested sludge	15 mg/L	93%
Chen *et al.*, 2000	Anaerobic Digester	PCP-acclimated WWTP digested sludge	45 mg/d	97%
Montenegro *et al.*, 2001	Hybrid (UASB/AF)	Granular	21 mg/L	1.07 mg PCP/g VS·d
Piringer and Bhattacharya, 1996	Batch tests	PCP-acclimated municipal anaerobic sludge	6-35 mg/L	18-38%

Table 8. (continued)

Source	Reactor Type	Sludge Type	Influent PCP conc.	Removal
Tham and Kennedy, 1994	UASB	Granular	5-120 mg/L	Not Available
Tsuno *et al.*, 1996	Biological GAC	Municipal anaerobic sludge	400 mg/L	>99%
Wu *et al.*, 1993	UASB	Granular, VFA degrading	1 mg/L	Inhibited
Wu *et al.*, 1993	UASB	Granular, PCP-acclimated	40-60 mg/L	99%

Materials and Methods

General

Chemical oxygen demand (COD), total and volatile suspended solids (TSS and VSS), volatile fatty acids (VFA), and total alkalinity determination followed procedures outlined in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). The closed-reflux titrimetric method was used for the COD tests (*Standard Methods,* Section 5220 C). The distillation method (*Standard Methods,* Section 5560 C) was used to determine VFA concentration in the SGBR effluent. Influent and effluent TSS and VSS determination was done with glass fiber filter paper (Whatman GF/C, 1.2 μm pore size) following the procedure outlined in *Standard Methods,* Section 2540 D and E. Influent and effluent total alkalinity determination followed the procedure in *Standard Methods,* Section 2320 B. Influent and effluent pH was measured using an electronic pH meter (Corning Instruments, Model No. 350).

Continuous reactor

A cylindrical Plexiglas reactor with an inside diameter of 11 cm (4.33 inches) and a total volume of 5.5 liters was used as the continuous SGBR in this study. The reactor configuration included four sampling ports spaced along the height of the reactor at the 1.0-liter, 2.0-liter, 3.25-liter, and 4.5-liter volumes, respectively. Five centimeters (approximately 2 inches) of 0.95-cm (3/8-inch) diameter pea gravel was used for the underdrain in the reactor. A Masterflex peristaltic pump connected to a ChronTrol programmable timer/controller was used to feed the reactor the desired amount of substrate every twenty minutes to maintain a 24-hour hydraulic retention time (HRT). The substrate used for the continuous reactor was non-fat dry milk (NFDM) with a concentration of 2,000 mg COD/L. The NFDM was supplemented with trace minerals and sodium bicarbonate and

was stored in a refrigerator at 4°C to prevent degradation. Table 9 summarizes the composition of the NFDM substrate used in the continuous reactor. A schematic diagram of the continuous reactor is shown in Figure 7.

The anaerobic granules used in the continuous SGBR were obtained from a UASB reactor at City Brew Brewery in LaCrosse, Wisconsin, treating brewery wastewater. The granules had been stored in a refrigerator at 4°C for approximately one year prior to use. The SGBR was seeded with 3.5 liters of biomass and allowed to reach steady state conditions before any testing with PCP began. Table 10 summarizes the operating parameters for the continuous SGBR.

The PCP used in the continuous reactor and batch experiments was obtained in crystalline form (Ultra Scientific, Cat. # RCP-019, North Kingstown, RI, USA) and dissolved in distilled water filtered through a Barnstead NANOpure II filter column to a concentration of 10 mg/L. For the continuous reactor experiments, a second Masterflex peristaltic pump connected to the programmable timer/controller was used to feed a NFDM/PCP mixture to the reactor. During the continuous reactor experiments the influent pump cycle times were adjusted accordingly to maintain the 24-hour HRT condition. The SGBR was spiked with a 50/50 (v/v) mixture of 10 mg/L PCP solution and NFDM solution for 24 hours. This design represented the type of toxicity loading to the SGBR that may be expected at a wastewater treatment plant, where only a portion of the influent wastewater contains the toxic compound and is diluted by the remaining flow. Secondly, a NFDM/PCP mixture was used because Wu *et al.* (1993) also noted that PCP cannot be used as a sole carbon source in the anaerobic degradation process.

Table 9. Composition of the NFDM substrate for the continuous SGBR

Component	Chemical Formula	mg per gram COD	mg/L for 2 gCOD/L
Non-Fat Dry Milk		961.5	1,923
Sodium Bicarbonate	$NaHCO_3$	1,500	2,000
Ferrous Chloride	$FeCl_2 \cdot 4H_2O$	3.56	7.12
Nickel Chloride	$NiCl_2 \cdot 6H_2O$	0.41	0.82
Cobalt Chloride	$CoCl_2 \cdot 6H_2O$	0.40	0.80
Manganese Chloride	$MnCl_2 \cdot 4H_2O$	0.36	0.72
Zinc Chloride	$ZnCl_2$	0.21	0.42
Ammonium Molybdate	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.16	0.32
Cuprous Chloride	$CuCl_2 \cdot 2H_2O$	0.13	0.26
Sodium Selenite	Na_2SeO_3	0.04	0.08
Boric Acid	H_3BO_3	0.02	0.04

Table 10. Operating parameters for the continuous SGBR

Operating Temperature	25.3 ± 0.5 °C
Hydraulic Retention Time (HRT)	24 hours
Organic Loading Rate (OLR)	2 g COD/L·d
Reactor Volume	5.5 L
Granule Volume	3.5 L
Substrate composition	2 g COD/L of NFDM + Sodium Bicarbonate + Trace Minerals

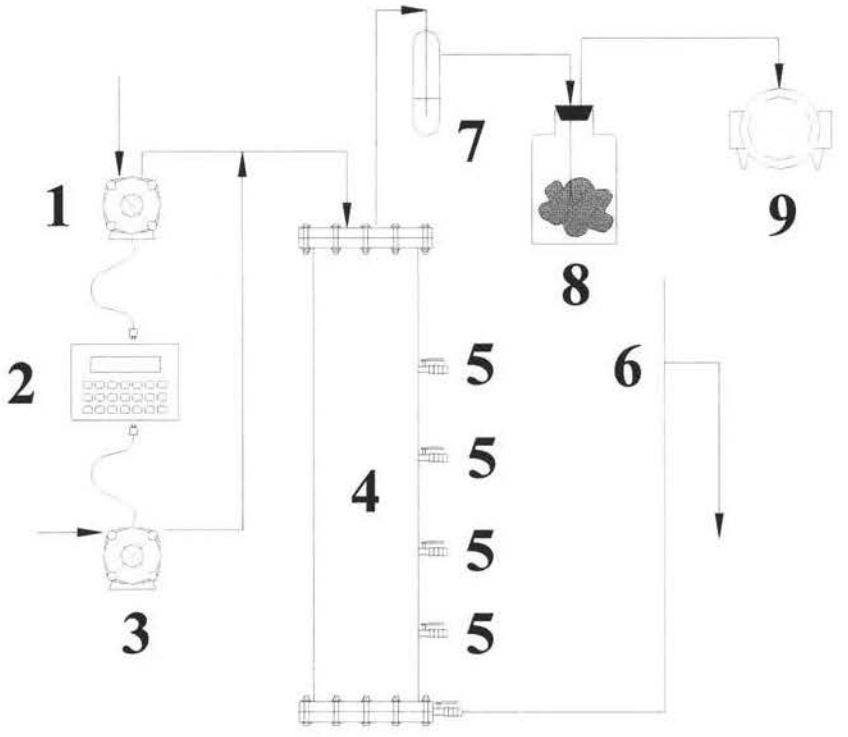

Figure 7. Schematic of the continuous SGBR system. 1, influent NFDM pump; 2, programmable timer/controller; 3, influent PCP pump; 4, SGBR; 5, sampling port; 6, T-connector; 7, gas indicator tube; 8, steel wool H_2S scrubber; 9, gas meter

Batch tests

The batch tests employed in this study were a modification of the SMA test by Rinzema *et al.* (1988). All batch tests were performed in duplicate under anaerobic conditions using 250-mL glass serum bottles, each capped with a rubber septum. The tests were performed in a constant temperature room (35 ± 1°C) using a shaker table (New Brunswick Innova Model 2000) set at 180 rpm. Five milliliters of biomass (anaerobic granules) was used in each bottle, and was obtained from the continuous SGBR. Acetic acid (1 M) was used as the base substrate. To allow adequate headspace the liquid volume of each bottle was maintained around 150 mL. A description of the SMA test procedure is outlined in Appendix C.

Biogas composition in the headspace of each bottle was measured at regular intervals throughout each test. The increase of methane in the headspace over time was measured

using a GOW-MAC gas chromatograph (GC) (Model 69-350 Thermal Conductivity Gas Chromatograph). Settings for the GC are outlined in Table 11. The sample size for each injection was 0.5 mL and the gas chromatograph was calibrated with standard gas (55% N_2, 30% CH_4, 15% CO_2) before each test. The methane content was plotted against reaction time and fit with a regression line. The rate of methane production (% CH_4/day) was calculated using the headspace volume of the bottle and the slope of the regression line. Solids measurements performed followed the appropriate procedures outlined in *Standard Methods*. Table 12 summarizes the batch tests performed during this study.

Table 11. GOW-MAC GC settings.

Parameter	Setting
Injection port temperature	160°C
Detector temperature	200°C
Column temperature	70°C
Bridge current	200 mA
Helium carrier gas flowrate	40 psig (60 mL/min)

Table 12. Summary of SMA batch test experiments.

Biomass Source	Experiment Designation	Experiment Objective
Port 1 thru 4	A	Determine SMA profile of continuous reactor prior to addition of PCP
Port 4	B	Evaluate the inhibitory effects of PCP on anaerobic granules at concentrations of 0.01, 0.1, & 1 mg/L
Port 1	C	Evaluate the inhibitory effects of PCP on anaerobic granules at concentrations of 0.01, 0.1, & 1 mg/L
Port 1	D	Evaluate the inhibitory effects of PCP on both anaerobic granules and anaerobic dispersed culture at concentrations of 6 &10 mg/L
Port 1 thru 4	E	Evaluate any changes in SMA profile of continuous reactor after the addition of PCP

Size analysis

Size analysis completed during the study was performed in the Materials Analysis and Research Laboratory of the Civil, Construction and Environmental Engineering Department at Iowa State University using a Pixera Pro CCD camera mounted on a light microscope combined with Noesis Vision's Visilog image processing and analysis software.

The image and size analysis equipment and software used in the granule size analysis had limitations that may have inaccurately portrayed the true size distribution of the samples examined. The major limitation with the analysis software was that the software first calculates the area of each object in the image, and then back-calculates the object's diameter assuming the object is spherical in shape. However, many of the granules were not spherical as shown in Figure 11, and this limitation could not be removed from the size analysis testing. Other limitations included the inability of the software program to consistently distinguish between contrasts in the color of the biomass making it easier to quantify and measure the denser, darker biomass than the flocculent biomass. The analysis software also cropped any granules around the frame of the image not representing the true size of these granules. Manual editing of the images was used to minimize these two limitations of the software during the analysis.

Results and Discussion

Batch test experiments

A total of five batch experiments were performed to evaluate the SMA of the granules from the master SGBR. Batch test A was performed after the master SGBR had reached steady-state to establish the SMA profile within the reactor prior to dosing with PCP. The results of batch test A, shown in Table 13, found that the average SMA of the granules decreased from top to bottom in the SGBR. The activity of the granules taken from the top of the reactor (Port 1) had an average SMA of 0.406 g COD-CH_4/gVS·d compared to granules in the bottom of the reactor (Port 4), which had an average SMA of 0.122 g COD-CH_4/gVS·d.

This trend could have been the result of the organic loading rate used in this study. Combined with the relatively long hydraulic retention time and relatively low wastewater strength, the granules in the upper half of the reactor may have consumed the majority of the influent COD. If such conditions existed, the granules in the lower half of the reactor may have entered a dormant state, resulting in decreased SMA. Therefore, given the influent wastewater characteristics, the volume of biomass used to seed the master reactor could have been reduced to better utilize the entire depth of the granule bed for treatment of the wastewater. Using a smaller quantity of granular biomass would most likely increase the

SMA of the granules themselves, given the biomass was not subjected to overloading conditions.

Table 13. Batch test A results

Sample	Reactor Location	SMA ($gCOD\text{-}CH_4/gVS\cdot d$)	Average SMA ($gCOD\text{-}CH_4/gVS\cdot d$)
1-1	Port 1 (Top)	0.413	0.406
1-2		0.399	
2-1	Port 2	0.179	0.131
2-2		0.083	
3-1	Port 3	0.090	0.09
3-2		0.089	
4-1	Port 4 (Bottom)	0.142	0.122
4-2		0.103	

Batch tests B through D were used to examine the inhibitory effects of PCP at different concentrations. The goal of these tests was to find the concentration of PCP that caused 50% reduction in the SMA of the granules relative to the control samples without PCP. This reduction in SMA would be attributed to inhibition caused by the PCP, and the corresponding concentration would be referred to as the 50% inhibition concentration (IC_{50}). The results of batch tests B through D are shown in Table 14.

Figure 8 is a plot of relative SMA values at the various concentrations of PCP examined in batch tests B, C, and D. There was no clear correlation between PCP concentration and relative SMA found from the results of batch tests B, C, and D. Therefore, the IC_{50} for PCP was not determined as stated above. The best correlation was found from batch test B, which exhibited a decrease in relative SMA as PCP concentration increased. However, this same trend was not observed when batch tests C and D were performed using granules from Port 1 of the master SGBR. During batch test C, samples with PCP concentrations of 0.01, 0.1, and 1 mg/L exhibited relative SMA values greater than 100% of the control samples, indicating the anaerobic granules could co-digest the PCP and acetic acid to produce methane at these concentrations. PCP concentrations of 6 and 10 mg/L used in batch test D began to show signs of inhibition with relative SMA values less than 100% of the control samples. These values however were not close to 50% of the SMA values for the control samples. Additional batch tests with higher concentrations of PCP are needed to establish the IC_{50} caused by PCP to the master SGBR. The anaerobic dispersed culture samples in batch test D were obtained by crushing granules from the continuous SGBR. Methane production was not observed from these samples. It was unclear if methane production was inhibited by the presence of PCP or the disruption of the biomass structure.

Table 14. Batch tests B through D results

Batch Test	Sample	Reactor Location	PCP Concentration (mg/L)	SMA (gCOD-CH_4/gVSS·d)	Average SMA (gCOD-CH_4/gVSS·d)	Relative SMA (% of Control)
B	Control 1	Port 4	0	0.0340	0.0528	
	Control 2	Port 4	0	0.0397		
	Control 3	Port 4	0	0.0847		
	2PCP1	Port 4	2	0.0000	0.0000	0.00
	2PCP2	Port 4	2	0.0000		
	1PCP1	Port 4	1	0.0000	0.0196	37.1
	1PCP2	Port 4	1	0.0196		
	0.1PCP1	Port 4	0.1	0.0386	0.0386	73.1
	0.1PCP2	Port 4	0.1	0.0000		
	0.01PCP1	Port 4	0.01	0.0000	0.0354	67.0
	0.01PCP2	Port 4	0.01	0.0354		
C	1PCP1	Port 1	1	0.7432	0.7107	175[a]
	1PCP2	Port 1	1	0.6781		
	0.1PCP1	Port 1	0.1	0.4480	0.4640	114[a]
	0.1PCP2	Port 1	0.1	0.4801		
	0.01PCP1	Port 1	0.01	0.8919	0.6785	167[a]
	0.01PCP2	Port 1	0.01	0.4651		
D	CG1	Port 1	0	0.0301	0.0278	
	CG2	Port 1	0	0.0259		
	6G1	Port 1	6	0.0174	0.0222	80.0
	6G2	Port 1	6	0.0270		
	10G1	Port 1	10	0.0095	0.0235	84.7
	10G2	Port 1	10	0.0376		
	CDC1	Port 1	0	0.0000	0.0000	
	CDC2	Port 1	0	0.0000		
	6DC1	Port 1	6	0.0000	0.0000	0.00
	6DC2	Port 1	6	0.0000		
	10DC1	Port 1	10	0.0000	0.0000	0.00
	10DC2	Port 1	10	0.0000		

[a] Average SMA of Port 1 samples from Batch test A used to calculate relative SMA

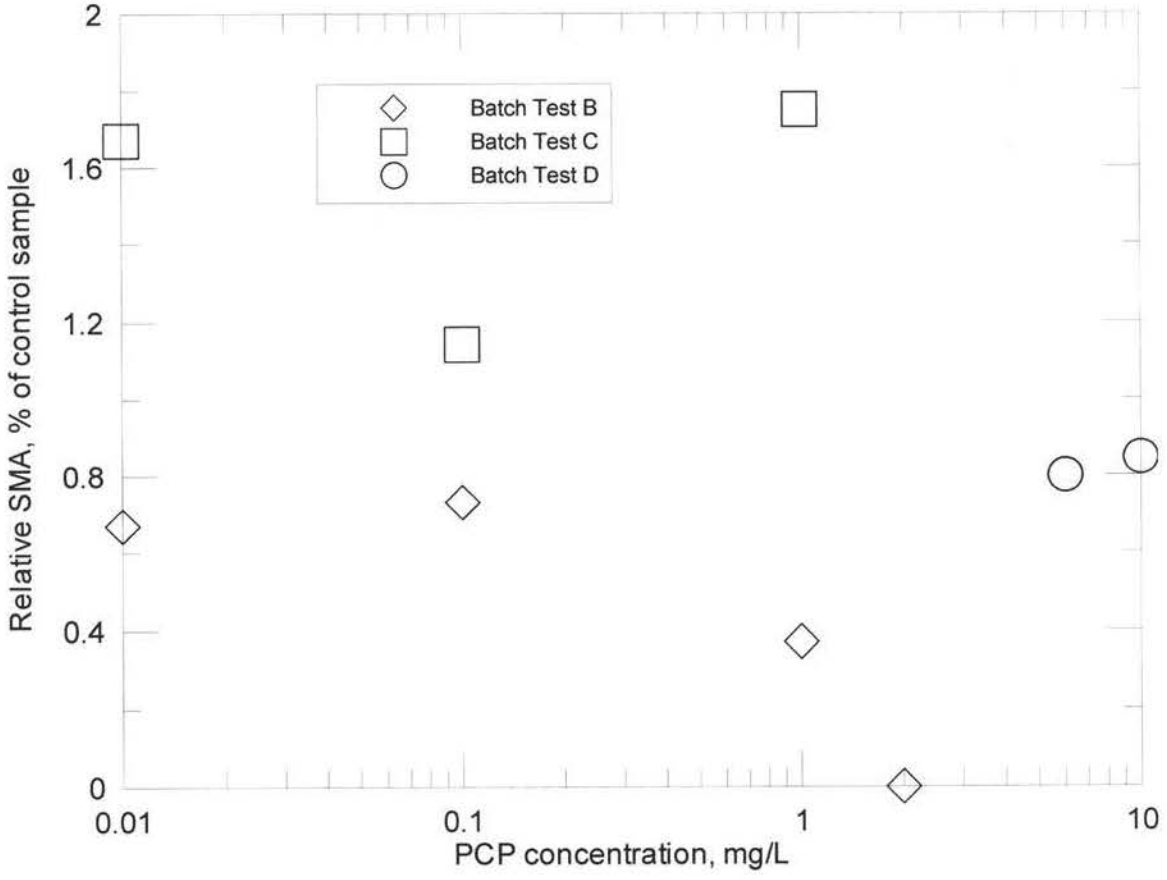

Figure 8. Relative SMA vs. PCP concentration for batch tests B through D.

Batch test E evaluated the SMA profile in the master SGBR after the additions of PCP during the continuous reactor experiments. Again, a distinct profile existed in the master SGBR with SMA values decreasing from top to bottom in the reactor. Table 15 summarizes the results of Batch test E. These results showed that the addition of PCP from the continuous reactor experiments did lower the average SMA of the granules throughout the entire reactor. Granules from Ports 3 and 4 (bottom half of the reactor) experienced a greater reduction in SMA than granules from the Ports 1 and 2 (top half of the reactor). Granules from the bottom half of the reactor may have been more susceptible to inhibition by PCP due to their low initial SMA. Removal of PCP by adsorption to the biomass, as found by Piringer and Bhattacharya (1999), would cause inhibition to unacclimated bacteria on the surface of the anaerobic granules. Assuming a layered granular structure, presence of PCP adsorbed to the surface of the granules would inhibit subpopulations of anaerobic bacteria on the exterior surface of the granules from degrading the influent wastewater into intermediates used by the various methane-producing subpopulations located in the interior of the granules.

The reduction in SMA of the anaerobic granules was the result of a lower quantity of the influent wastewater COD being converted into methane-COD.

The concentration of PCP used in the continuous reactor experiments was not high enough to cause complete inhibition of the anaerobic granules in the master SGBR. The high biomass concentration in the SGBR may have also minimized the effects of PCP addition and prevented complete inhibition of the granules. The low SMA of the granules from the bottom half of the SGBR may have been a result of a smaller population of various anaerobic bacterial subpopulations on the surface of the granules compared to granules from the top half of the reactor. Again, this could have been a result of the substrate characteristics as described previously. Therefore, a larger percentage of the bacteria on the exterior of the granules may have been inhibited by the PCP added in the continuous reactor experiments, resulting in lower relative SMA values when compared to granules from the top of the reactor as noted in Table 15.

Table 15. Batch test E results

Sample	Reactor Location	SMA (gCOD-CH_4/gVS·d)	Average SMA (gCOD-CH_4/gVS·d)	Relative SMA (% of Batch test A)
1-1	Port 1 (Top)	0.207	0.287	70.77
1-2		0.399		
2-1	Port 2	0.125	0.098	74.70
2-2		0.083		
3-1	Port 3	0.090	0.047	52.47
3-2		0.089		
4-1	Port 4 (Bottom)	0.142	0.034	27.73
4-2		0.103		

Continuous reactor experiments

Influent and effluent COD, suspended solids, pH, alkalinity, and VFA concentrations were determined on a regular basis prior to additions of PCP to establish the treatment efficiency and performance of the master SGBR. Regular analysis of the methane content in the biogas from the system was also measured using the GOW-MAC GC. Daily measurement of these parameters was conducted during the additions of PCP to the system. Table 16 lists the results from these analyses.

