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Acetamide Degradation by a Continuous-Fed Batch Culture of *Bacillus sphaericus*

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ABSTRACT

The methanogenesis of acetamide occurs through a two-step reaction in methanogenic sludges. First, acetamide is hydrolyzed to acetate and ammonia by a strict aerobic bacterium *(Bacillus sphaericus)*, then acetate is used by *Bacillus* as carbon source or converted to methane by methanogens. In this work, the kinetics of acetamide degradation by *B. sphaericus* was studied in a continuous reactor with biomass accumulation, fed with acetamide. The oxygen supplied was dissolved in the feed (6.4 mg/L) to resemble conditions in an anaerobic wastewater treatment reactor. A reaction in series model (acetamide → acetate → biomass) was used to find the kinetic parameters. Results show that *B. sphaericus* can hydrolyze acetamide in a second-order reaction with K_1 = 1.1 L/g/d, implying that the amount of biomass determines the rate and that no reaction will take place at specific loading rates greater than 35 gAm/gX/d. Growth parameters on acetate, as carbon source, under limiting O_2 conditions, are μ_{max} = 0.102/d, K_s = 37 mg/L, Y = 0.081 gX/gAm.

Index Entries: Acetamide; hydrolysis; *Bacillus sphaericus*; continuous culture with biomass accumulation; UASB reactor.

INTRODUCTION

Acetamide is a highly toxic xenobiotic compound widely used in the lacquer, cosmetic, explosive, textile, and pharmaceutical industries *(1)*, and it is also produced by acetonitrile biodegradation *(2)*.

Acetamide degradation has been studied in an upflow anaerobic sludge blanket (UASB) reactor *(7)*. It was found that at low acetamide loading rates (B_v = 1 g. L/d), 86% removal efficiencies were obtained;

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Table 1
Experimental Design for Growth of *B. sphaericus* in Continuous Culture

Am_o (mg/L)	1000	1500	2000	3000	3000	3000	3000
HRT (d)	1	1	1	1	2	3	0.5

Am_o inlet acetamide concentration; HRT, hydraulic retention time.

Table 2
Kinetic Equations for Acetamide Uptake, Acetate Production, and Biomass Growth

$Am \overset{\nu_1}{\underset{MN}{\rightarrow}} Ac \overset{\nu_2}{\underset{MN}{\rightarrow}} X$	Sequence reaction (2)
$\frac{dAm}{dt} = rAm = -K_1 \cdot Am\ X$	Second-order reaction (3) Acetamide Hydrolysis
$\frac{dAc}{dt} = +K_1 \cdot Am\ X \frac{\mu_{max} \cdot Ac}{K_{Ao} + Ac} \frac{X}{Y}$	Acetate production and uptake (4)
$\frac{d\chi}{dt} = \frac{\mu_{max} \cdot Ac}{K_{Ac} + Ac} X$	Monod (5)

increasing B_v caused lower removal efficiencies. It was also noticed that acetamide caused inhibition of acetate methanization.

It was later found *(6)* that this degradation was possible through a synergistic association between a sporulating, Gram-positive, strictly aerobic rod, which transformed acetamide to acetate, and ammonia and methanogens, which transformed acetate to methane *(6)*.

$$CH_3\,CONH_2 + H_2O \xrightarrow{\textit{Bacillus sphaericus}} CH_3COO^- + NH_4^+ \quad (1)$$

$$CH_3COO^- + H_2O \xrightarrow{\textit{Methanogens}} CH_4 + HCO_3^-$$

The coexistence of facultative aerobes with strict anaerobes is possible when aerobic bacteria take up the available oxygen in the media *(3–8)*.

Acetamide degradation can be modeled in a two-step consecutive reaction, in order to find out if the rate limitations are caused by dissolved oxygen limitations or by the acetamide substrate inhibition. Therefore, the acetamide hydrolysis kinetics by *B. sphaericus* were studied in a continuous tubular reactor, with biomass accumulation, under limited amounts of oxygen.

