ELSEVIER

Journal of Hazardous Materials B84 (2001) 253–264

Journal of Hazardous Materials

www.elsevier.nl/locate/jhazmat

Microorganism selection and biosurfactant production in a continuously and periodically operated bioslurry reactor

Daniel P. Cassidy*, Andrew J. Hudak

Department of Geosciences, Western Michigan University, Kalamazoo, MI 49008, USA

Received 30 December 2000; received in revised form 15 April 2001; accepted 20 April 2001

Abstract

A continuous-flow reactor (CSTR) and a soil slurry-sequencing batch reactor (SS-SBR) were maintained in 8 l vessels for 180 days to treat a soil contaminated with diesel fuel (DF). Concentrations of *Candida tropicalis, Brevibacterium casei, Flavobacterium aquatile, Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* were determined using fatty acid methyl ester (FAME) analysis. DF removal (biological and volatile) and biosurfactant concentrations were measured. The SS-SBR encouraged the growth of biosurfactant-producing species relative to the CSTR. Counts of biosurfactant-producing species (*C. tropicalis, P. aeruginosa, P. fluorescens*) relative to total microbial counts were 88% in the SS-SBR and 23% in the CSTR. Biosurfactants were produced in the SS-SBR to levels of nearly 70 times the critical micelle concentration (CMC) early in the cycle, but were completely degraded by the end of each cycle. No biosurfactant production was observed in the CSTR. DF biodegradation rates were over 40% greater and DF stripping was over five times lower in the SS-SBR than the CSTR. However, considerable foaming occurred in the SS-SBR. Reversing the mode of operation in the reactors on day 80 caused a complete reversal in microbial consortia and reactor performance by day 120. These results show that bioslurry reactor operation can be manipulated to control overall reactor performance. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bioslurry; Biosurfactants; CSTR; FAME; Foaming; SS-SBR

1. Introduction

Bioslurry treatment is an effective treatment for contaminated sediments, soils, and sludges [1], and consists of mixing excavated material with water in an aerobic reactor.

* Corresponding author. Tel.: +1-616-387-5543; fax: +1-616-387-5513.
E-mail address: daniel.cassidy@wmich.edu (D.P. Cassidy).

0304-3894/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0304-3894(01)00242-4

Different modes of bioslurry operation tested include batch systems by Jerger et al. in 1994, soil slurry-sequencing batch reactor (SS-SBR) [2], continuous-flow reactor (CSTR) [3], and tanks-in-series by Kleijntjens in 1991. However, in field systems SS-SBR and CSTR operation are the most common [4].

Eweis et al. [4] discuss the advantages of SS-SBR and CSTR operation. The CSTR dilutes contaminants upon entry to the reactor, which is undesirable for concentration-dependent biodegradation rates. The CSTR may require only one tank, but abrasion caused by continuous pumping results in high operation and maintenance costs relative to the SS-SBR. For a given retention time, a CSTR provides little operational flexibility to vary the contaminant concentrations in the reactor. The SS-SBR is filled during a discrete period of time (Fill), and then operated as a batch reactor to achieve the desired level of contaminant biodegradation (React). A fraction of treated slurry is then removed (Draw) and replaced with the same volume of untreated slurry to complete the SBR cycle. With a fixed retention time in the SS-SBR, the Fill volume and cycle time can be adjusted to provide optimal concentrations of contaminants and acclimated microorganisms. A significant advantage of the SS-SBR in hazardous waste treatment is that each batch can be tested before its release.

Contaminant degradation rates and foaming are two important factors in bioslurry reactor performance, and both are affected by biosurfactants. Biosurfactants are produced by many microbial genera grown on hydrophobic substrates [5–7]. Above the critical micelle concentration (CMC), surfactants emulsify hydrophobic contaminants, which can increase rates of microbial uptake [5]. However, the accompanying reduction in surface tension also stabilizes bubbles, which promotes foaming [8].

Little is known about the microbiology of bioslurry systems and how different modes of reactor operation can affect microorganism selection, biosurfactant production, foaming, and overall reactor performance. Cassidy et al. [9] compared the performance of a CSTR and a SS-SBR treating the same contaminated soil used in this study, and found marked differences in biosurfactant production and reactor performance with the two modes of operation. DF removal was 75% in the CSTR compared with 96% in the SS-SBR, and biosurfactant production was greater in the SS-SBR. The authors suggested that the SS-SBR encouraged the growth of microbes with a greater ability to produce biosurfactants and degrade DF than the CSTR. However, individual microbial species were not identified, so this hypothesis could not be tested. Wastewater studies have shown that SBRs and CSTRs can produce different microbial consortia [10,11]. This research was conducted to determine how SS-SBR and CSTR operation affect microorganism selection in soil, and whether such differences can explain differences in biosurfactant production and overall bioslurry reactor performance.

