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Fundamentals of the static granular bed reactor

Kristin Mach Evans
Iowa State University

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Fundamentals of the static granular bed reactor

by

Kristin Mach Evans

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Timothy G. Ellis, Major Professor
Larry J. Halverson
Thomas E. Loynachan
Shihwu Sung
Hans van Leeuwen

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Kristin Mach Evans

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Major Professor

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For the Major Program

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Abstract

The static granular bed reactor (SGBR) has been shown to be a highly efficient anaerobic system. High organic removal, low effluent suspended solids (TSS), and high methane content in the off gas were consistently obtained with all SGBRs operated. Two SGBRs treating non-fat dry milk, or synthetic wastewater, were operated continuously for more than four years at hydraulic retention times (HRTs) ranging from 36 h to 4 h, equivalent to over three solids retention time (SRT) periods. During the four years, effluent chemical oxygen demand (COD) concentrations were less than 50 mg/L and TSS concentrations were less than 30 mg/L under most conditions.

Because of the exceptional performance when the SGBR treated synthetic wastewater, the performance was examined under more stressful operating situations. SGBR performance was evaluated while treating wastewater with high sulfate concentrations, during HRT transitions, and at fractional bed volumes. In stressed circumstances, the SGBR continued to operate efficiently. The addition of sulfate had little effect on the SGBR system, and the overall COD removal was greater than 90% and effluent TSS concentrations were less than 70 mg/L.

During HRT transition, the SGBR was relatively unaffected immediately after the HRT was changed from 36 h to 5 h. A slight decrease in COD and TSS removal was observed during the first 12 hours after the HRT change, but operation returned to greater than 90% COD removal in about 24 hours. The SRT of the SGBR appears to be relatively unaffected by changes in HRT.

The SGBR also showed outstanding organic removal when operated at a fraction of the design bed volume. Four SGBRs were operated at 25%, 50%, 75%, and 100% of the design bed volume for a SGBR with a volume of 1L. At 1 g COD/L and 2 g COD/L, all four had similar organic removal. At 2 g COD/L and higher influent concentrations, the effluent TSS concentration was higher in the 25% bed depth reactor. In the event a full bed of granules are not available during start up, the SGBR could be operated effectively at a partial bed volume.

General Introduction

Current status of anaerobic digestion

Currently many forms of anaerobic digestion are in operation. Typically anaerobic digestion is used for high strength wastewater (Dangcong *et al.*, 1994; Punal *et al.*, 1998; Perez *et al.*, 1998; Rebac *et al.*, 1997; Yu *et al.*, 1998). Aerobic digestion of high strength wastewater requires large amounts of oxygen and larger volumes for oxygen transfer making the system less cost effective. In addition, aerobic organisms have a high yield resulting in large amounts of biomass requiring disposal or reuse. By operating in optimal temperature ranges, anaerobic digestion can produce a high quality effluent, as in the TPAD process (Han *et al.*, 1997) or anaerobic filters operated at thermophilic and mesophilic temperatures (Harris and Dague, 1993).

One potential drawback of anaerobic systems is the requirement of complex operation and control equipment. For example, some systems require mixing devices, gas or feed recirculation lines, or gas/liquids/solids separators. Although necessary, the additional components lead to additional operating problems. Simplicity is a factor that could greatly affect a decision to select an anaerobic treatment process.

Effluent from anaerobic processes usually requires costly post-treatment, typically at a municipal treatment facility. In addition to upkeep of the on-site treatment facilities, there is usually a charge for municipal treatment based on biochemical oxygen demand (BOD_5), total suspended solids (TSS) and nutrients (such as phosphorus and nitrogen). However, if a high rate anaerobic system could be coupled with another system to create a high quality effluent, the final effluent could be reused for nonpotable uses or discharged to a receiving stream.

Biomass and granules

An ideal biological treatment process would be easy to operate and produce a high quality effluent in a relatively small reactor volume. To achieve a high degree of organic removal at short hydraulic retention times (HRTs), many anaerobic processes take advantage of anaerobic bacteria's property to form a dense agglomeration of particles called granules. For instance, the Anaerobic Sequencing Batch Reactor (ASBR), Upflow Anaerobic Sludge Blanket (UASB), Anaerobic Migrating Blanket Reactor (AMBR), and other systems produce microbial granules during normal operation (Hulshoff Pol *et al.*, 1983; Alibhai and Forster, 1986; Angenet, 1998). These dense microbial granules incorporate the complex synotrophic microbial communities responsible for the breakdown of organic matter to CO_2, CH_4, and additional biomass.

Several theories on how granules break down organics exist. The more common thought is in anaerobic granules the outer layer breaks down complex substrate components into volatile fatty acids which then are broken down to acetate and methane deeper inside the granule (Fang *et al.*1994 and 1995). However, a wide consortia of organisms can be found on the surface of the granule (J.W. Morgan *et al.*, 1991) including methanogens, which leads to another mechanism of organic degradation by granules. With its wide array of organisms, the granules are assumed to be fairly adaptable to many wastewater sources and temperatures.

Static granular bed reactor

A stable and dependable anaerobic process has been developed for the treatment of low to medium strength wastewater. The Static granular bed reactor (SGBR) was developed in the Iowa State University environmental laboratory and incorporates highly active, dense microbial granules in a simple downflow configuration (Patent pending, U.S. Serial No. 60/302,504). The SGBRs were operated at ambient temperatures and achieve high organic removal. The system is not complicated and easy to operate with no mixers, solids/liquid separators, or other mechanical devices. In addition to a feed pump, the only extra equipment needed is gas or effluent recirculation line to reverse the feed flow temporarily to dislodge any granules trapped in the underdrain system. The effluent from the system is low in chemical oxygen demand (COD), total suspended solids (TSS), and volatile acids which may allow it to be discharged to a surface water with no additional treatment in some instances (i.e. the system routinely meets a 30 mg/L BOD_5 and 30 mg/L TSS effluent standard).

Several experiments were set up in the studies in this dissertation to examine the viability and performance of the SGBR. The initial part of the research was to determine if a new reactor configuration consisting of a dense bed of anaerobic granules would be feasible for the treatment of low to medium strength wastewater. Because of the set up of the reactor, it was named the static granular bed reactor (SGBR). Although later it was discovered that the bed may not be truly static, and granules appear to move around some in the bed. It was shown effective for the treatment of low to medium strength synthetic wastewater at ambient temperatures.

The feasibility study was conducted using two SGBRs with different inner diameters but equal working volumes. Each system had a one liter working volume. SGBR 1 had an inner diameter of 4 inches and SGBR 2 had an inner diameter of 2 ½ inches resulting in different height to width ratios (H/W) for each SGBR. Using the first two SGBRs, several hydraulic retention times (HRTs) were examined, ranging from 36 to 5 hours. Each SGBR was operated independently until steady state conditions were achieved at each operating condition. High organic removal resulted from both systems, even at short HRTs.

During the project, the 2 ½ inch diameter reactor (H/W = 7) performed slightly better than the 4 inch diameter reactor (H/W = 2). It was thought that results from the two reactors would provide information towards ideal sizing requirement for the SGBR. The SGBR with the larger H/W ratio had too small a diameter as evidenced by operational difficulties. During start up, the granules climbed the walls of the reactor due to buoyancy from gas production. Based on experience with the two lab scale SGBRs, optimal design of the reactor is thought to have a height to width ratio between the two ratios examined.

SGBR performance

SGBR performance is comparable to other systems treating similar wastewaters. Ndon and Dague (1997) examined the performance of an ASBR at different HRTs and operating temperatures. At an HRT of 24 and 12 hours the soluble COD removal was 93 and 81% respectively while operating at 35°C. Collins *et al.* (1998) treated primary clarifier effluent with an expanded bed reactor and achieved greater than 90% COD removal at 20°C. Similar to the SGBR, the system also had very low VFA concentrations. Orozco (1996) achieved an optimum of 92% COD removal using an anaerobic plug flow reactor operated at 13 to 17°C with synthetic wastewater.

A long SRT is indicative of a high biomass concentration, giving a large and more mature microbial population capable of degrading substrate to a lower concentration. As with other biological treatment systems, the SGBR performance was primarily a function of SRT. The SRT was estimated to be greater than 300 days for both SGBR systems, which is greater than similar systems. Dague *et al.* (1998) operated an ASBR at SRTs ranging from 30 to 180 days at 20°C. Since all of the biomass granules were retained in the SGBR, the SRT remained relatively constant despite changes in hydraulic and organic loading. The only loss of biomass was believed to be through granule attrition and measured as TSS in the effluent. At increased loadings, other systems tend to experience a loss of biomass. An ASBR treating low strength synthetic wastewater observed decreases in mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) at decreased HRT conditions (Dague *et al.* 1998). This loss was due to the greater decant volumes required in each cycle.

Treatment of other waste streams by the SGBR

Demonstrating that the SGBR was capable of treating synthetic wastewater was only the first step in confirming the SGBR as a viable anaerobic technology. Additional work was required to find suitable applications for the system. A study using pork slaughterhouse wastewater compared the performance of a SGBR and an ASBR (Jung and Ellis, 2001). Both the ASBR and SGBR performed well at long HRTs. Both exceeded 85% COD removal. However, the SGBR was capable of operation at lower HRTs because of reactor configuration. At a 10 h HRT, the decant volume was too great for the ASBR, and significant amounts of biomass were lost. The SGBR's down flow system and underdrain maintained biomass at both 10 and 8 h HRTs.

Another problem for anaerobic systems is sulfate that exists in wastewater streams. Sulfate is reduced to hydrogen sulfide by sulfate reducing bacteria (SRB). In addition to odor problems, hydrogen sulfide is toxic to methanogens and can interfere with methane production. The SRB also compete with methanogens for substrate, in particular acetate. Methanogens and SRB can coexist, but at high sulfate concentrations and lengths of time greater than a year, SRB can out-compete methanogens for substrate eliminating the population (Visser *et al.* 1993). In a SGBR system treating a high sulfate waste stream, it was thought there will be no detrimental effect because hydrogen sulfide (H_2S) will be produced at the top of the reactor. The hypothesis was H_2S will be in highest concentration at the top of the reactor, not affecting the majority of the granules.

Performance limitations of the SGBR

The original SGBRs were operated for 48 months to serve two purposes by examining failure and transition in the system. Once performance decreased as evident by low COD removal, low gas production, or high solids concentration in the effluent, the experiment was concluded. HRT was stepped down gradually and organic removal was monitored to determine if failure was a result of excessive organic loading or a result of too high of a flow rate through the reactor, washing out biomass. Periodic clogging or drainage problems occurred about every six weeks, but were quickly resolved. These problems were an indication that the SGBR reached its limit based on physical limitations, not necessarily biological performance limitations.

Once operational parameters were identified, other information was needed to progress the development of the SGBR technology. Research to examine transition states was performed, and observations on how the SGBR adapted to increased organic loading were recorded. Once steady state was achieved, a new HRT and thus new flow rate was determined and set. As the HRT changed, samples were taken every six hours over a two day period and testing was performed to identify trends in acclimation. As the HRTs are lowered, a second study was being performed which examined the transition period as the SGBR adjusts to the new loading condition. The transition period, for this study, was defined as the first 36 hours after the HRT was lowered. During this period, samples were taken every 6-8 hours and analyzed for COD removal, volatile fatty acid (VFA) concentration, total suspended solids (TSS) concentration, and pH. Gas composition was also monitored during the change. Two transitions were examined: 36 to 8h and 8 to 5h. Both transitions showed that the SGBR adapted to the new conditions within 24 hours.

Fatty acid methyl ester analysis

Throughout various segments of the study, fatty acid methyl ester (FAME) analyse were performed on the granules from several different reactors. Comparisons were made from granules from the following systems: two SGBRs with different height to width ratios treating synthetic wastewater, two different anaerobic treatment systems (one SGBR and one ASBR) treating pork slaughter house wastewater, and two SGBRs of similar height to width configurations (one treating synthetic wastewater and the other treating pork slaughter house wastewater). The other FAME samples were taken from a SGBR that was fed synthetic wastewater with the addition of sulfate. Other researchers have shown that with the addition of sulfate, sulfate reducing bacteria can out-compete methanogens for necessary substrate which affects COD removal and methane production. During the operation of the reactor, FAME profiles were examined and a shift in community structure may indicate an increase in sulfate reducing bacteria population. FAME profiles were analyzed using the similarity index created by Werker and Hall (2002). Werker and Hall's index is based on statistical analysis, using the entire profile or certain fatty acids found in the sample.

FAME analysis has been deemed useful in comparing a single reactor and its microorganism profile changes over time. In observing a single reactor over time, one may use the profile to detect changes in microbial population resulting from stressed environment, toxic effects, or age of the reactor. Using the procedures developed and data obtained, FAME work will be used to compare community structures.

Another FAME analysis was used to compare a SGBR with an UASB, looking for similarity or differences in the microbial community structure. The profiles obtained here and the profiles from the ASBR and SGBR comparison gave some indication as to the performance of the SGBR. If profiles are similar between the SGBR and other reactors, the performance of the SGBR may be contributed to the reactor configuration. If the profiles are different, the SGBR configuration may select for particular microorganisms which enhance the performance. However, a difference in performance may not be solely attributed to differences in microbial communities. Because of the reactor configuration, differences may be a function of flow type, mixing characteristics, mass transport or several other factors that weren't examined here.

Bed height study

Another investigative study examined scale up considerations of the SGBR. For a full scale SGBR, seeding the entire bed may be costly. If operation can begin with less than full bed volume, capital cost will be less. An examination of the bed depth required at start up of the SGBR was conducted. Four SGBRs were set up with identical dimensions but with varying depths of granules. One SGBR was operated as a control reactor with 100% of the design bed depth (1L). The other three reactors were operated at 25, 50, and 75% of design bed depth. Performance was observed during this time to determine how bed depth affects organic removal, but more importantly, how quickly granules grew or accumulated in the reactor. All four reactors had the same liquid level in the reactor, controlled by a T-connector. This set up is thought to be most similar to how a full scale SGBR may be started. If acceptable performance can be achieved at less than full bed depths, initial costs can be minimized. Several different runs were performed at various feed strengths for comparison.

