Inhibitory Effects and Biotransformation of Acrylic Acid in Computer-Controlled pH-Stat CSTRs

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Abstract: In this study, the inhibitory effects and anaerobic biotransformation of acrylic acid in computer-controlled pH-stat completely stirred tank reactors (CSTRs) with two different cultures, namely unacclimated and acrylate-acclimated acetate-enriched Methanosarcina and homogenized (crushed) granular cultures, were investigated. The microbial inhibition, influent concentration, and loading rate of acrylic acid were studied in the experiments. The experimental results revealed that methanogenic cultures at a concentration of 3200 ± 80 mg/L as volatile suspended solids (VSS) could be acclimated to acrylic acid up to a loading rate of 220 mg/L per day (0.068 g acrylic acid/g VSS per day) in the presence of a constant acetate concentration of 200 ± 200 mg/L as the primary substrate after 300 days of acclimation. The same cultures (680 ± 80 mg/L as VSS), after 80 days of acclimation to acrylic acid as the sole carbon source, transformed acrylic acid up to the loading rate of about 200 mg/L per day (0.29 g acrylic acid/g VSS per day) almost completely (>99%) to acetic and propionic acid, but could not effectively metabolize these intermediate products. Acrylate-acclimated homogenized granular cultures (6900 ± 80 mg/L as VSS) effectively metabolized 2200 mg/L per day (0.32 g acrylic acid/g VSS per day) of acrylic acid, as the sole carbon source, after 50 days of severe inhibition. © 1999 John Wiley & Sons, Inc. Biotechnol Bioeng 62: 200–207, 1999.

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INTRODUCTION

Acrylic acid is a hazardous compound (U.S. EPA) used mainly in the manufacture of water-soluble acrylic resins, dispersants, and flocculants, and is a byproduct in the petrochemical industry (Howard, 1989; Stig, 1985). The U.S. EPA has imposed regulations on the effluent limit of acrylic acid into navigable waters and publicly owned treatment works not to exceed 92 mg/L as 5-day biochemical oxygen demand (BOD₅) for any 1-day period and 34 mg/L as BOD₅ for a monthly average (Federal Register, 1987).

Acrylic acid, reported to be amenable to anaerobic treatment (Speece, 1983), was degraded with more than 75% of theoretical methane being produced in 8 weeks of incubation in a batch anaerobic screening study utilizing 10% sludge from a secondary digester as an inoculum (Shelton and Tiedje, 1984). However, toxicity is an important concern for the anaerobic treatment of acrylic acid. Chou et al. (1978a) reported that 12 mM (860 mg/L) of acrylic acid resulted in 50% inhibition of methanogenic activity in an anaerobic Warburg respirometer study. Furthermore, only 21% of acrylic acid was metabolized after 90 days of acclimation in a continuously mixed anaerobic reactor (Chou et al., 1978b). Stewart et al. (1995) studied the biodegradability of acrylic acid using serum-bottle anaerobic toxicity assays and biochemical methane potential experiments, and reported that acrylic acid can be treated up to 100 mg/L without inhibiting methanogenic cultures. However, a certain time period was necessary for acclimation and subsequent degradation of higher concentrations. Demirer and Speece (1998a) similarly stated that acrylic acid is significantly toxic to acetate-enriched Methanosarcina cultures above slug doses of 20 mg/L (0.019 mg acrylic acid/mg VSS) in a batch study. Dohanyos et al. (1988) reported that anaerobic upflow biofilters could be adapted to remove acrylic acid. Treatment efficiencies of about 98% were obtained for an influent concentration of 10 to 12 g/L of acrylic acid at a chemical oxygen demand (COD) loading rate and HRT of 3.8 g/L per day and 6 days, respectively. They also observed that the system failed due to the toxic effect of acrylic acid when the HRT was decreased to 1.6 days. Qu and Bhattacharya (1996) reported that maximum acrylic acid loading attained in an acclimated methanogenic chemostat was found to be 66.7 mg/L per day. In a recent study, Demirer and Speece (1998b) evaluated the performance of a two-staged upflow anaerobic sludge blanket (UASB) reactor and reported that 97% to 98% chemical oxygen demand (COD) removal was achieved for an influent acrylic acid concentration and loading rate of 3000 mg/L and 1.8 g/L per day, respectively. The importance of acclimating the anaerobic cultures to acrylic acid was noted by several researchers (Chou et al., 1978b; Demirer and Speece, 1997, 1998a,b; Qu and Bhattacharya, 1996; Stewart et al., 1995).