2. Materials and methods

2.1. The feed slurry and the reactors

The test soil, feed slurry preparation, and reactor setup are described by Cassidy et al. [9]. The total volume was 8 l and the working volume was 6 l (18 cm freeboard). Reactor 1 was operated as a CSTR for the first 80 days of the study period, and was then switched to

SS-SBR operation for the next 100 days. Reactor 2 was operated as a SS-SBR for the first 80 days, and was then switched to CSTR operation for the next 100 days. Both reactors had an 8-day retention time, and the SS-SBR was fed 3 l on 4-day cycles. The time for Fill and Draw was 5 min, so essentially, the entire cycle time was React.

2.2. Microorganism quantification and identification

The numbers of total DF-degrading microorganisms and individual species were determined in quadruplicate, 5 ml effluent slurry samples. Cells were extracted using the procedure described by Van Elsas and Smalla [12]. Dilutions were plated on Noble agar (Difco) prepared with DF. The plates were incubated for 5 weeks at 28°C and the colony forming units (CFU) on each plate were counted. These plates were sent to Microcheck, Inc. (Northfield, VT) for species identification using fatty acid methyl ester (FAME) analysis. Concentrations are reported as CFU/g.

2.3. Biosurfactant production

All biosurfactant measurements were done on filtered (0.45 μm) slurry samples, according to Cassidy et al. [9]. Surface tension (ST) was measured on duplicate 5 ml filtrate samples with a Tantec CBVP-Z Surface Tensiometer. Emulsification capacity (EC) was quantified on duplicate 15 ml filtrate samples. DF was added in 0.5 ml increments to filtrate until a separate phase was observed on top of the liquid. The EC is the percent of volume of DF emulsified per volume of filtrate. Surfactant concentration was quantified on triplicate 5 ml samples using critical micelle dilution (CMD). ST remains constant near 25–30 dynes/cm when surfactant concentrations are above the CMC. The CMD is the dilution required to bring the ST above 30 dynes/cm. Foam layer thickness was measured visually.

2.4. Quantification of diesel fuel (DF)

DF was extracted and quantified with GC/FID according to the methods described by Cassidy et al. [9]. Slurry DF concentrations were measured in the whole (unfiltered) slurry, and reflect DF present in all phases. Aqueous DF was measured in filtered slurry (0.45 μm) and only measured DF in the aqueous phase (i.e. soluble and emulsified).

3. Results and discussion

3.1. The feed slurry

The average concentrations of solids and DF were 12% (w/v), and 21.8 g/kg, respectively (Table 1). The aqueous DF concentration was below detection (0.1 mg/l). The aqueous solubility of fresh DF is approximately 5 mg/l [4]. The low aqueous DF concentrations in the slurry are due weathering during the 10–30 years of contamination. Biodegradation, volatilization, and leaching preferentially remove more soluble compounds [4]. The ST was

Table 1
Characterization of the feed slurry for the reactors[a]

Analyte	Result	Units
Solids concentration	12.0 ± 0.7 (25)	% (w/v)
Slurry DF concentration	21.8 ± 0.5 (46)	(g/kg)
Aqueous DF concentration	<0.1 (20)	(mg/l)
Surface tension	71.7 ± 0.5 (22)	(dynes/cm)
pH	7.8 ± 0.1 (44)	
Microbial concentration	$2.74 \times 10^8 \pm 6.4 \times 10^6$ (22)	CFU/g

[a] Mean ± standard deviation (number of measurements).

71.7 dyne/cm, near the value of 72 dyne/cm for pure water, which shows that surfactants were not present at measurable levels. The pH was 7.8, and the total concentration of microorganisms was 2.68×10^8 CFU/g.