Addition of PCP to the SGBR in the form of two shock loading events was not detrimental to the performance of the system. A PCP concentration of 10 mg/L caused the greatest degree of inhibition, measured as relative SMA, to methanogens in the biomass

Table 16. Summary of analytical testing for the master SGBR

Parameter (mg/L except pH & as noted)	Day		
	Period I 105-211	Period II 212-221	Period III 222-229
	Normal operation NFDM feed only	PCP addition #1 50/50 (v/v) PCP/NFDM feed on Day 212; NFDM feed only Day 213-221	PCP addition #2 50/50 (v/v) PCP/NFDM feed on Day 222; NFDM feed only Day 223-229
Influent TCOD	1985 ± 195	1818 ± 14	1854
Influent SCOD	1687 ± 136	1699 ± 24	1657 ± 31
Effluent TCOD	31.2 ± 11.6	25.3 ± 9.7	22.9 ± 6.8
Effluent SCOD	17.8 ± 7.5	19.1 ± 9.3	9.1 ± 5.4
Influent TSS	128 ± 28	103	NM[a]
Influent VSS	118 ± 26	92	NM[a]
Effluent TSS	8.8 ± 3.2	8.5 ± 2.9	9.3 ± 2.8
Effluent VSS	7.6 ± 4.6	8.2 ± 2.6	8.5 ± 2.9
Influent Alkalinity (as $CaCO_3$)	172 ± 53	100	NM[a]
Effluent Alkalinity (as $CaCO_3$)	825 ± 340	600	NM[a]
Effluent VFA (as HAc)	18.6 ± 21.8	12.2 ± 3.8	12.6 ± 10.7
Influent pH	6.36 ± 0.60	6.19 ± 0.62	6.14 ± 0.53
Effluent pH	6.92 ± 0.20	7.13 ± 0.13	7.10 ± 0.06
Biogas CH_4 (%)	59.0 ± 11.6	63.79 ± 0.02	66.36 ± 0.03
Daily CH_4 @ STP (L)	1.82 ± 0.64	1.63 ± 0.36	1.60 ± 0.38

[a] NM = Not Measured

during batch test D, and was used for the continuous reactor experiments. Since the concentration of PCP was below the solubility limit of PCP in water (14 mg/L), any PCP that passed through the SGBR would have been measured as soluble-COD (SCOD) in the effluent. As the results show, effluent SCOD increased slightly during Period II, which may have been an indication of PCP washout through the system. During Period III effluent SCOD was lower than both Period I and II, indicating possible acclimation to the PCP. Given the relatively short exposure time and nature of PCP loading to the system acclimation and degradation of PCP by the granular biomass in the SGBR was not expected. Methane content in the biogas increased and effluent VFA concentrations decreased during Periods II and III. Daily methane production decreased slightly during Periods II and III after the additions of PCP. Effluent pH also increased during these two time periods, but was still within the acceptable range for methanogens.

Figures 9 through 13 show trends in effluent parameters before, during, and after additions of PCP to the continuous reactor. Effluent SCOD, VFA, and suspended solids concentrations increased on the first day of Period II and three days following the addition of PCP at the beginning of Period III. Increased effluent SCOD indicated PCP washout through the system. Elevated effluent VFA concentration signaled inhibition to methanogens in the biomass. Increased effluent suspended solids resulted from washout of biomass from the SGBR. Figures 9, 10, and 11 show that these effluent parameters quickly returned to normal concentrations during the rest of the time period, respectively. However, both average SCOD removal and methane concentration in the biogas increased following the additions of PCP to the system. Overall, these results indicated little, if any, lasting inhibition by PCP occurred to methanogens in the biomass.

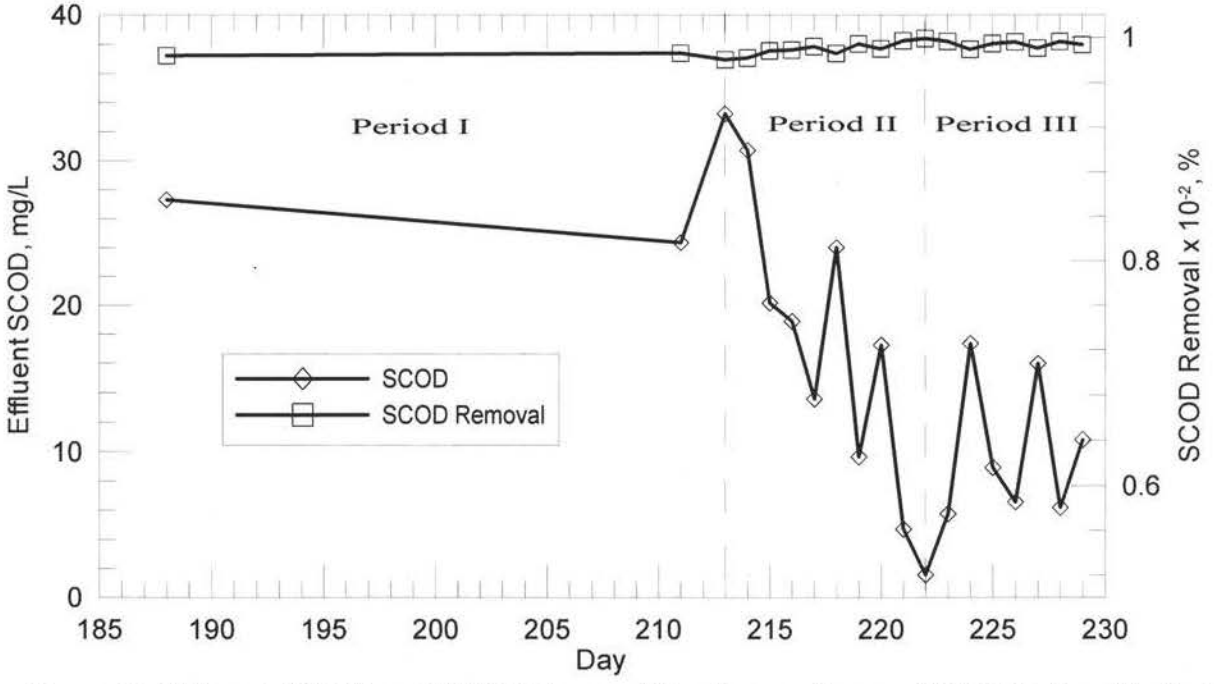

Figure 9. Effluent SCOD and SCOD Removal for the continuous SGBR before (Period I), during, and after PCP addition (Periods II & III). PCP was added on the first day of Periods II & III.

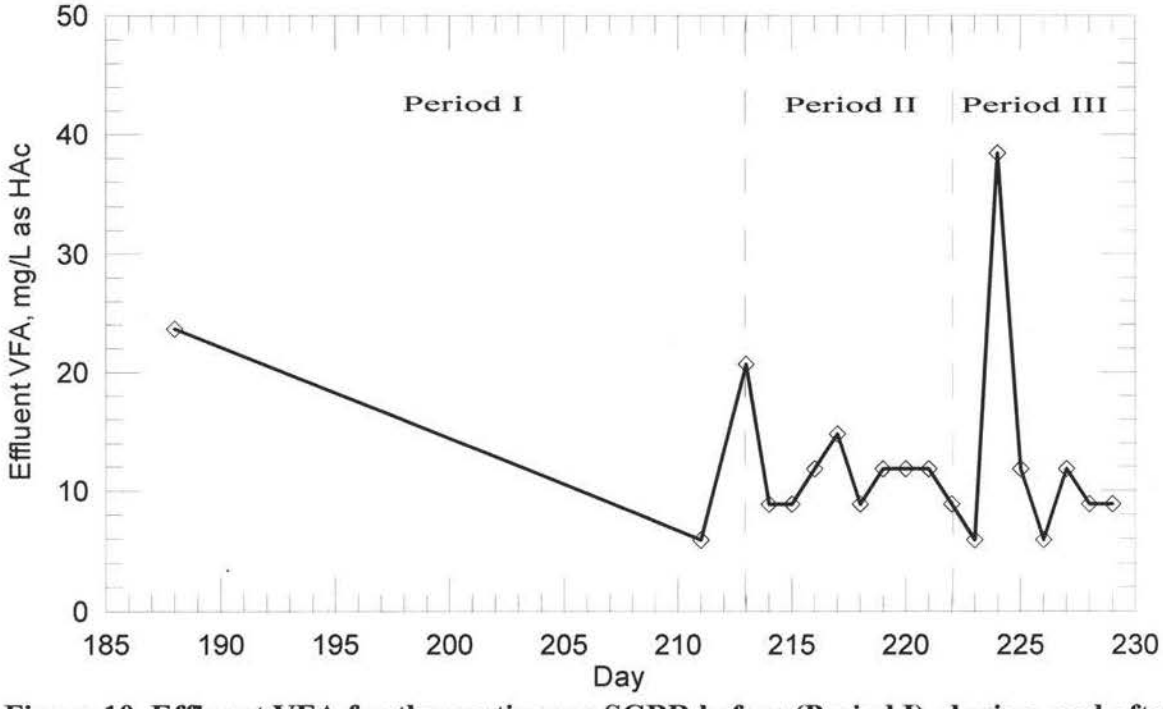

Figure 10. Effluent VFA for the continuous SGBR before (Period I), during, and after PCP addition (Periods II & III). PCP was added on the first day of Periods II & III.

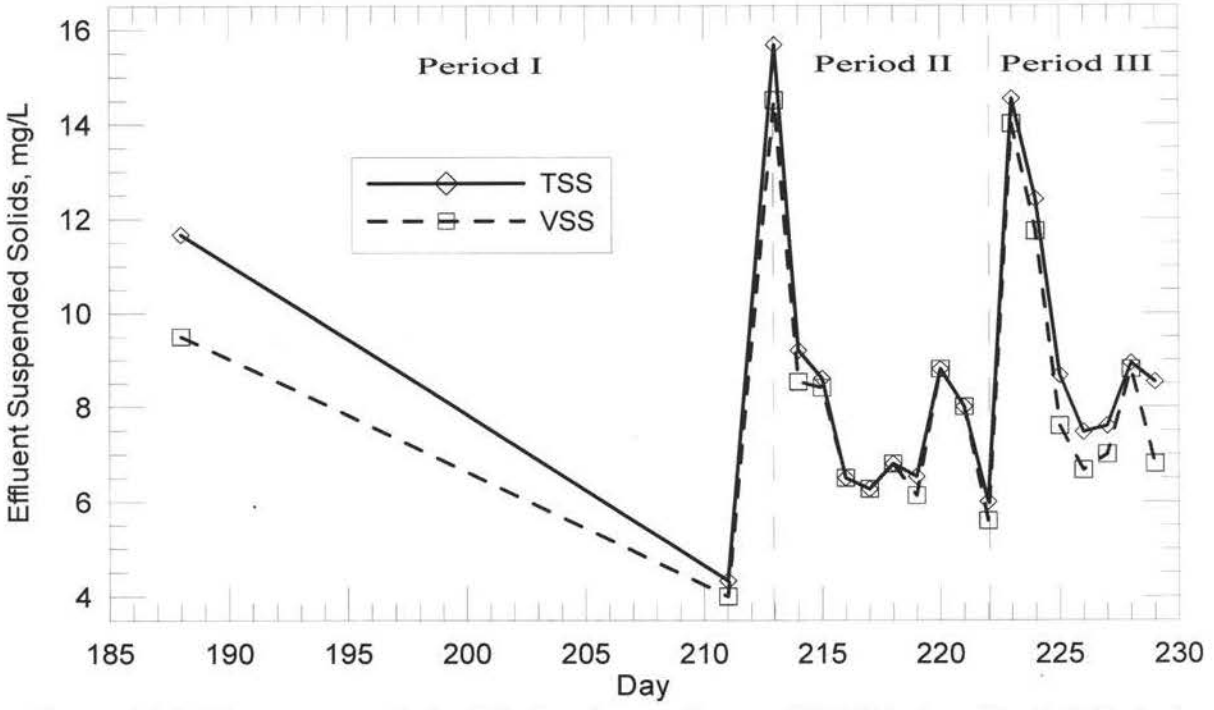

Figure 11. Effluent suspended solids for the continuous SGBR before (Period I), during, and after PCP addition (Periods II & III). PCP was added on the first day of Periods II & III.

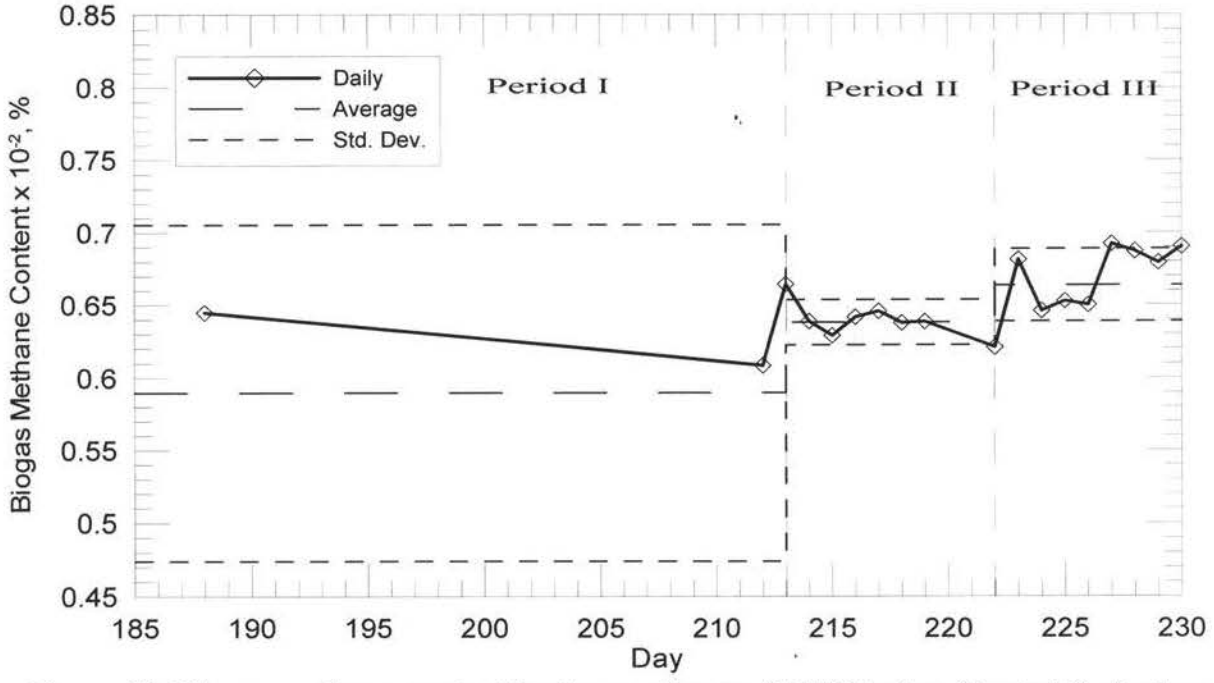

Figure 12. Biogas methane content for the continuous SGBR before (Period I), during, and after PCP addition (Periods II & III). PCP was added on the first day of Periods II & III.

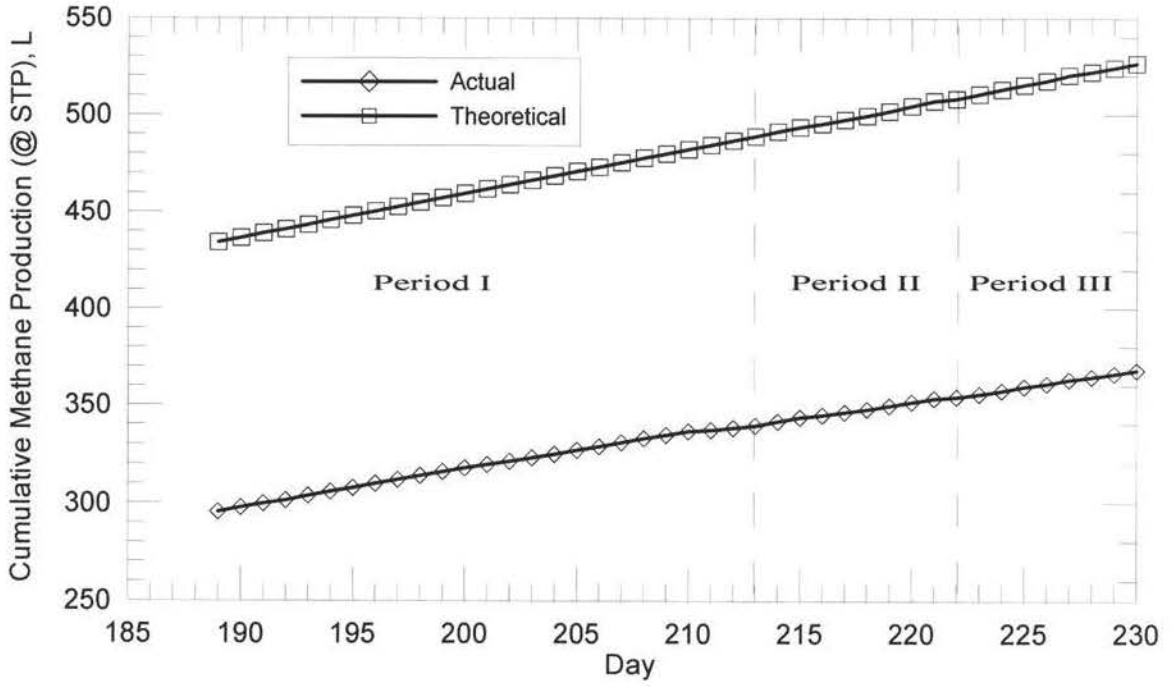

Figure 13. Cumulative methane production for the continuous SGBR before (Period I), during, and after PCP addition (Periods II & III). PCP was added on the first day of Periods II & III.

Size analysis

Prior to the additions of PCP to the SGBR a granule size distribution along the entire height of the reactor was determined. Figure 14 shows the size distributions as a function of count fraction along the profile of the reactor. The mean granule diameters from the top (Port 1) to the bottom of the reactor (Port 4) were 0.74 mm, 0.93 mm, 0.98 mm, and 0.78 mm, respectively. From these results the largest granules were located in the middle of the reactor (Ports 2 & 3); however, the volume-weighted mean diameter results showed that the largest granules were located at the top of the reactor (Port 1). The volume-weighted mean diameters from top to bottom of the reactor were 2.46 mm, 1.63 mm, 1.35 mm, and 1.34 mm, respectively. The granule size distribution along the reactor profile as a function of the area fraction is shown in Figure 15. The mean diameter and volume-weighted mean diameter for the seed granules were 0.90 mm and 1.30 mm, respectively.

The mean granule diameter at the top of the reactor was skewed due to the high count fraction of small diameter particles in this sample, as shown in Figure 14. These small diameter particles were newly synthesized biomass from biological yield and accumulation inside the SGBR. Figure 15 shows that the larger granules are located in the top half of the

SGBR (Ports 1 & 2). The results of the size analysis show that granules throughout the entire reactor have increased in size when compared to the seed granules. The greatest increase in size due to growth was in the top of the granule bed (Port 1). These results coincide with the results of batch tests A and E that the most biologically active granules were in the top of the bed. As previously discussed, these results could be due to the influent wastewater characteristics used in this study.

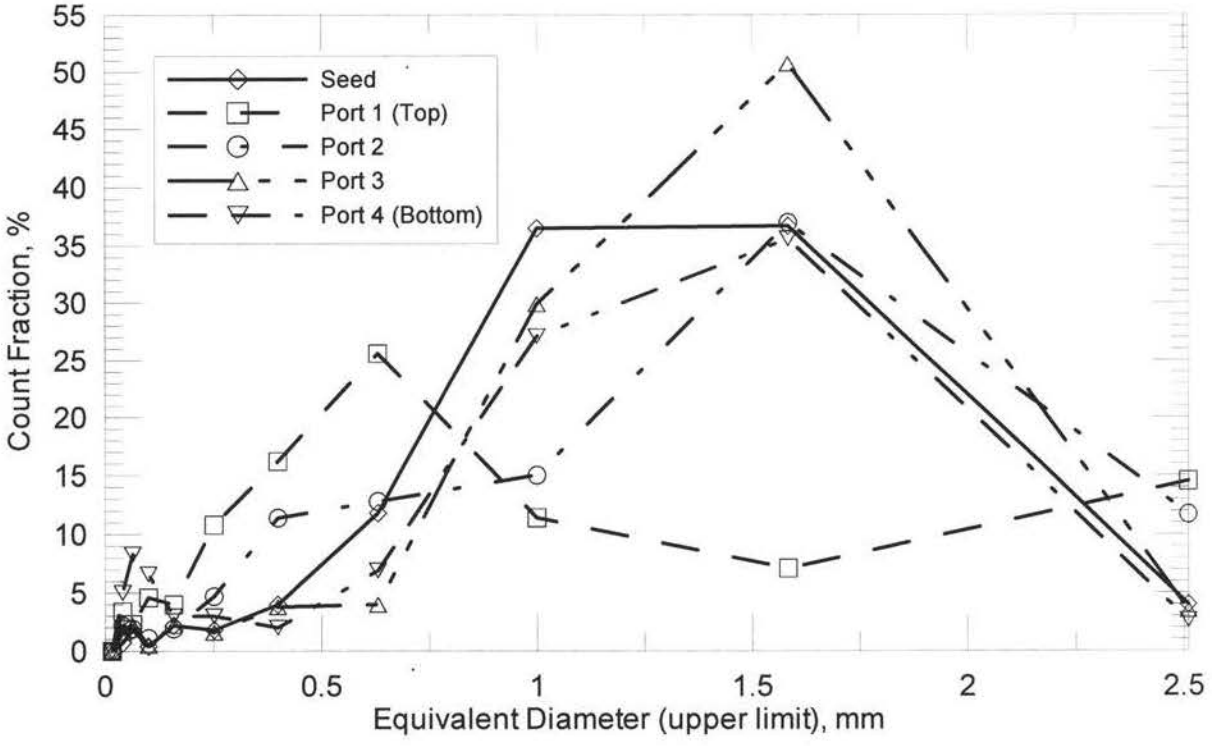

Figure 14. Size distribution (count fraction) of granules along the profile of the SGBR.