Wastewaters from the production of acrylic acid contain...
mainly acrylic acid, light esters of acrylic acid, and also short-chain fatty acids, mainly acetic and propionic acids. When aerated, the esters of acrylic acid are air-stripped before being decomposed biologically, which makes the aerobic treatment of these wastewaters without contaminant release to the atmosphere virtually impossible. Anaerobic treatment, on the other hand, facilitates biological decomposition of organic substances in sealed reactors without exposure to air. The esters of acrylic acid are hydrolyzed in anaerobic medium to acrylic acid, methanol, ethanol, and butanol and then decomposed to substrates suitable for methanogenic microorganisms (Dohanyos et al., 1988).

Janssen (1991) enriched a Gram-positive, obligately anaerobic bacterium, 19acr3, belonging to the species Clostridium propionicum from anaerobic sediments, which was able to ferment acrylate as follows:

$$3\text{CH}_2\text{CHCOO}^- + 3 \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + 2\text{CH}_3\text{CH}_2\text{COO}^- + \text{HCO}_3^- + \text{H}^+ \quad (1)$$

The anaerobic metabolism of acetate and propionate (the intermediate products of acrylate) at SRT = 20 days are given by Speece (1996) as follows:

$$0.125 \text{CH}_3\text{COO}^- + 0.119 \text{H}_2\text{O} + 0.002 \text{CO}_2 + 0.002\text{NH}_4^+ + 0.121 \text{CH}_4 + 0.123 \text{HCO}_3^- \quad (2)$$

$$\text{CH}_3\text{CH}_2\text{COO}^- + 0.407 \text{H}_2\text{O} + 0.036 \text{NH}_4^+ + 0.036 \text{C}_4\text{H}_6\text{O}_2\text{N} + 0.949 \text{CH}_3\text{COO}^- + 0.711 \text{CH}_4 + 0.196 \text{CO}_2 + 0.016 \text{HCO}_3^- \quad (3)$$

Even though anaerobic treatment is a very promising treatment option for acrylic acid, the number and the extent of the studies on anaerobic metabolism of acrylic acid in the literature are limited. The majority of these studies are short-term batch experiments which yielded very limited information for a particular set of experimental conditions. Therefore, this study was conducted, as a part of series of studies on anaerobic acrylic acid metabolism, to investigate the toxic effects and biotransformation of acrylic acid in computer-controlled pH-stat CSTRs with acetate-enriched methanogenic and homogenized (crushed) granular cultures. The toxic effects of acrylic acid on enriched Methanosarcina culture were studied in the first pH-stat CSTR experiment in which acrylic acid was added with acetate. Anaerobic metabolism of acrylic acid as the sole carbon source was investigated in the remaining two pH-stat CSTR experiments with acrylate-acclimated Methanosarcina and homogenized granular cultures. The significance of microbial acclimation, influent concentration, and loading rate of acrylic acid were evaluated in the experiments.

**MATERIALS AND METHODS**

Two types of cultures were employed in the experiments: (1) acetate-enriched Methanosarcina and (2) mixed UASB granules. The acetate-enriched Methanosarcina culture was cultivated in a pH-stat CSTR operating for about 6 months at a constant pH of 6.8 ± 0.2 and an acetate utilization rate (AUR) of 28 ± 6 g/L per day. Homogenized granular cultures were obtained by physical homogenization (crushing) of the mixed UASB granules in a blender. The granular culture was obtained from the Smucker’s Jelly UASB reactor (Orrville, OH) treating carbohydrate wastewater with HRT and organic loading rate of 24 h and 15 kg/m³ per day, respectively.