3.2. Diesel fuel removal

Fig. 1 shows the results of DF measurements made in the feed and the effluent slurry from the reactors throughout the entire 180-day study period. DF concentrations began to decrease immediately in both reactors, showing that DF-degrading microorganisms were present and active in the soil. Operation of Reactor 1 as a CSTR caused the DF concentration to decrease to levels of approximately 5 g/kg within the first 20 days, where concentrations remained until day 80. Operation of Reactor 2 as a SS-SBR caused the DF concentration to decrease to <1 g/kg within the first 24 days. The DF concentration then remained at stable levels of approximately 0.5 g/kg until day 80. The mode of operation in the two reactors was

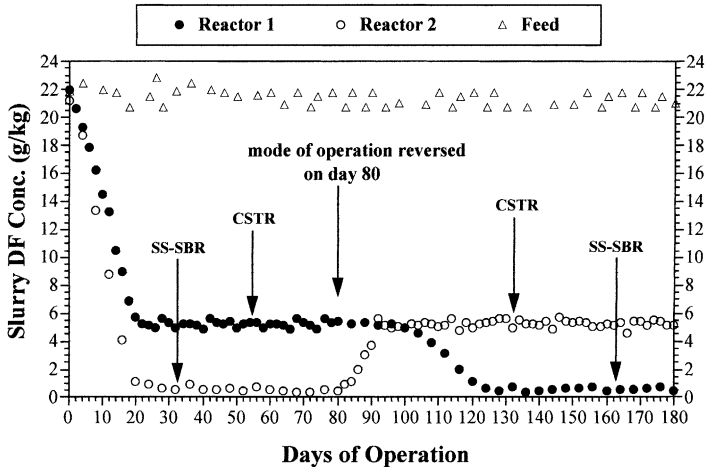

Fig. 1. Average values of diesel fuel (DF) concentration in effluent slurry from the two reactors during the 180 days of operation. The mode of operation was reversed on day 80.

reversed on day 80 (Fig. 1). Reactor 1 was converted from a CSTR to a SS-SBR, and Reactor 2 was converted from a SS-SBR to a CSTR. Reversing the mode of operation resulted in a reversal of the relative DF removal observed in the reactors. Converting Reactor 1 from a CSTR to a SS-SBR resulted in a decline in DF concentrations from the previous steady state value of 5 g/kg to a new steady state value of approximately 0.5 g/kg within 50 days after changeover. During the first 20 days after changing the mode of operation, there was no significant decline in DF levels in Reactor 1. However, between 20 and 44 days after the changeover (i.e. between days 100 and 124), the concentration of DF in the effluent began to decline to the new steady state values. Converting Reactor 2 from a SS-SBR to a CSTR caused an increase in DF levels to a steady state concentration of approximately 5 g/kg within 16 days after changeover.

Table 2 lists reactor measurements and performance calculations for the different modes of operation during steady state conditions (days 40 and 80 and days 140–180). These periods provide more than 3 retention times (24 days) for steady state conditions to be reached in the reactors from the beginning of the experiment and after the reversal on day 80. Three retention times are typically required to reach steady state conditions in biological reactors (Eweis et al. [4]). DF concentrations (Fig. 1) were certainly at steady state levels during these periods. The pH was 7.8 with both modes of operation, buffered by the high carbonate content of the soil. Effluent DF concentrations were over 10 times greater with CSTR operation than with SS-SBR operation. The aqueous DF concentrations below detection and ST values near 72 dynes/cm show that surfactants were not present in significant amounts in effluent from either mode of operation. Effluent OUR was nearly four times lower in the SS-SBR (5.5 mg/l h) than the CSTR (21.6 mg/l h), which indicates that biodegradation reactions had more nearly reached completion in the SS-SBR. The concentration of microorganisms was 26% higher with SS-SBR operation (1.30×10^9 CFU/g) than with CSTR operation (1.03×10^9 CFU/g). Total DF removal efficiency was 98% in the SS-SBR,

Table 2
Reactor measurements and performance calculations during steady state operation (days 40–80 and 140–180)

Parameter	CSTR	SS-SBR
Reactor measurements		
Effluent pH	7.8 ± 0.06 (22)[a]	7.8 ± 0.1 (22)
Effluent slurry DF (g/kg)	5.2 ± 0.4 (22)	0.5 ± 0.1 (22)
Effluent aqueous DF (mg/l)	<0.1 (22)	<0.1 (22)
Effluent ST (dyne/cm)	71.6 ± 0.5 (22)	71.7 ± 0.6 (22)
Effluent OUR (mg/l h)	21.6 ± 1.0 (22)	5.5 ± 0.3 (22)
Effluent microbial concentration (10^9 CFU/g)	1.03 ± 0.2 (32)	1.30 ± 0.2 (32)
Performance calculations		
Removal efficiency (%)	76	98
DF removed (mg/d)	1494	1917
DF stripped (mg/d)	164	38
DF biodegraded (mg/d)	1330	1879
DF stripped (%)	11	2
Overall biodegradation rate (mg/l d)	222	313

[a] Mean ± standard deviation (number of measurements).

and only 76% in the CSTR. The DF removal due to stripping was over five times higher in the CSTR (11%) than in the SS-SBR (2%). Overall biodegradation rates were 41% higher in the SS-SBR (313 mg/l d) than in the CSTR (222 mg/l d).