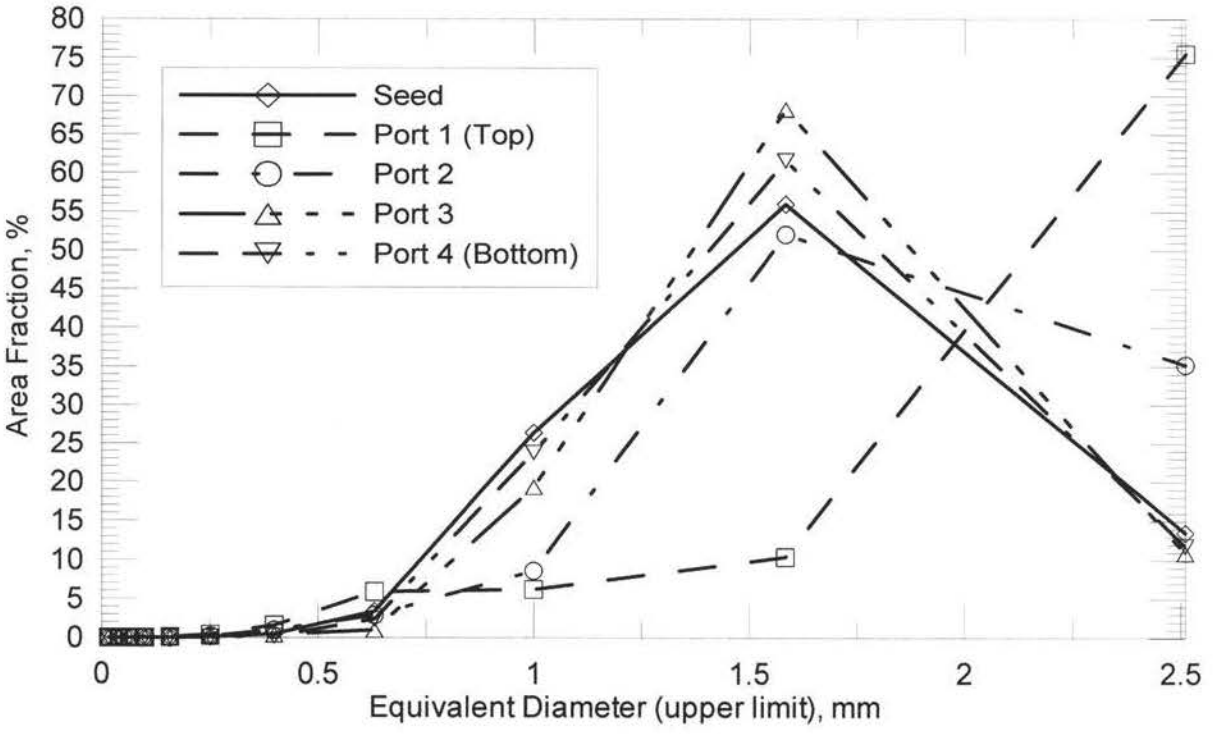

Figure 15. Size distribution (area fraction) of granules along the profile of the SGBR.

Conclusions

The SMA batch tests showed greater inhibition to methanogens in the biomass from the bottom half than the top half of the reactor. Average SMA values in the top and bottom half of the SGBR before the PCP additions to the system were 0.268 g COD-CH_4/g VS·d and 0.125 g COD-CH_4/g VS·d, respectively. After the PCP additions these same values were 0.192 g COD-CH_4/g VS·d and 0.041 g COD-CH_4/g VS·d. Despite the results of the batch tests, the continuous reactor experiments during this study showed the addition of PCP up to concentrations of 10 mg/L had little effect on the overall performance of the SGBR system. Effluent SCOD, VFA, and suspended solids concentrations did increase following shock loading of PCP to the system, but quickly returned to normal concentrations after a few days. Little, if any, lasting inhibition to methanogens in the biomass occurred during or after the addition of PCP. Methane content in the biogas and SCOD removal efficiency increased following the addition of PCP to the system. Removal of PCP from the system was accomplished by one of the following: adsorption to the biomass; degradation to intermediates and conversion to methane; washout through the system into the SGBR effluent. Additional research is necessary to determine the fate of PCP in the SGBR.

SMA batch tests performed to measure the inhibitory effect of PCP to methanogens in the biomass did not yield a concentration of PCP that caused 50% inhibition (IC_{50}) relative to the control samples. PCP concentrations of 6 and 10 mg/L began to show signs of inhibition with relative SMA values less than 100% of the control samples. Additional SMA testing with higher concentrations of PCP is necessary to determine the IC_{50} for methanogens in the biomass from the SGBR system.

Batch tests both before and after the PCP additions to the SGBR system showed that the granules from the top of the reactor had the highest SMA values. These results indicated that the organic loading rate was too low to utilize the entire bed depth of the reactor. Therefore, granules in the bottom of the reactor were under-loaded, in terms of substrate concentration, and went into a dormant state. Evidence of this condition was exhibited in the batch tests. All batch test samples with granules from the bottom half of the reactor (Ports 3 & 4) experienced a pronounced lag phase during the SMA tests. Therefore the system can be subjected to higher organic loadings if necessary. Otherwise, a smaller quantity of biomass can be utilized in the SGBR at the organic loading rate used in this study to more effectively utilize the entire granule bed.

Size analysis prior to the additions of PCP found that the largest granules were located in the top half of the reactor. Granules in the top of the reactor also experienced the greatest increase in size compared to the seed granules used start the SGBR system. The dramatic growth of these granules coincide with the results of the batch tests that showed the greatest SMA values at the top of the reactor as well. These findings were the result of the organic loading rate and wastewater characteristics used in this study.

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CHAPTER 4. ENGINEERING SIGNIFICANCE

On-site Pilot Demonstration Project

The practical and engineering significance of this project was to demonstrate the SGBR on a large scale basis treating Hormel Foods wastewater. The on-site demonstration project utilized the first-ever pilot-scale SGBR system to demonstrate this new anaerobic treatment system. A laboratory comparison study previously showed that effective treatment of Hormel Foods wastewater was possible using the SGBR. The biological performance results from this study echoed the results from the laboratory comparison study, indicating that scale-up of the system did not impact treatability of the wastestream. The excellence performance over a range of OLRs and simple design of the SGBR, make this new anaerobic system advantageous compared to many conventional anaerobic systems treating slaughterhouse wastewaters.

During this study the pilot-scale system operated over HRT and OLR ranges of 48- to 16-hours and 1.09 to 4.55 g COD/L·d, respectively. The total COD, BOD_5, and TSS removal efficiencies throughout the entire study averaged over 90%. The SGBR used in the laboratory comparison study was capable of operating at an 8-hour HRT and OLR of 7.23 g COD/L·d, achieving a total COD removal efficiency of 89% under these conditions. The significance of these results is that the lower limits, in terms of HRT and OLR, for the SGBR have not been found. To date, excellent removal efficiencies have been measured at all the HRT and OLR conditions examined utilizing the SGBR to treat Hormel Foods wastewater.

In terms of full-scale design, the benefit of high removal efficiencies at increased OLRs and shorter HRTs translates into smaller reactor size requirements compared to other systems. However, full-scale SGBR sizing cannot be based on OLR and HRT alone, but must also incorporate additional space for biomass accumulation inside the system. Plans to increase the OLR to the anaerobic system in the future will have a significant effect on biomass accumulation in either the current anaerobic contact system or a full-scale SGBR. Tripling the OLR to the anaerobic system, accounting for a slight decrease in organic removal efficiency due to higher loads, may double or triple the rate of biomass accumulated per reactor volume. Because of the effectiveness of the SGBR to retain biomass solids, this increase would be evident as additional biomass accumulation inside the reactor, whereas increased biomass washout may be experienced with the current anaerobic contact system. Under the current conditions, effluent VSS concentrations from the anaerobic contact system were already higher than that from the pilot-scale SGBR.

Significant lessons were learned about the physical operation of the SGBR that were not experienced during the laboratory comparison study. From this, recommendations for hydraulic control and biomass handling were included with the results of the project.

Hydraulic control of the SGBR is paramount for the success of the system, and is maintained by controlling the biomass solids inside the reactor. Unfortunately, this only became an issue at the end of the project period. Therefore, biomass was wasted only twice toward the end of the project and the recommended backwashing procedure was not attempted during the operation of the pilot SGBR system. Additional research incorporating this backwashing procedure in order to waste accumulated solids from the reactor is recommended for the development of a full-scale system. The amount of biomass that accumulates inside the reactor will depend on the OLR, removal efficiency, biomass washout, fixed solids accumulation, and methane production from the system. Balancing these parameters over time will determine the amount of accumulated biomass in the system. Given the backwash water solids concentration, the required volume of backwash water can then be calculated in order to remove the necessary quantity of accumulated biomass. Ultimately, by combining the calculated backwash volume and backwash flowrate, the system operator can determine the length of time required for backwashing to waste accumulated solids from the system. Biomass solids settled out in the clarifier can be returned to the reactor if needed or stored for reseeding in the event of a system failure.

The granular biomass must also be handled by means that minimize the risk of exposure to excessive shear and compressive forces and agitation. Exposure to these elements destroys the structural integrity of the granules, resulting in physical failure of the system. Although the fragile characteristics of the granular biomass were known, the problem associated with using a peristaltic pump to transfer the granules was not expected at the beginning of the project. Multiple transfers of the original biomass using a peristaltic pump crushed the granules. The pump head design was not conducive to properly handling the fragile biomass. The finely crushed biomass pieces settled inside the reactor forming a dense sludge layer at the bottom of the reactor inhibiting the system from draining normally.

Using a diaphragm or progressive cavity pump pushes the biomass through the pump in discrete volumes. Excessive forces or agitation are not imparted on the biomass during this process. The fluid and solids are moved through the pump by means of positive displacement. This is the ideal and the most effective method for transferring fragile materials.

Inhibition Study utilizing PCP

The significance of this study was that little, if any, lasting inhibitory effects to a SGBR system were measured in the laboratory following the additions of PCP up to concentrations of 10 mg/L. Prior to this research the SGBR had not been subjected to loadings with inhibitory compounds. Given the characteristics of PCP and its effects on other anaerobic systems, the addition of PCP to the unacclimated granules in the SGBR was

expected to cause inhibition to methanogens in the biomass at low PCP concentrations. The high biomass concentrations maintained in the SGBR might have mitigated the inhibitory effects of PCP addition to the system. Therefore, recommendations for future research included dosing higher concentrations of PCP and determining the fate of PCP in the system.

CHAPTER 5. GENERAL CONCLUSIONS

On-site Pilot Demonstration Project

Results from the on-site pilot demonstration of the SGBR showed effective biological treatment of Hormel Foods slaughterhouse wastewater was possible using anaerobic granules. Limitations typically found in treating slaughterhouse wastewater were not experienced during this study. Analytical testing showed excellent results for treatment of this wastestream using the SGBR. Not only did the results show consistent performance of the system at different HRT and OLR conditions, but the ability of the SGBR to withstand daily changes in influent wastewater characteristics was also demonstrated. The SGBR was able to recover from long periods without sustained feeding. Treatment efficiency was nearly identical before and after breaks in the operation of the system. This study also showed that rapid startup of the SGBR was possible. When compared to other high-rate anaerobic systems treating slaughterhouse wastewater, the total COD removal efficiency of the SGBR system was more consistent and better than all of these systems. During this study additional experience was gained in biomass handling and transfer as well as hydraulic control of the SGBR. Such experiences were invaluable for the development of a full-scale SGBR system, as these two areas were not a concern in the smaller, laboratory-scale systems.

Inhibition Study utilizing PCP

Results from the inhibition study demonstrated that there was little, if any, lasting inhibitory effects due to PCP up to concentrations of 10 mg/L to the SGBR. Further research using higher concentrations of PCP (above the solubility limit in water) or other potentially inhibitory compounds is recommended to further test the limits of the system. This research should also focus on determining the fate of such compounds added to the system.

The research also found distinct SMA and granule size profiles existed within the SGBR. These SMA and size profiles were the result of the OLR used in this study. Additional research focusing on the minimal acceptable granule bed depth for effective wastewater treatment will also provide insight into the limitations of this new system.

Overall Objective

The overall objective of this research was to determine limitations and evaluate performance of the SGBR under various conditions. Not only did both separate research

studies achieve their respective objectives, but both studies added to the foundation for the future development of a full-scale SGBR system. Both studies incorporated conditions that will or could be experienced at full-scale operation. Understanding the impact of these conditions to the SGBR system today, will ensure the success of a full-scale SGBR system tomorrow.

APPENDIX A:

DIMENSIONED REACTOR DRAWINGS AND SYSTEM SCHEMATICS

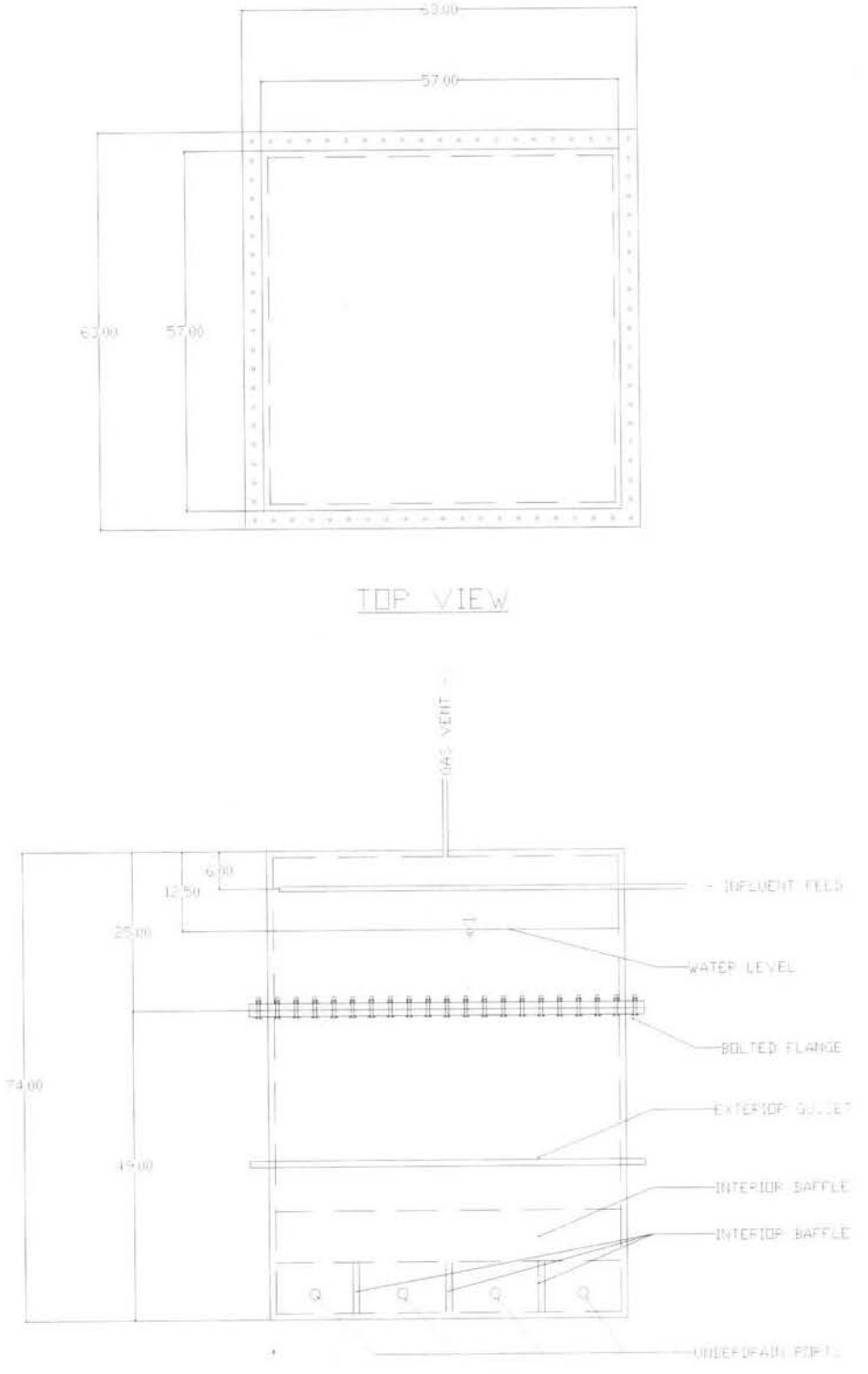

Figure A1. Dimensioned drawing of Reactor #1

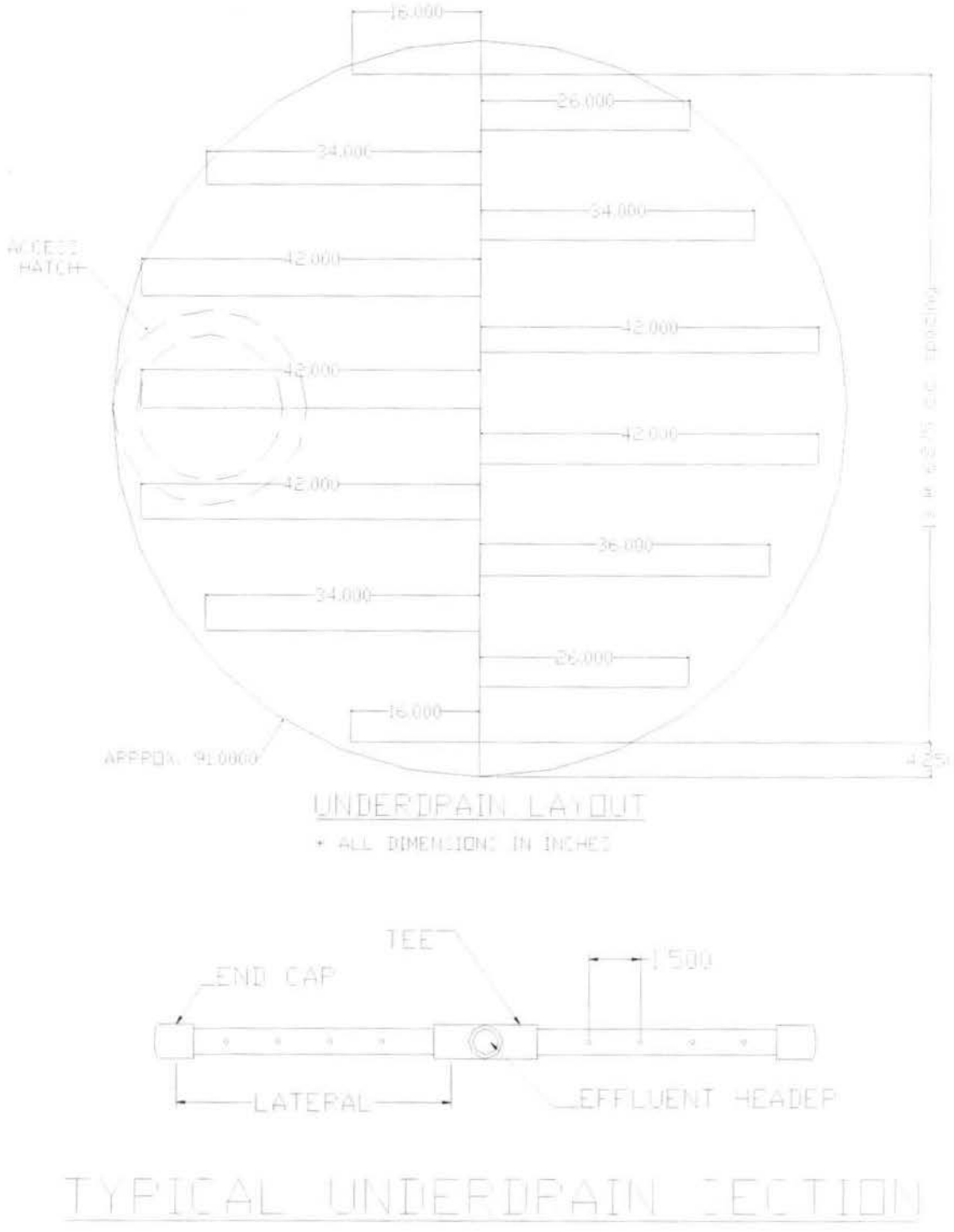

Figure A2. Dimensioned drawing of Reactor #2

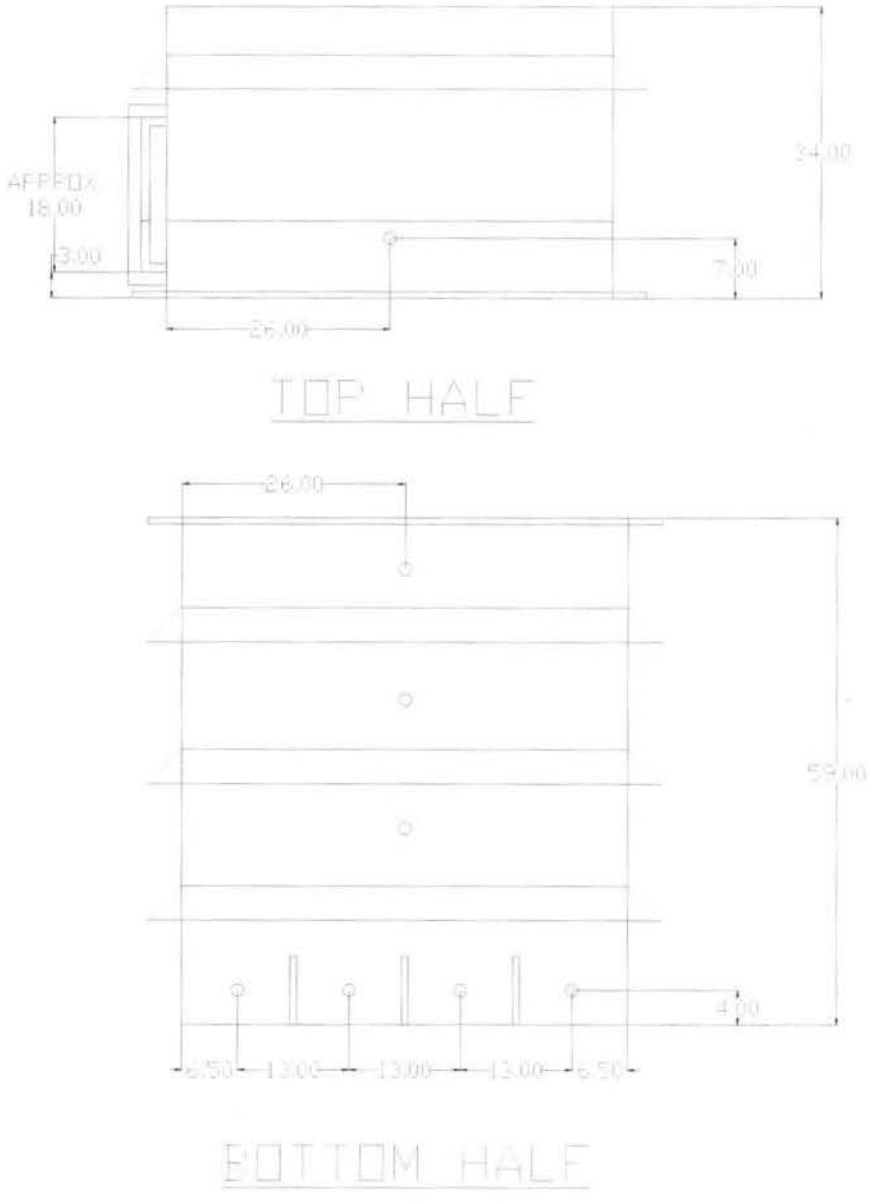

Figure A3. Dimensioned drawing of Reactor #3

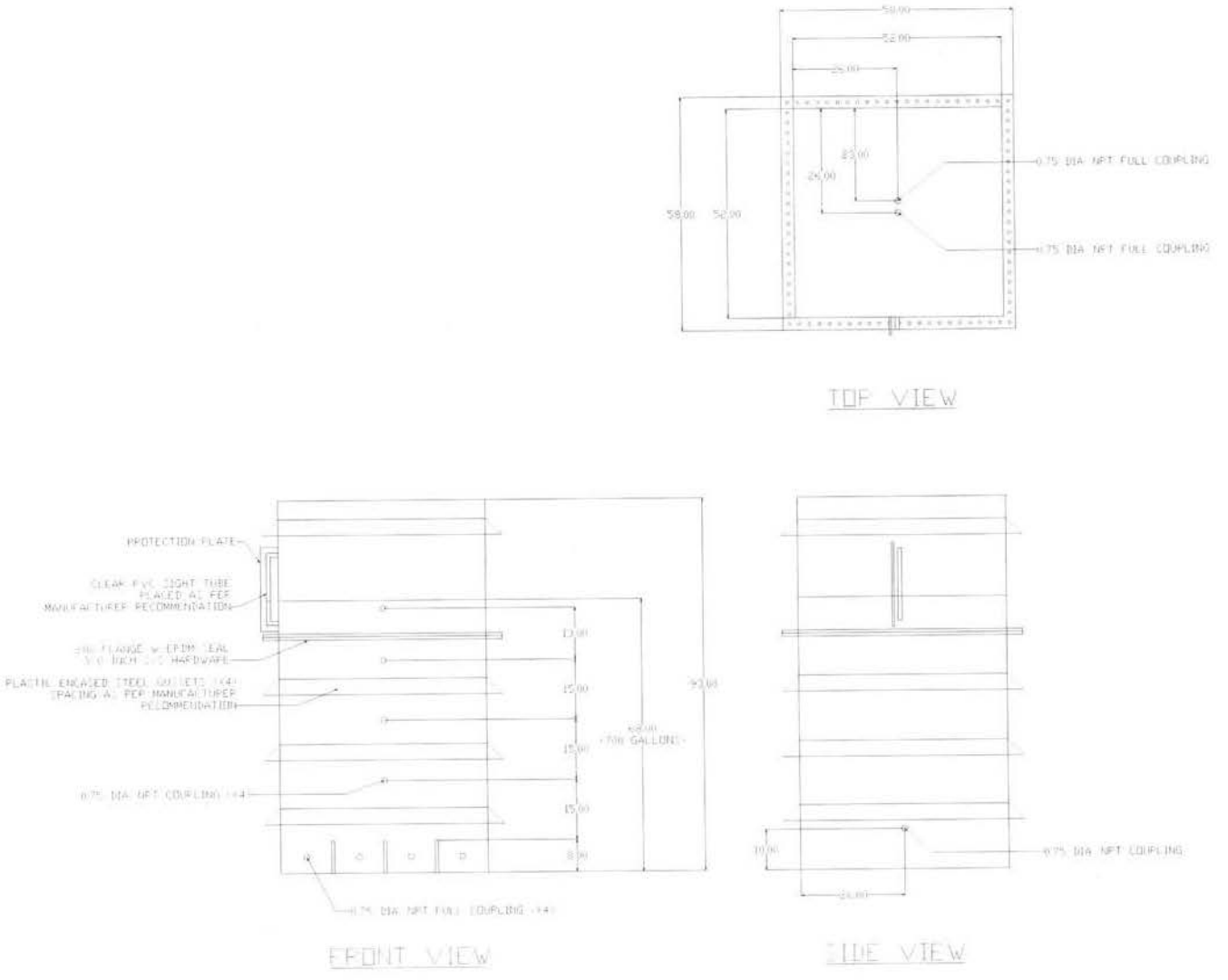

Figure A4. Dimensioned drawing of Reactor #3

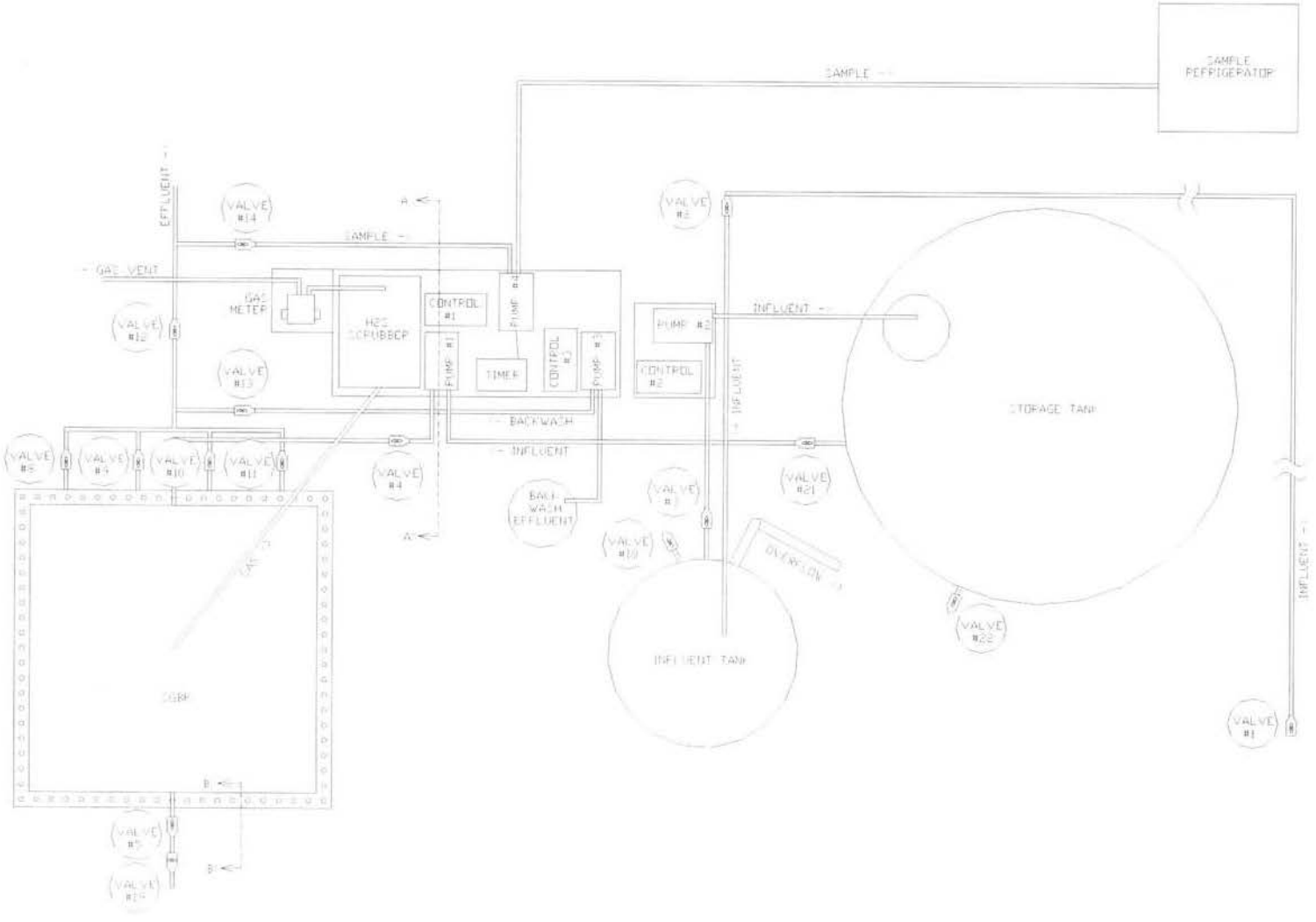

Figure A5. Reactor #1 system schematic

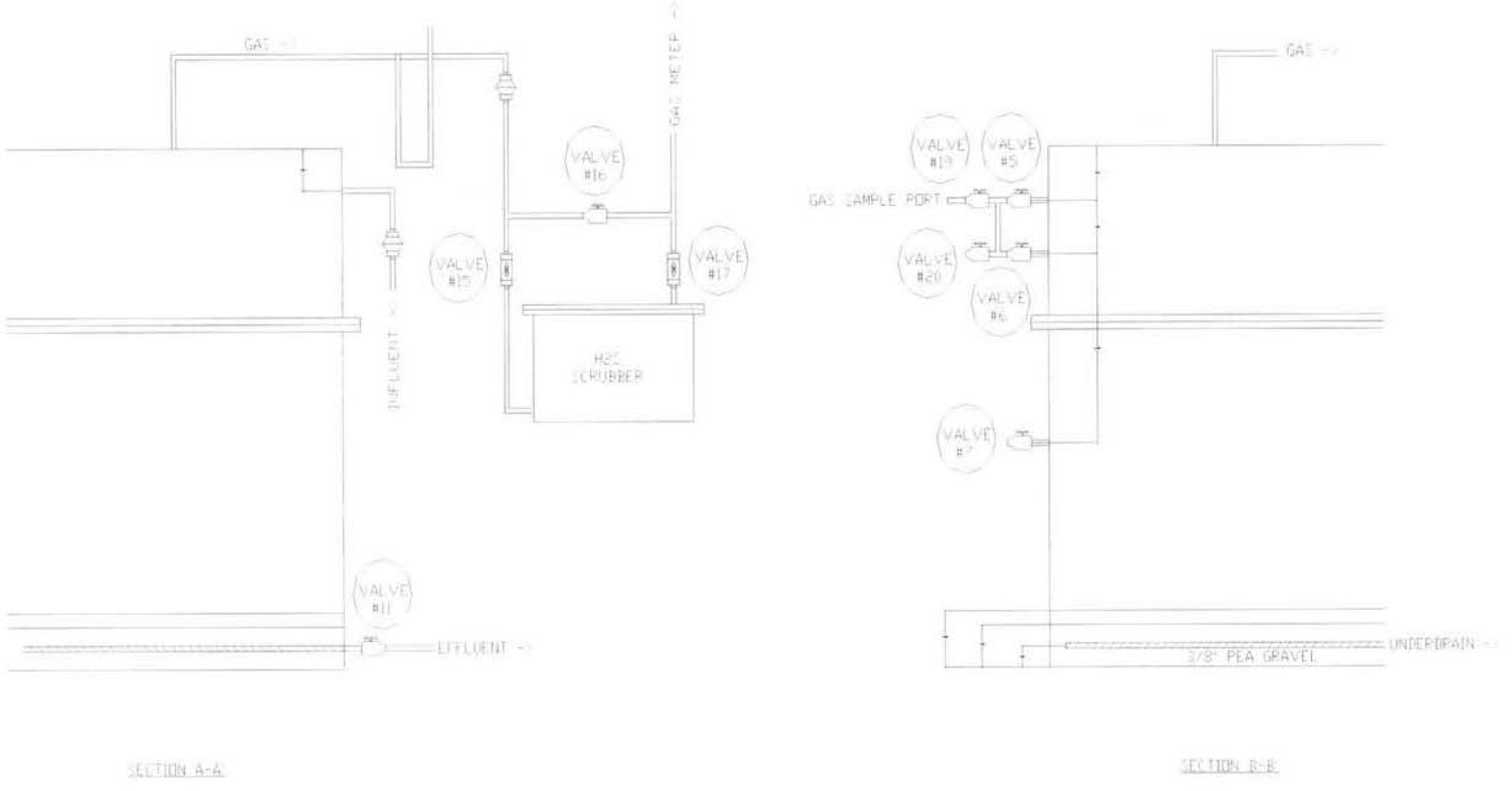

Figure A6. Reactor #1 system schematic section details

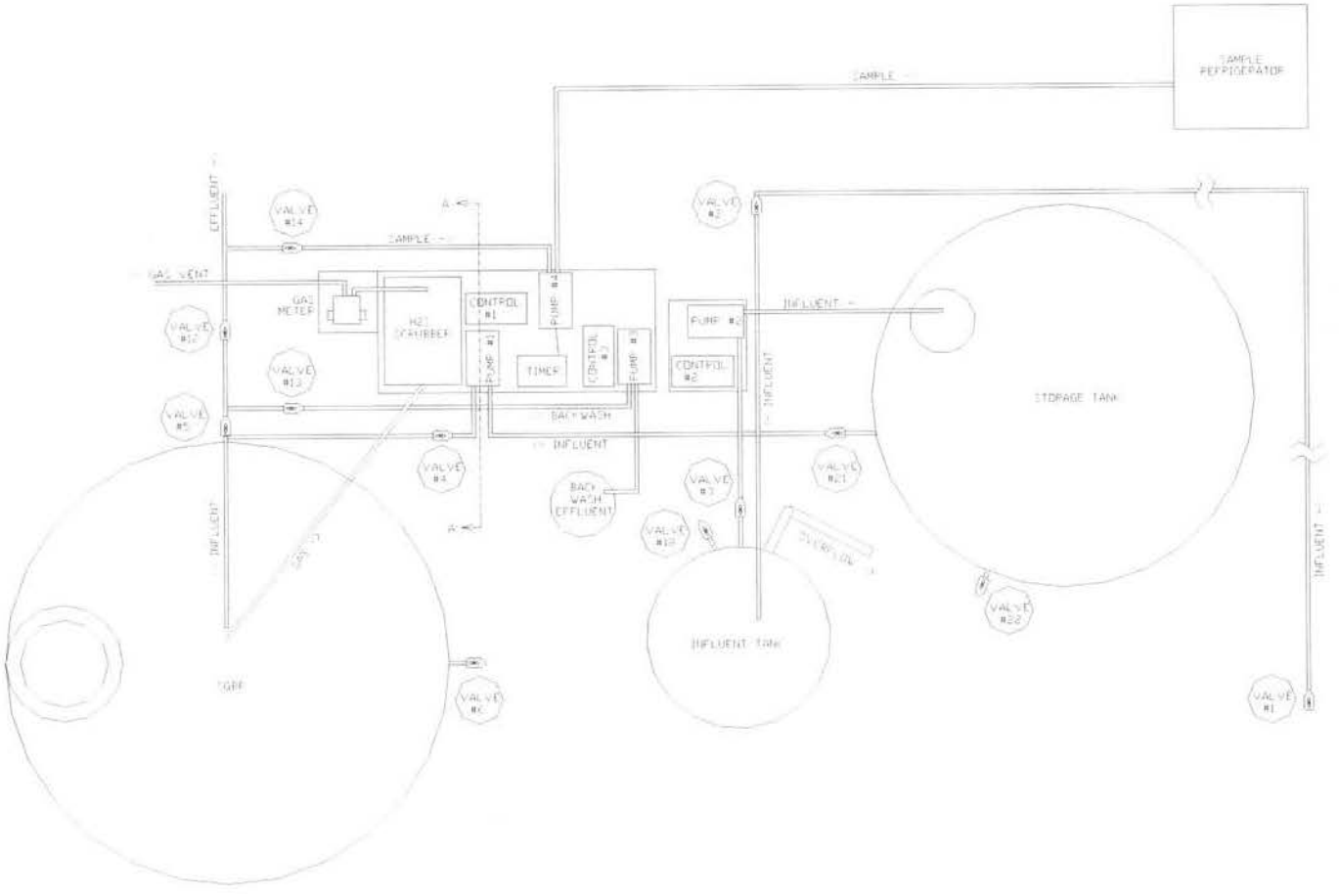

Figure A7. Reactor #2 system schematic

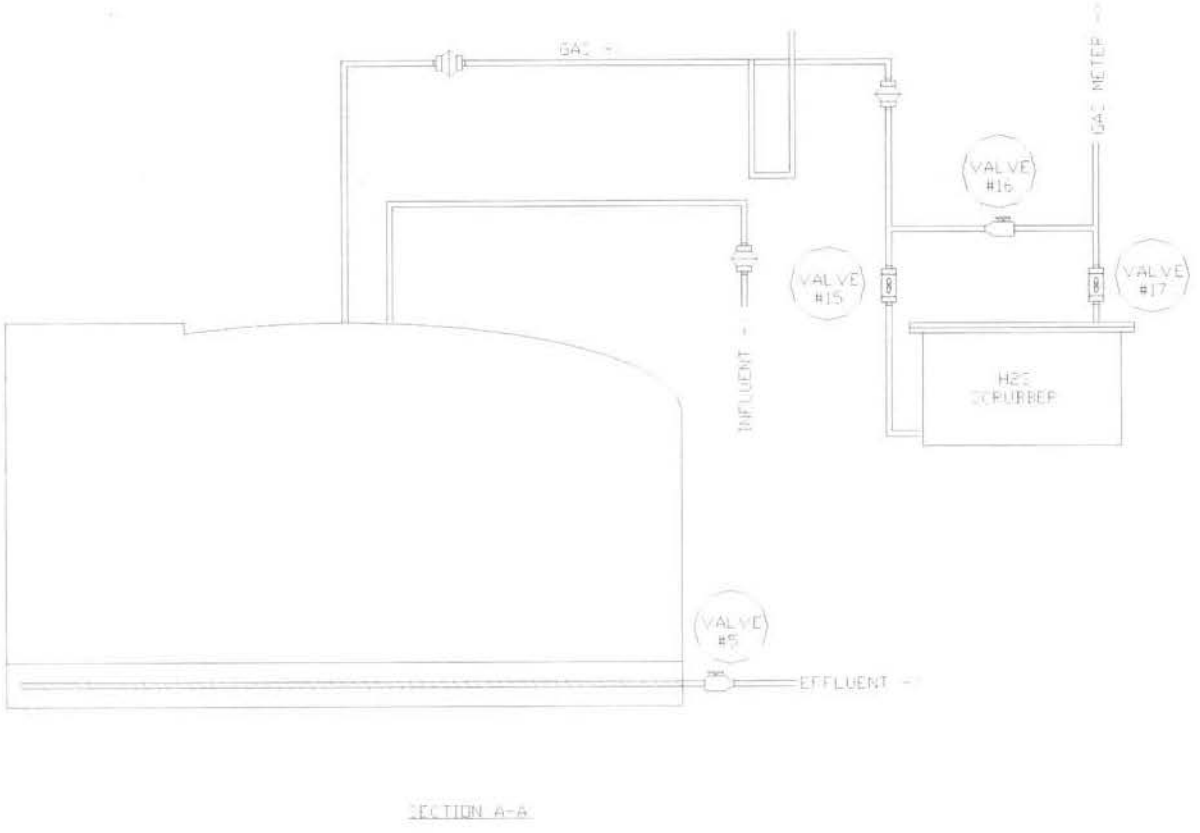

Figure A8. Reactor #2 system schematic section details

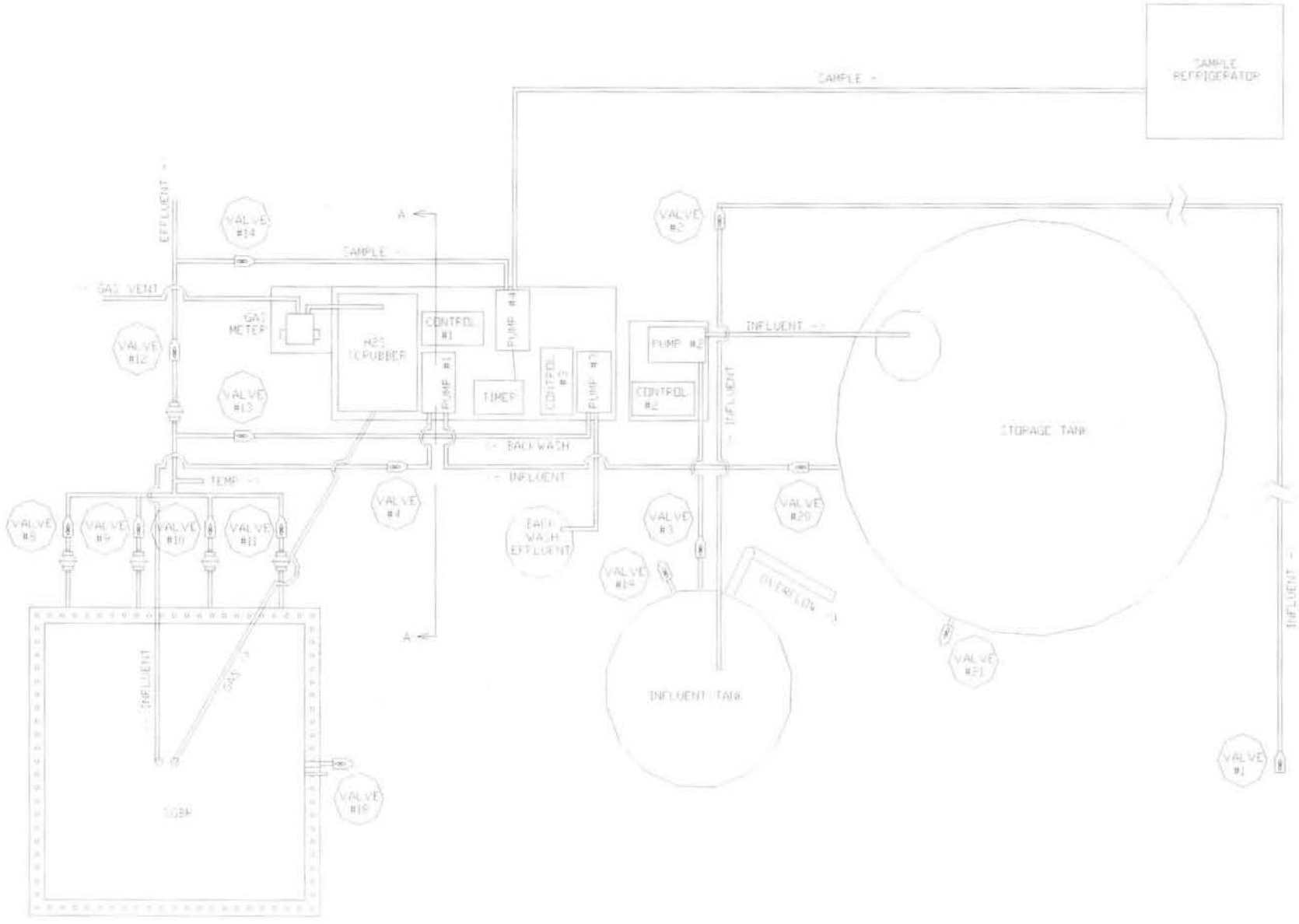

Figure A9. Reactor #3 system schematic

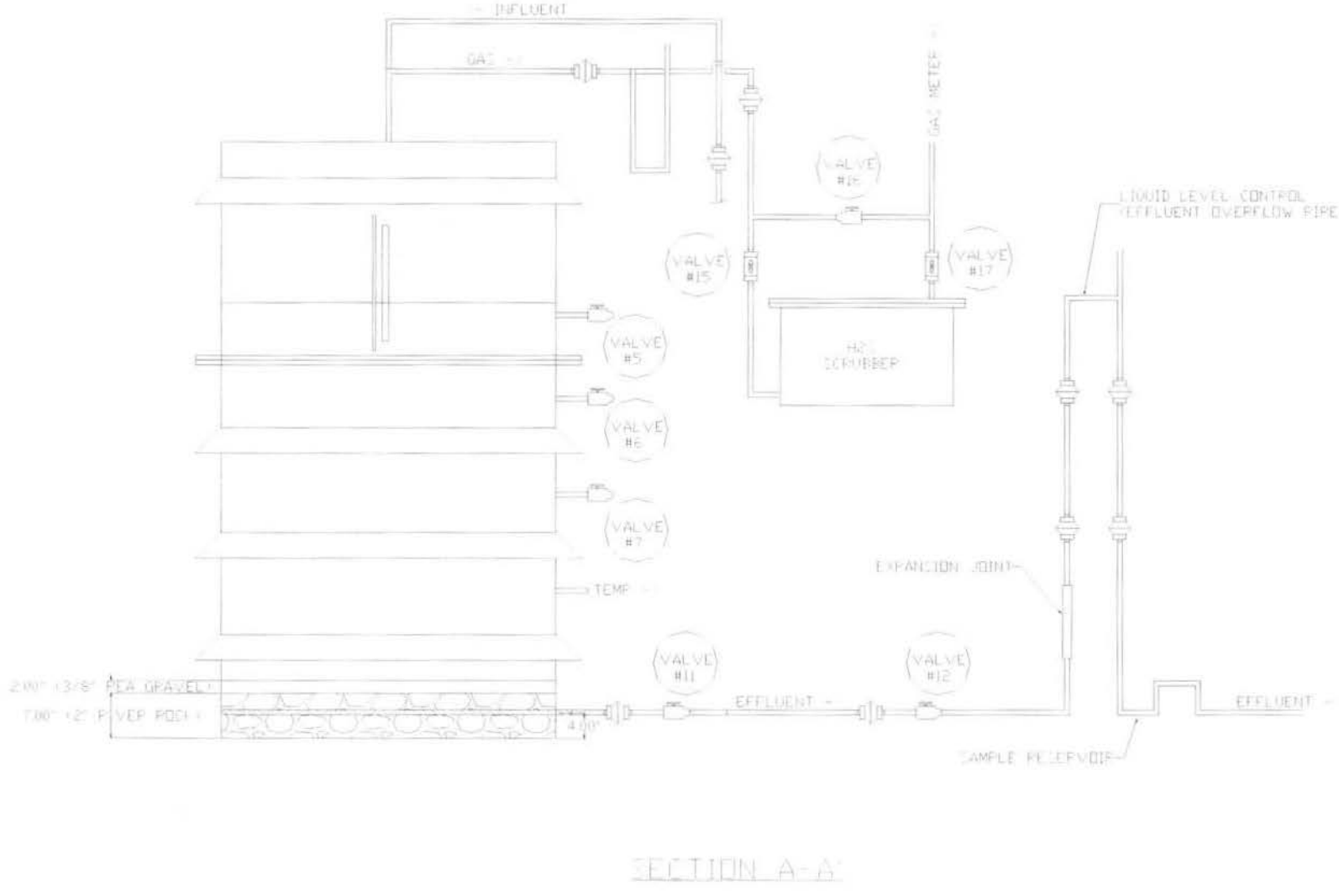

Figure A10. Reactor #3 system schematic section details

APPENDIX B:

DAILY INFLUENT AND EFFLUENT OPERATING DATA

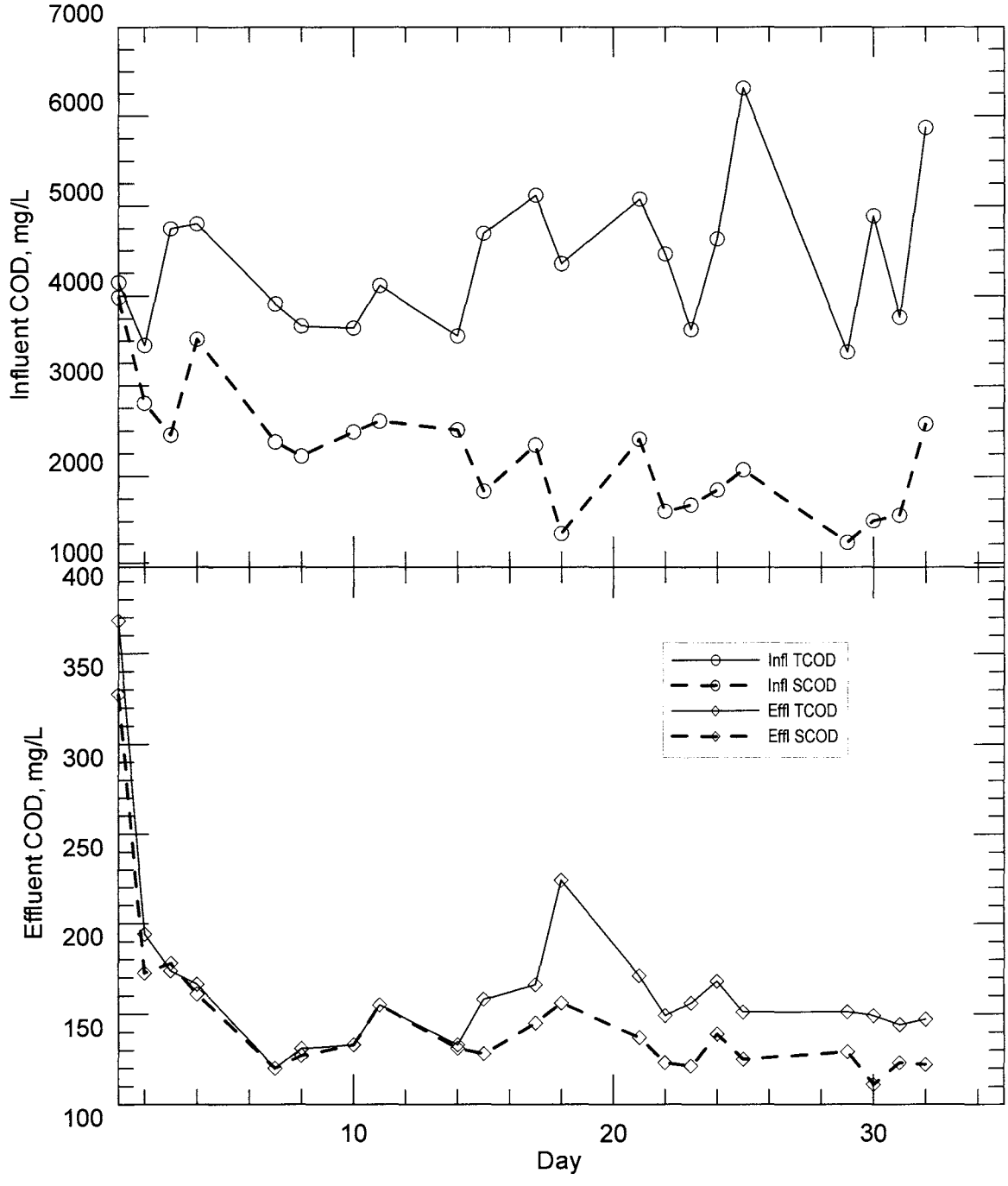

Figure B1. Influent and Effluent Total and Soluble COD for Day 1-32 (April 2 – May 3, 2002). HRT for Day 1-32 was 48 hours.

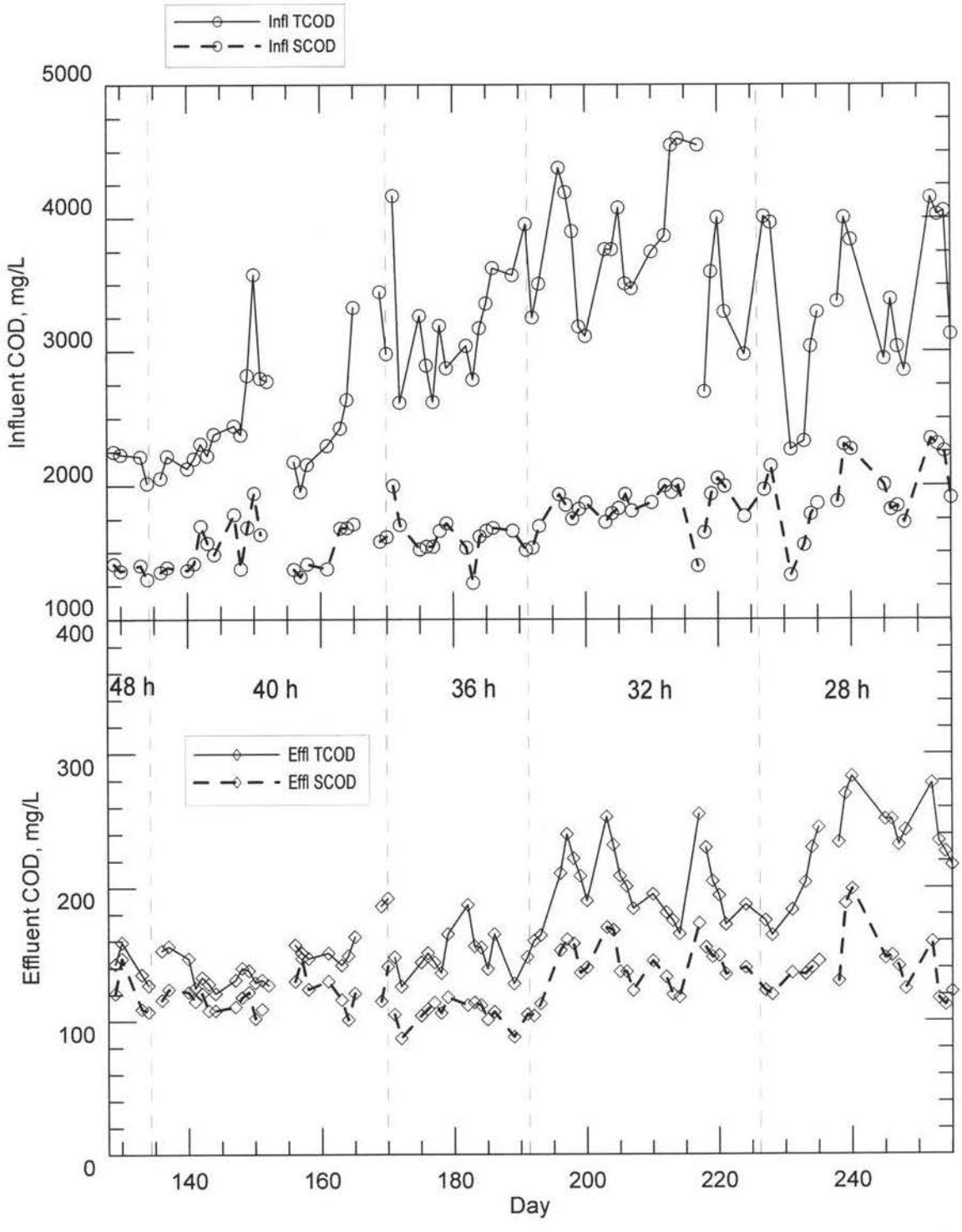

Figure B2. Influent and Effluent Total and Soluble COD for Day 128-255 (August 7 – December 12, 2002). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

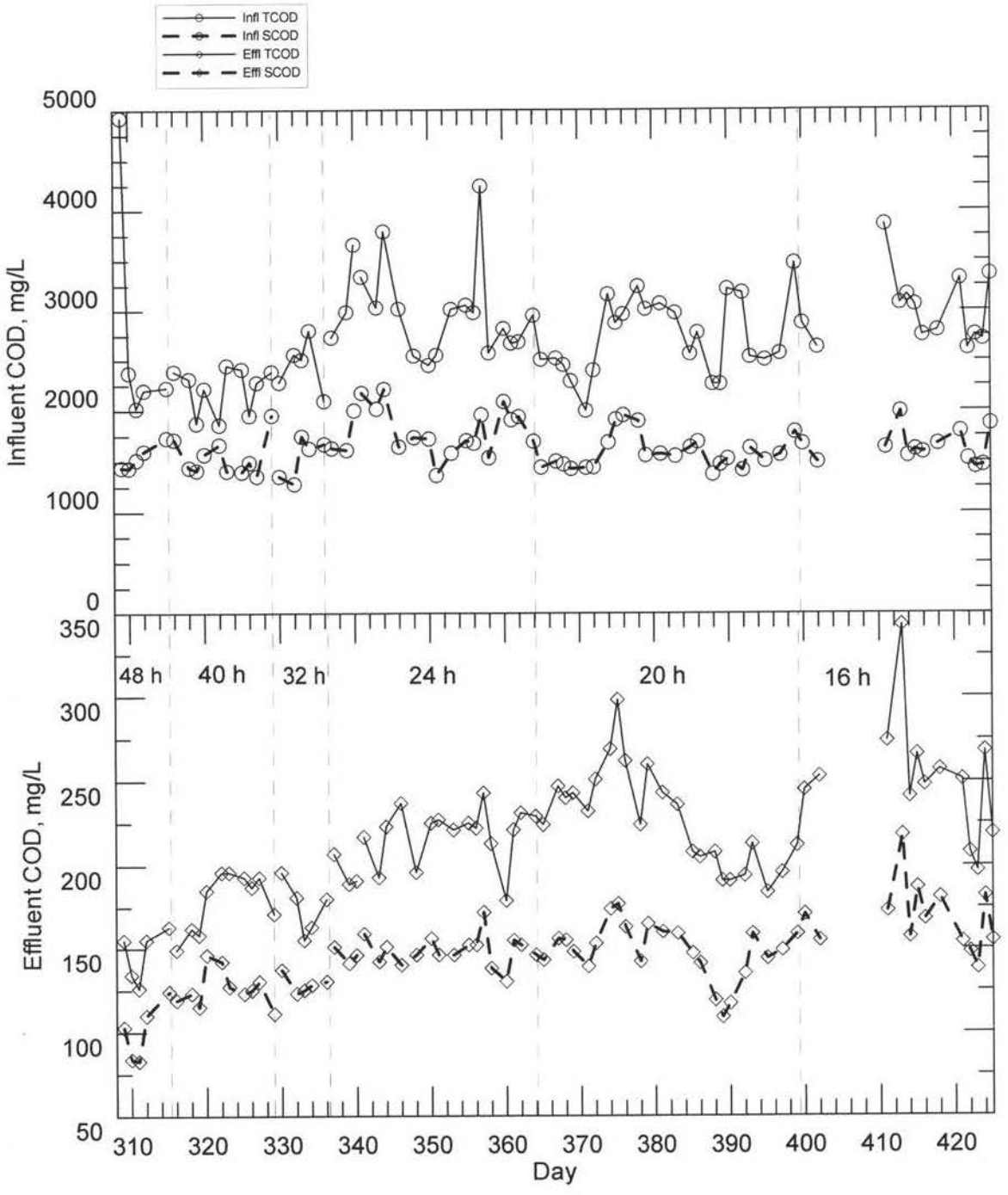

Figure B3. Influent and Effluent Total and Soluble COD for Day 308-425 (February 3 – May 31, 2003). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

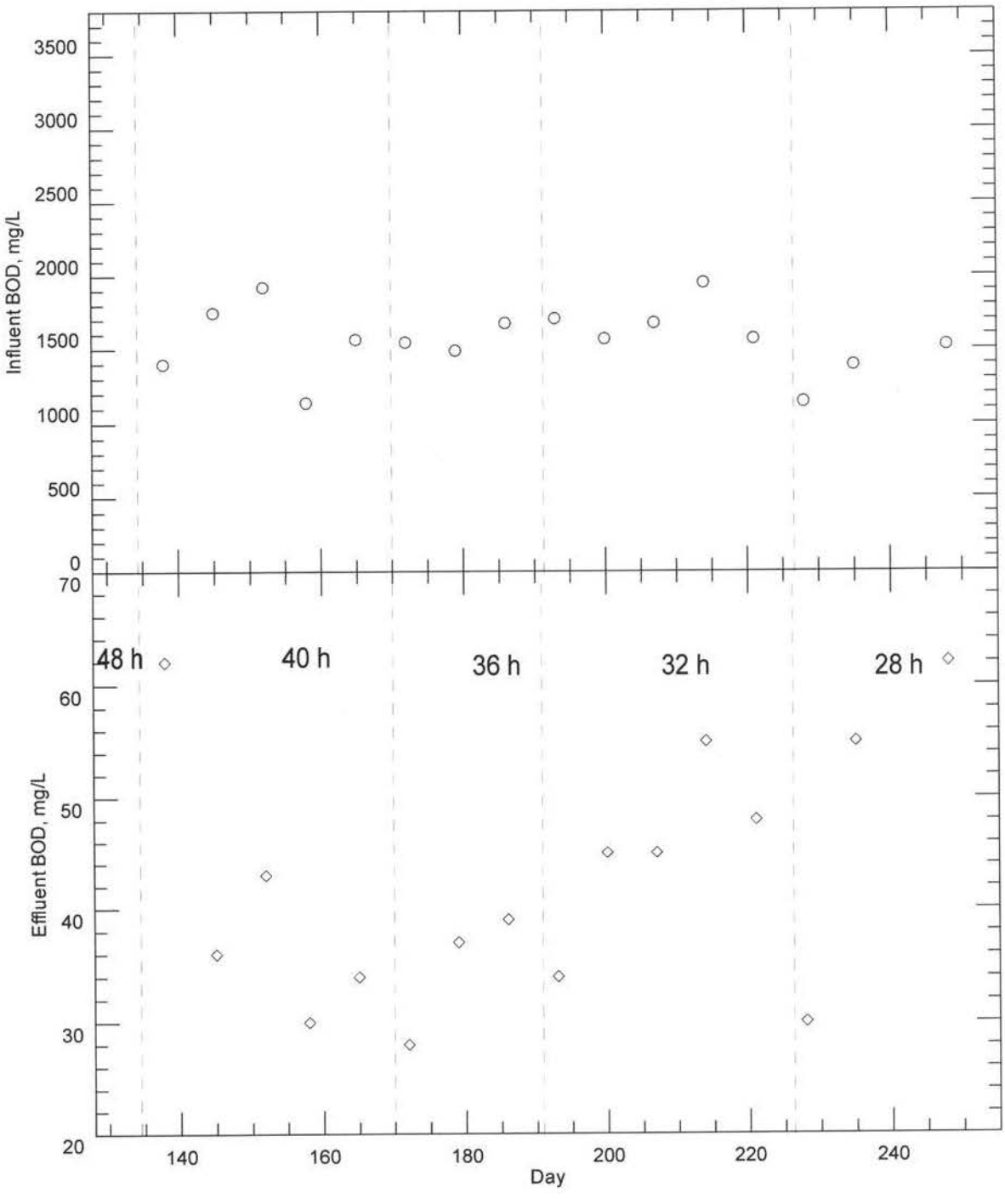

Figure B4. Influent and Effluent BOD for Day 128-255 (August 7 – December 12, 2002). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

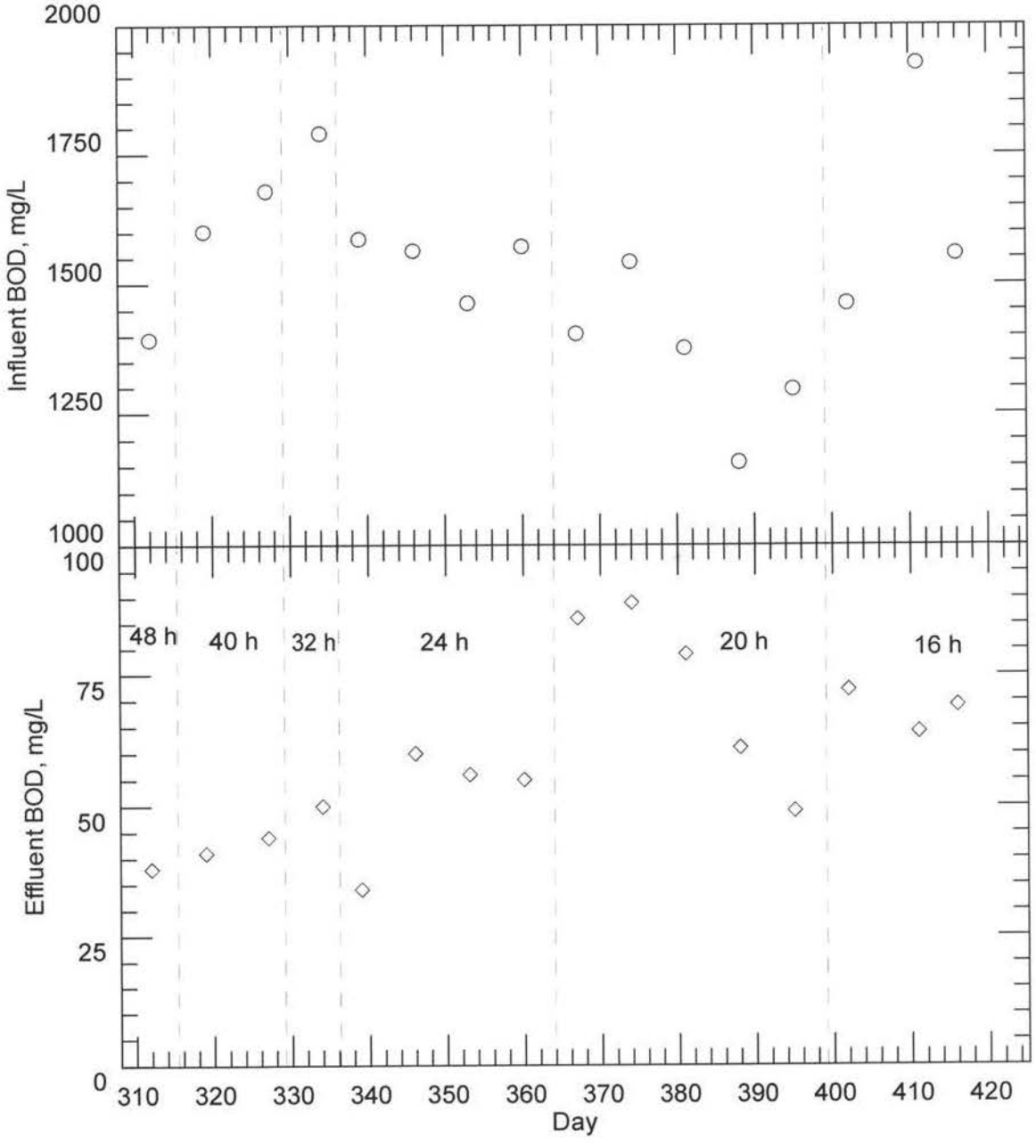

Figure B5. Influent and Effluent BOD for Day 308-425 (February 3 – May 31, 2003). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

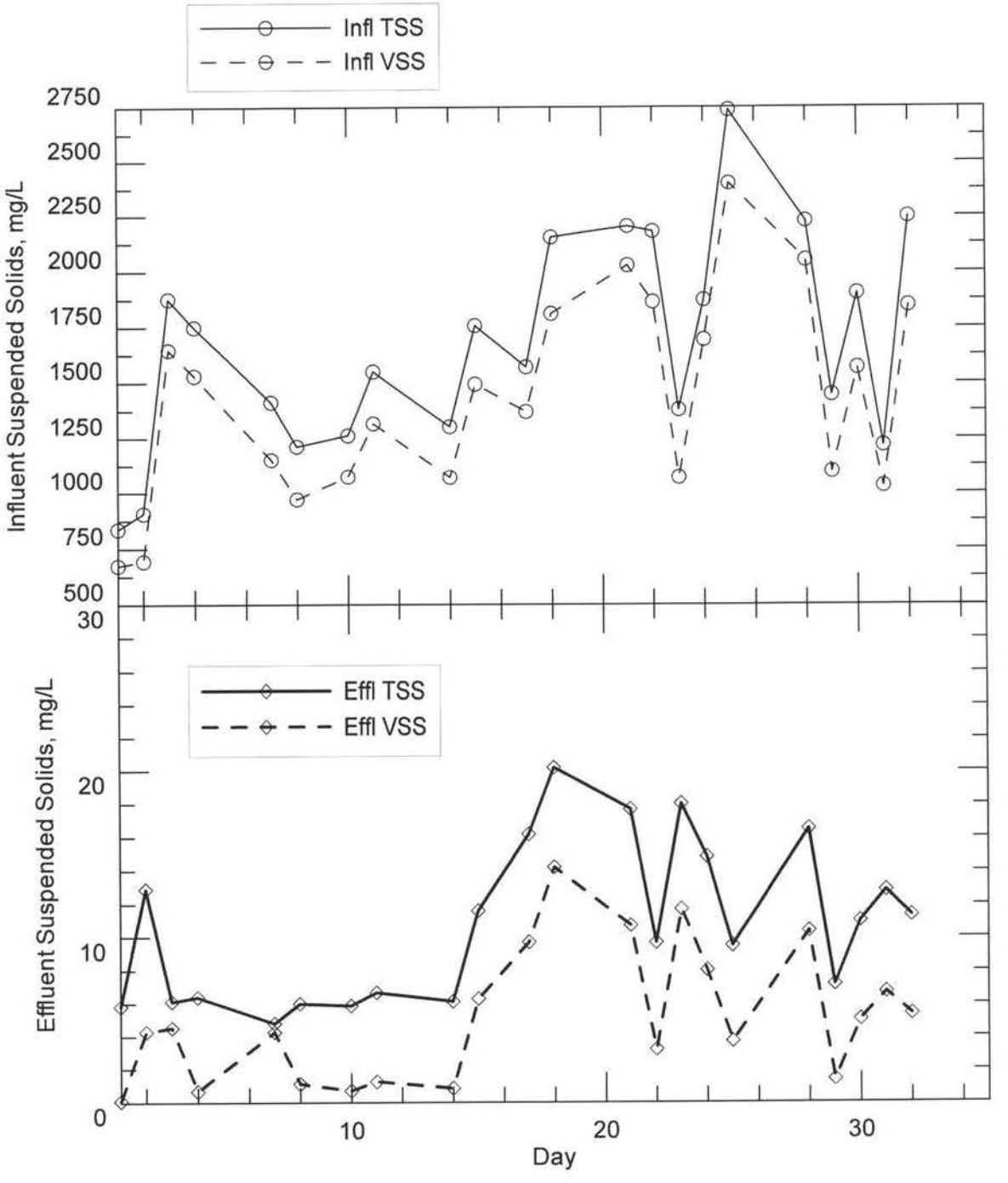

Figure B6. Influent and Effluent Total and Volatile Suspended Solids for Day 1-32 (April 2 – May 3, 2002). HRT for Day 1-32 was 48 hours.