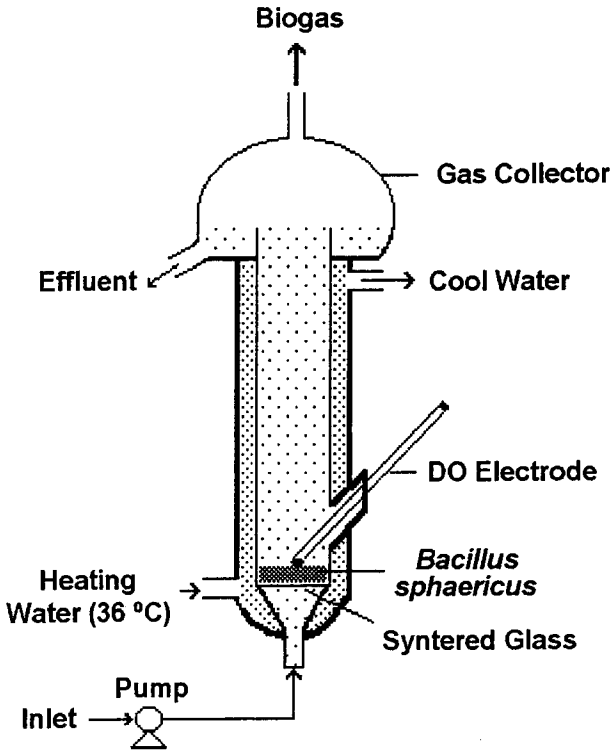

Fig. 1. Tubular reactor with syntered glass to retain the *B. sphaericus* biomass, and a gas separator.

MATERIAL AND METHODS

Continuous Cultivation

A 0.8-L continuous-operation volume tubular reactor (Fig. 1) was operated at 34–36°C to hydrolyze acetamide. It was inoculated with 80 mL of a pure strain of *B. sphaericus* in the exponential growth phase isolated from a UASB reactor *(7)*.

The feed was a solution consisting of Balch medium *(9)*, oligoelements, 0.5 g/L yeast extract, 0.1 g/L of casein peptone and acetamide, and saturated with air to get an oxygen concentration of 6.4 mg/L. Acetamide concentration and hydraulic retention times were varied, as shown in Table 1, to assess the kinetics constants. The media was adjusted to pH 7.0, and sterilized in an autoclave.

Analyses

Acetamide and acetate were determined in a Varian gas chromatograph with a flame ionization detector, using a capillary column (0.22 mm × 30 m) (At-1000, Altech), with helium as carrier gas. One-mL samples were pretreated with 50 μL of formic acid by centrifugation at 3000 rpm

Table 3
Transient Mass Balances in *B. sphaericus* Growth in Continuous Culture with Biomass Accumulation

$$\frac{dAm}{dt} = D(Amo - Am) - K1AmX \quad (6)$$

$$\frac{dAc}{dt} = - Dac + AmX - \frac{\mu^*Ac}{K_{Ac} + Ac}\frac{X}{Y_{XS}} \quad (7)$$

$$\frac{dX}{dt} = \mu X \quad (8)$$

for 15 min. Biomass was measured by $OD_{600\ nm}$ (Bausch & Lomb, Spectronic 20), and calibrated against known concentrations of *B. sphaericus*. Oxygen concentration was measured with a HACH meter (HACH, Loveland, CO).

Kinetic Characterization of the Strain

The affinity (K_s), the maximum specific growth rate (μ_m), and the yield (Y) constants were determined with the sequence reaction kinetic equations and mass balances shown in Tables 2 and 3, respectively. The acetamide hydrolysis is modeled as a second-order reaction (Eq. 3), in which acetate is an intermediate product in a sequence reaction (Eq. 2 and 4). Biomass growth follows Monod kinetics (Eq. 5). The liquid phase is in continuous flow while biomass is retained within the vessel.

RESULTS AND DISCUSSION

Figure 2 shows the acetamide loading rates (B_v) applied to the reactor, together with the outgoing rates of acetamide and acetate. The largest acetamide uptake efficiency was observed at a B_v = 1 gAm L/d. At Bv = 1.5, acetate accumulates and the biomass remains constant (Fig. 3). It is only in the B_v = 2 when biomass starts to accumulate, while acetate and acetamide start to be consumed. By B_v = 3, acetate is being totally consumed, while biomass growth rate is the highest. When the B_v is again reduced to 1.5 by increasing the HRT, biomass builds up to 900 mg/L. With this high biomass concentration, acetamide and acetate are both at almost zero. During this period, there is no acetamide hydrolysis and no growth, probably because of the sevenfold specific acetamide load to the bacteria (Fig. 4).