Performance Evaluations of Two Static Granular Bed Reactors

K. F. Mach and T. G. Ellis
Department of Civil and Construction Engineering, Iowa State University,
Ames, Iowa 50011-3232, U.S.A.

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Abstract

Two lab scale static granular bed reactors (SGBRs) with different height to width ratios, but identical working volumes, were operated over a range of organic and hydraulic loading rates. SGBR 2 consistently performed better than SGBR 1 with higher chemical oxygen demand (COD) removal, lower volatile fatty acid (VFA) concentrations, and lower suspended solids (SS) concentrations. Examination during a transition period from a 36 h hydraulic retention time (HRT) to a 5 h HRT showed SGBR performance was relatively unaffected. A separate study on a SGBR treating wastewater containing 5g COD/L of ethanol confirmed the exceptional performance, especially during hydraulic and organic loading rate changes. The SGBRs performance was attributed to its stable and long solids retention time (SRT).

Keywords

granular biomass, high rate anaerobic process, static granular bed reactor (SGBR)

Introduction

Anaerobic digestion of low to medium strength wastewater requires the use of a high rate system such as an upflow anaerobic sludge blanket (UASB) reactor, an anaerobic sequencing batch reactor (ASBR), or a suspended growth (e.g., anaerobic contact) or attached growth (e.g., anaerobic filter) system. With any high rate system, care must be taken not to wash out the biomass during hydraulic or organic loading rate changes. In this project, a new high rate system, the static granular bed reactor (SGBR), was developed and was shown to be highly effective in removing organics from low to medium strength wastewater. In an effort to maximize the density and retention of active anaerobic granular biomass, the SGBR was developed as a downflow reactor without flow recirculation. The resulting high granule density optimized the contact between the microorganisms and the wastewater and maintained long SRT (81 to 300 d) which were relatively independent of the hydraulic and organic loading rate. The simplicity of the SGBR operation offered a significant advantage over other systems which require recirculation pumping, elaborate solids/liquid/gas separation devices, or sophisticated underdrain and backwashing systems. Two SGBRs with different reactor configurations were evaluated during this study.

Materials and methods

Two laboratory scale SGBRs with working volumes of 1 L were constructed from plexiglass cylinders, and each had additional head space to accommodate granule growth and gas collection. A stainless steel mesh (1/16") was placed in the bottom of the SGBRs to retain the biomass. A "T" connector was installed in the effluent line to maintain the 1 L working volume in the reactor and to keep the granules wet.

Seed granules were obtained from an operating UASB at Heileman's Brewery in LaCrosse, WI. The difference between the two SGBRs was the height to width ratios. SGBR 1 had an inner diameter of 101 mm and a granule height of 135 mm. SGBR 2 had an inner diameter of 64 mm and a granule height of 432 mm. Both were operated for approximately two years under several HRT conditions at ambient temperature (22 ±2°C).

Synthetic feed consisting of nonfat dry milk and nutrients was fed on a semi-continuous basis (2-4 times per hour) using a peristaltic pump. Feed strength was approximately 1000 mg COD/L throughout the study. Feed and effluent were both stored at 4°C. Analytical parameters (alkalinity, COD, suspended solids) were determined using Standard Methods and performed weekly. The VFA concentration was measured by the titration method (Method 5560 C in Standard Methods) with an assumed efficiency of 70%. Gas composition was analyzed by a Gow Mac gas chromatograph and was tested bi-weekly. BOD_5 concentration was measured once during each HRT condition.

The goal of the research was to develop an optimal reactor configuration, maximizing the biomass to volume ratio. Reactor configuration was examined to determine if one reactor configuration consistently performed better than the other (e.g., higher gas production or higher COD removal). Both SGBRs were started at a 36 h HRT, and after steady state was achieved, the HRT was lowered. After approximately one month, the reactors appeared to be at steady state, however each HRT condition was maintained for 65-70 days to ensure steady state.

During start up, gas bubbles caused the granule bed in SGBR 2 to rise above the liquid level. However, after acclimation the granules settled and remained in the liquid area. Both SGBRs required very little maintenance, with the exception that SGBR 1 experienced occasional (less than once a month) clogging during the 5 h HRT that was resolved by reversing the feed flow for approximately 1 min. and then returning to normal operation.

After two months at the 5 h HRT, the clogging problems ceased, and reversing the flow was not necessary.

Table 1. Operating Data.

	HRT (h)	SRT (days)	Feed Conc. (mg COD/L)	Eff. Conc. (mg COD/L) ± std deviation	Eff. BOD (mg/L)	Total Suspended Solids (mg/L)	VFA (mg/L) as acetic acid [1] ± std deviation	H_2S (ppm)[2]
SGBR 1 Days of operation								
0-45	36	81	1040	523 ± 120	NA[3]	130	24 ± 5	NA[3]
46-140	24	350	989	45 ± 28	10	20	14 ± 5	650
141-230	16	377	1102	30 ± 15	10	6	14 ± 6	600
231-315	12	321	893	30 ± 19	26	16	18 ± 7	600
316-422	8	319	947	79 ± 29	47	8	17 ± 2	1800
423-515	6	312	949	38 ± 24	27	10	19 ± 1	1400
516-598	5	320	910	39 ± 15	22	12	17 ± 5	1200
599-664	36	375	975	46 ± 18	13	8	16 ± 2	600
665-	5	318	1009	40 ± 22	10	19	9 ± 1	300
SGBR 2 Days of operation								
0-91	36	381	1145	52 ± 23	8	5	12 ± 3	1700
92-150	24	350	971	59 ± 15	NA[3]	18	17 ± 5	500
151-212	16	353	931	44 ± 24	26	3	17± 5	NA[3]
213-320	12	342	819	50 ± 21	21	6	17 ± 1	850
321-450	8	327	958	33 ± 15	14	5	14 ± 1	600
451-510	5	321	964	24 ± 12	12	7	12 ± 5	800
511-576	36	379	997	41 ± 19	10	5	11 ± 1	1100
577-	5	319	1038	41 ± 18	9	10	9 ± 1	500

[1] as measured by titration. GC analysis showed non-detect limits for acetic, proprionic, n-butyric acid
[2] in gas phase
[3] not analyzed

Results

During all HRT conditions, the reactors had excellent results with respect to COD and BOD_5 removals, and low effluent VFA, BOD_5, and SS concentrations. Table 1 shows the results from both SGBRs during HRTs ranging from 36 to 5 h.

During the study, the poorest performance occurred at the initial 36 h HRT, because the granules were not acclimated to the wastewater. It was decided to return the SGBRs to a 36 h HRT condition and reevaluate the performance once the granules had been acclimated and were more efficient. After the datum was collected at the second 36 h steady state, the SGBRs were returned to a 5 h HRT. Throughout the research period, SGBR 1 and SGBR 2 maintained greater than 92% and 94% total COD removal respectively. Soluble COD removal in both SGBRs was 95-97%. The BOD_5 data confirm the high organic removal.

Throughout the research period, SGBR 2 consistently had lower measured VFA concentrations. Gas chromatographic (GC) analysis, however, indicated that the titration method might have overestimated the VFA concentration. By GC analysis, acetic, propionic, butyric, and valeric acid concentrations were each measured at or below the detection limit of 1 mg/L. Suspended solids and BOD_5 consistently met (with the exception of the 8 hour HRT condition for SGBR 1) the NPDES requirement of 30 mg/L which would allow the effluent to be discharged to surface water in areas where nutrient removal was not required.

In order to more fully evaluate the SGBR, specifically the organic loading turndown ratio, the transition period from a 36 h HRT to a 5 h HRT was examined. After the retention time was shortened to 5 h, analytical testing was performed every 12 h to observe the non-steady state performance. Figure 1 shows the COD and total suspended solids (TSS) during the transition period. After the HRT was lowered (resulting in a corresponding increase in organic loading), the effluent total COD increased from 46 mg/L to 144 mg/L in SGBR 1 (the wider and shorter reactor). However, SGBR 2 showed no significant increase in effluent COD concentration. Both reactors showed an increase in TSS, but the concentration for both remained below 30 mg/L. Soluble COD increased initially, but returned to low levels (<30 mg/L) within the 36 h period. Other parameters such as the VFA concentration were not greatly affected. VFA concentration remained low (<12 mg/L by titration method) during the transition period. During the transition period, the hydrogen sulfide concentration was less than 150 ppm which was low for both SGBRs compared to other sampling times (see Table 1).

The operation at the 5 h HRT remained exceptional. The organic removal remained high. COD removal in SGBR 2 improved slightly, but not significantly (95% at 36 h to 97% at 5 h). Gas production increased with the higher organic loading, and the methane content was 81% for both SGBRs.

Discussion

Overall, the performance of the SGBRs was consistent and did not vary with changes in HRT conditions as shown in Table 1. VFA concentrations were extremely low. By comparison, an ASBR operating at 20°C and 16 h HRT treating a synthetic substrate (non-fat dry milk) had effluent VFA concentrations of 30 mg/L (Ndon and Dague 1997).

As with other biological treatment systems, the SGBR performance was primarily a function of SRT. As seen in Table 1, the SRT was greater than 300 days for both SGBR systems. Dague *et al.* (1998) operated an ASBR at SRTs ranging from 30 to 180 days at

20°C. Since all of the biomass granules were retained in the SGBR, the SRT remained relatively constant despite changes in hydraulic and organic loading. The only loss of biomass was through granule attrition and was measured as TSS in the effluent. At increased loadings, other systems tend to experience a loss of biomass. An ASBR treating low strength synthetic wastewater observed decreases in mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) at decreased HRT conditions. This loss was due to the greater decant volumes in each cycle (Dague *et al.* 1998). In the present study, the performance of the two SGBRs during the transition period (7:1 organic loading turndown ratio) was further evidence that the performance was relatively immune to abrupt changes in loading (see Figure 1).

In addition to analytical performance testing, size analysis was performed on the granules using microscopic photography. Size analysis confirmed the change in granule structure. At the start of the experiment, the majority (60%) of the granules ranged in size from 0.7 to 1 mm in diameter. Eight months later, 89% of the granules measured greater than 1.0 mm in diameter. The growth of the granules was also noticed in the volume occupied in the reactor. As expected due to the narrower diameter, SGBR 2 experienced a greater increase in bed height (127 mm increase) within the reactor. Granule growth benefits the reactor by increasing the mass of organisms present thereby lowering the F/M ratio and increasing efficiency. Clogging problems were believed to be caused by granules trapped in the underdrain system. An increase in granule size may have been an important factor in the decreased clogging as the research progressed.

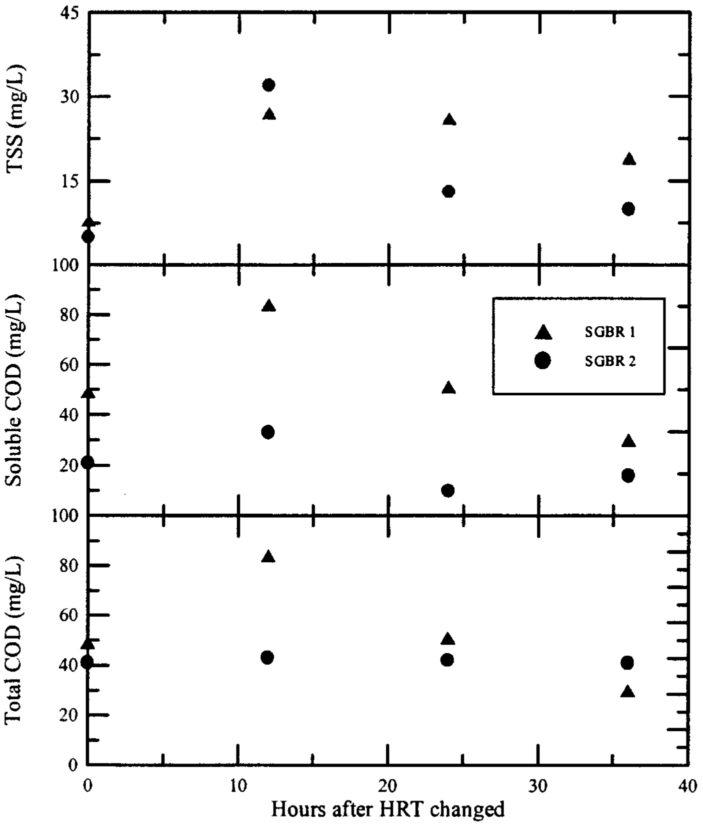

Figure 1. Effluent TSS and COD during transition period from 36 to 5 h HRT.

The SGBR has significant advantages over other anaerobic treatment processes. Anaerobic filters have inert media occupying space in the reactor, where the SGBR has only active granules creating a higher mass of degrading organisms per unit volume. The SGBR's dense granule bed allows for a higher microbial concentration in a smaller volume. Compared with the upflow anaerobic sludge blanket (UASB) and the anaerobic sequencing batch reactor (ASBR), the SGBR can achieve longer SRT. UASBs can experience SRTs ranging from 7-212 d (Speece, 1996; Tay and Yan, 1997; Lay and Cheng, 1998). The SGBR is a downflow reactor that maintains the organism population by preventing the washout of biomass. Ndon and Dague found that an ASBR operated at short HRTs (16 and 12 h) lost significant amounts of biomass due to larger volumes decanted during the cycles as a result of increased hydraulic loading (1997).