The results in Fig. 1 and Table 2 clearly show that SS-SBR operation resulted in greater DF biodegradation and less DF stripping than CSTR operation. Reversing the mode of operation in the reactors caused a reversal in relative performance, demonstrating that differences in performance were not vessel-specific, but were rather due to differences in mode of operation. Likewise, the steady state DF concentrations achieved were the same for SS-SBR and CSTR operation, regardless of the reactor. The reversal in performance after the changeover also showed that 80 days of reactor operation as a CSTR did not result in the loss of the characteristics of DF degradation expressed with SS-SBR operation, and vice versa. These results are very similar to those reported by Cassidy et al. [9].

3.3. Biosurfactant production in the SS-SBR

Although biosurfactants were not present in effluent from either mode of operation (Table 2), biosurfactants were produced during the SS-SBR cycle. Several track studies were performed during steady state SS-SBR operation in Reactors 1 and 2 to monitor DF removal and surfactant-related properties during the SS-SBR cycle. Data from the track study on days 60 to 64 are shown in Figs. 2 and 3. Results from the other track studies before and after the reversal in operation on day 80 were similar to Figs. 2 and 3, and to results from previous studies on the same soil [9,13].

Fig. 2 shows track study results of DF concentrations (slurry and aqueous) and OUR. The concentration of DF in the slurry decreased from approximately 12 g/kg after Fill to a concentration of approximately 0.5 g/kg by the end of the 4-d cycle. The concentration of DF in the aqueous phase increased exponentially from below detection to above 1500 mg/l

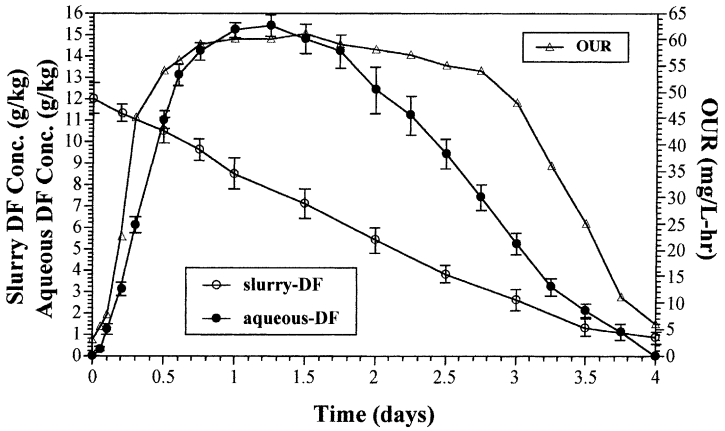

Fig. 2. Average values of slurry and aqueous diesel fuel (DF) concentrations and oxygen uptake rate (OUR) during one cycle in the SS-SBR during steady state operation (days 60–64 of operation). Error bars show standard deviation.

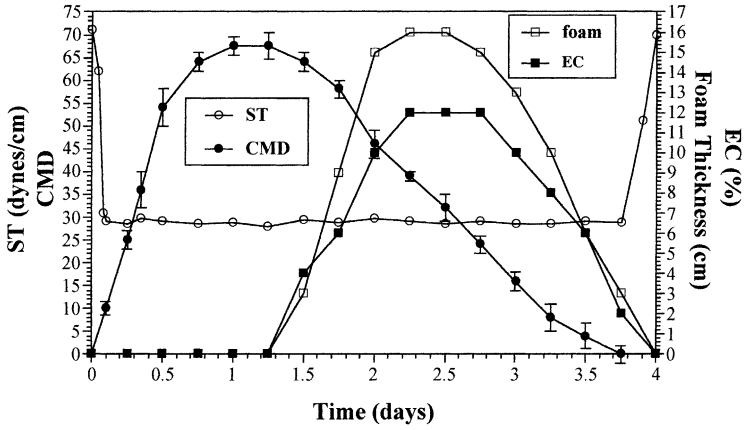

Fig. 3. Average values of surface tension (ST), biosurfactant concentration (CMD), foam thickness, and emulsification capacity (EC) during one cycle in the SS-SBR during steady state operation (days 60–64 of operation). Error bars show standard deviation.