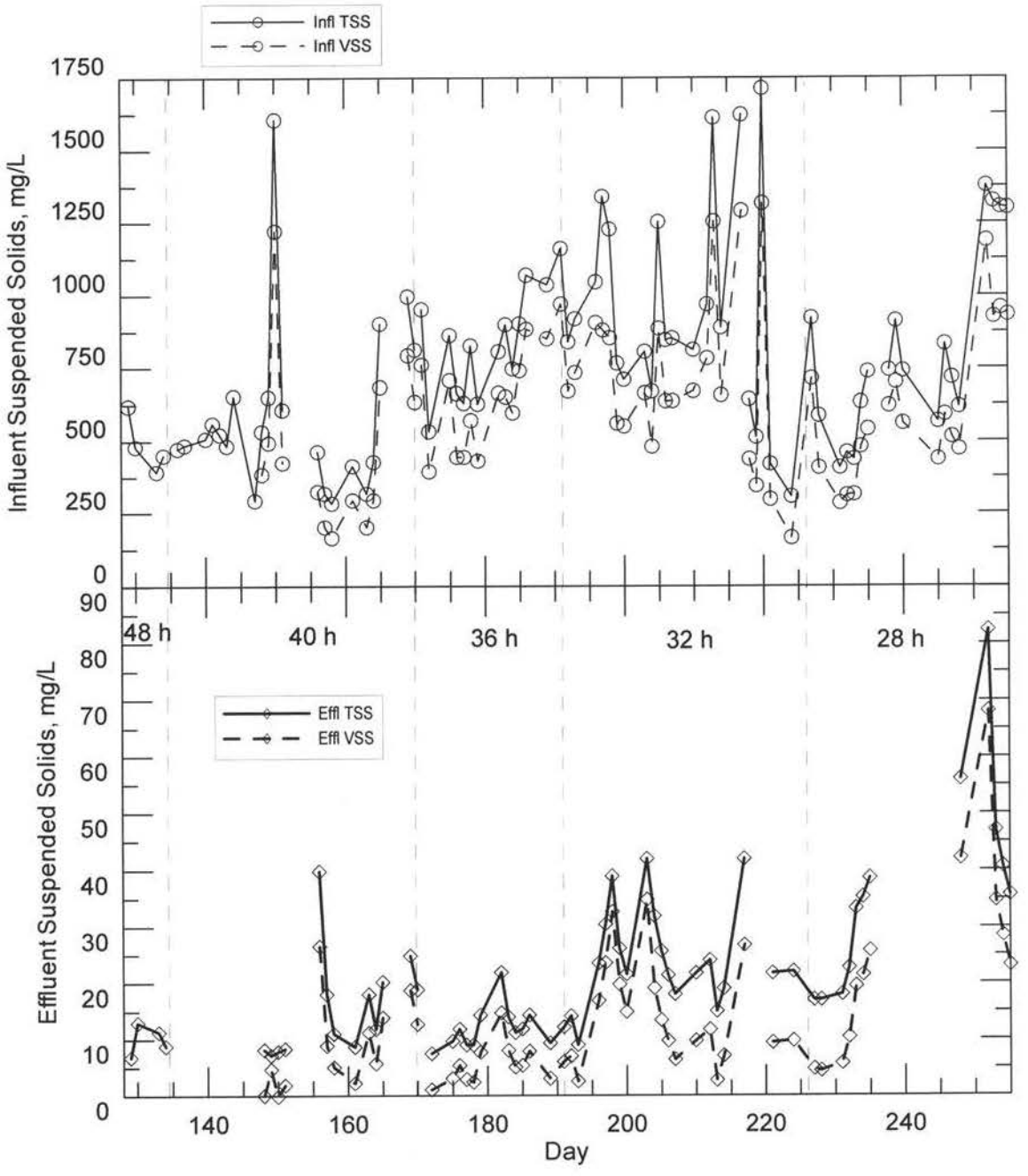

Figure B7. Influent and Effluent Total and Volatile Suspended Solids for Day 128-255 (August 7 – December 12, 2002). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

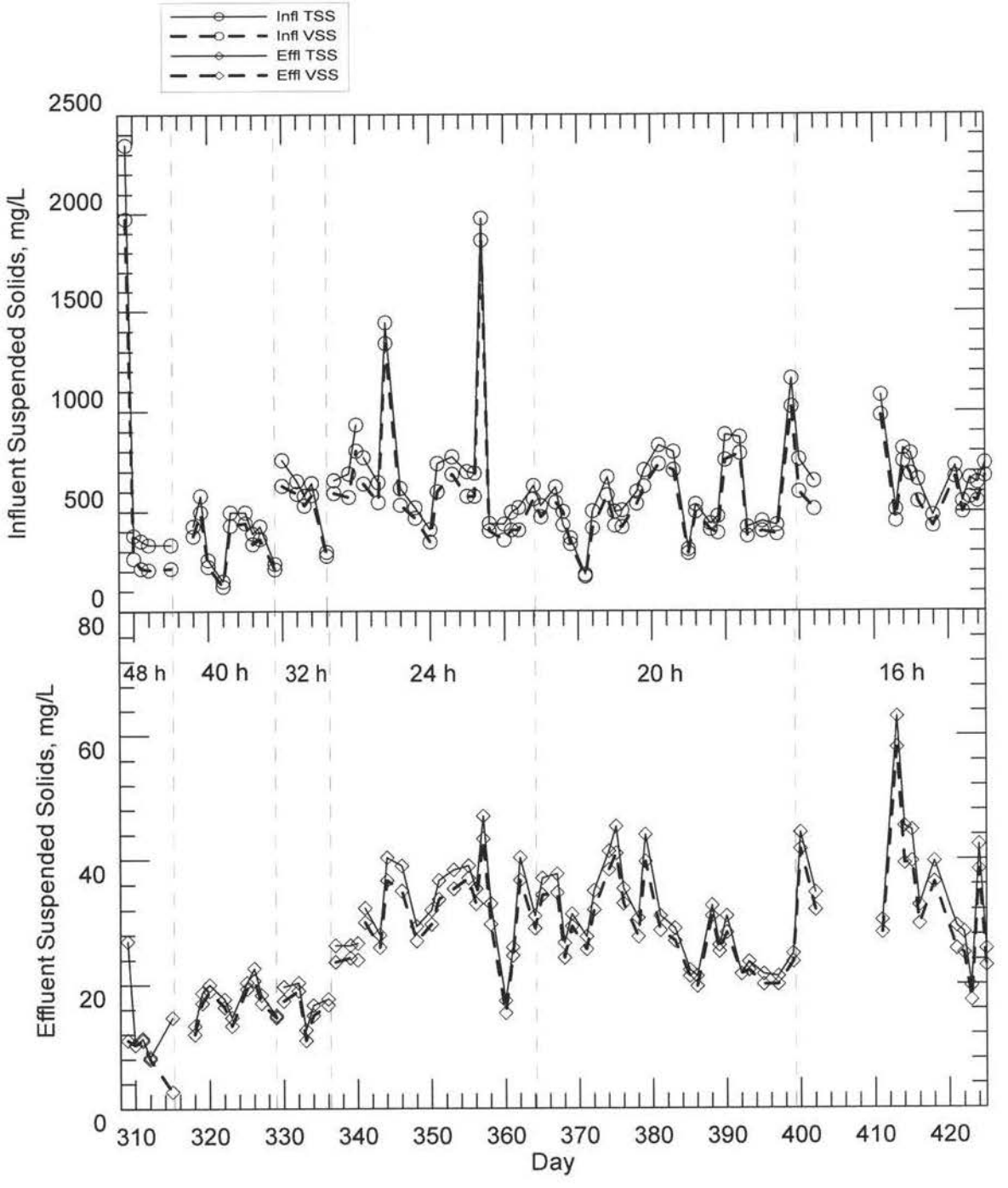

Figure B8. Influent and Effluent Total and Volatile Suspended Solids for Day 308-425 (February 3 – May 31, 2003). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

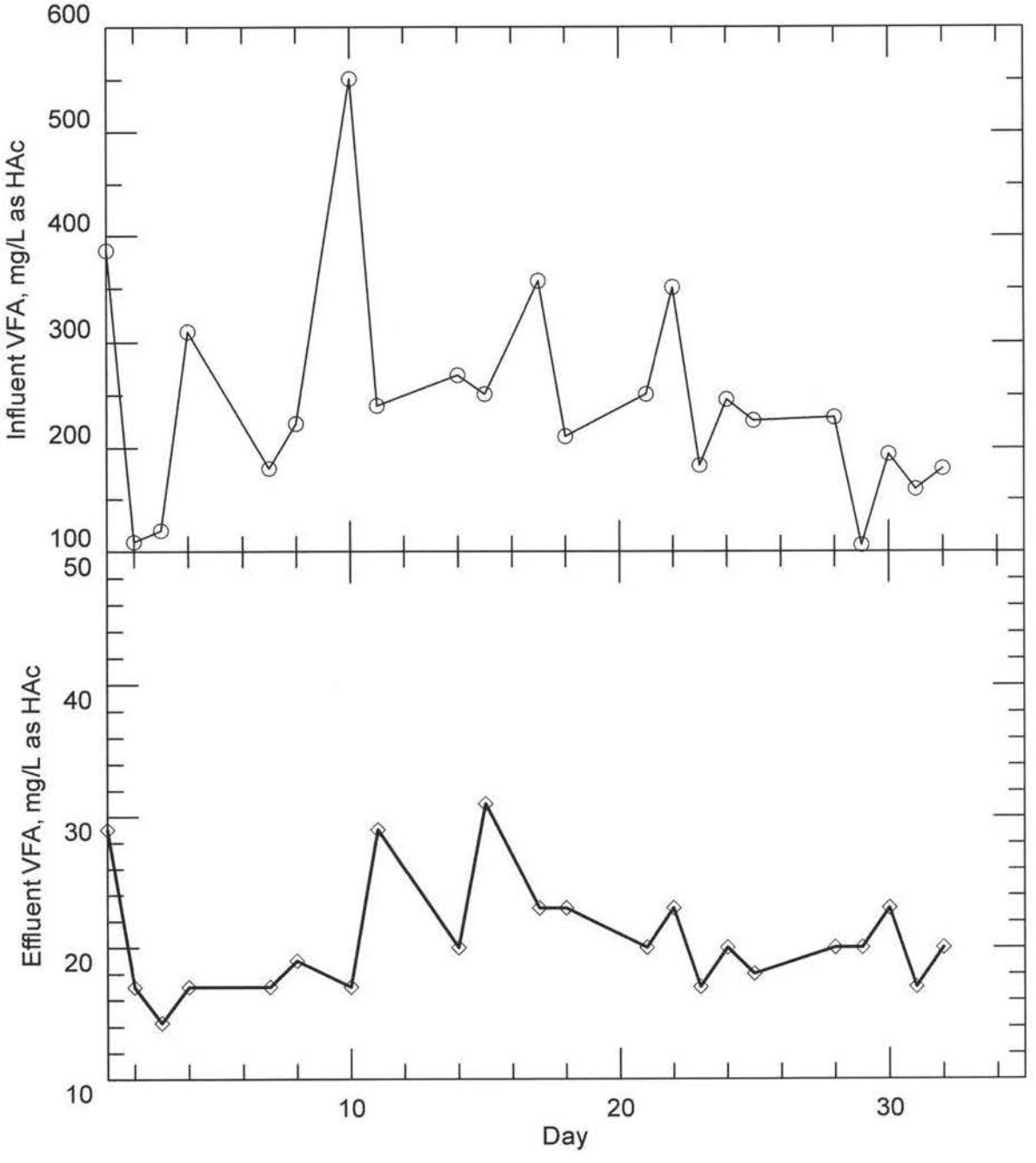

Figure B9. Influent and Effluent VFA for Day 1-32 (April 2 – May 3, 2002). HRT for Day 1-32 was 48 hours.

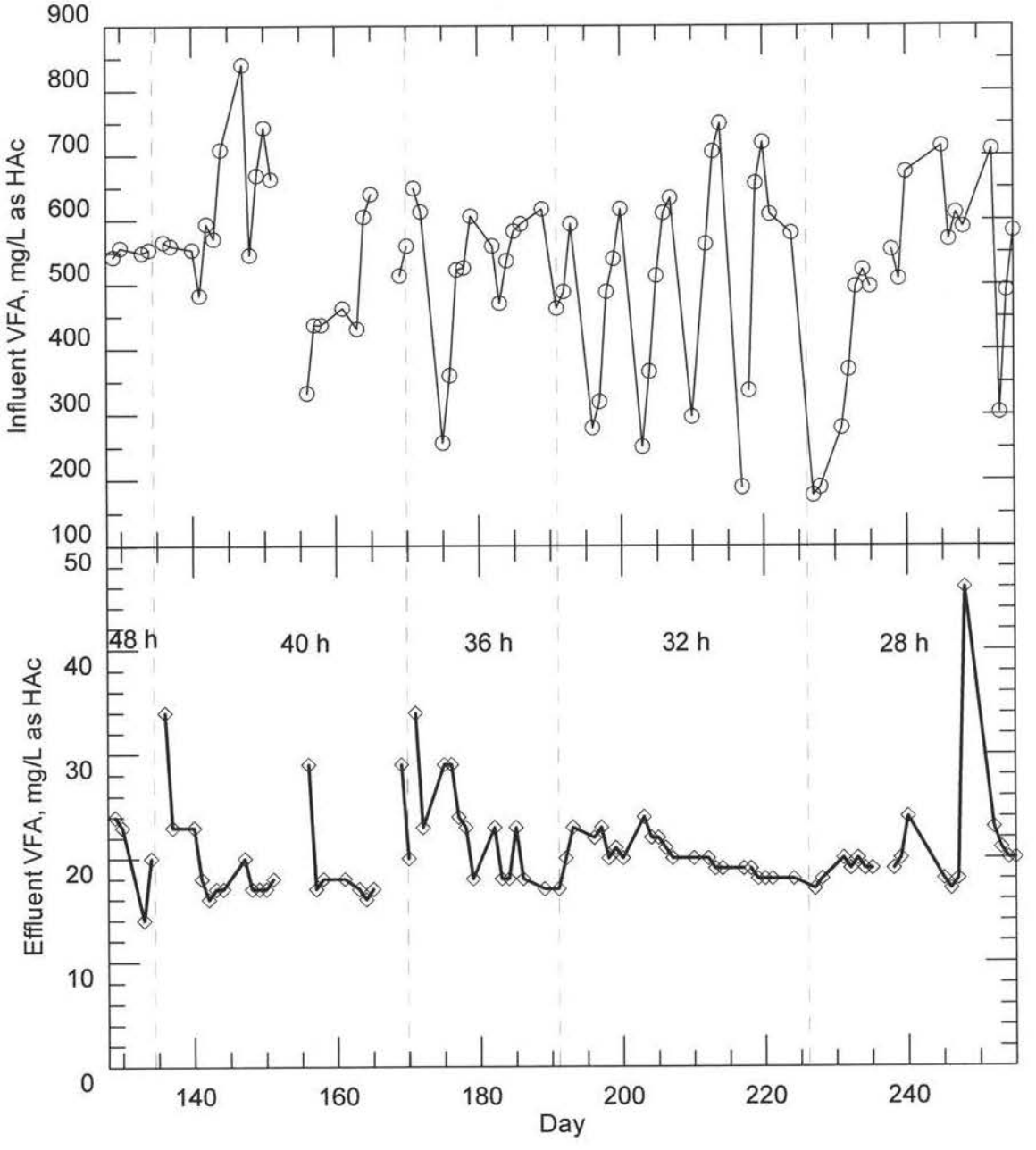

Figure B10. Influent and Effluent VFA for Day 128-255 (August 7 – December 12, 2002). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

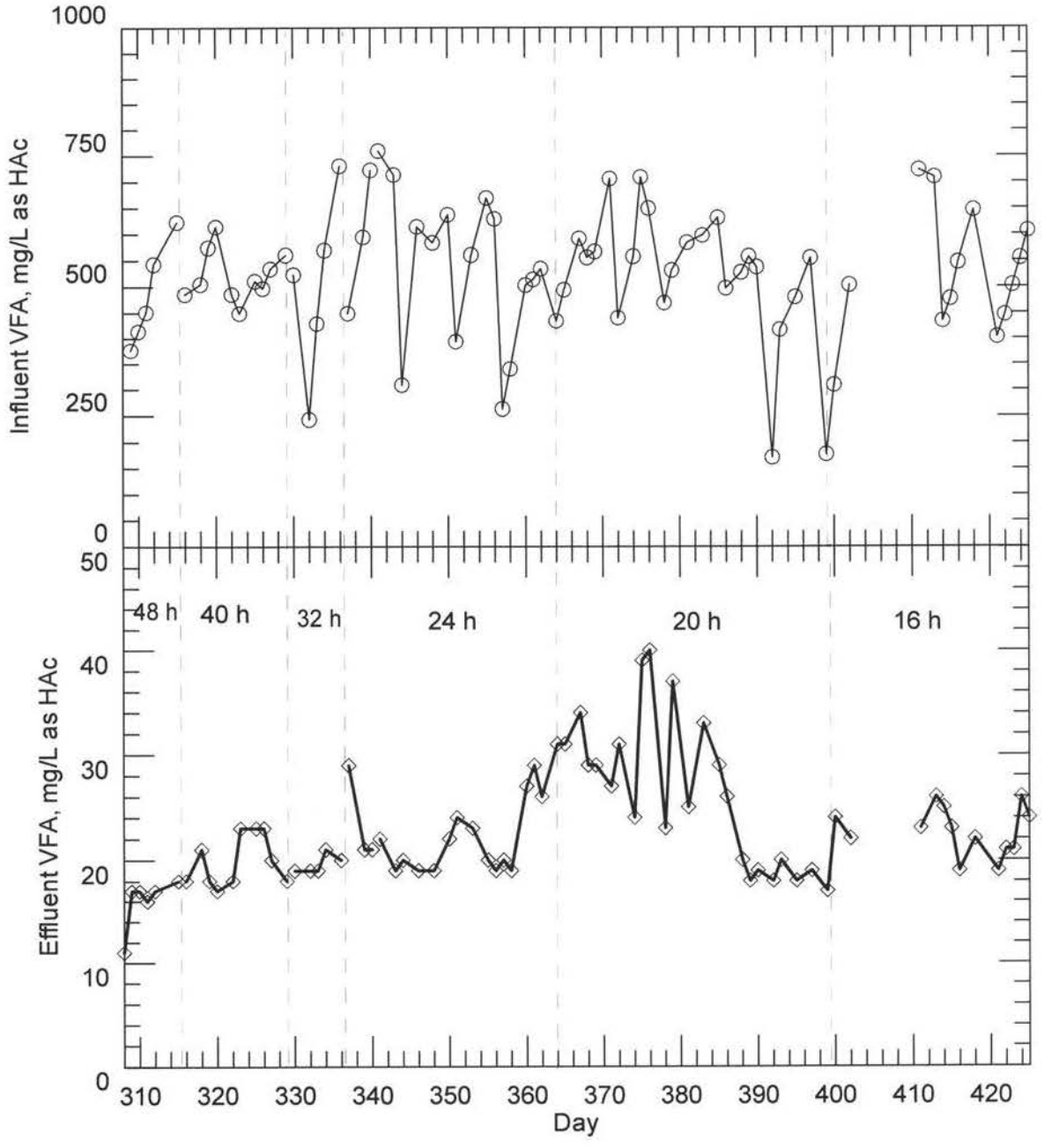

Figure B11. Influent and Effluent VFA for Day 308-425 (February 2 – May 31, 2003). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