Another SGBR in the same laboratory (Venzke, 2001) study confirmed that COD removal is relatively independent of the HRT. A SGBR treating ethanol wastewater with a 1 L working volume was operated for 2 years at 35°C (±2°C). This system was operated at HRTs ranging from 48 to 12 h, and feed strength was approximately 5g COD/L throughout the study. COD removal, VFA concentrations, and SS concentrations were measured during the study and were monitored during HRT changes. During steady state operation, COD removal was 95 to 98%. As HRT was lowered, COD removal decreased from 98% at a 48 h HRT to 95% at 12 h HRT. Effluent VFA concentrations were low throughout the study (<12mg/L). As the HRT was changed, the SGBR was monitored and results show that HRT has little effect on organic removal. Both COD removal and VFA concentrations remained relatively constant during the transition period and therefore were relatively unaffected by the HRT. SGBR had slightly higher VFA concentrations during transition periods but returned to low levels (<12 mg/L) once steady state was achieved. Figure 2 compares the results from steady state (week 0) to transition period (week 1) and new steady state (week 2).

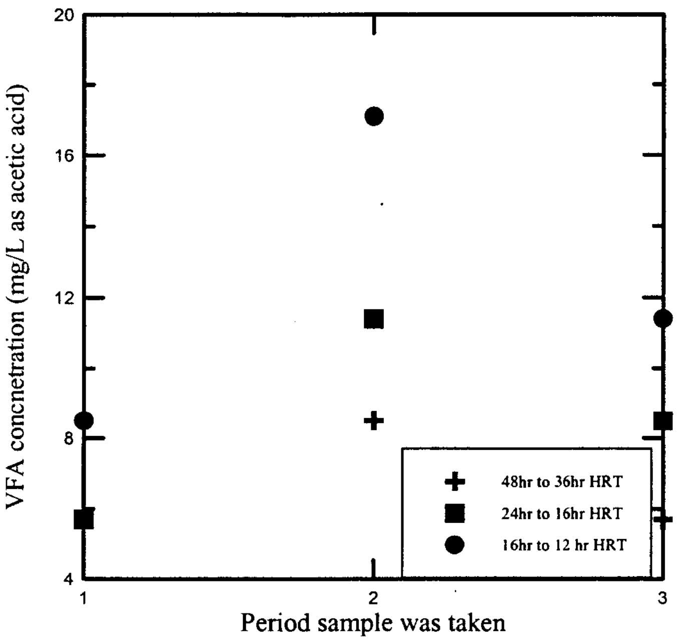

Figure 2. VFA concentration changes in EtSGBR

The SGBR was effective at low HRT conditions where other systems may fail. An ASBR operated at various HRTs and a feed strength of 1000 mg COD/L showed significantly decreased performance as HRT was lowered. At 20°C and 48 h HRT COD removal was 95%, but performance decreased to 70% removal at a 12 h HRT (Ndon and Dague 1997). An anaerobic filter and a fluidized bed reactor experienced decreased COD removal at higher organic loadings due to washout of biomass. COD removal was 83% at a 1.2 d HRT, and when HRT was lowered to 0.65 d COD removal decreased to 74% (Perez *et al.,* 1998).

In a side by side comparison treating meat processing wastewater, the SGBR performed slightly better than an ASBR (Jung, 2001). An ASBR and a SGBR were set up to compare the performance of the two systems while treating a meat processing wastewater. Both reactors had greater than 90% COD removal, however the SGBR consistently had greater than 94% COD removal. The SGBR had lower SS concentrations in the effluent compared to the ASBR, 20 mg/L and 28 mg/L respectively.

Conclusion

The following conclusions can be made from this study.

- The taller reactor (SGBR 2) had better performance with respect to COD removals, effluent TSS concentrations, and effluent VFA concentration.
- The SRT remained fairly constant despite changes in hydraulic loading.
- The high turndown organic loading ratio (7:1) did not affect performance even at short (5 h) HRTs.
- The SGBR systems adjusted quickly to changes in the hydraulic and organic loading and returned to stable operation within 36 h.
- The SGBR systems achieved higher COD removal and lower effluent TSS concentration than the ASBR in a comparison study.
- The SGBRs achieved a long SRT (~300 d) that was unaffected by changes in hydraulic and organic loading.

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Fatty Acid Methyl Ester (FAME) Analysis of High Rate Anaerobic Wastewater Treatment Systems

Kristin F. Mach and Timothy G. Ellis
Department of Civil and Construction Engineering
Iowa State University, Ames, Iowa 50011
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Abstract

The purpose of this research was to use fatty acid methyl ester (FAME) analysis to examine the microbial community structure of different SGBRs in various anaerobic environments. Three reactors were examined under various operating conditions. Granule samples were taken from an anaerobic sequencing batch reactor (ASBR) and a static granular bed reactor (SGBR) treating pork slaughterhouse wastewater operated under identical conditions. The third reactor was a SGBR treating synthetic wastewater containing non-fat dry milk. Using FAME, fatty acids were extracted and a profile or "fingerprint" of the community structure was created. Profiles of the different reactors were compared, and the community differences among the reactors were compared. FAME profiles also indicate that the microorganism structure did not change significantly as the hydraulic retention time (HRT) was lowered.

Keywords

FAME, high rate anaerobic systems, microorganism community structure

Introduction

All microorganisms contain lipids within the cell membrane. Different strains of organisms have different proportions of fatty acids (FAs) within the lipids which can be isolated and identified creating a "fingerprint" of the organism, or community, in mixed cultures. Originally FA profiles were used to estimate biomass, but recently it has been used to give information regarding the diversity of a microbial community and the conditions (environmental, nutritional, or otherwise) surrounding the community. Microbial FA profiles change with alterations in environmental or nutritional factors. Analyses can also be used to detect stressor conditions or changes in community structure. Specific organisms have signature FA which appear in the profiles. For example, monounsaturated FA are considered signature acids for anaerobic gram negative bacteria (Steinberger *et. al.*, 1999), including sulfate reducing bacteria and methanogens, and branched chain fatty acids are signature for gram positive bacteria.

Whole community fatty acid methyl ester (FAME) analysis is a simple procedure to identify and characterize FAs. The Microbial ID, Inc. (MIDI) procedure was developed to extract fatty acids from pure bacterial cultures, but has been successfully applied to microbial communities such as soils and anaerobic lagoons (Pankhurst *et al.*, 2001 and Merril, 1999). In the MIDI protocol, microbial cells are saponified by heat and a strong base. Once FAs are free from the lipids, they are methylated to form FAMEs which can be analyzed by gas chromatography (GC) and summarized by MIDI's Sherlock data analysis system. The summary is what generates the FA profile. A potential drawback of the MIDI procedure is that several FAs have similar retention times and cannot be identified individually, thus are presented as a summed group. Another procedural problem is that during the saponification step, the strong base and other tube contents may boil and leak out of the sample tube making it difficult to rely on results obtained from the particular tube. Consequently, duplicate or triplicate copies of a particular sample must be analyzed to ensure satisfactory results.

Although FAME traditionally is used to extract FAs from pure cultures grown under closely controlled lab conditions, the procedure is becoming more popular with mixed cultures in environmental and lab reactor settings. Werker and Hall (2002) examined changes in microbial communities in wastewater treatment systems with the intent of examining community instability and performance of the treatment system. Pankhurst *et al.* (2001) determined if FAME was appropriate for use in soil microbial communities and examined community structure changes as environmental conditions changed. Total ester linked FAME and phospholipid linked FAME analysis were compared. Their research showed that although the profiles were different, both types of FAME analysis were equally capable of identifying the environmentally influenced community structure changes. MIDI-FAME has also been used to characterize groundwater communities. Glucksman *et.al.* (2000) used FAME analysis after concentrating microorganism cells through a membrane filter to obtain enough biomass. They found the procedure suitable for groundwater communities despite its traditional inception on pure cultures.

FAME is a relatively quick and simple procedure that can be used as an analytical tool to describe biological systems. The analysis creates a profile of the microorganism community. By comparing the profiles over time, they can show when changes occur to the reactor population. Populations may change with age of the system (e.g., SRT), when loading or operating conditions change, or for other reasons. Changes in performance may also be an indication that the microorganism population has shifted. For example, in anaerobic systems, methane is the major component of the off gas with small amounts of other compounds such as hydrogen sulfide. If the hydrogen sulfide concentration increases, the FAME profile may show a change that indicates an increase in sulfate reducing bacteria population.

Another use for whole community FAME would be in an early warning system for biological systems. FAME can detect when a microbial community is operating under stressed conditions, especially when aromatic or toxic compounds are present or when nutritional deficiencies appear. Early detection of a stressor may prevent poor performance or even failure of a biological treatment process and allow time for corrective action to take place. Lab scale studies would provide valuable information for an early warning detection system. Perey *et al.* (2000) used FAME analysis to examine nitrifying communities in a lab scale sequencing batch reactor (SBR). For a one day inhibition experiment, thiourea was fed to the SBR and samples were collected at 1, 4, 8, and 24 hours after addition of the inhibition chemical. Their results show that the inhibitor caused a shift in the FAME data. It was concluded that a "steady state" or consistent microbial FAME profile would need to be established first before inhibition could be detected.

In this study community structures were examined from several different reactors to try and correlate performance and microbial community structures. The first two reactors were a static granular bed reactor (SGBR-P) and an anaerobic sequencing batch reactor (ASBR). Both systems treated pork slaughterhouse wastewater and were operated at the same temperature and HRT. A second study compared two SGBRs, the one treating pork slaughterhouse wastewater (SGBR-P) at 35 °C and the other treating a synthetic wastewater of non-fat dry milk (SGBR-M) at 23 °C. FAME samples were taken at various hydraulic retention times (HRTs) and profiles were compared between reactors and between different HRTs.

Materials and methods

Samples analyzed were from reactors which had been in operation from 8 months (ASBR and SGBR-P) to 3 years (SGBR-M). Chemical oxygen demand (COD), total suspended solids (TSS) concentration, and pH were measured throughout operation. During this study, the ASBR and SGBR-P were operated at 14, 12, and 10 h hydraulic retention times (HRTs). Additionally, SGBR-P was operated at an 8 h HRT. During the 10 h HRT, the ASBR lost significant amounts of biomass and COD removal decreased. Consequently, it was not operated at HRTs lower than 10 h. Granules were obtained from sampling ports at approximately the same height in both reactors throughout the study. The SGBR-M was operated at a 5 and 8 h HRT during this project. Similar analytical parameters were measured for both systems. Samples for SGBR-M were taken from a sampling port in the side of the reactor and occasionally additional granules were withdrawn from a small tube inserted into the feed line. Granules were sampled from approximately the same location for all sampling events.

Within one hour of collection, FAMEs were extracted using the Microbial Identification System anaerobic extraction protocol (Microbial ID, Inc., Newark, DE). Cells in the samples were saponified by heat and the presence of a strong base. In this step, fatty acids were separated from lipids. After the separation, the remaining FAs were methylated to form FAME and extracted into an organic solvent. Following extraction, FAME samples were analyzed on a HP 6890 (Hewlett Packard, Rolling Meadows, IL) gas chromatograph. MIDI's Sherlock data analysis system (Microbial ID, Inc., Newark, DE) was used to identify the fatty acid methyl esters and to generate a community profile for each sample based on the quantity of FAMEs present.

The difficulty with obtaining FAME profiles from the SGBRs and the ASBR was the anaerobic granules that gave the reactors the high microbial biomass. The dense granules contained a large number of microorganisms throughout the granule. The saponification step did not break down the entire granule structure, did not yield high amounts of FAs, and thus did not produce reliable analyses. The problem was overcome by crushing the granules in the sample tube before the saponification step creating greater surface area for release of FAs.

Data from FAME analysis is presented in units of percent of the total area of named FAMEs in a sample using standard fatty acid nomenclature. The length of the FA chain is reported first followed by a colon and then the number of double bonds in the chain. Positions of the double bonds are counted from the methyl end of the chain (ω). Included in the description can be the cis or trans configuration of the molecule. Branching in the FA and any hydroxyl groups are also indicated relative to the ω end of the chain.

Because of the large number of FAs present in each sample, principal component (PC) analysis was used to observe any changes in the FAME profiles. Principal component analysis explains the maximum variation in the data based on theoretical components, or principal components in the data. For this study, PC analysis was done by MIDI FAME's Sherlock program (Microbial ID, Inc., Newark, DE).

Results

The SGBR-P had better performance with lower suspended solids concentration in the effluent and higher organic removal than the ASBR. A summary of operating and performance results are found in Tables 1 and 2. FAME analysis was used to determine if performance differences were the result of different microbial populations.

Table 1. Performance parameters for the SGBR-M (standard deviation) during this study's sample periods.

HRT (hours)	5	8
Days at HRT	76	91
Operating Temp (°C)	23 (2)	23 (2)
COD influent (mg/L)	1001 (74)	964 (119)
COD effluent (mg/L)	41 (18)	77 (50)
pH effluent	6.80 (0.17)	6.74 (0.15)
Effluent TSS (mg/L)	10 (3)	6 (4)

The FAME profiles generated indicate the similarities or differences in the FA content of each sample. These profiles can be correlated to the microbial population during the different sampling times and conditions.

Table 2. Performance parameters for the ASBR and SGBR-P (with standard deviation) during this study's sample periods.