The composition of the Vanderbilt Media (VM) used as the basal media in all experiments was as follows (concentrations of the constituents are given in parentheses as mg/L): NH₄Cl (400), MgSO₄ 7H₂O (400), KCl (400), Na₂S.9H₂O (300), CaCl₂.2H₂O (50), (NH₄)₂HPO₄ (80), FeCl₃.4H₂O (40), CoCl₂.6H₂O (10), KI (10), (NaPO₃)₆ (10), MnCl₂.4H₂O (0.5), NH₄VO₃ (0.5), CuCl₂.2H₂O (0.5), ZnCl₂ (0.5), AlCl₃.6H₂O (0.5), NaMoO₄.2H₂O (0.5), H₃BO₃ (0.5), NiCl₂.6H₂O (0.5), NaWO₄.2H₂O (0.5), Na₂SeO₃ (0.5), cysteine (10), and NaHCO₃ (6000).

The pH-stat CSTRs (Fig. 1) consisted of a magnetically stirred widemouth glass bottle of 2 L of effective (liquid) volume, a headspace of about 100 mL, and no recycle. The bottles were sealed with black rubber stoppers with ports for probe penetration, feeding, sample withdrawal, and gas venting. The pH-stat CSTRs incorporated a multichannel pH-control system that consisted of a computer (Apple Ile, Apple Computer Co.), an amplifier, and a pH probe (G-05990-55, Cole Parmer Instrument Co.).

In this system, substrate control and alkalinity were interrelated and controlled by a computer as follows: First, a signal was generated by the pH-probe in the reactor and transferred to the computer through an amplifier. The computer read the amplified signal once every 45 seconds, then compared it with a preset value of 6.8. The pH increased when the substrate was consumed by the microorganisms. After detecting a signal above the pH setpoint, the computer sent a signal to turn on the peristaltic substrate feed pump for 1 second. Then, a fixed amount of acetate and/or acrylate substrate was delivered to the reactor and lowered the
pH by no more than 0.2 pH units. After another 45 seconds, the computer checked the pH value and started the cycle again if needed. In this way, the pH value in the reactor was kept at 6.8 ± 0.2 and the acetate concentration was automatically maintained at about 2000 ± 200 mg/L so acetate was never limiting methanogenesis during the experiments. This system overcame the disadvantage of the general batch method in which high substrate levels can be maintained only for a short period, and of the general chemostat system in which high loading rates and high substrate concentrations are difficult to achieve due to pH changes of the system.

In pH-stat CSTR studies, either a supplemental acetic feed containing 315 g/L of acetic acid or a supplemental acrylic acid feed containing 50 g/L of acrylic acid was used. In addition to acetic or acrylic acids, the supplemental feeds also contained (concentrations of the constituents given in parentheses as mg/L): NH4Cl (9000), MgSO4.7H2O (1200), FeCl3.4H2O (150), CoCl2.6H2O (6), NiCl2.6H2O (6), and yeast extract (10).

The supplemental feeds supplied carbon-energy source (acetate and acrylate) as well as excess amounts of nutrients and trace metals. In using supplemental feeds, the rationale was to overcome possible temporary deficiencies of the nutrient and trace metals contained in the VM. Especially high concentrations of the trace metals can frequently be fed to reactors in free or available form due to the low pH of the supplemental feeds, unlike in VM, in which a large proportion of the metals was precipitated with sulfide.