Although a limited amount of oxygen is being fed to the reactor, Table 4 shows that the reaction itself is not limited by oxygen, because the amount available for the bacteria is inversely proportional to the biomass concentration.

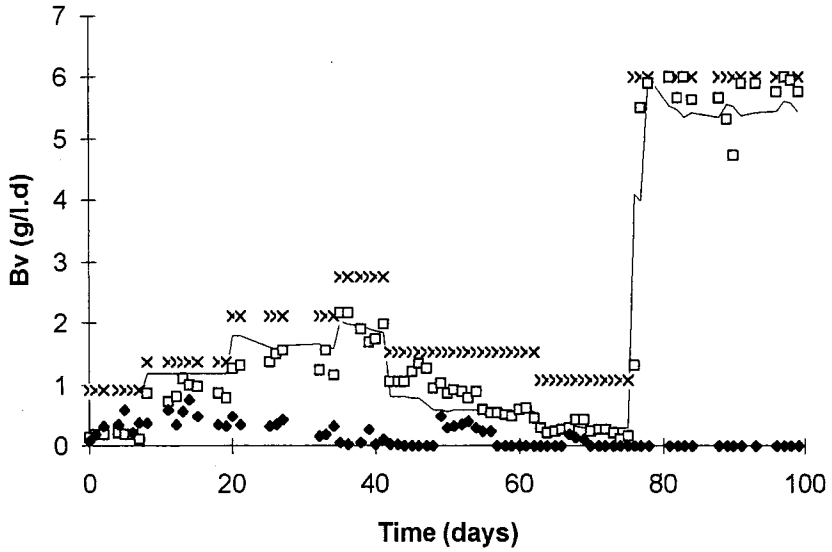

Fig. 2. Continuous culture of *B. sphaericus;* acetamide loading rate (X), acetamide (□), and acetate (◆) accumulation rates. The acetamide predicted behavior (−) as described in Eq. 3 with K_S = 37 mg/L and μm = 0.10 per d.

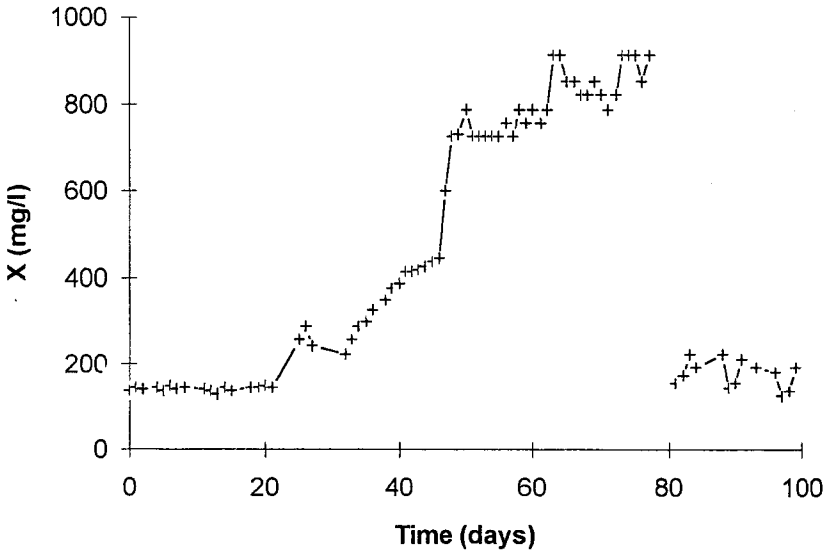

Fig. 3. *B. sphaericus* biomass accumulation in the tubular reactor exposed to several loading rates. The brokes line is a reinoculation of the reactor.