Reactor and HRT (h)	SGBR – 14	ASBR – 14	SGBR – 12	ASBR – 12	SGBR – 10	ASBR – 10	SGBR – 8
Days at HRT	99	99	116	116	65	65	30
Operating Temp (°C)	35 (2)	35 (2)	35 (2)	35 (2)	35 (2)	35 (2)	35 (2)
COD influent (mg/L)	2188 (713)	2188 (713)	2355 (719)	2355 (719)	2340 (681)	2340 (681)	2534 (501)
COD effluent (mg/L)	88 (22)	360 (276)	106(48)	258 (141)	159 (41)	537 (247)	189 (29)
pH effluent	6.89 (0.19)	6.85 (0.18)	6.89 (0.23)	7.07 (0.3)	6.69 (0.34)	6.57 (0.24)	6.67 (0.34)
Effluent TSS (mg/L)	17 (9)	251 (265)	28 (12)	102 (89)	34 (17)	264 (180)	31 (8)

Principal component analysis was performed on the FAME profiles of the two reactors treating slaughterhouse wastewater. The analysis showed that the ASBR had a slightly more homogenous population profile which is consistent with the mixing and settling during the operation of the system as shown in Figure 1. The SGBR profile was more random. This could be expected because of less movement of the granules in the system which leads to greater microbial diversity. The concentration of several FAs were significantly different between the reactors. Both reactors had a high distribution of FAs with no single group consisting of more than 20% of the sample. The wide variety of FAs allowed for easy identification and comparison of changes in sample profiles. Because of the wide array of FAs in the samples, only FAs that made up more than 5% of the total area were compared. See Table 3 for examples of the FAs observed.

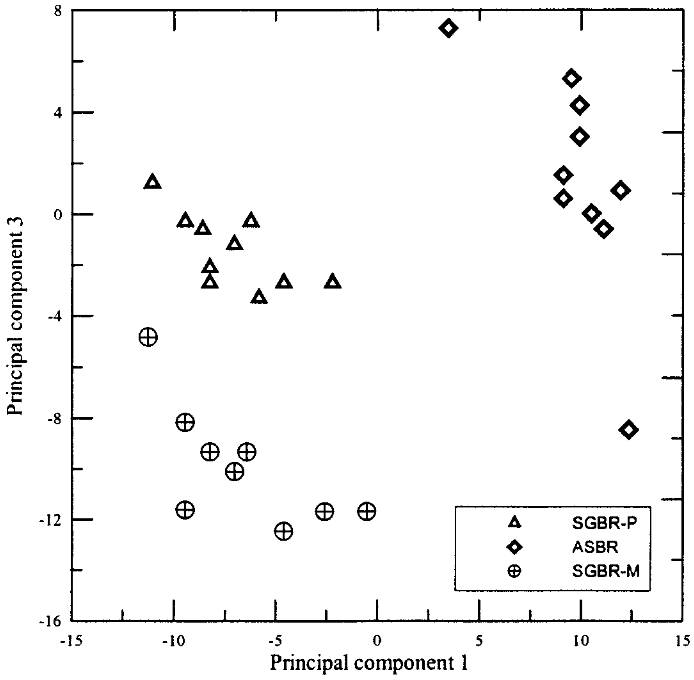

Figure 1. Two dimensional plot of FAME profiles of two reactors treating pork slaughterhouse wastewater and a SGBR treating synthetic wastewater generated by principal component analysis.

Table 3. Average fatty acid concentrations in percent of named area (with standard deviation) for ASBR and SGBR-P as determined by FAME analysis.

Reactor and HRT (h)	SGBR - 14	ASBR - 14	SGBR - 12	ASBR - 12	SGBR - 10	ASBR - 10	SGBR - 8
Number of samples	12	9	15	12	12	12	11
14:0	6.03 (0.15)	5.91 (0.29)	5.92 (0.16)	5.18 (0.211)	6.17 (0.09)	5.12 (0.15)	6.14 (0.12)
15:0 iso	9.86 (0.85)	13.07 (0.52)	9.00 (0.18)	12.27 (0.45)	8.97 (0.28)	12.06 (0.45)	8.98 (0.27)
15:0 anteiso	8.96 (1.38)	8.03 (0.14)	7.54 (0.21)	6.28 (0.13)	7.65 (0.16)	6.4 (0.22)	7.63 (0.16)
16:0	13.97 (1.06)	11.85 (0.51)	15.95 (1.07)	12.58 (0.76)	15.90 (0.69)	12.42 (0.82)	15.90 (0.71)
Sum feature 3[2]	nd[1]	4.66 (0.49)	nd[1]	5.13 (0.34)	nd[1]	4.61 (0.42)	nd[1]
Sum feature 5[3]	4.31 (0.08)	nd[1]	4.39 (0.23)	nd[1]	4.37 (0.26)	nd[1]	4.36 (0.26)

[1] not detected

[2] Sum of 16:1 w7c and 15 iso 2OH

[3] Sum of 18:2 w6,9c and 18:0 anteiso

The most notable difference between the two different reactor systems is the lack of summed feature 3 (various of 15 and 16 carbon chained FAs) in the SGBR and lack of summed feature 5 (various 18 carbon chained FAs) in the ASBR.

As anticipated, results of the two SGBRs treating different wastewaters show differences in the microbial community structures. As with the pork slaughterhouse reactors, the profiles from the SGBRs contained a wide distribution of FAs. The SGBR-M profile did not have several of the FAs that SGBR-P contained. For example, SGBR-P contained 18:1w9c, 18:0, and summed feature 5 (as described earlier). It can be generalized that SGBR-P had a higher quantity of larger (>18 carbons) chained FAs than SGBR-M. Table 4 lists the FA concentration found in SGBR-M.

Table 4. Average fatty acid concentrations in percent of named area (standard deviation) in SGBR-M as determined by FAME analysis.

HRT (hours)	5	8
Number of samples	9	9
15:0 iso	10.14 (1.30)	11.27 (1.66)
15:0 anteiso	22.01 (0.92)	23.21 (4.35)
16:0	10.17 (0.78)	10.75 (0.76)
18:1 w7c	4.51 (0.24)	4.70 (0.42)

Discussion

Because of the different reactor configurations, differences in microbial community structure were expected between the ASBR and SGBR-P. The ASBR is a four phased batch reactor with mixing occurring during one of the phases. The SGBR is a bed of granules operated in a down flow manner. While dynamics of the granule bed is still unclear, previous work has shown that movement of the granules is limited, and therefore, a more diverse microbial population exists throughout the depth of the bed (Mach and Ellis, 2001). Because of the limitations of FAME and the incomplete anaerobic library of microbial profiles, only general conclusions can be made about the differences in community structure. Based on the differences in microorganism community structure found with principal component analysis, the SGBR may have better performance because of the large variety of organisms existing in the reactor. However, the success of the technique to identify differences between the two systems highlights the potential to use FAME as an analytical tool to characterize anaerobic microbial community structure.

FAME profiles did not change as the HRT was decreased for either the ASBR or the SGBR-P. One explanation for the constant community structure is both systems are designed for long solids retention times (SRTs) and therefore retain biomass well. It has been shown that the SRT in the SGBR system is unaffected by changes in HRT (Mach and Ellis, 2001) resulting in a very stable microbial population.

Comparison of the FAME profiles for the two SGBRs show a difference in microbial structure which is most likely a result of the different wastewaters. Different organism populations grew at different proportions because of the different substrate available. Further work should be performed to determine specific organisms present in the granules.

Along with the MIDI-FAME program, there is a library of organisms and their FA fingerprint. Unfortunately, the library was developed mostly with easy to culture aerobic organisms and therefore the anaerobic library is very limited. Further work is needed to create fingerprints of anaerobic organisms.

Because the FAME procedure was developed for lab cultures, there are several concerns for application of the technique to mixed culture samples. The first is the extraction procedure uses high temperature and a strong base to hydrolyze lipids and form FAs. This step does not distinguish between lipids from biomass cells and lipids from substrate or other non-biomass origins creating an artificially high amount of FA in the sample which in turn may affect the profile. One way to eliminate the effect of FAs from substrate is to complete a FAME profile and subtract out the "baseline" or substrate affect. In this study, FAs in the substrate were insignificant (no FA consisting of more than 3%) and did not affect the profiles.

MIDI-FAME is a simple procedure to extract FAs from all cells in the sample. MIDI-FAME may over-estimate the quantity of FA in a sample because of the procedure by incorporating lipids from biomass and other sources. Although phospholipid fatty acid analysis also targets FAs associated with lipids, it is more specific as to which lipids are targeted. The drawback is the phospholipids FA procedure is very time consuming (Werker and Hall, 2000). It has been shown for environment and mixed culture samples, MIDI-FAME is quite accurate. Werker and Hall (2000) found in a comparison of the two procedures that seven specific FA were similar in proportion in both tests. It was also shown that whole cell fatty acid analysis was able to detect –OH groups commonly found in gram negative bacteria while the phospholipid procedure did not detect these groups. In this project the granules were the only source of biomass and no additional lipids influenced the FAME profile.

Conclusions

Using FAME analysis, differences in microbial community structure between two different reactors treating the same substrate has been shown in this study. FAME has also shown differences in two SGBRs treating different wastewater. Reactor configuration appears to be the reason for different FAME profiles between the ASBR and SGBR-P. The difference in the two SGBRs may be described by the theory that the difference in wastewaters will encourage the growth of different microorganisms, but temperature, age of the reactor system and other operating variables should also be considered.

Because of the FAME procedure and results obtained, it would be more beneficial for research to use FAME results to monitor changes within a particular reactor or setting. Using FAME in this manner allows for creation of a steady state or baseline profile that can be used to observe changes.

Nonetheless, this research has shown FAME analysis to be successful in distinguishing between different microbial communities. FAME profiles imply each reactor had its own unique microorganism structure. These structures can be summarized by FAME profiles which over time may indicate a change in performance of the anaerobic system.

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Fundamentals of the static granular bed reactor

Kristin F. Mach, Eric A. Evans, Michael J. Roth, Timothy G. Ellis

Department of Civil, Construction, and Environmental Engineering,

Iowa State University, Ames, Iowa 50011 U.S.A.

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Abstract

The Static Granular Bed Reactor (SGBR) has demonstrated all of the valuable traits of high rate anaerobic systems with limited drawbacks. Fundamental operations and performance characteristics of the SGBR were examined. The biomass retention mechanism results in high sustained solids retention times (SRTs) typically over 300 days. Chemical oxygen demand (COD) removal efficiencies exceed 90% often with the effluent concentrations less than 100 mg/L. Methane is generated near the theoretically expected volume based on COD removal. Effluent contains low BOD_5, total suspended solids (TSS) and volatile fatty acids (VFAs) concentrations.

Keywords

Static Granular Bed Reactor (SGBR), granular biomass, high rate anaerobic process

Introduction

A stable and dependable anaerobic process has been developed for the treatment of industrial wastewater. The SGBR, invented in the Iowa State University environmental laboratory, incorporates highly active, dense microbial granules in a simple downflow configuration (U.S. Patent No. 6,709,591). Treatment with the SGBR is intended to simplify anaerobic reactor design, operation, and maintenance without sacrificing effluent quality.

Several experiments were set up to examine the viability and performance of the SGBR. The initial part of the research was to determine if a new reactor configuration consisting of a dense bed of anaerobic granules would be feasible for the treatment of low to medium strength wastewater. It was shown effective for the treatment of synthetic wastewater at ambient laboratory temperatures (Mach and Ellis, 2000). Upon successfully demonstrating its reliability, optimal SGBR dimensions were examined, and wastewater treatability was researched for both lab and pilot scale systems. Startup for SGBR systems has been fast (often within a month) and efficient. Long-term performance has shown the SGBR reliably removes high percentages of COD at various hydraulic retention times (HRTs) and organic loading rates (OLRs). The purpose of this paper was to examine the fundamental operations and performance characteristics of the SGBR.

Anaerobic granule bed

To achieve a high degree of organic removal at short HRTs, many anaerobic processes take advantage of anaerobic bacteria's property to form a dense agglomeration of particles called granules. For instance, the anaerobic sequencing batch reactor (ASBR), upflow anaerobic sludge blanket (UASB), Anaerobic Migrating Blanket Reactor (AMBR), and other systems produce microbial granules during normal operation where selective pressure and upflow velocities are prevalent (Alibhai and Forster, 1986; Hulshoff Pol and Lettinga., 1986; Angenet, 1998). These dense microbial granules incorporate the complex syntrophic microbial communities responsible for the breakdown of organic matter to CO_2, CH_4, and biomass.

A long (SRT) is indicative of a high biomass concentration, giving a large and more mature microbial population capable of degrading substrate to a lower concentration. As with other biological treatment systems, SGBR performance was primarily a function of SRT. Solids retention time was estimated to be greater than 300 days for SGBR systems treating non-fat dry milk (NFDM), which is greater than similar systems. Dague *et al.* (1998) operated an ASBR, with substrate of NFDM, at SRTs ranging from 30 to 180 days at 20°C. An anaerobic sludge blanket treating domestic sewage operated with a SRT greater than 500 days (Elmitwalli *et al.*, 1999), with removal efficiencies around 70%.

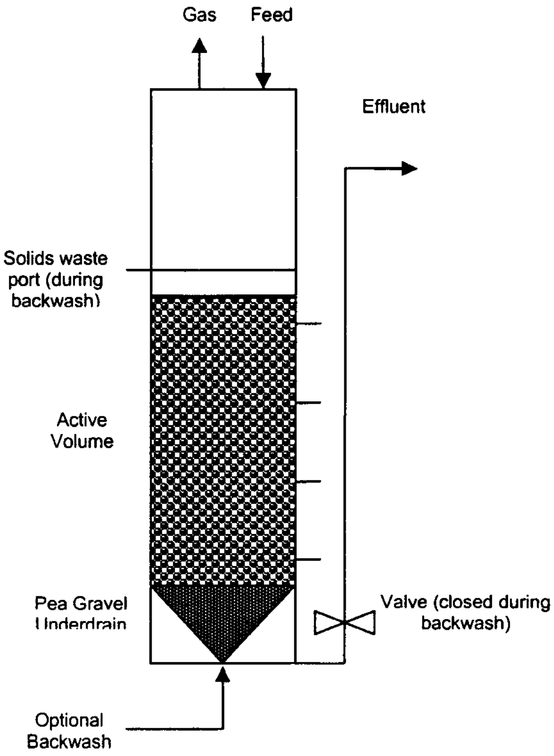

Figure 1. SGBR diagram.