between 1 and 1.25 d. After 1.5 d, the aqueous DF concentration began to decrease, and was below detection again by the end of the cycle. OUR increased exponentially during the first 4 h, reaching values between 50 and 60 mg/l h between days 1 and 3. OUR then decreased over the last day of the cycle to the effluent value of 5 mg/l h. The results in Fig. 2 show that DF was emulsified during biodegradation. Aqueous DF concentrations above 1500 mg/l are more than 2 orders of magnitude greater than DF solubility (5 mg/l), providing evidence of biosurfactant production. A well-mixed oil-in-water emulsion consists of droplets smaller than 0.1 μm in diameter [5,8], which apparently allowed emulsified DF to pass through the 0.45 μm filter.

Fig. 3 shows track study results for ST, CMD, foam thickness, and EC. ST decreased immediately after Fill from nearly 72–30 dynes/cm within the first 4 h of the cycle. ST values remained between 28–30 dynes/cm until 3.75 d, after which ST increased rapidly back to near 72 dynes/cm. CMD measurements increased from zero to 10 within the first 4 h of the cycle. CMD values reached nearly 70 by day 1. After 1.25 d, CMD began to decrease steadily until it reached zero again between days 3.75 and 4. Foam thickness was zero during the first 1.25 d of the cycle, and then increased to a maximum of 16 cm after 3.5 d. Foam completely disappeared by the end of the cycle. The profile of EC mirrored that of foam thickness. EC was zero during the first 1.25 d, rose to a maximum value of near 12% between 2.25 and 3.25 d, and then decreased to zero.

The results in Fig. 3 show that biosurfactants were produced in the SS-SBR to levels as high as 70 times the CMC, but were completely removed by the end of the cycle. The drop in ST to 30 dynes/cm is indicative of biosurfactant concentrations above the CMC [7]. The decrease in ST coincided with surfactant concentrations above the CMC (Fig. 2), as shown by non-zero values of CMD. Emulsification of DF (Fig. 1) is also consistent with surfactant levels above the CMC. Since biosurfactants are biodegradable and sorb readily to soil [6,7], the accumulation of surfactants early in the cycle indicates that the rate of

surfactant production exceeded rates of degradation and sorption. Likewise, the decrease in CMD after 1.5 d shows that rates of removal were greater than rates of production during this time. The increase in ST coincident with CMD values dropping to zero at 3.75 d show that biosurfactants were removed by the cycle's end. This can be attributed to biodegradation. Volatile biosurfactant removal was not quantified, but is likely to be small considering the very large molecular weight of these compounds [6].

Foaming was not a simple function of surfactant concentration (Fig. 3), and did not occur until the surfactant concentration began to decline from the maximum value. EC is a measure of the relative amount of free (non-contaminant bound) surfactant molecules in solution [8,14]. Therefore, the identical time profiles of foam thickness and EC indicate that foaming resulted from the temporary accumulation of free surfactant molecules. EC values were zero when surfactant concentrations were greatest, indicating that all the surfactants were bound to DF at that time. The accumulation of free surfactants after 1.25 d is explained by surfactant production in excess of DF available for emulsification, and/or by greater degradation rates of DF than surfactants during this time. Similarly, after 2.5 d free biosurfactants were apparently degraded faster than they were produced or released from emulsified DF. The positive relationship between foaming and EC suggests that adding untreated slurry would occupy free surfactant molecules and decrease foaming. Preliminary studies (data not presented) indicate that this indeed true.

3.4. Species concentrations

It is clear from the results shown above that SS-SBR operation enhances biosurfactant production, DF biodegradation, and foaming relative to CSTR operation, suggesting that different microbial consortia were developed. To test this hypothesis, quadruplicate samples were taken periodically to quantify and identify individual species in the effluent slurry. Five species were positively identified with FAME analysis in all the samples from the feed slurry and the reactors; *Candida tropicalis* (a yeast), *Brevibacterium casei, Flavobacterium aquatile, Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*. Positive FAME identification consists of a ratio of similarity index to standard deviation (SI/SD) greater than 0.500. All of these microorganisms degrade DF, as it was the sole carbon source in the agar. *C. tropicalis*, *P. aeruginosa*, and *P. fluorescens*, produce biosurfactants when grown on petroleum hydrocarbons. *C. tropicalis* produces a mannan-fatty acid surfactant [15], *P. aeruginosa* produces a rhamnolipid surfactant [16], and *P. fluorescens* produces a lipopeptide surfactant [17]. *Flavobacterium* and *Brevibacterium* are not known to produce biosurfactants.