Speece (1996) reported that Methanosarcina (Ks 400 mg/L, maximum specific substrate utilization rate 6 to 10 g COD/g VSS per day) and Methanothrix (Ks 20 mg/L, maximum specific substrate utilization rate 2 to 4 g COD/g VSS per day) species are the only known methanogens capable of acetate metabolism. Predominance of Methanosarcina is favored at high acetate concentrations. However, the predominance shift to the Methanosarcina does not occur unless specific trace metal bioavailability is satisfied. Significantly high specific substrate utilization rates and acetate concentrations achieved in this study were considered as proof of the dominance of Methanosarcina over Methanothrix cultures and no microbial characterization was performed.

Takashima and Speece (1989) also reported that, if the acetate utilization rate (AUR) was over 15 g/L per day, 6000 mg/L of NaHCO3 in the VM was not sufficient to keep the acetate acid concentration of 2000 ± 200 mg/L in the pH-stat reactor, and extra alkalinity addition was required. Therefore, if the AUR was over 15 g/L per day, extra alkalinity was added in accordance with the stoichiometric ratio of 0.013 mol of HCO3− per mole of acetate utilized, defined as follows:

\[
\text{CH}_3\text{COOH} + 0.013 \text{ HCO}_3^- + 0.013 \text{ NH}_4^+ + 0.968 \text{ CO}_2 + 0.051 \text{ H}_2\text{O} \]

The pH-stat reactors were operated either in fed-batch or continuous mode:

- **Fed-batch operation.** No biomass was wasted intentionally. However, the supplemental acetic and acrylic acid was fed to the reactors by the computer when the pH in the reactor was >6.8, and a corresponding volume was wasted from the reactor contents. This is called "unintentional" biomass wastage.

- **Continuous operation.** An SRT/HRT of 5 days was maintained in the reactor by daily wasting of 400 mL of the reactor contents. The "unintentional" biomass wastage was subtracted from the 400 mL, and the remaining volume was replaced with VM.

**Analytical Methods**

Acrylic, acetic, and propionic acids were measured by a Shimadzu gas chromatograph (GC-6AM), equipped with a flame-ionization detector and a 1.7-m glass column packed with 0.3% Carbowax 20M/0.1% H3PO4, 60/80 Carbopack-C (Supelco, Inc.). The column temperature was 125°C, and the injector/detector temperature was 200°C; the carrier gas was nitrogen with a flow rate of 40 mL/min. Data integration was achieved with a Dionex 4290 integrator. Samples were prepared by centrifuging (Model GP, Beckman Instruments Co.) for 10 min at 4000 rpm and filtering 3 mL of the supernatant through 0.45-μm filter paper (Whatman Co.). The filtered samples were acidified with 10% formic acid to lower the pH to less than 3 to convert the acrylate, acetate, and propionate to acrylic, acetic, and propionic acids, respectively, so that they were volatile and 1 μL of the acidified samples was injected into the GC. The detection limit of GC for acrylic, acetic, and propionic acids was 10 to 15 mg/L.

Biomass was approximated as mixed liquor volatile suspended solids (VSS) by following Standard Method 2540 E (Standard Methods, Methods, 1992). Alkalinity measurements were carried out as described in Standard Method 2320 B (Standard Methods, 1992). Manual pH measurements were taken with a pH-meter (Model 107, Fisher Scientific Co.) and pH-probe (G-05992-55, Cole Parmer). Automatic pH measurements were taken with a pH-meter/controller and pH-probe (5656-00 and G-05992-55).
respectively; Cole Parmer). All analytical measurements were conducted in duplicate samples. The values reported in the figures are the averages with error bars indicating the standard deviation of the measurements.

RESULTS AND DISCUSSION

Toxicity of Acrylic Acid on Methanosarcina Enrichment Culture

The objective of this experiment was to observe the toxic effects of continuous acrylic acid addition on acetate-enriched Methanosarcina cultures utilizing acetate as the primary substrate with acrylic acid in a pH-stat CSTR. Methanosarcina cultures were acclimated to acrylic acid with the following strategy: unless inhibition was observed at the previous concentration, a higher acrylic acid concentration was maintained. However, a particular acrylic acid concentration resulted in inhibition of the AUR of the system, and an increase in the acrylic acid concentration was not maintained in the feed until the AUR of the system recovered to 50% of the baseline value (the average AUR value prior to acrylic acid feeding). With this strategy, the cultures were not exposed to sudden increases of acrylic acid in the feed, allowing acclimation. Because acrylic acid was fed to the pH-stat reactor only with the supplemental acetic acid feed, the acrylic acid administration was coupled with the AUR of the system.