Materials and methods – reactor design

The SGBR utilizes a simple downflow reactor configuration with a bed of granulated biomass resting on an underdrain (Figure 1). Since wastewater flows down through the bed, maximum contact between wastewater and the granular anaerobic consortia is ensured. The gas that is produced causes the granules to rise, creating significant mixing intensity and an upflow granule velocity. A complicated gas, liquid, solids separation device is not required since the gas and wastewater are countercurrent.

Lab scale studies with the SGBR have used various working volume sizes ranging from one to 12 liters. Research involving other systems used similar sized reactors. Elmitwalli and colleagues (1999) examined a UASB and an anaerobic hybrid reactor both were 3.84 liters with the wastewater 60 cm high in the reactor, giving an approximate height to width ratio (H/W) of 13.

At increased loadings, other systems tend to experience a loss of biomass. An ASBR treating low strength synthetic wastewater observed decreases in mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) at HRTs less than 6 hours (Dague *et al.* 1998). This loss was due to the greater decant volumes required in each cycle. Solids retention time in the SGBR remained relatively constant despite changes in hydraulic and organic loading since all biomass granules were retained. The only loss of biomass was believed to be through granule attrition measured as TSS in the effluent or through intentional wasting.

Granule movement in the SGBR may be attributed to bed buoyancy as observed in other anaerobic treatment systems. In a UASB treating domestic sewage at 13°C, the sludge bed would float during periods of high influent COD concentrations (Elmitwalli *et al.*, 1999). In another UASB operated at 34°C, sludge appeared to develop a biofilm layer and have poor settling ability possibly due to a greater quantity of gas trapped in the sludge when treating non-acidified substrate (Elias *et al.*, 1999). Rebac and colleagues (1999) attributed the sludge floating to the higher yield of acidogens within the system. Low strength brewery wastewater was treated by a pilot scale EGSB with the dimensions 7.5 m tall and 20 cm inner diameter (Kato *et al.*, 1999). An upflow anaerobic filter treating municipal wastewater had a height of 50 cm and an inner diameter of 7 cm (H/W = 7.1) (Bodík *et al.*, 2002).

Flow through the SGBR is not fully understood. As the name suggests, it was assumed that the bed of granules was static and wastewater flowed through channels created by gas. As research continued, some movement of the granules was observed but not to the extent of a completely mixed system. Wastewater movement in the SGBR appears to be through a combination of flow channels created by gas production and movement of the granules.

An underdrain system is necessary to provide support for the granules and to provide phase separation for the SGBR system. In research with the SGBR, both stainless steel mesh and small diameter gravel have been used. Both underdrain systems worked well however, gravel was the more practical choice for pilot scale and larger systems.

All SGBRs were operated at ambient temperatures except a laboratory comparison study between an SGBR and an ASBR treating pork slaughterhouse wastewater. The SGBR is uncomplicated and easy to operate with no mixers or gas, liquids, solids separators. In addition to a feed pump, the only extra equipment needed is gas or effluent backwash line to reverse the feed flow temporarily to dislodge any granules trapped in the underdrain system, waste solids, and free entrapped gas.

Wastewater treatability with SGBR

Simple synthetic wastewater

Preliminary results have shown the SGBR (SGBR 3) was capable of treating a simple sucrose wastewater. Wastewater composed of ½ sucrose and ½ non-fat dry milk at a concentration of 5g COD/L was treated by SGBR 3. Effluent quality was good with roughly 90% COD removal and suspended solids concentrations varying from less than 10 mg/L to above 200 mg/L. Wastewater, however, accumulated in the reactor due to reduced effluent flow. Reduced flows through SGBR 3 at higher organic loadings with simple wastewaters appear to be caused by a combination of accumulated fines in the reactor and entrapped gas in the granule bed, and may be corrected by regularly backwashing the reactor to waste fine solids.

Complex synthetic wastewater

Static granular bed reactor 1 and SGBR 2 effectively treated non-fat dry milk while operating under identical conditions with the only difference being the height to width ratios. Static granular bed reactor 1 and SGBR 2 had height to width ratios of two and eight, respectively. Both SGBRs were fed 1000 mg COD/L synthetic wastewater consisting of non-fat dry milk and essential minerals. The SGBRs achieved in excess of 95% total COD removal with effluent TSS concentrations less than 20 mg/L after startup. SGBR performance was comparable to other systems treating similar wastewaters. Collins *et al.* (1998) treated primary clarifier effluent with an expanded granular sludge bed reactor (EGSB) and achieved greater than 90% COD removal at 20°C. At 35°C, an anaerobic baffled reactor treating 500 mg COD/L at a 10 hour HRT achieved higher than 90% COD removal (Langenhoff and Stuckey 2000). However, the COD efficiency decreased to 70% when the temperature was lowered to 20°C. Similar to the SGBR, the system also had very low VFA concentrations. Orozco (1997) achieved an optimum of 92% COD removal using an anaerobic plug flow reactor operated at 13 to 17°C with synthetic wastewater.

Pork slaughterhouse wastewater

Separate lab and pilot studies showed the high-rate anaerobic treatability of Hormel Foods wastewater in the SGBR. The lab study compared the performance of the 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).

A performance comparison was made between the pilot-scale SGBR (SGBR 4), laboratory-scale SGBR (SGBR 5), 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. Chemical oxygen demand removal efficiency was nearly identical for SGBR 4 and SGBR 5, with SGBR 5 having slightly higher COD removal efficiencies at the higher OLR conditions. Chemical oxygen demand removal efficiency for SGBR 4 and SGBR 5 was between 82-96%.

Sulfate rich wastewater

Another problem for anaerobic systems is sulfates in wastewater streams. Sulfate is reduced to hydrogen sulfide by sulfate reducing bacteria (SRB). In addition to odor problems, hydrogen sulfide is toxic to methanogens and can interfere with methane production. The SRB also compete with methanogens for substrate, in particular acetate.

Methanogens and SRB can coexist but given high sulfate concentrations and periods of a year or more, SRB can out-compete methanogens for substrate eliminating the methanogens (Visser *et al.,* 1993). Sulfide accumulation in anaerobic systems decreases the activity of methanogenic populations (Yamaguchi *et al.*, 1999).

In a SGBR system (SGBR 6) treating a high sulfate waste stream, it is thought there will be no detrimental effect because hydrogen sulfide (H_2S) will be produced early in the digestion process. Since sulfate reducing bacteria are believed to be thermodynamically favored (Rinzema and Lettinga, 1988) and the liquid/gas flow was countercurrent, the prediction was H_2S will be in highest concentration at the top of the reactor where it was released. Deeper in the bed the organics will be converted to the final product of methane. It has been observed that methanogens and sulfate reducing bacteria can co-exist in anaerobic domestic sewage digesters (Fukui *et al.*, 2000) and in a lab scale UASB treating volatile acids and sulfate (Omil *et al.*, 1997).

Static granular bed reactor 6 treated a 3 g COD/L synthetic (2/3 non-fat dry milk and 1/3 sucrose) wastewater with sulfate added (COD to sulfate ratio 3:1). The reactor had a sulfate loading rate of 1.33 g S/L, but consistently removed greater than 90% of the COD. Hydrogen sulfide concentrations in the biogas averaged 20,000 ppm when operated at an 18 hour HRT. Parkin *et al.* (1991) treated sulfate rich synthetic (propionate) wastewater in an upflow anaerobic filter and found a reduction in COD removal efficiencies at COD to sulfate ratios of 8:1 and 9:1 corresponding to a sulfate loading rate of 625 mg S/L.

Municipal wastewater

A SGBR (SGBR 7) was used to treat municipal wastewater. No additions were made to the wastewater, which had an average COD of 333 mg/L and an average five day carbonaceous biological oxygen demand ($CBOD_5$) of 130 mg/L. The reactor was operated at HRTs of 48, 36, 24, 18, 12, and 8 hours at 23°C. The system operated exceptionally down to an 18 hour HRT during which effluent COD averaged 71 mg/L, effluent TSS averaged 8 mg/L, and effluent $CBOD_5$ averaged 31 mg/L. A number of anaerobic systems have been used to treat municipal wastewater: the UASB reactor (Lettinga *et al.,* 1983), the anaerobic filter (AF) (Bodík *et al.,* 2001), the ASBR (Bodík *et al.,* 2001), and the anaerobic fluidized sludge blanket (AFSB) reactor (Tanaka *et al.,* 1991). Chemical oxygen demand removal for municipal wastewater ranged 50-90% for these systems depending on HRT and temperature.

Start up and Transition Performance

Startup of SGBR 1 was completed in 45 days at an HRT of 36 hours. At the end of the startup, the effluent COD concentration averaged 45 mg COD/L and effluent TSS concentration was 20 mg/L. Static granular bed reactor 2 had too small a diameter. During initial start up, the granules climbed the walls of the reactor due to buoyancy from gas production. After 91 days, SGBR 2 startup was complete with 95% COD removal. Viraraghavan and Kikkeri (1990) used an anaerobic filter to treat dairy wastewater, and it was started up at room temperature over a period of 160 days.

Rapid startup of the SGBR 4 treating pork slaughterhouse wastewater was shown to be possible. The reactor 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 total COD 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. An expanded granular sludge bed (EGSB) reactor was acclimated to slaughterhouse wastewater over a four week period (Núñez and Martínez, 1999). The sludge in the EGSB was activated with mixture of sucrose and slaughterhouse wastewater. During the start up process, the sucrose concentration decreased and was eventually eliminated.

Static granular bed reactor 7 was started treating municipal wastewater at an HRT of 48 *h* at 23°C. By day 13, the effluent COD was 34mg/L, which was near the average operational COD. On day 20, the effluent $CBOD_5$ concentration was measured at 13mg/L. An upflow AF treating municipal wastewater supplemented with glucose and sodium acetate, to give a total influent COD concentration of 300 mg/L, was started up at 23 °C with an HRT of 20 *h* and achieved efficient organics removal within a few weeks (Bodík *et al.*, 2001). Bodík *et al.* (2001) also found that an ASBR fed with the same substrate efficiently removed COD a couple days after start-up, but had poor sedimentation until day 10-20. Tanaka *et al.* (1991) treated municipal sewage in an AFSB and an AF seeded with predigested sludge and the startup required a few months at an HRT of 48 hours.

Long Term Performance

Studies involving the SGBR were operated for periods ranging from one to five years. A study examining an EGSB treating brewery wastewater at various temperatures lasted for 60 days (Kato *et. al*, 1999). For SGBR 1 and SGBR 2, several HRTs were examined ranging from 36 to 5 hours over a period exceeding five years. Effluent COD and TSS concentrations are shown along with HRTs and OLRs in Table 1. Ndon and Dague (1997) examined the performance of an ASBR at different HRTs and operating temperatures. At an

HRT of 24 and 12 hours the soluble COD removal was 93 and 81% respectively while operating at 35°C.

Table 1. SGBR performance data

	HRT, h	OLR, g/(L·d)	Effluent			% Removal		
			COD, mg/L	BOD$_5$, mg/L	TSS, mg/L	COD	BOD$_5$	TSS
SGBR 1	36	0.7	46 ± 18	13	8	95.3	N.A.	N.A.
	24	1.0	45 ± 28	10	20	95.5	98.4	N.A.
	16	1.5	30 ± 15	10	6	97.3	98.8	N.A.
	12	2.0	30 ± 19	26	16	96.6	95.8	N.A.
	8	3.0	79 ± 29	47	8	91.7	90.9	N.A.
	6	4.0	38 ± 24	27	10	96.0	95.9	N.A.
	5	4.8	40 ± 22	16	12	96.0	96.5	N.A.
SGBR 2	36	0.7	52 ± 23	9	5	95.5	99.0	N.A.
	24	1.0	59 ± 15	N.A.[a]	18	93.9	N.A.	N.A.
	16	1.5	44 ± 24	26	3	95.3	95.7	N.A.
	12	2.0	50 ± 21	21	6	93.9	95.9	N.A.
	8	3.0	33 ± 15	14	5	96.6	97.8	N.A.
	5	4.0	41 ± 18	11	7	96.1	98.0	N.A.
SGBR 3	48	2.5	297.0 ± 108	N.A.	132.9 ± 82.2	94.5	N.A.	N.A.
	36	3.3	202.1± 68.8	N.A.	123.3 ± 46.1	95.3	N.A.	N.A.
	24	5.0	240.0 ± 84.0	N.A.	127.0 ± 41.9	94.0	N.A.	N.A.
	18	3.3	169.0 ± 88.4	N.A.	108.7 ± 36.5	93.5	N.A.	N.A.
SGBR 4	48	1.37	147	38	13	94.2	97.3	97.6
	40	1.33	179	43	18	92.3	97.4	94.5
	32	1.84	175	50	17	92.8	97.2	96.7
	24	3.01	214	51	33	92.8	96.7	95.0
	20	3.25	228	73	31	91.8	94.7	93.9
	16	4.55	249	68	36	92.1	95.8	94.6
SGBR 5	48	0.44	134±65	10	43±18	83.7	98.5	42.3
	36	0.58	76±30	16	24±11	91.5	97.7	68.3
	24	1.87	90±22	13	20±7	95.0	98.6	86.5
	18	2.06	86±9	N.A.	13±7	94.5	N.A.	90.8
	14	3.7	88±21	8	17±9	95.7	99	90.9
	12	4.67	110±47	N.A.	30±14	95.4	N.A.	89.0
	10	5.8	161±41	10	35±17	92.7	98.8	97.8
	8	7.05	244±51	7	31±8	92.3	99.0	66.2
SGBR 6	18	4.0	63	N.A.	44	97.3	N.A.	75
SGBR 7	48	0.079	68.1 ± 23.7	17.4 ± 6.5	17.4 ± 6.5	56.5	39.8	72.6
	36	0.23	69.3 ± 33.4	23.4 ± 2.9	12.9 ± 3.9	81.6	86.2	93.1
	24	0.50	65.0 ± 18.3	25.6 ± 9.0	11.7 ± 2.4	87.0	81.1	96.1
	18	0.76	70.9 ± 15.3	31.3 ± 5.8	8.2 ± 3.5	87.5	62.7	95.0
	12	0.59	76.5 ± 14.8	56.8 ± 9.4	7.8 ± 4.1	74.1	66.0	96.7

[a] = not available

Static granular bed reactor 3, with an 11.78 liter active volume, was used to treat synthetic wastewater for 350 days in a comparison study. Wastewater strength was varied from 5 g COD/L (days 0-227) to 2.5 g COD/L (days 227-350) with a 50/50 mixture (based on concentration) non-fat dry milk and sucrose mixture and 100% non-fat dry milk, respectively. Hydraulic retention times were 48, 36, 24, 28, and 12 hours. Effluent quality from SGBR 3 showed the system capable of high COD removal with low effluent TSS concentrations (Table 1).