Fig. 4 shows the log of the concentrations of the biosurfactant producers *C. tropicalis* and *P. fluorescens* throughout the study period. *P. aeruginosa* concentrations were not plotted because they did not vary significantly with mode of operation. Concentrations of both *C. tropicalis* and *P. fluorescens* reached stable, steady state values within 24 to 32 days after startup. Concentrations of both species were significantly greater with SS-SBR operation than with CSTR operation. After the mode of operation was reversed on day 80 new steady state concentrations were again reached within 40 days for *P. fluorescens* and 52 days for *C. tropicalis*. However, reversing the mode of operation also reversed the relative concentrations of both species also reversed. Steady state concentrations of both species

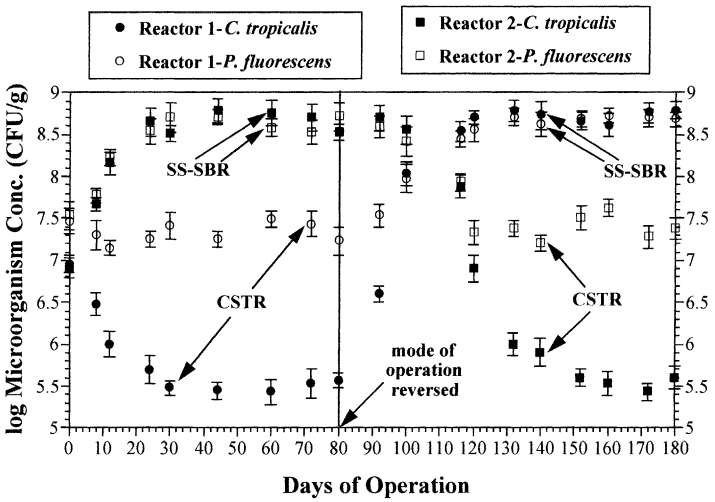

Fig. 4. Average log values of the concentrations of *Candida tropicalis* and *Pseudomonas fluorescens* (known biosurfactant producers) in the reactors during the entire 180 days of operation. The mode of operation was reversed on day 80. Error bars show standard deviation.

were very similar with SS-SBR and CSTR operation before and after reversing the mode of operation. These results show that the growth of *C. tropicalis* and *P. fluorescens* were favored with SS-SBR operation relative to CSTR operation.

Fig. 5 shows the log of the concentrations of *B. casei* and *F. aquatile* throughout the study period. Neither of these genera is known to produce biosurfactants. Concentrations of both *B. casei* and *F. aquatile* reached steady state values within 12 to 24 days after startup, and again within 52 to 60 days after reversing the mode of operation. Reversing the mode of operation reversed the relative concentrations of both *B. casei* and *F. aquatile*, as was observed with the two biosurfactant-producing species (Fig. 4). However, in contrast to the two biosurfactant producers, concentrations of *B. casei* and *F. aquatile* were significantly greater with CSTR operation than with SS-SBR operation. Steady state concentrations of both species were very similar with SS-SBR and CSTR operation before and after reversing the mode of operation. These results show that the growth of *B. casei* and *F. aquatile* were favored with CSTR operation relative to SS-SBR operation.

Table 3 lists average concentrations of all five of the FAME-identified species in the feed slurry and in reactor effluent during steady state conditions. Days 40 to 80 and 140 to 180 were considered the two steady state periods before and after reversing the mode of operation, which seems justified by concentrations of individual species (Figs. 4 and 5) and DF (Fig. 1). Concentrations are reported as 10^6 CFU/g in order to simplify comparisons. Only *P. aeruginosa* had significantly greater concentrations in effluent from both modes of operation than in the feed slurry, indicating that growth occurred with both modes of operation. *P. fluorescens* experienced no significant growth in the CSTR, and concentrations of *C. tropicalis* were reduced more than an order of magnitude by CSTR treatment. However, both *C. tropicalis* and *P. fluorescens* showed considerably growth in the SS-SBR.