As seen in Figure 2c, the AUR increased gradually and reached to 26 g/L per day after 36 days. Starting from day 37, the mode of operation was changed to continuous from fed-batch. The reason was to make the Methanosarcina (vs. Methanothrix) the dominant species in the methanogenic culture.

Acrylic acid administration was started on day 56. The initial influent acrylic acid concentration of 200 mg/L was maintained in both supplemental acetic acid feed and VM. In order not to washout the biomass from the reactor, biomass wastage was not performed if the AUR was less than 5 g/L per day the previous day. Immediately after onset of acrylic acid addition, the AUR started to decrease and dropped to less than 1 g/L per day after 3 days (day 58). This was due to inhibitory effects of acrylic acid on the methanogenic cultures, as reported by Stewart et al. (1995), Qu and Bhattacharya (1996), and Demirer and Speece (1998b). Beginning from day 59, the AUR started to recover gradually as seen in Figure 2c. The rise in the AUR continued and the reactor recovered to its background activity (the steady-state AUR before the onset of acrylic acid addition) after 12 days indicating that cultures was acclimated to 200 mg/L acrylic acid. As soon as this recovery was observed, the influent concentration of acrylic acid was increased to 400 mg/L on day 67. Again, this increase led to an abrupt decline in the AUR of the reactor. The propionic acid concentration was less than 100 mg/L before acrylic acid addition to the system, and increased to 940 mg/L on day 102. The activity of the system recovered suddenly to its original level after 5 days with an acrylic acid loading rate of 160 mg/L per day (day 124). Concurrently, the propionic acid level in the reactor dropped to 470 mg/L level on day 136. This observation was in agreement with the findings of Qu and Bhattacharya (1996) who reported that acrylic acid was more toxic to propionate utilizers than to acetate utilizers in an anaerobic system and propionate was not effectively utilized when acrylate was present in the system.

From the beginning of acrylic acid addition to the reactor (day 56), the acrylic acid concentration in the reactor was between 22 and 190 mg/L for the influent acrylic acid concentration of 200 to 800 mg/L until day 118. Even though there might have been some acrylic acid metabolism before day 118, it was not significant. On day 134, the AUR started to increase and reached 28 g/L per day on day 136 when the acrylic acid concentration in the feed was raised to 2000 mg/L. Meanwhile, assuming that Methanosarcina enrich-
ment cultures was the dominant species in the reactor (as signified by high AUR and low SRT values), the operational mode of the reactor was changed to fed-batch on Day 135.

Following the increase of influent acrylic acid concentration to 2000 mg/L in the supplemental acetic acid feed, the AUR increased for 3 days (days 136 to 138), and then started to decrease and eventually leveled off at approximately 27 g/L per day, which corresponded to an acrylic acid loading rate of approximately 170 mg/L per day (days 141 to 148).

As can be seen in Figure 2a, the influent acrylic acid concentration in the supplemental acetic acid feed was increased to 5000 mg/L on day 148. The AUR continued to increase for 2 more days (days 150 to 151). After reaching 39 g/L per day, the AUR started to decline. Starting from day 148 when the influent acrylic acid concentration was raised to 5000 mg/L, the acrylic acid loading rate to the reactor ranged between 380 to 650 mg/L per day for 13 days (days 149 to 161), and the acrylic acid concentrations measured in the reactor were less than 24 mg/L, corresponding to 99.99% bioconversion of acrylic acid (Fig. 2d). On day 163, the AUR and acrylic acid loading rate dropped to 3.4 g/L per day and 27 mg/L per day, respectively. Concurrently, the propionic acid concentration increased to around 1100 mg/L on day 167 but acrylic acid concentrations were still very low (>20 mg/L). From then on, a gradual recovery was observed and the AUR leveled off to approximately 5 to 6 g/L per day, which corresponded to an acrylic acid loading rate of about 100 mg/L per day.