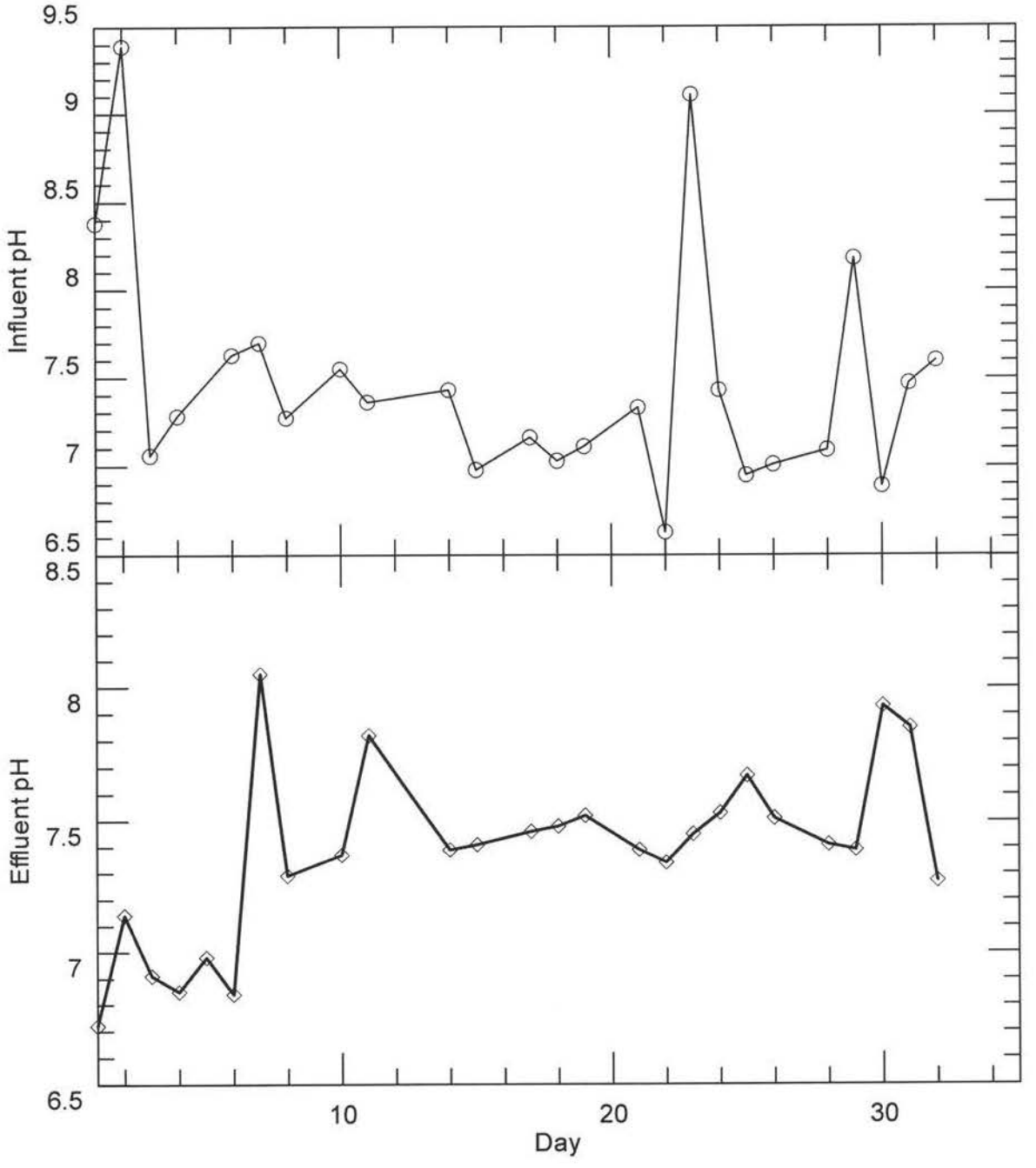

Figure B12. Influent and Effluent pH for Day 1-32 (April 2 – May 3, 2002). HRT for Day 1-32 was 48 hours.

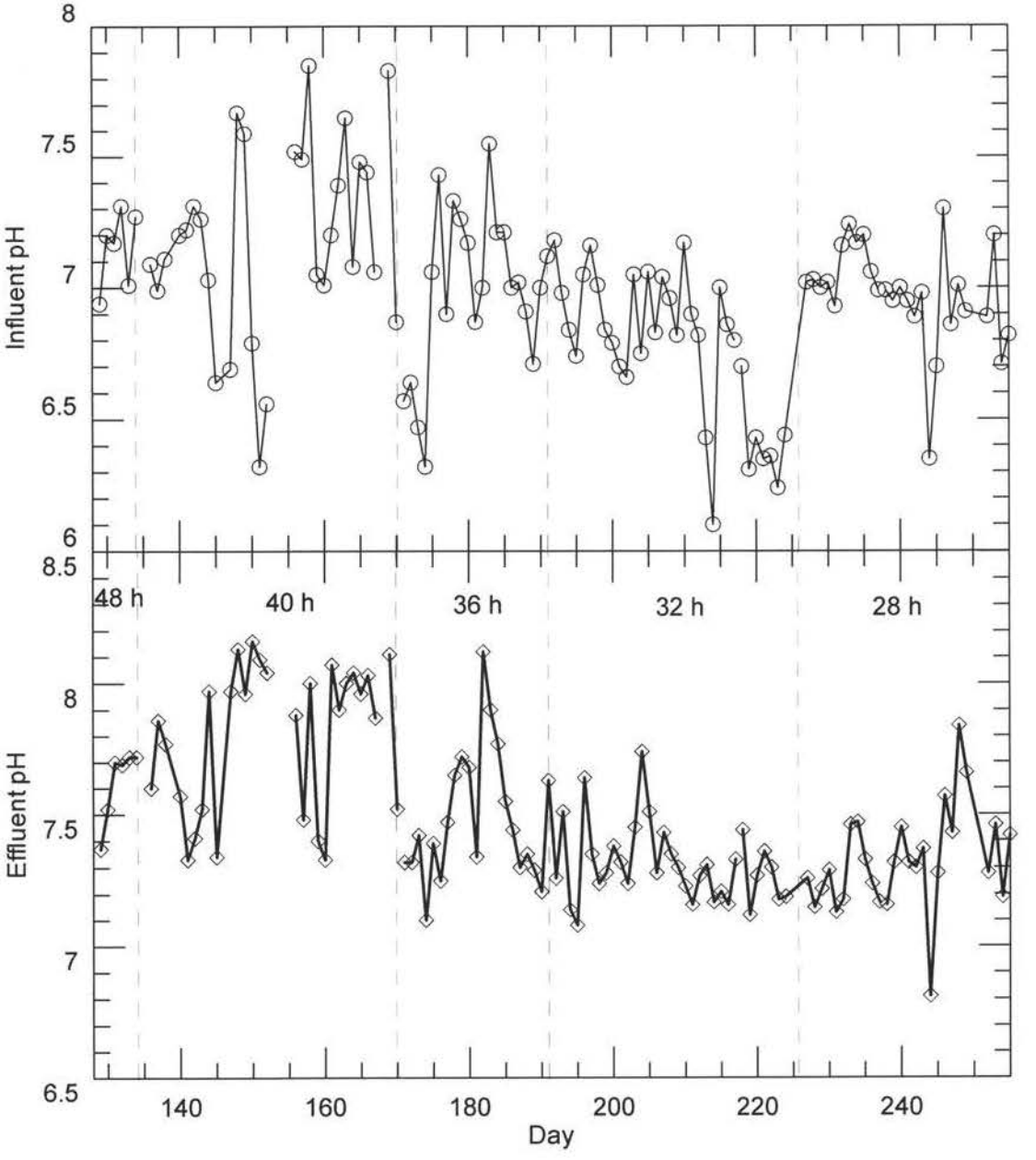

Figure B13. Influent and Effluent pH for Day 128-255 (August 7 – December 12, 2002). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

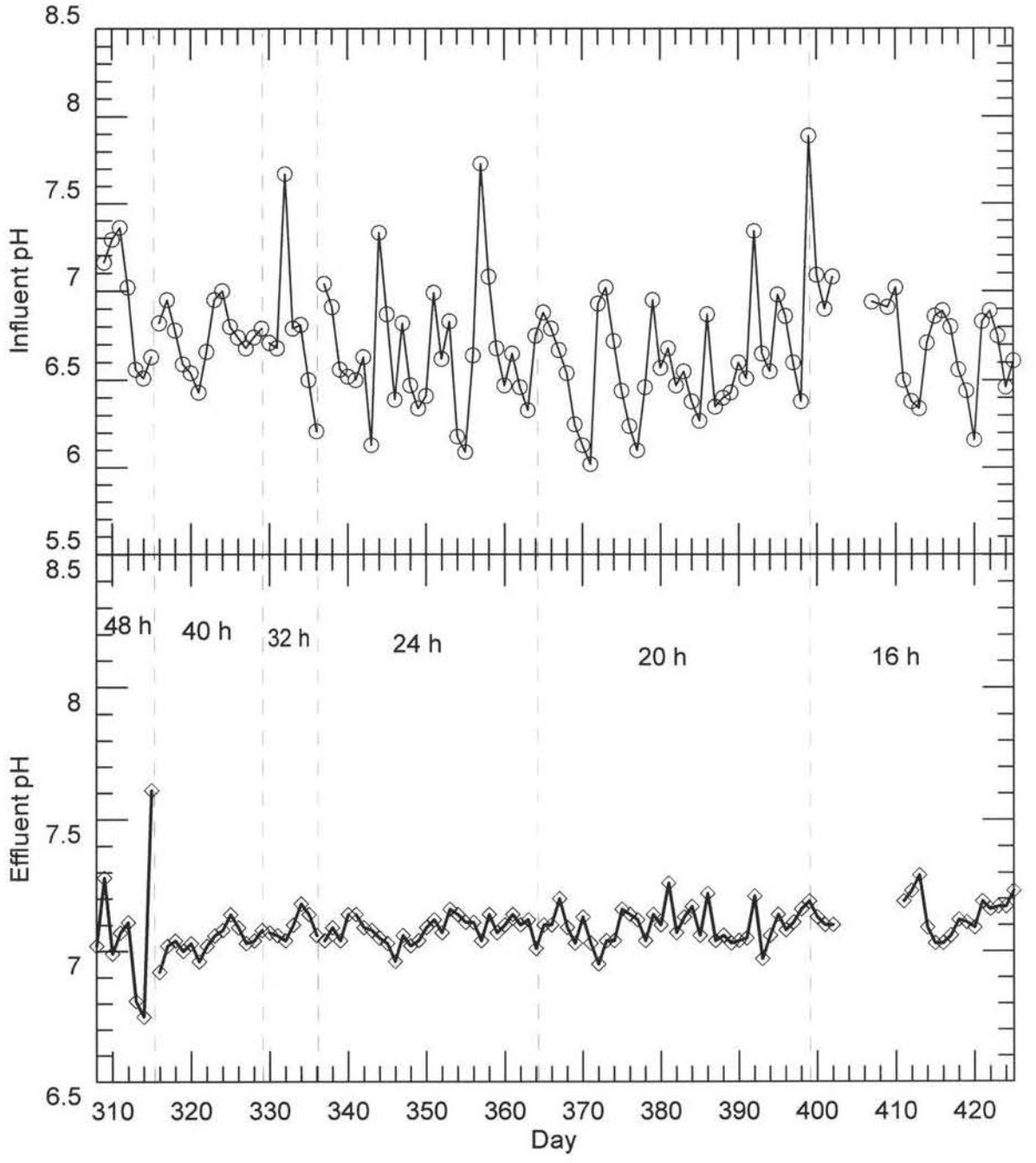

Figure B14. Influent and Effluent pH for Day 308-425 (February 3 – May 31, 2003). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

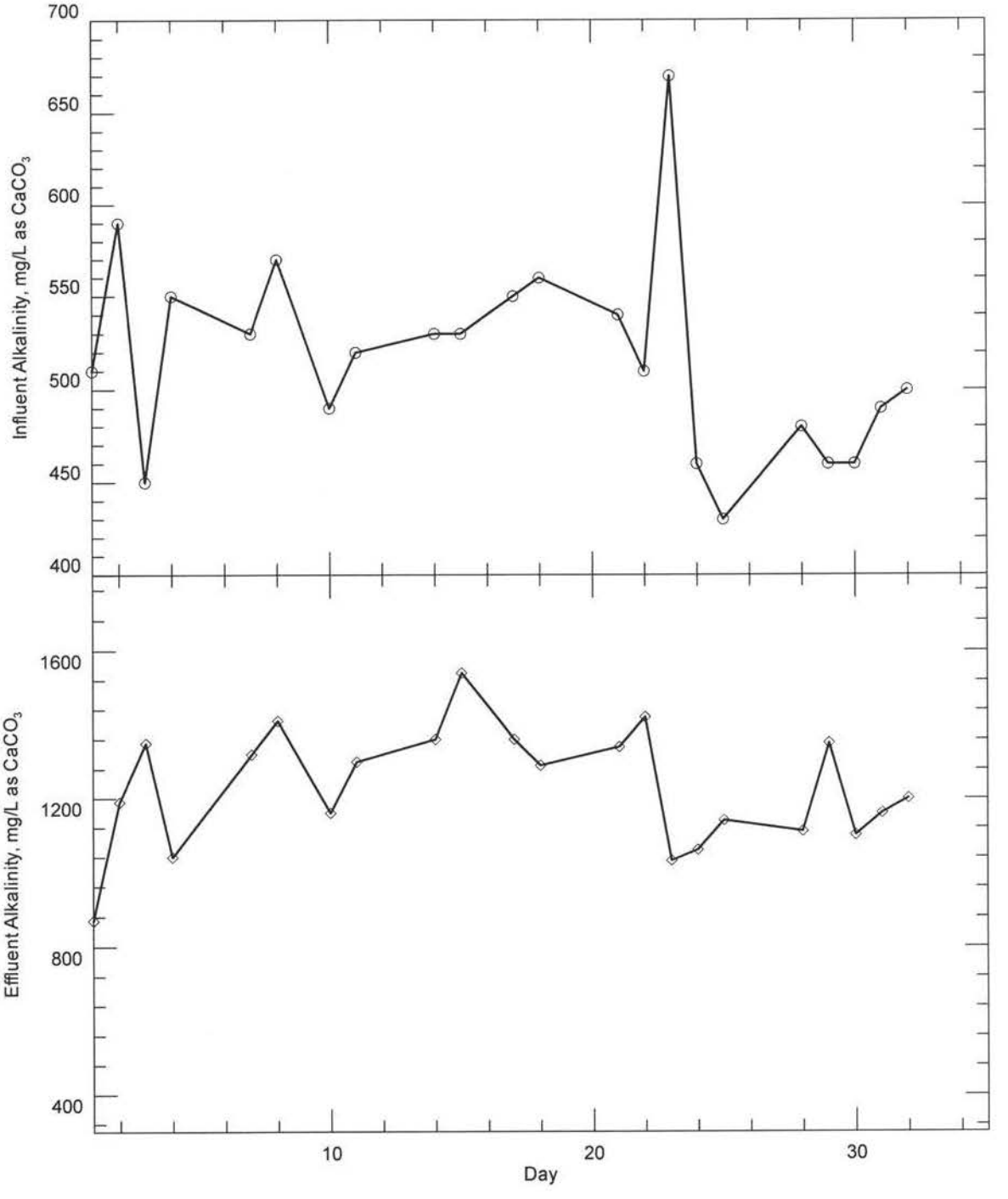

Figure B15. Influent and Effluent Alkalinity for Day 1-32 (April 2 – May 3, 2002). HRT for Day 1-32 was 48 hours.

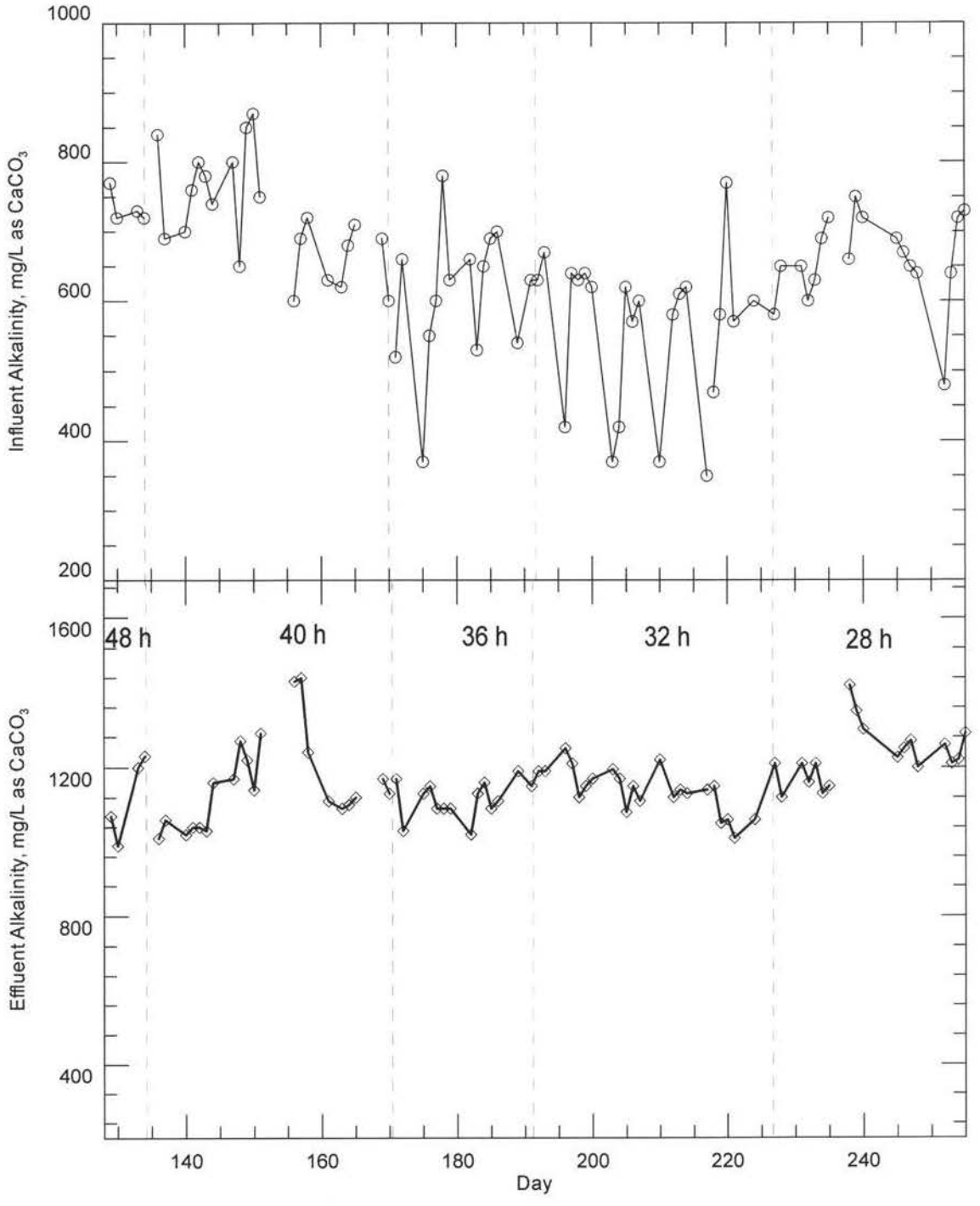

Figure B16. Influent and Effluent Alkalinity for Day 128-255 (August 7 – December 12, 2002). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

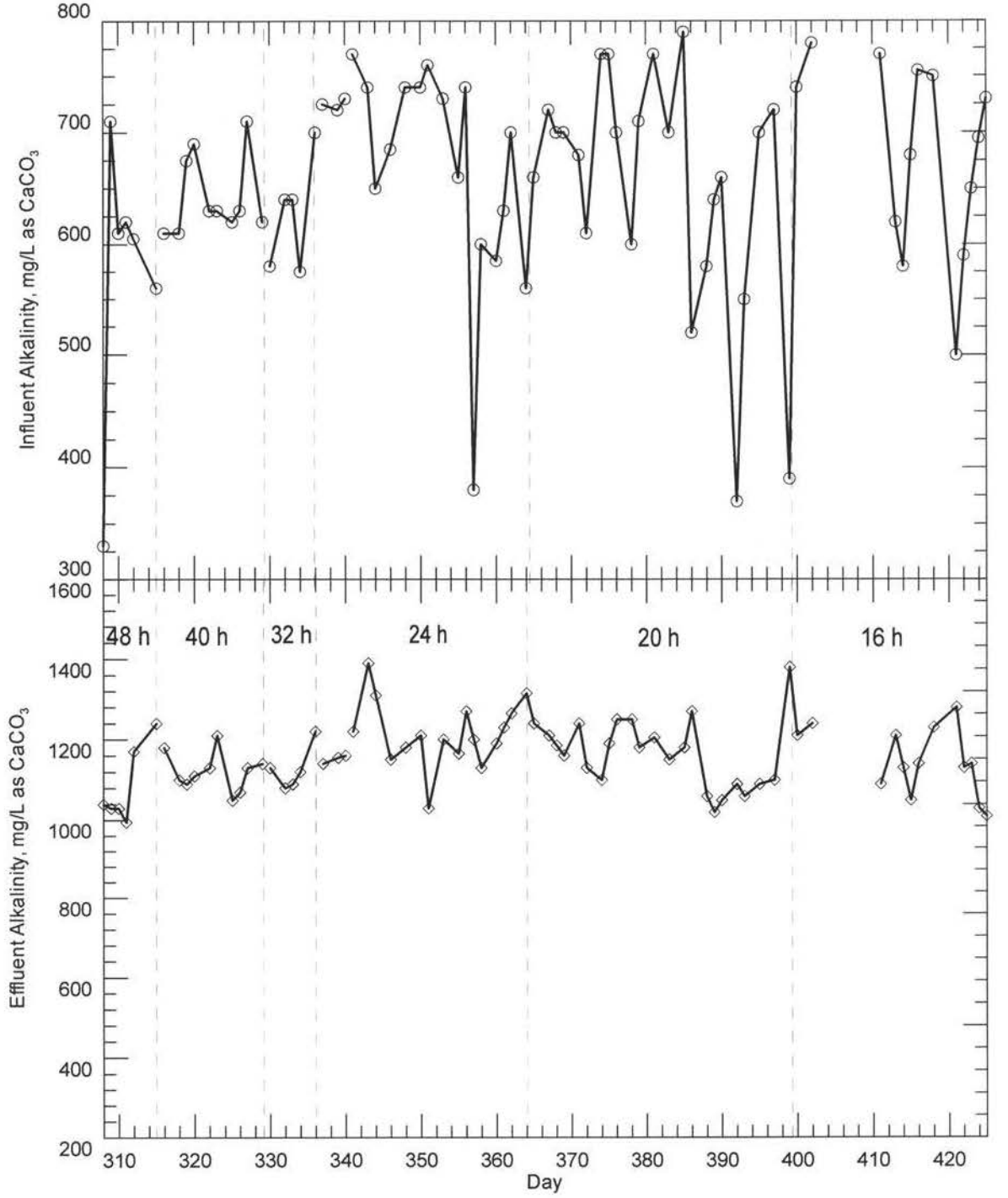

Figure B17. Influent and Effluent Alkalinity for Day 308-425 (February 3 – May 31, 2003). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

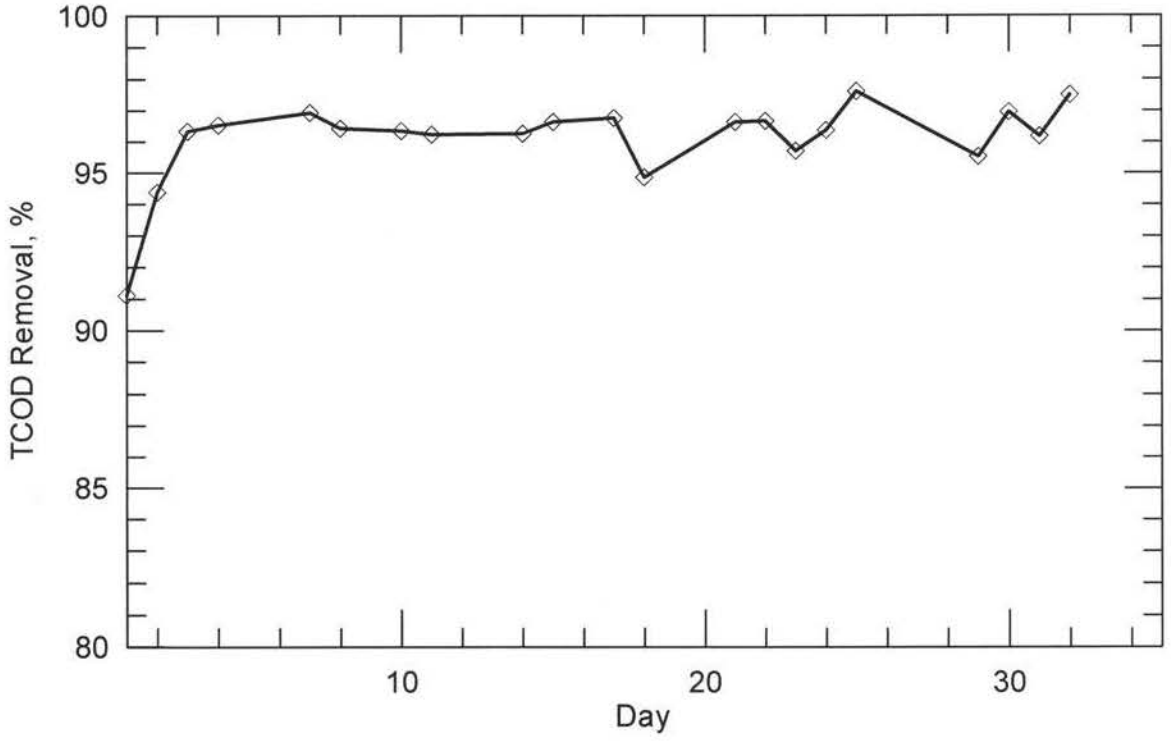

Figure B18. Total COD Removal for Day 1-32 (April 2 – May 3, 2002). HRT for Day 1-32 was 48 hours.