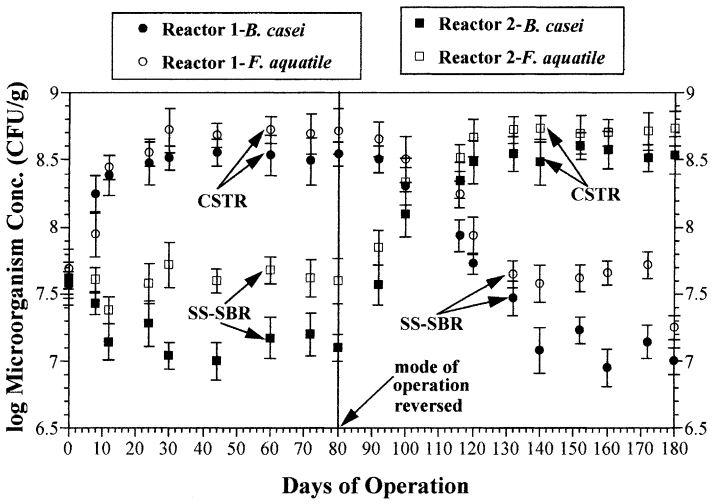

Fig. 5. Average log values of the concentrations of *Brevibacterium casei* and *Flavobacterium aquatile* (not known to produce biosurfactants) in the reactors during the entire 180 days of operation. The mode of operation was reversed on day 80. Error bars show standard deviation.

Table 3
Concentrations of individual species in the feed slurry and in effluent from the reactors during steady state operation (days 40–80 and 140–180)

Measurement	Feed	CSTR	SS-SBR
Microorganism concentration (10^6 CFU/g)			
Candida tropicalis[a]	8 ± 4 (32)[b]	0.3 ± 0.1 (32)	501 ± 84 (32)
Pseudomonas aeruginosa[a]	97 ± 11 (32)	221 ± 68 (32)	203 ± 53 (32)
Pseudomonas fluorescens[a]	19 ± 6 (32)	20 ± 11 (32)	437 ± 95 (32)
Brevibacterium casei	47 ± 27 (32)	214 ± 41 (32)	12 ± 5 (32)
Flavobacterium aquatile	52 ± 3 (32)	479 ± 66 (32)	44 ± 13 (32)
∑ Identified species	223	934	1197
∑ Biosurfactant producers	124	241	1141
Ratios of microbial concentrations (%)			
∑ Identified species/total[c]	81	91	92
∑ Biosurfactant producers/total[c]	45	23	88

[a] Species known to produce biosurfactants.
[b] Mean ± standard deviation (number of measurements).
[c] Values of total microbial concentrations are from Tables 1 and 2.

Conversely, *B. casei* and *F. aquatile* both showed marked growth in the CSTR, and no growth or a decrease in numbers in the SS-SBR. Lower numbers in reactor effluent than the feed indicates antagonistic conditions in the reactor towards the species. Numbers of all five species were significantly different with SS-SBR and CSTR operation except *P. aeruginosa*. *C tropicalis* concentrations achieved with SS-SBR operation were more than

3 orders of magnitude greater than with CSTR operation. Concentrations of *P. fluorescens* were more than an order of magnitude greater in effluent from SS-SBR operation than CSTR operation. In contrast, concentrations of both *B. casei* and *F. aquatile* were more than an order of magnitude greater with CSTR operation than with SS-SBR.

Several other species were occasionally identified by FAME analysis, but not consistently. The sum of the five FAME-identified species comprised 81% of the total microorganism concentration in the feed and 92% in the two reactors (Table 3). This shows that as a group the five species were the most abundant microorganisms in the system. The ratio of the total concentration of biosurfactant producers (i.e. the sum of *C. tropicalis, P. aeruginosa*, and *P. fluorescens*) to the total microorganism concentrations was nearly four times greater with SS-SBR operation (88%) than with CSTR operation (23%). In the feed, biosurfactant producers comprised 45% of the total microbial concentration.

The FAME identification results in Figs. 4 and 5 and Table 3 clearly demonstrate that SS-SBR and CSTR operation developed microbial consortia with drastically different population distributions of individual species. Concentrations of four of the five individual species varied more than an order of magnitude with the two modes of operation. The results also show that SS-SBR operation favored the growth of biosurfactant-producing microorganisms in the soil relative to non-biosurfactant producers. These results explain the enhanced biosurfactant production and DF biodegradation with SS-SBR operation relative to CSTR operation observed in this study and by Cassidy et al. [9].