Conclusion

The SGBR has demonstrated that it is a viable high-rate anaerobic system under numerous operating conditions. Simplicity in design, operations and maintenance make it an attractive technology. High solids retention time ensures a high degree of treatment in terms of both organics and suspended solids removal. Simple and complex synthetic wastewaters, pork-slaughterhouse wastewater, sulfate-rich wastewater, and municipal wastewater have all been successfully treated with the SGBR. Start-up of the SGBR can be achieved in short time periods, as little as one month, since it is seeded with highly active granules. Long-term performance has shown the SGBR can operate for up to five years with high COD removal efficiency and low effluent TSS concentrations at a number of HRTs and OLRs.

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The SGBR, a transition study

Kristin M. Evans[1], Joseph C. Ward[2], Timothy G. Ellis[1]

[1]Department of Civil, Construction, and Environmental Engineering,
Iowa State University, Ames, Iowa 50011
[2]McClure Engineering Company, 705 First Avenue N.
Fort Dodge, Iowa 50501

Introduction

Lab scale bioreactors are typically operated at a steady state conditions, consistent performance at a particular loading condition. However in full scale applications, hydraulic and organic loading changes are common. In an industrial setting, a bioreactor may be expected to operate under highly variable flow during any operational setting caused by changes in production, down time, clean up, or other reasons. The change in loading may be a result of decreased hydraulic retention time (HRT) or an increase in the organic concentration. This study examined the effect of increased hydraulic loading (i.e. shorter HRT) at a constant chemical oxygen demand (COD) concentration.

One distinguishing characteristic of the SGBR is its ability to achieve steady state performance in a very short time period, regardless of the HRT conditions. Because of the quick adaptation to a new HRT, it was decided to more thoroughly examine the SGBR performance during the 24 hour period following a change. It was hypothesized that the SGBR performance would return to high organic removal (>95% removal) within several days. The following transitions were observed: 36 to 8 h HRT, 8 to 5 h HRT, 5 to 4 h HRT. The trend in the SGBRs was to acclimate quickly and return to near optimum performance within 24 hours. At the 4 h HRT steady state condition, both SGBRs experienced extreme bed expansion filling the entire reactor volume. Regular backwashing with effluent appeared to redistribute the bed, releasing any gas or solids trapped in the bed, and allowing it to settle better. Although performance was still acceptable (COD removal >90%) the SGBRs may have reached physical limitations of operation.

The transition study was defined as examining the adaptation of the SGBR as HRT was decreased under a constant feed composition, thus increasing the organic loading rate (OLR). Steady state was established before the transition occurred as a means of measuring performance. Once HRT was changed, samples were taken at regular intervals to observe adaptation of the reactor. Sampling continued for up to 48 h after the HRT change. The SGBR was operated at the new HRT condition for a short period to verify that the reactor had completely acclimated (at least 3 consistent data results).

Although the research presented is compared to research that examines shock loading, this may not be a fair comparison. Often shock loading studies increase the COD concentration or reduce the HRT (or a combination of both) for a short period. Performance is observed during the overloading interval and then monitored once original conditions are resumed. The SGBR, however, was examined as the operating conditions were changed to a new steady state condition at an increased organic loading rate.

Materials and methods

Over the course of the study, two different SGBR reactors were examined. The SGBRs were designed and operated as described in previous work. In this study, all had a 1 liter working volume and were operated at ambient temperatures. The only difference between the SGBRs was the reactor configuration (various height to width ratios, Mach and

Ellis, 2000). A synthetic wastewater consisting of non-fat dry milk (NFDM) and micronutrients was used during the study. The bioreactors were fed on a semi-continuous basis at intervals depending on the HRT desired (e.g. fifteen seconds every five minutes).

The experiments observed two reactors with a 1 liter working volume, but had different dimensions. These two SGBRs (SGBR 1 and SGBR 2) were fed a 1 g COD/L simulated wastewater as described previously (Mach and Ellis, 2000). After more than 2 years of operation, SGBR 1 and SGBR 2 were transitioned between several HRT conditions. The three transition periods were 36 h to 5 h, 8 h to 5 h, and 5 h to 4 h (starting and ending HRT, respectively).

All analytical parameters, such as COD, BOD_5, alkalinity, pH, etc., were measured by Standard Methods (1995). Samples were collected and analyzed immediately or stored according to Standard Methods (1995). Methane content was measured using a Gow-Mac gas chromatograph.

Results

The first condition examined was a change in HRT from 36 h to 5 h, which changed the organic loading rate (OLR) from 0.667 g COD/L/d to 4.8 g COD/L/d. SGBR 1 and SGBR 2 both performed extremely well with respect to COD, TSS and BOD removal. Table 1 shows performance parameters as measured during the steady state condition prior to the transition period, but does not include transition data. When operated at a 36 h HRT, both SGBRs removed greater than 96% of the COD and maintained an effluent TSS concentration below 10 mg/L. The HRT was changed, and effluent samples were collected every 12 h and analyzed immediately or stored until analysis occurred. As seen in Figure 1, a relatively quick adaptation occurred with respect to organic removal. Within 36 h effluent COD concentrations returned to "pre-transition" levels (i.e. performance at 36 h HRT).

After the transition period, performance was monitored while the SGBRs were operated at 5 h HRT. Effluent COD concentrations did not increase in SGBR 1 or SGBR 2. In SGBR 1, the COD concentration decreased from 46 mg/L to 39 mg/L, as evident in Table 1 when comparing 36 h HRT to 5 h HRT data. Effluent TSS concentrations increased slightly, but this could be a result of increased hydraulic forces on the reactor and granules.

Table 1. Steady state parameters for SGBR 1 and SGBR 2.

	SGBR 1				SGBR 2		
	HRT (h)				HRT (h)		
	36	8	5		36	8	5
Days at HRT	599-664	726-752	665-725		511-576	637-663	577-637
Effluent COD (mg/L)	46±18	79±29	39±22		41±23	33±15	41±18
Effluent TSS (mg/L)	8±2	8±1	12±5		5±1	5±1	10±3
VFA (mg/L as acetic acid)	16±4	17±2	17±6		11±3	14±1	12±4

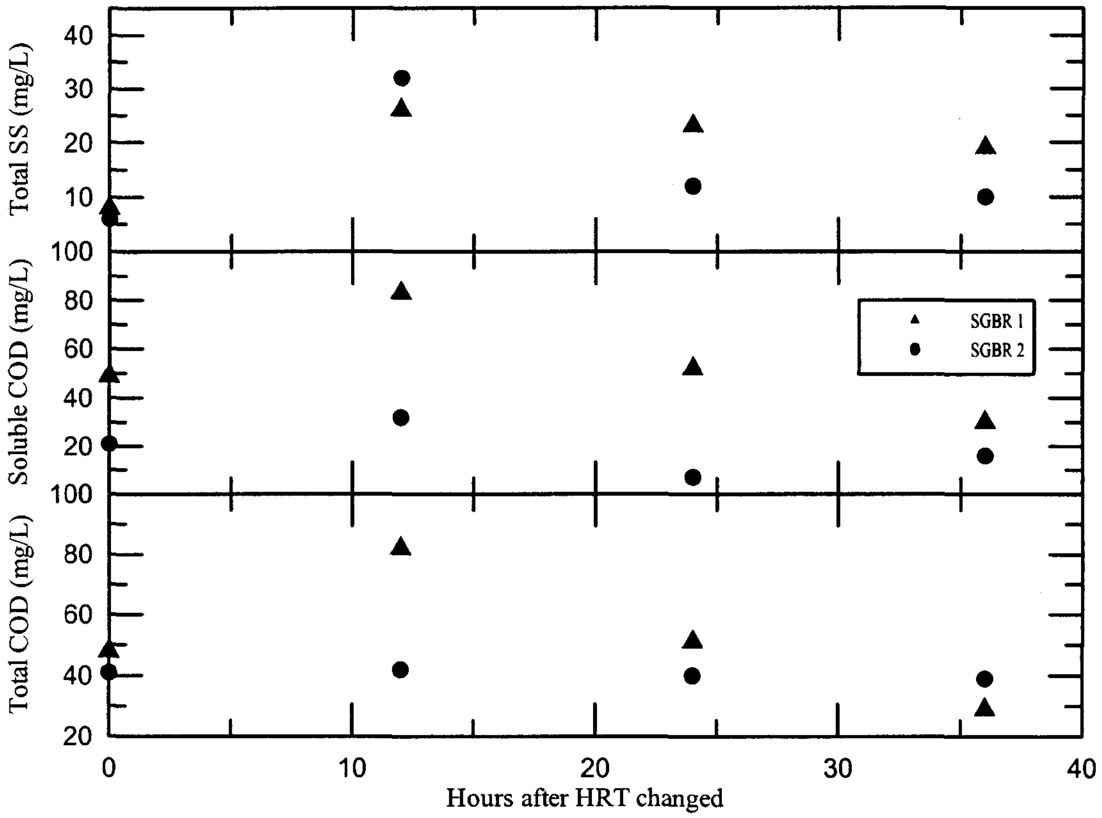

Figure 1. Performance of SGBR 1 and SGBR 2 as HRT was changed from 36 h to 5 h.

A transition was also made from 8 h to 5 h HRT with the same SGBRs (several months after the first experiment concluded) resulting in a change in the OLR from 3 g COD/L/d to 4.8 g COD/L/d. Steady state data from an 8 h HRT began to show performance differences between SGBR 1 and SGBR 2. SGBR 2 maintained exceptional results (slightly higher COD removal at 8 h than 36 h HRT). COD removal decreased but remained above 92% in SGBR 1.

Performance during the HRT transition decreased. Effluent COD concentration in SGBR 1 reached as high as 350 mg COD/L one day after the change but returned to steady state conditions (>90% TCOD removal) within 3 days. SGBR 2 performed much better during the transition. Effluent COD concentrations peaked at 140 mg COD/L and returned to expected performance within 48 h after HRT was changed (see Figure 2).

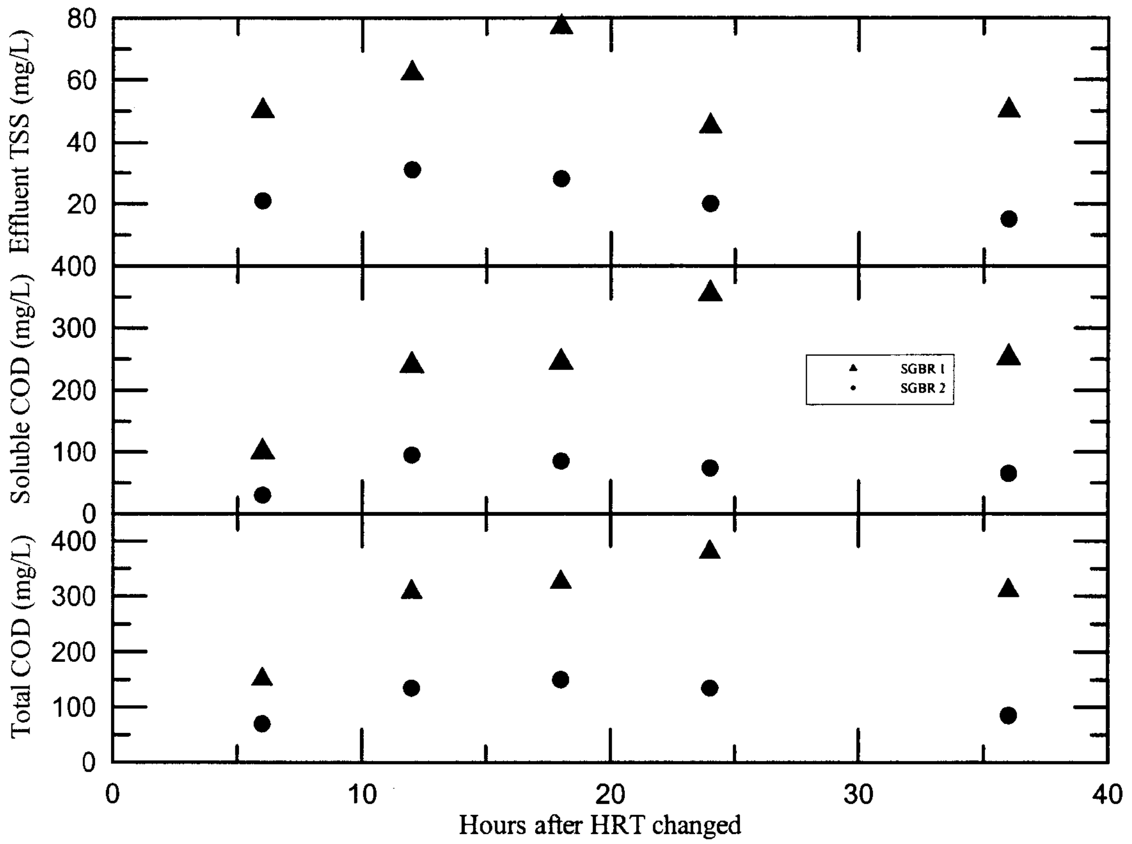

Figure 2. Performance of SGBR 1 and 2 as HRT was changed from 8 h to 5 h.