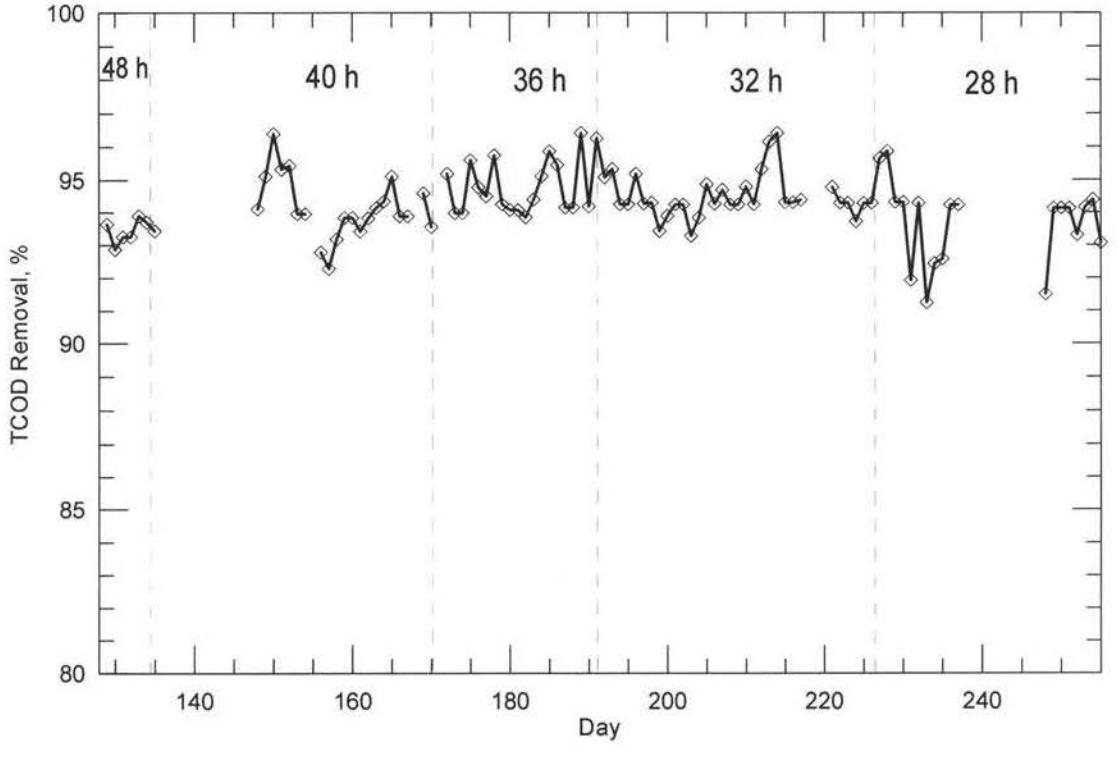

Figure B19. Total COD Removal for Day 128-255 (August 7 – December 12, 2002). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

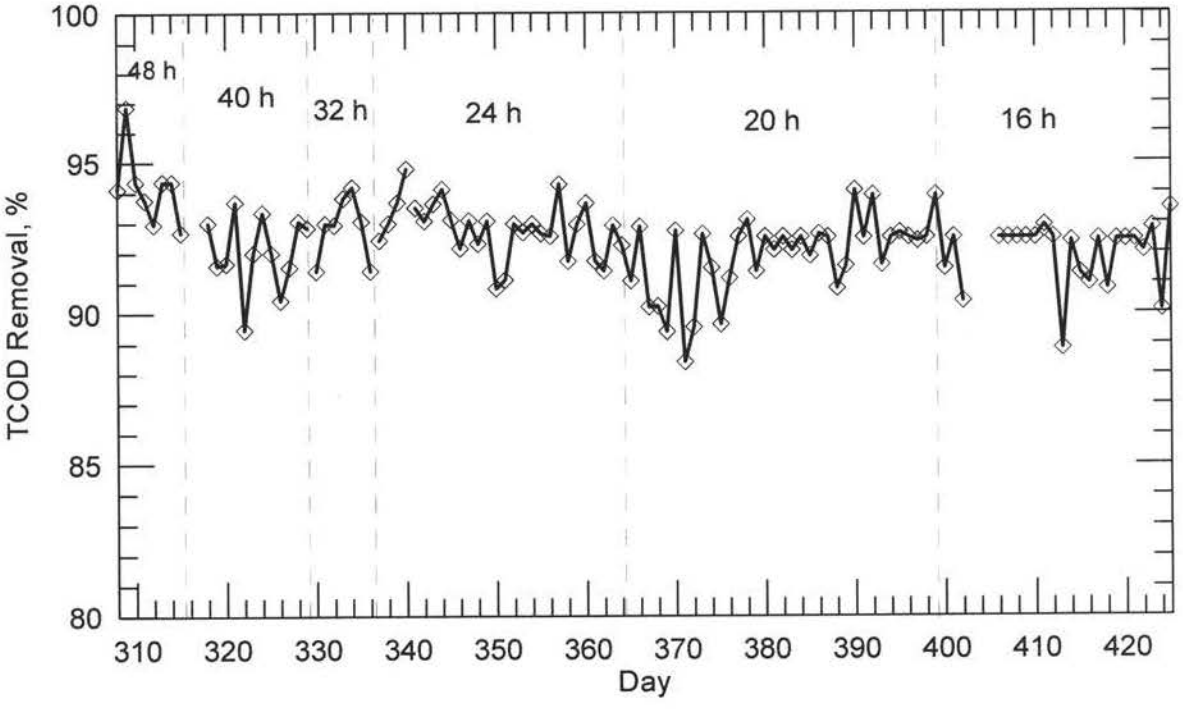

Figure B20. Total COD Removal for Day 308-425 (February 3 – May 31, 2003). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

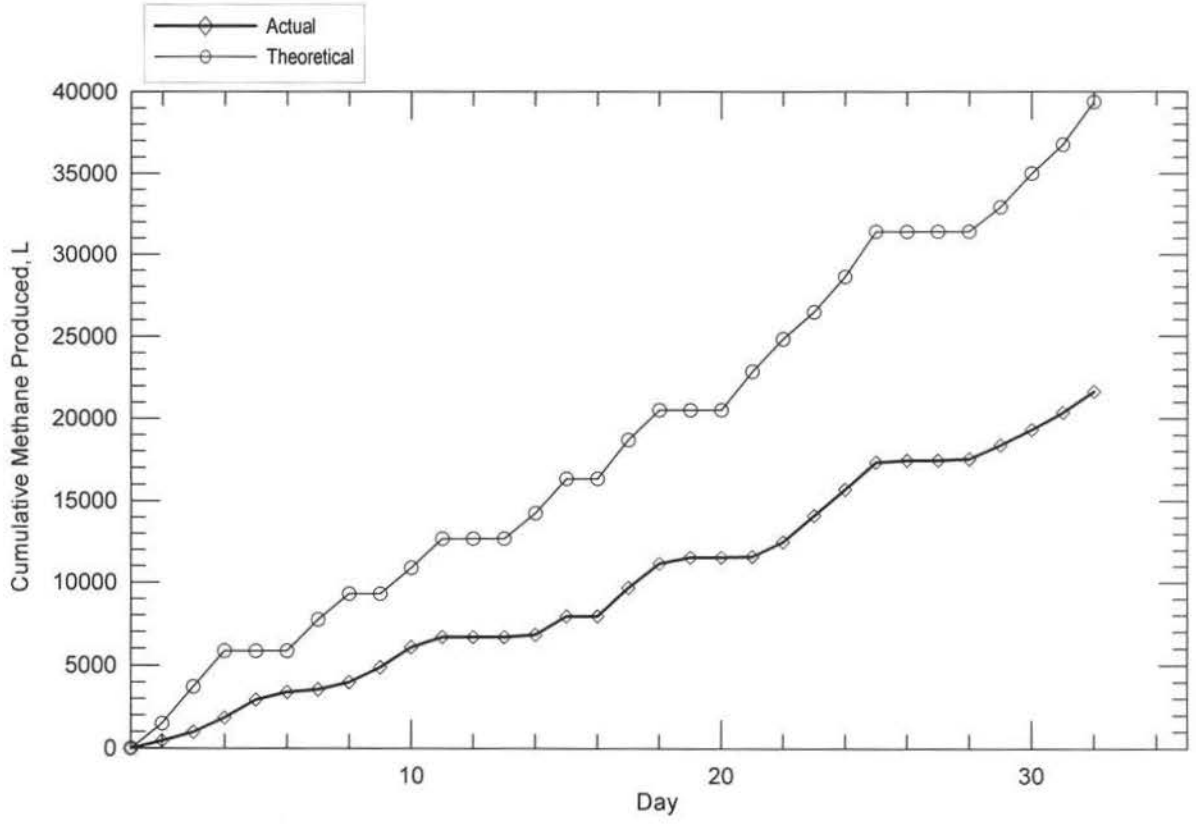

Figure B21. Cumulative Methane Production for Day 1-32 (April 2 – May 3, 2002). HRT for Day 1-32 was 48 hours.

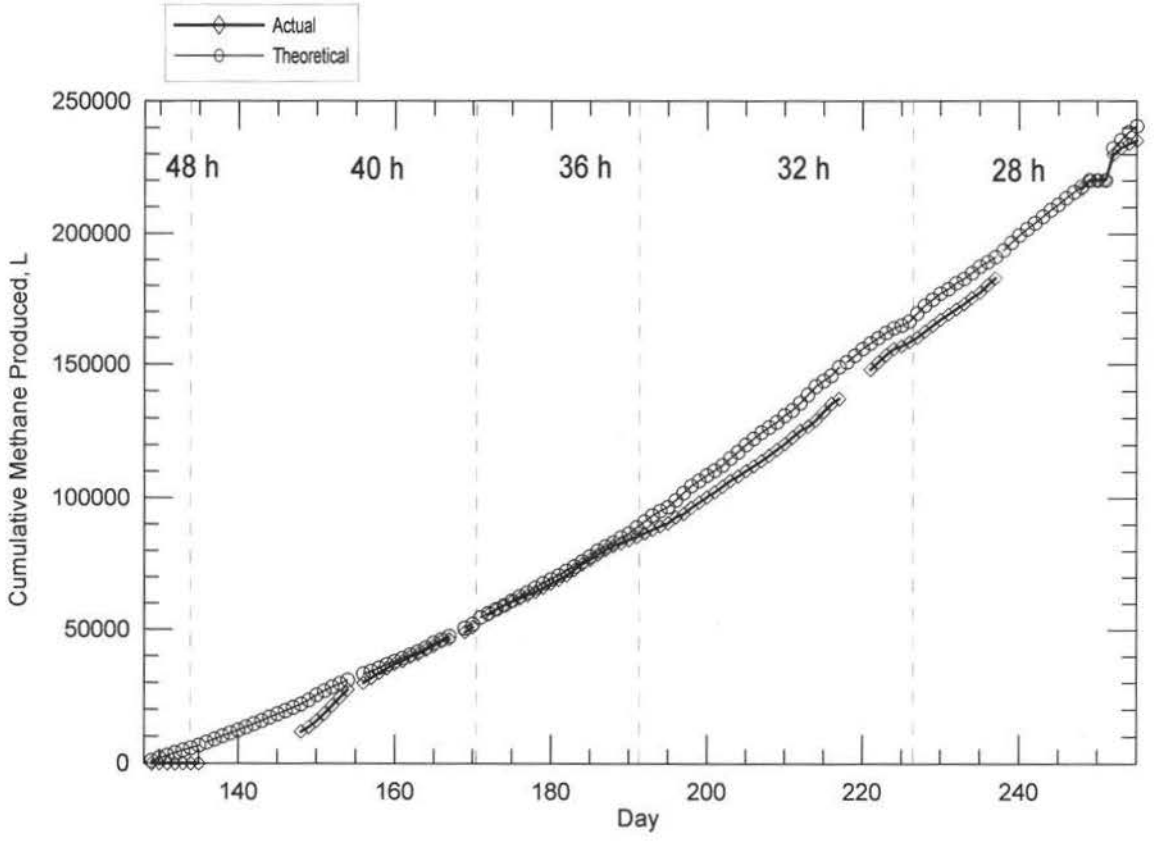

Figure B22. Cumulative Methane Production for Day 128-255 (August 7 – December 12, 2002). The numbers (i.e. 40 h) refers to the HRT condition for the time period.

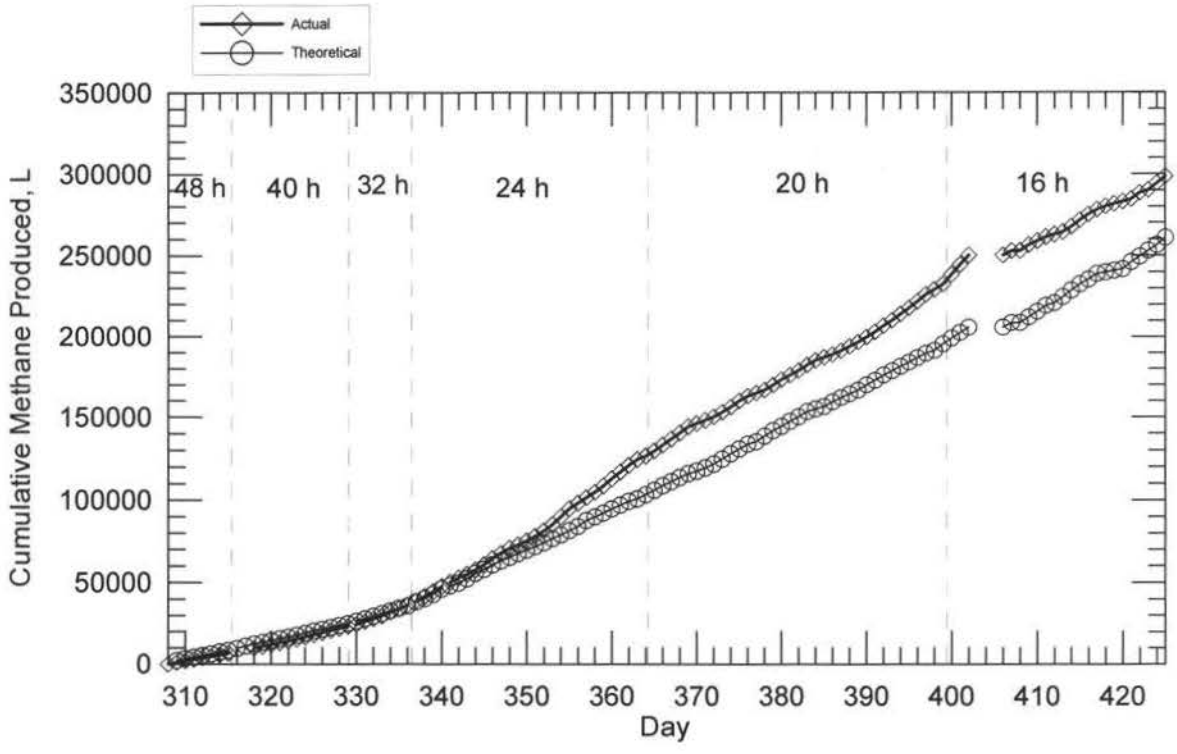

Figure B23. Cumulative Methane Production for Day 308-425 (February 7 – May 31, 2003). The numbers (i.e. 40 h) refers to the HRT condition for the time period. The discrepancy between the actual and theoretical cumulative methane production was due to the malfunction of the gas meter.

APPENDIX C:

SPECIFIC METHANOGENIC ACTIVITY TEST

Procedure (Angenent, 1998)

Day 1:

1. Weigh the empty serum bottle
2. Fill the bottle completely with water and weigh
3. Make anaerobic water by bubbling distilled, filtered water (nanopure water) with nitrogen gas
4. Add 15 mL of nutrient stock solution to each bottle
5. Add 5 mL of 1 M acetic acid to each bottle
6. Add anaerobic water until the bottle's volume is around 140 mL
7. Correct the pH to 6.85-6.9 by adding 3% NaOH (flushing the bottle with N_2 will increase the pH to 7)
8. Add 5 mL of granular biomass to each bottle
9. Flush the liquid with N_2 gas for 15-30 seconds at high flow rate
10. Flush the headspace in the capped bottle using two needles for 2 minutes
11. Add 0.5 mL of 0.25 M Na_2S
12. Place bottle on shaker table and leave overnight. If the solution is still pink after 30 minutes, the bottle was open to the atmosphere too long. Therefore, add a little more Na_2S or flush the liquid and headspace again with N_2 gas.

Day 2:

1. Determine VFA concentration using spare batch bottle and calculate how much 1 M acetic acid to add to each bottle to obtain an acetate concentration of 2 g/L (alternatively, add 2.5 mL of 1 M acetic acid to each bottle without determining VFA concentration)
2. Add desired quantity of 1 M acetic acid and/or PCP to each bottle
3. Correct pH to 6.85-6.9 with 3% NaOH and record pH
4. Cap the bottle with rubber septum
5. Flush the headspace in the capped bottle using two needles for 2 minutes
6. Place bottles on shaker table for one hour
7. After one hour, begin to measure the methane concentration in the headspace of the bottles at regular intervals until the end of the test

8. Remove the rubber septum and measure the pH

9. Measure the weight of the bottle with the solution and calculate the volume of the headspace using the weight of the full bottle from Day 1

10. Measure the solids content of the granular biomass in each bottle (either VS or VSS)

11. From the results of the GC analysis, plot the increase in methane percentage over reaction time. Fit a regression line to the data with the slope of the line representing the % CH_4/d. Correct this value using the calculated headspace volume, solids content, and a factor of 0.388 to yield the SMA (gCOD-CH_4/gVSS·d at STP) for each bottle.

Table A1. Batch medium stock solution for batch tests[a]

Compound	Chemical Formula	Concentration	
Sodium Phosphate Monobasic	$NaH_2PO_4 \cdot H_2O$	7.95	g/L
Potassium Phosphate	K_2HPO_4	6.0	g/L
Ammonium Chloride	NH_4Cl	2.8	g/L
Magnesium Sulfate	$MgSO_4 \cdot 7H_2O$	1.0	g/L
Yeast Extract		1.0	g/L
Calcium Chloride	$CaCl_2 \cdot 2H_2O$	0.1	g/L
Trace Element Solution		10	mL/L

[a] Will be diluted ten times with anaerobic water and biomass

Table A2. Trace element stock solution for batch tests

Component	Chemical Formula	Concentration	
Ferrous Chloride	$FeCl_2 \cdot 4H_2O$	10,000	mg/L
Nickel Chloride	$NiCl_2 \cdot 6H_2O$	142	mg/L
Cobalt Chloride	$CoCl_2 \cdot 6H_2O$	2,000	mg/L
Manganese Chloride	$MnCl_2 \cdot 4H_2O$	500	mg/L
Zinc Chloride	$ZnCl_2$	50	mg/L
Ammonium Molybdate	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	50	mg/L
Cuprous Chloride	$CuCl_2 \cdot 2H_2O$	38	mg/L
Sodium Selenite	Na_2SeO_3	123	mg/L
Boric Acid	H_3BO_3	50	mg/L
EDTA		1,000	mg/L
Resazurin		200	mg/L
Aluminum Chloride	$AlCl_3 \cdot 6H_2O$	90	mg/L
Hydrochloric Acid	HCl (37.7%)	1	mL/L

References

Angenent, L.T. (1998). Development of a new high-rate anaerobic process for the treatment of industrial and domestic wastewaters: the anaerobic migrating blanket reactor (AMBR). Thesis (Ph.D.). Iowa State University, Ames, IA.

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