The hydrolysis rate of acetamide follows a second-order reaction, as shown in Eq. 3. Reaction constants were evaluated by both algebraic (selecting steady states) and differential methods (taking discrete increments in differential Eq. 6), in order to take all the experimental points into ac-

Table 4
Acetamide Hydrolysis by *B. sphaericus*

B_v (g/l/d)	Am_i (mg/L)	η_m (%)	Ac (mg/L)	η_c (%)	X (mg/L)	B_{OD} (mgOD/gχd)	B_x (gAm/gχd)
1	1000	88	411	41	157	32.6	6.4
1.5	1500	44	536	36	163.25	31.4	9.2
2	2000	37	354	18	288.25	18	7
3	3000	28	31.2	1	413.25	12.4	7.26
1.5	3000	43	0	0	788.25	3.2	1.9
1	3000	78	0	0	913	2	1.1
6	3000	4.4	0	0	207	49.46	29

B, acetamide loading rate; Am, acetamide concentration; η, conversion efficiencies; Ac, acetate concentration; X, biomass; subindex: v = volumetric; x = specific; m = acetamide; c = acetate; and OD = oxygen dissolved.

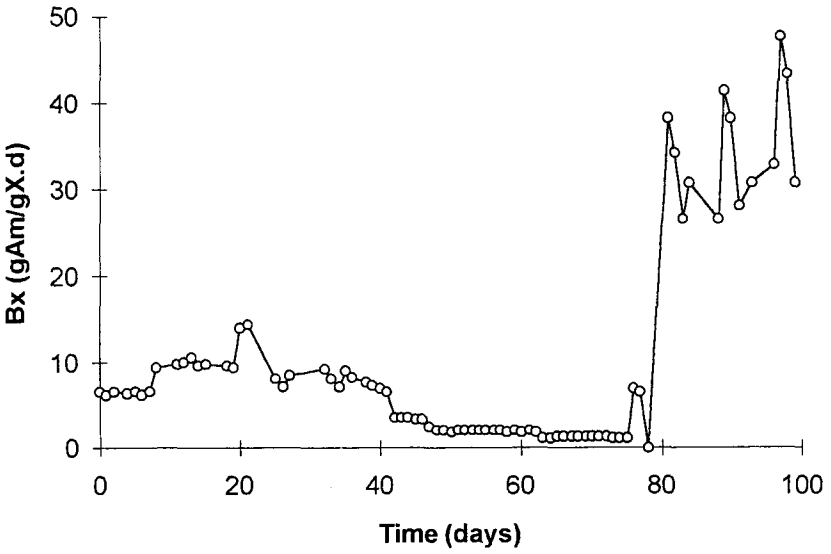

Fig. 4. Specific acetamide loading rate (gAm/gX/d).

count. The results obtained by these two methods were similar: K_i = 1.1 and 0.8 L/gX/d for the algebraic are differential methods, respectively. The continuous line in Fig. 4 shows the predicted values obtained by Eq. 6.

Solving Eqs. 7 and 8 simultaneously, the growth yield coefficient was found to be $Y_{X/Ac}$ = 0.018 gX/gAc.

Figure 3 shows a period of exponential growth between d 30 and 48. It was used to estimate the kinetic parameters of Monod equation. By equating Eqs. 5 and 8, and solving for a linear regression, the values of μ_m and K_s were estimated to be 0.102 per d and 0.0367 gAc/L, respectively.

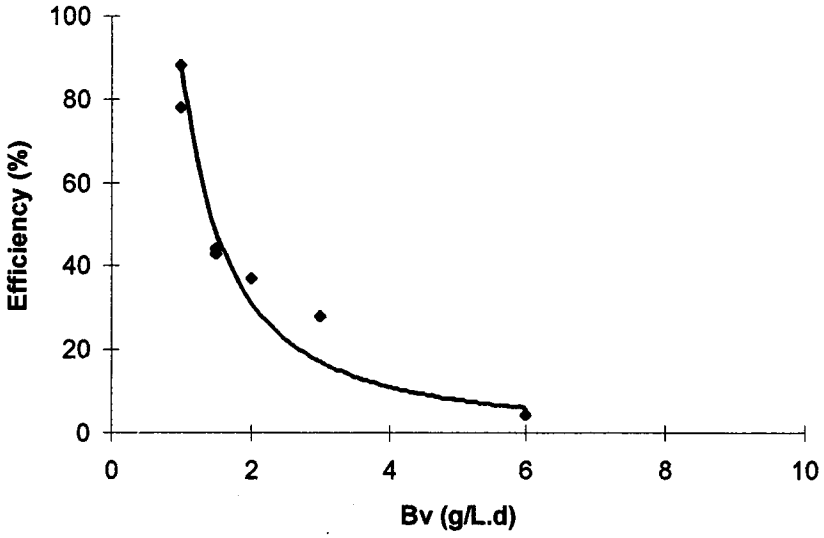

Fig. 5. Hydrolysis efficiency as a function of B_v. Large efficiencies are obtained at low rates.