The final phase of this project involved transitioning SGBR 1 and SGBR 2 from a 5 h HRT to a 4 h HRT. The organic loading was increased from 4.8 g COD/L/d to 6 g COD/L/d. This was a smaller increase than the other transitions, but it may have been the near the edge of practical operation of the SGBRs. Both reactors did not perform well, but the decreased performance was most likely due to a combination of physical constraints and decreased biological performance. Volatile fatty acids VFAs increased significantly causing a drop in pH, decreased or no methane production, and high effluent COD and solids concentration.

The 5 h to 4 h transition experiment in SGBR 1 was discontinued after 18 hours due to uncontrolled bed expansion. It was not feasible to continue operation as both reactor beds expanded greatly during this transition. SGBR 2 continued operation through the increased bed depth, but COD removal decreased to less than 90%. Although there was some head space above the granules in both SGBRs, if more space was available, the problem in SGBR 1 may not have occurred.

The other problem may have been the higher flow through the reactor. At the lower HRTs, the feed cycle could have been adjusted better (shorter non-feed times) becoming even closer to a continuous fed system. Instead, large quantities of wastewater were fed to the SGBR for short periods of time. This created a pool of influent above the granule bed that took time to flow through the granules. Because these were the original SGBRs, factors like this were unknown and future research should take these observations into consideration.

As shown in Figures 1 and 2, SGBR 2 had more consistent performance and was not affected as much by the changes in HRT. Both SGBRs quickly adjusted to the new HRT condition and continued operation.

Discussion

As stated earlier, these experiments most closely resemble shock loading scenarios. However, instead of decreasing the organic load after 24 hours like most shock loading, the SGBR continued to operate at the higher loading condition. During shock loading experiments, it was expected that performance would decrease during the shock load segment. Once the system returned to normal operation, there was still a period of adjustment before the system returned to expected performance. In the SGBR, the shock load occurred 24-48 hours after the HRT was decreased. After the shock load in the SGBR, the shorter HRT was maintained. Unlike shock studies with other systems (Chua *et al.*, 1997; Nachaiyasit and Stukey, 1997; Elias *et al.*, 1999), COD and TSS removal in the SGBR remained high throughout the entire transition.

Increased VFA concentrations and decreased performance during the 5 to 4h HRT transition periods in the SGBRs were most likely a result of over loading. Other systems experienced similar results. Elias *et al.* (1999) observed decreased performance (or instability periods) related to increased hydraulic loading among other things. In the instability period, VFA concentrations increased creating low pH and therefore a toxic environment. The researchers noted that organic loading needed to be decreased for the VFAs to decrease and performance to return to "normal" levels. During all transition conditions in the SGBR, the VFAs increased then decreased within 24-48 h indicating quick adaptation by the microorganisms in the system.

Another observation made was that other systems, such as the ASBR, observed decreased COD removal as HRT decreases for a given temperature and constant COD concentration (Dague *et al.*, 1998). The SGBRs excelled in this area also. The transition studies were all conducted at ambient temperature. Even as HRT decreased, satisfactory COD removal was observed regardless of HRT.

Other studies have shown that anaerobic systems are very sensitive to overloading leading to, ultimately, failure of the system. An anaerobic fixed film reactor was operated at constant organic loading but various HRT conditions (Chua *et al.*, 1997). A two fold increase in HRT caused a decrease in performance for three days. After this period, performance began to recover. It was observed that the biofilm was not washed out. The SGBR does not wash out biomass at lower HRTs also. Because of the down flow configuration, one would expect to lose large amounts of biomass and solids as HRT was decreased. During a transition to a shorter HRT, the effluent solids concentration increased during the sample period, but decreased within 36-48 hours.

Reactor configuration and operation have one of the greatest impacts on changes in either hydraulic or organic load changes. An anaerobic baffled reactor of a plug flow type system, observations were made as the feed changed from 4 to 8 g COD/L for 8 days and as the feed changed from 4 to 15 g COD/L for 13 days (Nachaiyasit and Stukey, 1997). During the study, the system was maintained at a 20 h HRT during different loading conditions. During the first loading condition, the system adjusted within 2 days. The design of the reactor seemed to mute effects of increased loading which did not have as much of an effect lower in the reactor bed. In the second loading study, distress to the reactor was observed. High VFA concentrations were observed 12 days after the change. During the study, COD removal remained above 90%.

The SGBR system configuration emulates a plug flow system. Because of the gas production, the granules move in the bed creating a slight mixing effect, but not a completely mixed bed. This stratification appears to make the system more tolerant of operational changes within the reactor. The exceptional performance of the SGBR appears to be a function of the long SRT which is maintained regardless of the HRT. For other reactor configurations the SRT is adversely affected by increased hydraulic loading (i.e., decreased HRT).

Conclusions

In summary, SGBR 1 and SGBR 2 adjusted with 36 hours to the HRT conditions even when the HRT was decreased to 5 h. Overall SGBR 2 performed better than SGBR 1 especially during the transition from an 8h to 5h HRT. Finally, physical failure of both SGBRs occurred during the 5h to 4h HRT transition for both SGBRs.

Organic removal trends showed a return to steady state removal at the 36 hour point in repeated data sets. Solids, VFA, and alkalinity reached maximum observed concentrations between 18 and 24 hours for each of the transition conditions, but data showed a quick return to steady state levels.

Both reactors filled with granules to the top of the column during the 5h to 4h HRT transition. Although physical failure appeared to be the cause, very high levels of VFA and VFA/alkalinity indicated the reactors were creating VFA at a greater rate than the methanogens could convert them. These data and the increase in granule washout could be interpreted as the beginning of an organic failure in SGBR 2.

Both SGBRs performed extremely well, both in the steady state performance and in quick acclimation during a transition period. The SGBR, its unique configuration, its simple operation, and rapid adjustment to new operating conditions make this system ideal for industrial wastewater treatment.

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Additional Results

Bed height study

Objective

The objective of the bed height study was to observe the start up of the SGBR with different fractions of the design bed volume.

Materials and methods

Four 1 liter SGBRs were operated with different granule bed volumes. The four bed volumes were 250 ml, 500 ml, 750 ml, and 1 liter. Each reactor was fed from the same wastewater container to assure similar conditions. Synthetic wastewater was used to control influent parameters (i.e., COD, TSS, alkalinity, etc). The SGBRs were operated for approximately 8 weeks at each feed concentration. All analytical parameters were measured using Standard Methods (1995). After each run was completed, the reactors were emptied, the SGBRs were reseeded with new granules, and the SGBRs were re-started with a different feed concentration. Feed concentrations of 1 g COD/L, 2 g COD/L, and 4 g COD/L were used. For each run, the HRT was held constant at 20 h. Granule growth was not observed but it was not expected that the granules would grow during the short start up time period.

Results

Organic removal and TSS were the primary parameters observed during the study. COD removal did not vary between reactors at the low strength wastewaters (1 g COD/L and 2 g COD/L). During the last trial (4 g COD/L), the SGBRs showed a decrease in performance. The 25% and 50% bed volume did not perform well in terms of TSS removal. At the higher loading the smaller bed volumes were less effective at entrapping solids within the bed. The 75% and 100% bed volume performed better, but not as well as at the lower strength wastewaters (see Tables 1 and 2). In terms of COD removal at 4 g COD/L, the 25% and 100% performed better than the other two SGBRs. Figures 1, 2, 3, and 4 visually compare the four SGBRs for a specific loading event with error bars. The food to microorganism (F/M) ratio became an important performance factor. The approximate F/M for the 25%, 50%, 75%, and 100% bed volume at 1 g COD/L were 0.07, 0.035, 0.024, and 0.017, respectively. The two smaller bed depth SGBRs had a higher F/M ratio indicating they were not operating in a "starved state" which resulted in higher COD levels in the effluent while treating wastewater with concentrations of 1 g COD/L and 2 g COD/L. At 4 g COD/L, the 25% and 100% bed volume had comparable performance. However, due to the very large standard deviations of all SGBRs, the performance of the 25% and 100% bed volumes may not be similar.

Table 1. Effluent COD concentration for various loadings.

Fraction of design bed volume	1 g COD/L	2 g COD/L	4 g COD/L
25%	27.9±17	41.5±22	304±153
50%	33±22	35.1±21	1121.6±456
75%	21.9±14	14.8±11	1042.8±534
100%	14.3±17	33.5±27	294.4±176

Table 2. Effluent TSS concentration for various loadings.

Fraction of design bed volume	1 g COD/L	2 g COD/L	4 g COD/L
25%	26±14	40±15	146±12
50%	24±13	26±14	184±8
75%	21±7	25±10	113±9
100%	18±8	20±7	80±11

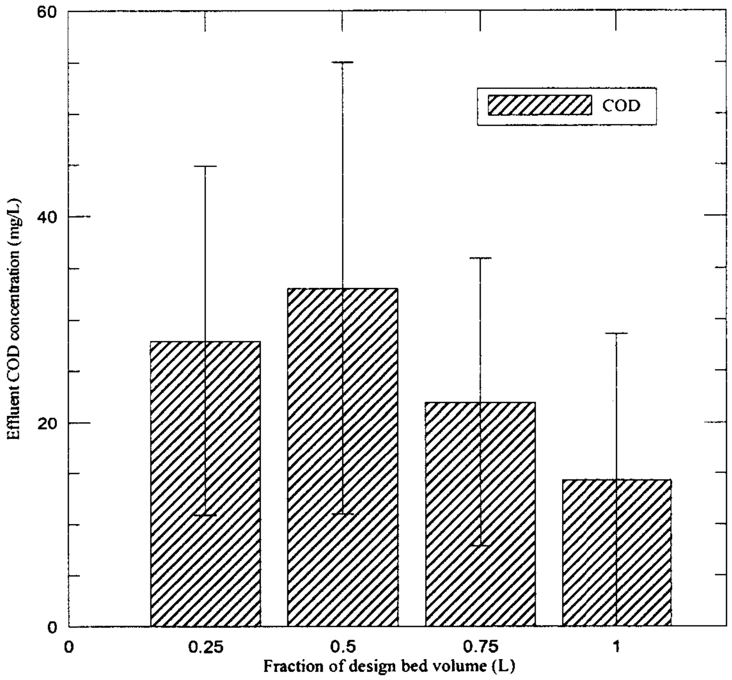

Figure 1. Effluent COD concentration at 1 g COD/L.

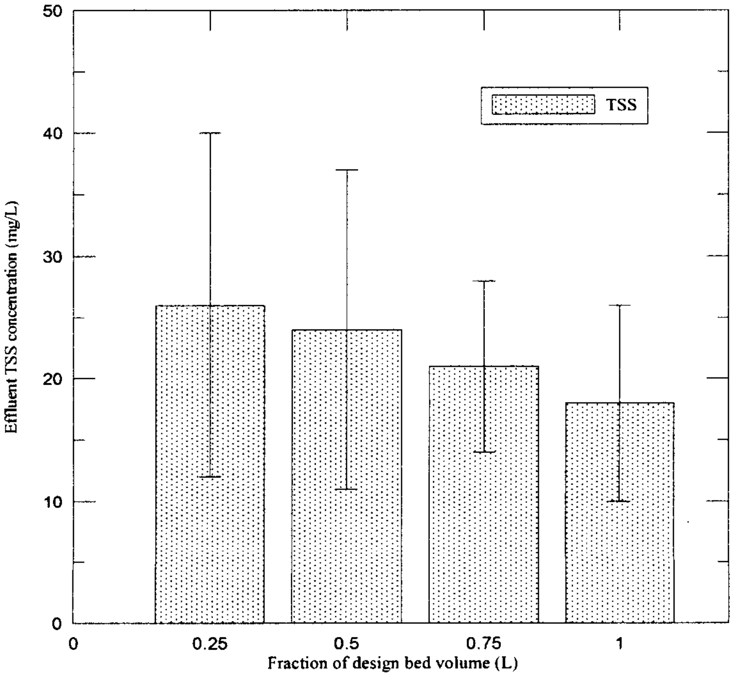

Figure 2. Effluent TSS concentration at 1 g COD/L.

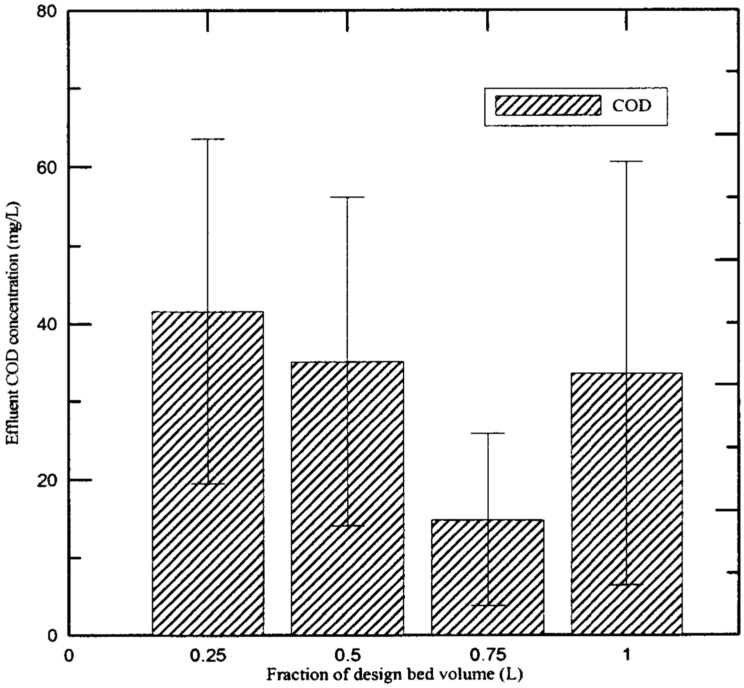

Figure 3. Effluent COD concentration at 2 g COD/L.