Starting from day 175, a gradual recovery in the activity of the system started. The acrylic acid utilization rate increased to 163 mg/L per day on day 182 parallel to an increase in the AUR (Fig. 2b). After day 268, the AUR varied between 17 to 22 g/L per day until day 311, corresponding to an acrylic acid utilization rate of 260 to 330 mg/L per day (Fig. 2b and c). In this period, VSS, acrylic, acetic, and propionic acid concentrations in the reactor were around 3200, less than 20, 1950 to 2300, and 300 to 370 mg/L, respectively. Note that these high acetic acid concentrations are related to the operation of the computer-controlled pH-stat CSTR, which automatically maintained this acetate level, and not to the acrylic acid bioconversion. After about 300 days of acclimation, the maximum acrylic acid loading rate that was attained with the originally unacclimated Methanosarcina cultures was approximately 220 mg/L per day (0.068 g acrylic acid/g VSS per day). This value was significantly higher than the corresponding value of 66.7 mg/L per day reported by Qu and Bhattacharya (1996). This difference was thought to be due mainly to the extended microbial acclimation performed in this study.

**Anaerobic Biotransformation of Acrylic Acid with Acclimated Methanosarcina Enrichment Culture**

In the previous experiment unacclimated Methanosarcina cultures was acclimated to utilize 220 mg/L per day of acrylic acid in the presence of 2000 ± 200 mg/L of acetic acid as the primary substrate after about 300 days of acclimation. The results of the previous experiment reveal that acclimation had a very significant affect on anaerobic acrylic acid biotransformation in pH-stat CSTRs.

Therefore, in this experiment, the anaerobic biotransformation of acrylic acid was evaluated by seeding a pH-stat CSTR with an acrylate-acclimated Methanosarcina enrichment culture. The acclimated cultures was taken from the effluent of the pH-stat CSTR used in the previous experiment. The Methanosarcina cultures were acclimated to acrylic acid as the sole carbon source for 80 days and the acrylic acid utilization rate was between 160 and 220 mg/L per day at the time of taking the acclimated Methanosarcina seed (between days 137 and 146). After seeding, the reactor was fed with only acrylic acid feed and operated in fed-batch mode (Fig. 3).

Acrylic acid utilization rates started around 400 mg/L per day in the first day, but declined gradually with time (Fig. 3a). This gradual decrease continued until day 36 when the operation of the reactor was stopped. In spite of the continuous daily uptake of acrylic acid (130 to 500 mg/L per day) during the operation of the reactor, the acrylic acid concentrations measured in the reactor were less than 20 mg/L (Fig. 3b). However, propionic acid concentration increased steadily and exceeded 3000 mg/L on day 22, which indicated that negligible propionate was being utilized. Propionic acid concentration leveled off at around 2400 to 2500 mg/L. The VSS of the reactor was around 1100 mg/L on day 5, but decreased to around 400 mg/L on day 29, as seen in

**Figure 3.** Anaerobic biotransformation of acrylic acid with acclimated Methanosarcina enrichment culture in a pH-stat CSTR.
Figure 3d. The acrylic, volatile fatty acid concentrations measured in the reactor (Fig. 3b and c) suggested that acrylic acid was transformed completely to acetic and propionic acids (>99%). However, although methanogens could metabolize acetic acid and convert it to CH₄ and CO₂ fairly well, propionic acid could not be transformed significantly. Similar observations were also reported for anaerobic acrylic acid biotransformation in UASB reactors (Demirer and Speece, 1998a). The reason for this may be the insufficient number of propionate utilizers in the reactor, because the culture used to seed this reactor had an acrylic acid utilization rate of less than 200 mg/L per day and the dominant culture was originally acetate-enriched Methanosarcina. Furthermore, the maximum propionic acid concentration that the seed culture was exposed to was around 1100 mg/L (day 167 in Fig. 2) in the previous experiment. However, propionic acid concentrations up to 2500 mg/L were detected in this experiment, which probably resulted in the observed inhibition.