It is not known why SS-SBR and CSTR operation selected for different microbial consortia, since the feed introduced the same relative concentrations of species and nutrients in both reactors. However, there are several possible reasons. The two modes of operation produce different conditions. Conditions in the CSTR are relatively constant, while SBR operation exposes microorganisms to fluctuating contaminant concentrations: high contaminant levels prevail after Fill and low levels after the contaminants are biodegraded. Wastewater studies have shown that SBR treatment can have a profound effect on the metabolic, morphologic, and settling properties of the microbial consortium selected for in the system relative to CSTR treatment [10,11]. Also, this investigation and a previous one with the same soil [9] showed that biosurfactant production was directly related to the maximum contaminant concentration achieved in the reactor [9]. Some biosurfactants have antibiotic properties [7], so that once biosurfactant production by one species is promoted it could produce antagonistic reactions in other species. Such ecological considerations merit further investigation.

4. Summary and conclusions

SS-SBR operation favored the growth of biosurfactant-producing species relative to the CSTR. Biosurfactant-producing species comprised 88% of the total microbial concentration with SS-SBR operation, with and 23% with CSTR operation. Concentrations of the yeast *C. tropicalis* were over 3 orders of magnitude greater with SS-SBR operation than in the CSTR. Biosurfactants were produced in the SS-SBR to levels of nearly 70 times the critical micelle concentration (CMC) early in the cycle, but were completely degraded (probably from biodegradation) by the end of each cycle. No biosurfactant production was observed

in the CSTR. DF biodegradation rates were over 40% greater and DF stripping was over five times lower in the SS-SBR than the CSTR. However, considerable foaming occurred in the SS-SBR, whereas none was observed in the CSTR. Reversing the mode of operation in the reactors on day 80 caused a complete reversal in microbial consortia and reactor performance by day 120, showing that the differences were not vessel-specific. Results from this and previous studies indicate that bioslurry reactor operation can be manipulated to control performance (e.g. foaming, and contaminant biodegradation and stripping). Plug flow reactors and tanks-in-series typically perform in a similar fashion to SBRs, so future research should investigate the performance of these reactor types.

References

[1] USEPA, Engineering Bulletin: Slurry Biodegradation, Center for Environmental Research, EPA/540/2-90/016, Cincinnati, OH, 1990.
[2] R.L. Irvine, P.S. Yocum, J.P. Earley, R. Chozick, Water Sci. Technol. 27 (1993) 97.
[3] M.J. Geerdink, Kinetics of the microbial degradation of oil-in-soil slurry reactors, Doctoral dissertation, Technical University of the Delft, The Netherlands, 1995, p. 103.
[4] J.B. Eweis, S.J. Ergas, D.P.Y. Chang, E.D. Schroeder, Bioremediation Principles, McGraw-Hill, New York, 1998, p. 296.
[5] C. Syldatk, F. Wagner, in: N. Kosaric, W.L. Cairns (Eds.), Marcel Dekker, New York, 1987, p. 89.
[6] R.M. Miller, in: H.D. Skipper, R.F. Turco (Eds.), Soil Science Special Publication No. 43, Soil Science Society of America, 1995, p. 33.
[7] J.D. Desai, I.M. Banat, Microbiol. Mol. Biol. Rev. 61 (1997) 47.
[8] P. Becher, Emulsions: Theory and Practice, Van Nostrand Reinhold, New York, 1965, p. 343.
[9] D.P. Cassidy, S. Efendiev, D.M. White, Water Res. 34 (2000) 4333.
[10] S.C. Chiesa, R.L. Irvine, Water Res. 19 (1985) 471.
[11] R.L. Irvine, L.H. Ketchum Jr., CRC Critical Rev. Environ. Control 18 (1988) 255.
[12] J.D. Van Elsas, K. Smalla, in: C.J. Hurst (Ed.), American Society for Microbiology, Washington, DC, 1994, pp. 388–392.
[13] D.P. Cassidy, Water Environ. Res. 73 (2001) 87.
[14] G.L. Ghurye, C. Vipulandan, R.C. Willson, A practical approach to biosurfactant production using mixed cultures, Biotechnol. Bioeng. 44 (1994) 661.
[15] O. Kappell, A. Feichter, Biotechnol. Bioeng. 18 (1976) 967.
[16] M. Roberts, M.E. Mercade, M.P. Bosch, J.L. Parra, M.J. Espuny, M. Manresa, J. Guinea, Biotechnol. Lett. 11 (1989) 871.
[17] T.R. Neu, T. Hartner, K. Poralla, Appl. Microbiol. Biotechnol. 32 (1990) 518.