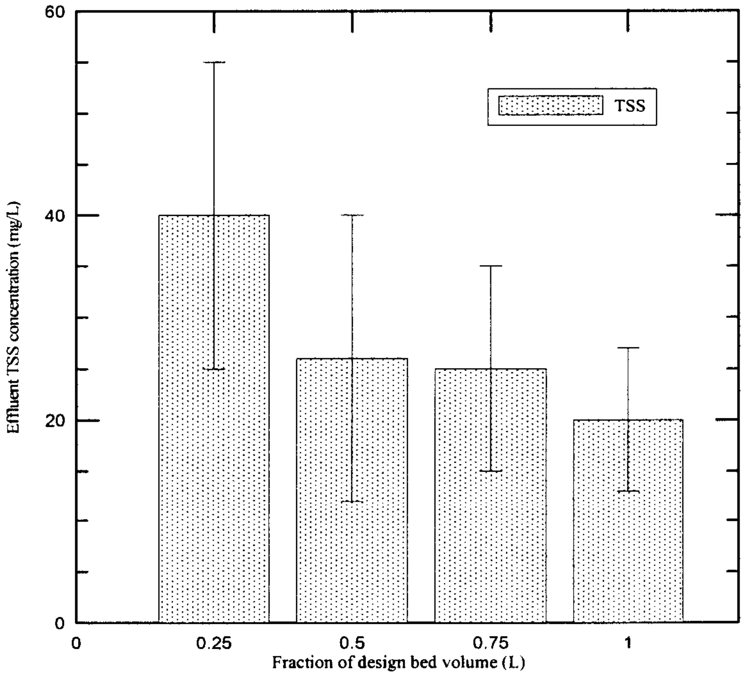

Figure 4. Effluent TSS concentration at 2 g COD/L.

The implication of this study indicates the full bed of granules may not be completely active at any time. Because the SGBR was operated in a "starved state", some of the granules may become dormant until food is available. This finding is consistent with results found in a SGBR toxicity study (Roth, 2003). In the toxicity study, granules lower in the bed had a delayed SMA response suggesting that the granules were dormant due to lack of substrate (i.e., the substrate had been consumed in the upper region of the bed).

Conclusions

These results suggest that a full bed of granules is not required for start up with low to medium strength wastewater (i.e., less than 4 g COD/L). In a full bed there was an indication that some of the granules in the lower portion of the SGBR were not active. If fewer granules are needed for start up, the initial capital cost is less. It also reduces the burden of seeding the entire bed volume at one time.

Finally, if dormant granules exist in a full bed volume, the SGBR would have a built in safety factor. The safety factor would be important when the system was organically overloaded, allowing the dormant organisms to become active as excess substrate reached the lower portion of the bed. The reserve or dormant granules would also potentially protect the SGBR from accidental shock loading of toxic chemicals.

Sulfate reducing reactor

Objective

The objective was to use FAME to observe any community structure changes and to observe the effect of sulfate concentrations on the performance of the SGBR. A SGBR was operated at a 24 h HRT with a feed strength of 3 g COD/L. The SGBR was seeded and fed with 2/3 non-fat dry milk and 1/3 sucrose synthetic wastewater, based on COD. After approximately 6 weeks, sulfate was added to the wastewater at a ratio of 3 g COD to 1 g SO_4. As the SGBR achieved steady state, the sulfate concentration was incrementally increased. Performance observations were recorded and FAME profiles compiled as the COD/SO_4 ratio was decreased.

Materials and Methods

The SGBR had a 1.5L working volume and was maintained at a 24h HRT. Synthetic wastewater was composed of 2 g COD/L of non-fat dry milk and 1 g COD/L sucrose. COD, TSS concentration, and pH were measured throughout operation. Effluent samples were collected, stored, and analyzied according to Standard Methods (1995).

Granules for FAME analysis were obtained from a sampling port on the side of the reactor. Granules were sampled from approximately the same location for all sampling events. Within one hour of collection, FAMEs were extracted using the Microbial Identification System anaerobic extraction protocol (Microbial ID, Inc., Newark, DE). Cells in the samples were saponified by heat and the presence of a strong base. In this step, fatty acids were separated from lipids. After the separation, the remaining FAs were methylated to form FAME and extracted into an organic solvent. Following extraction, FAME samples were analyzed on a HP 6890 (Hewlett Packard, Rolling Meadows, IL) gas chromatograph.

MIDI's Sherlock data analysis system (Microbial ID, Inc., Newark, DE) was used to identify the fatty acid methyl esters and to generate a community profile for each sample based on the quantity of FAMEs present.

As referenced in Mach and Ellis 2002, principal component (PC) analysis was used to observe any changes in the FAME profiles. Principal component analysis explains the maximum variation in the data based on principal components in the data. All PC analysis was done by MIDI FAME's Sherlock program (Microbial ID, Inc., 1996).

Results

It was hypothesized that as the ratio of COD/sulfate was changed there would be a change in the FAME profile as sulfate reducing bacteria (SRB) had more substrate available to them. Unfortunately FAME was not a good method to examine population change for this experiment. FAME profiles did not indicate a change in community structure. It is believed that the entire population increased therefore the fraction of SRB remained constant. COD and other analytical parameters did not change greatly as both methanogens and SRB degraded organics present in the wastewater. Others' research (Barber and Stuckey, 2000; Mizuno, *et al.*, 1998; Omil, *et al.*, 1998; Visser, *et al.*, 1993) has shown a year or more is needed for SRB to out-compete methanogens. Although the experiment lasted about 12 months, there was no evidence of SRBs as the predominant species.

Figure 5 shows the principal component comparison for three operating conditions. There is no clear separation of the three samples, indicating the samples were not distinctly different. If significan differences in the FAME profiles occurred, the samples would tend to be clustered.

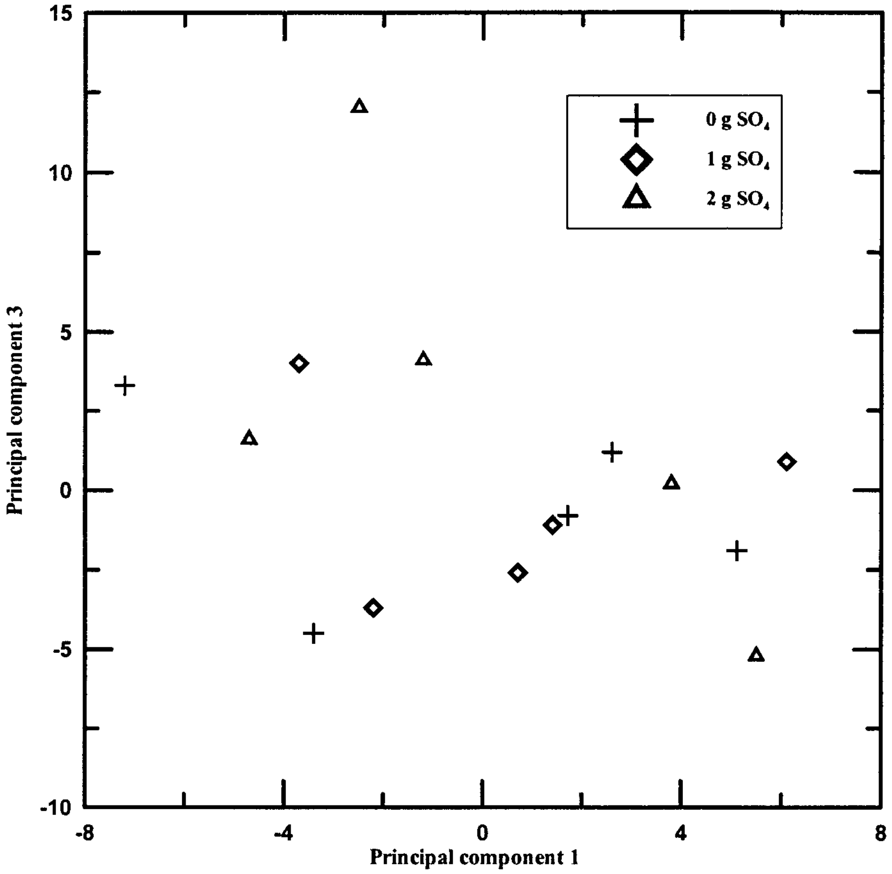

Figure 5. Principal component analysis for the SGBR treating wastewater with sulfate.

Although results from the FAME experiment were inconclusive, analytical data collected during the study provides some insight into the effects of sulfate on SGBR operation. During operation with a COD:SO4 ratio of 3:1, the SGBR averaged a concentration of about 60 mg COD/L in the effluent which is equivalent to 96% COD removal, . The effluent COD concentration was approximately 67 mg/L as the sulfate concentration was increased. Effluent solids concentration was slightly higher than other SGBRs, averaging 55 mg/L and 45 mg/L for sulfate concentrations of 1 g/L and 2 g/L. Sulfate reducing bacteria were present as indicated by the high hydrogen sulfide concentration in the off gas (15,000-26,000 ppm). Results for the three operating conditions are presented in Table 1.

The project also created problems with the hydrogen sulfide scrubbers. To protect the gas chromatograph equipment, hydrogen sulfide was removed before analyzing the gas composition. Lab scale anaerobic systems typically produce under 1000 ppm of hydrogen sulfide. A glass jar with steel wool was originally used for the scrubber. However, because of the large quantity of hydrogen sulfide generated, the steel wool was consumed too quickly and often caused clogging problems in the gas line. An aqueous solution of zinc acetate was used in place of the steel wool scrubber. The zinc sulfide precipitated out. This resulted in easier clean up and replacement of the aqueous scrubber.

Based on the data collected, it appeared that sulfate was reduced in the top of the reactor having little affect on the methanogens in the lower regions of the SGBR. As the sulfate was reduced, the hydrogen sulfide was released into the off gas. If hydrogen sulfide were produced lower in the granule bed, a decrease COD removal and methane production mayhave been observed.

Table 1. Results from a SGBR treating high sulfate wastewater.

$COD:SO_4$	Days at operating condition	Effluent COD (mg/L)	Effluent TSS (mg/L)	H_2S (ppm)	% Methane
3:0	40	22	15±1	600	78%
3:1	122	62	54.5±16	15,000	73%
3:2	134	67	45±22	26,000	72%

Conclusions and Recommendations

Engineering Significance

Since the creation of the SGBR, many valuable observations have been made. It was first observed that the dense bed of granules fed in a downflow manner was capable of high organic removal with a relatively small working volume. After more than four years of operation, lab results show that the effluent concentration of COD was less than 80 mg/L with the exception of the start up period in SGBR 1 (large diameter). During the four years of operation, the SGBR went through approximately 4 SRT periods. The SGBR is capable of operating under a wide range of HRTs with exceptional organic removal. Based on results from the transition study, the SGBR can operate under abrupt increases in organic loading with minimal effect on performance.

Although ideal operating conditions and reactor configurations are still under investigation, SGBR 2 (tall reactor) appeared to be the more effective reactor. It showed consistent COD removal and improved performance as HRT was lowered. SGBR 1 had exceptional performance also but was not as reliable as SGBR 2 which resembled a traditional plug flow reactor. The gas production within the reactor also aided in system operation. As gas was released, a slight mixing effect or movement of the granules occurred helping to prevent bed compaction and allowing the wastewater to flow through the bed.

After determining the SGBR would work with synthetic wastewater, other waste streams were tried including a synthetic wastewater consisting of nonfat dry milk, sucrose and sulfate. The SGBR treating wastewater containing high sulfate concentrations performed well with COD removal greater than 95%. It appeared that the hydrogen sulfide was produced in the top of the reactor, preventing inhibition to the methanogens deeper in the bed.

The SGBR had exceptional performance operating with only a fraction of the design bed volume. High organic removal during start up with less than full design bed volume was possible for wastewaters with low solids concentrations. If the bed was operated closer to design bed volume, the ability to entrap and retain solids would produce a lower effluent TSS concentration. This finding also supports the theory that at the design bed volume, the entire bed of granules was not active at one time. The inactive or reserve granules could provide a useful safety factor for sudden organic loading increases.

Full scale considerations

Operational data from the SGBRs will be useful as work progresses on potential pilot and full scale operations. In order to best scale up the SGBR, more information is needed about its performance under various operating conditions.

With full scale considerations in mind, the transition studies examined performance of the SGBR in a response to HRT changes. Knowing that the SGBR adapted to increased organic loading within 24 hours, slight variations in full scale operations should not affect overall performance of the SGBR. Additional information regarding full scale operation was the bed volume study. SGBRs with different percentages of design bed volumes were operated under identical conditions. COD results showed all bed volumes removed 90% of the COD, and the design bed volume (control reactor) removed 99% of the COD.

Recommendations for future work

The development of the SGBR is still in progress. Several key factors still need to be researched. In addition to determining ideal operating conditions such as HRT, ideal reactor size needs to be confirmed. To further advance the SGBR, additional waste streams need to be tested. The SGBR may not be applicable for all waste streams, but those treated with the SGBR produce a high quality effluent. Wastewaters with solids should be examined also; scheduled backwashing the SGBR has not been tried yet. Other research can be performed on the granules of the SGBR. Granules could be examined for acclimation capabilities; certain wastewaters may not be easily degraded by the microbial population. In addition to adaptability, granules could be examined for morphology changes as operating conditions are varied. To identify specific bacterial populations, it was suggested to use fluorescence microscopy to identify methanogens on the granules or to use SEM by examining thin slices of the granules to look for additional bacterial structures.

Although there are still areas to be examined, the current research suggests that the creation of the SGBR is a significant finding in the anaerobic treatment field.

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