The results of this experiment indicate that, in 80 days, acrylate-acclimated Methanosarcina cultures at a concentration of 680 ± 80 mg/L as VSS could biotransform acrylic acid with a rate of 0.29 g acrylic acid/g VSS per day almost completely (>99%) to its intermediate products (mainly acetic and propionic acids), but could not metabolize propionate significantly.

**Anaerobic Biotransformation of Acrylic Acid with Homogenized Granular Culture**

Immobilization of anaerobic bacteria in biofilms and/or granules may enhance the stability as well as toxicity resistance of the process due to the fact that the maximum conversion rate will most likely be determined by diffusion limitation of the substrate (Lens et al., 1993). However, mass transport limitations due to the granular structure of the biomass result in poor performance of these systems in terms of specific activity; that is, lower reaction rates and affinity toward substrate (Alphenaar et al., 1993; Morvai et al., 1992).

On the other hand, even though physical homogenization of the granules destroys the layered structure and the communal synergism existing in the granule microenvironment, it will also enhance the overall mass transfer. For example, a distinct increase in the butyrate conversion rate and decrease in apparent half-saturation constant ($K_s$) were observed upon homogenizing the granule structure in a UASB reactor (van Lier et al., 1996).

Therefore, anaerobic biotransformation of acrylic acid, as the sole carbon source, was evaluated with homogenized granular biomass in this experiment. For this purpose, a pH-stat CSTR was seeded with a homogenized granular culture and operated for about 3 weeks with acetate being the only carbon source. In this period, the culture was adapted to the computer-controlled pH-stat CSTR operation. After this interval, acetate addition was terminated and only acrylic acid feed was added as the sole carbon source. The reactor was operated in fed-batch mode.

![Figure 4](image-url)  
*Figure 4.* Anaerobic biotransformation of acrylic acid with homogenized granular culture in a pH-stat CSTR.

In the first 19 days of the operation, the acrylic acid utilization rate varied between 400 and 600 mg/L per day. On day 20, the activity of the reactor dropped to near zero (Fig. 4a), and it took almost 50 days for the system to recover back to 500 mg/L per day of acrylic acid utilization rate. The homogenized granular culture started to biotransform acrylic acid to acetic and propionic acids from the initial days of operation. The concentration of acrylic acid was 72 mg/L on day 15, but never exceeded 30 mg/L thereafter (Fig. 4b). The acetic acid level in the reactor was 2000 ± 200 mg/L when it was being fed as the only carbon source. However, after acrylic acid was fed as the sole carbon source (day 0 in Fig. 4a), the acetic acid concentration started to decline and dropped to 1300, 700, and 200 mg/L on days 23, 42, and 62, respectively (Fig. 4c). After day 62, the acetic acid concentration remained around 200 mg/L until the end of the experiment (Fig. 4c). The propionic acid concentration elevated abruptly as the system utilized acrylic acid and reached 1700 mg/L on day 23. The rate of increase of propionic acid in the system slowed down after day 23, because the acrylic acid utilization rate dropped to very low levels due to inhibition of the system.

As seen in Figure 4a, the acrylic acid utilization rate started to increase on day 70 after about 50 days of inhibition. This 50-day period probably corresponded to acclimation of the culture to acrylic acid as the sole substrate. The increase in the activity of the system lasted until day 81 at which time the acrylic acid utilization rate was 2800 mg/L per day. This high acrylic acid loading rate resulted in another inhibition period for 5 days (days 82 to 87), but the
system recovered to around 2200 mg/L per day of acrylic acid utilization rate at a biomass concentration of 6900 ± 60 mg/L as VSS (0.32 g acrylic acid/g VSS per day) on day 88 when it stabilized with less than 10% variation until the end of the experiment (Fig. 4a). The propionic acid that built-up in the system started to decline parallel to the onset of the recovery in the acrylic acid utilization rate on day 70 (Fig. 4c). It dropped to near 200 mg/L on day 98 and stayed at that level for the rest of the experiment.

Initially, the homogenized granular culture contained a significantly higher amount of acetogens (or propionate utilizers) relative to Methanosarcina enrichment cultures, because homogenized granules represent a mixed culture enriched on a carbohydrate-based feed. In spite of this fact, the homogenized granular culture needed about 50 days of acclimation, before it could start to biotransform the propionate that was produced as the intermediate product of acrylate. Therefore, microbial acclimation is as important as the presence of acetogens in effective biodegradation of acrylic acid.

**CONCLUSIONS**

Similar to acrylic acid biotransformation in batch and UASB reactors (Demirer, 1996; Demirer and Speece, 1998a, b), microbial acclimation was found to be very significant for the biotransformation of acrylic acid in computer-controlled pH-stat CSTRs. For example, after 300 days of acclimation, a Methanosarcina enrichment culture in a pH-stat CSTR operating with a constant acetate concentration of 2000 ± 200 mg/L, as the primary substrate, was acclimated to utilize an acrylic acid loading rate of about 220 mg/L per day (0.068 g acrylic acid/g VSS per day). This value was significantly higher than the corresponding value of 66.7 mg/L per day reported by Qu and Bhattacharya (1996). With the acclimation strategy used in the experiments, acrylic acid was fed to the system in proportion to its performance in terms of AUR. Therefore, the cultures were not exposed to sudden increases of acrylic acid in the feed, which minimized the inhibition periods and time needed for acclimation. Furthermore, the computer-controlled operation of the reactors overcame the disadvantage of the general batch method in which high substrate levels can be maintained only for short periods, and of the general chemostat system in which high loading rates and high substrate concentrations are difficult to achieve due to pH changes of the system. All of these factors are thought to have contributed to the higher level of performance observed.

The same pH-stat CSTR culture, after 80 days of acclimation to acrylic acid as the sole carbon source, transformed acrylic acid up to the loading rate of about 200 mg/L per day (0.29 g acrylic acid/g VSS per day) almost completely (>99%) to acetic and propionic acids, but could not effectively metabolize these intermediate products. For example, the propionic acid level reached to 2400 to 2500 mg/L during this period. Because the cultures used in this experiment were acclimated to acrylic acid, it is speculated that there were not enough acetogens in the reactor to utilize propionate.

To verify this speculation, acrylicate-acclimated homogenized granular cultures that contained both acetogens and methanogens since it was fed on a carbohydrate waste was used in the third experiment. The experimental results indicate that acrylicate-acclimated homogenized granular cultures in a pH-stat CSTR biotransformed acrylic acid, as the sole carbon source, to acetic and propionic acids at a very similar rate (0.32 g acrylic acid/g VSS per day). Moreover, unlike the acetate-enriched Methanosarcina cultures, the homogenized granular cultures efficiently biotransformed acetic and propionic acids. Therefore, it can be proposed that better performance of the acrylicate-acclimated homogenized granular cultures in propionic acid biotransformation may be attributed to the presence of higher amounts of acetogenic organisms in the original granular cultures than in the Methanosarcina enrichment cultures.

Finally, it can be concluded that a controlled acrylic acid administration/acclimation strategy in parallel with proper pH-control and unlimited substrate levels achieved through computer-controlled operation of the reactors, and presence of mixed (acetogens as well as methanogens) anaerobic cultures, represent key parameters in accomplishing high anaerobic acrylic acid biotransformation rates of up to 0.32 g acrylic acid/g VSS per day.

**References**