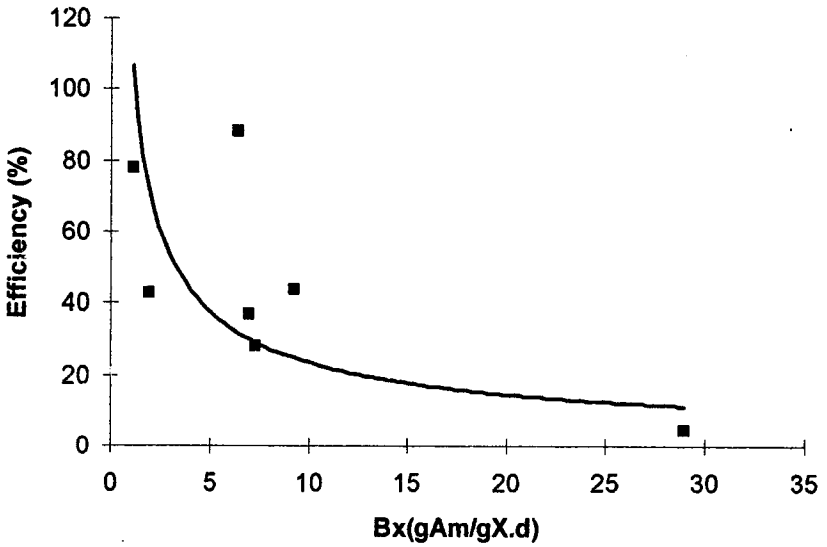

Fig. 6. The hydrolysis efficiency, as a function of the biomass loading rate, shows the same tendency as the B_v effect.

Table 4 shows the steady-state values of each run. From these data, Fig. 5 shows that the hydrolysis efficiency drops as B_v increases. This is also associated to the biomass loading rate (B_x = gAm /gX/d) (Fig. 6).

The specific acetamide hydrolysis rate (gAm/gX/d) is a negative function of acetamide concentration, thus suggesting acetamide substrate inhibition (Fig. 7). Two runs are out of this tendency, because the reaction rate

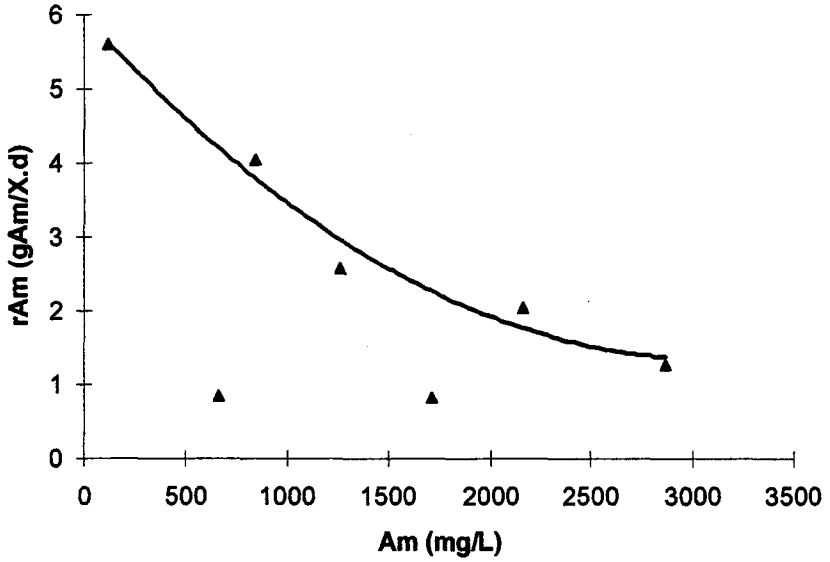

Fig. 7. The specific acetamide hydrolysis rate, as a function of the concentration, suggests a decreasing rate with increasing concentration. The two points out of the tendency are limited by loading rate.

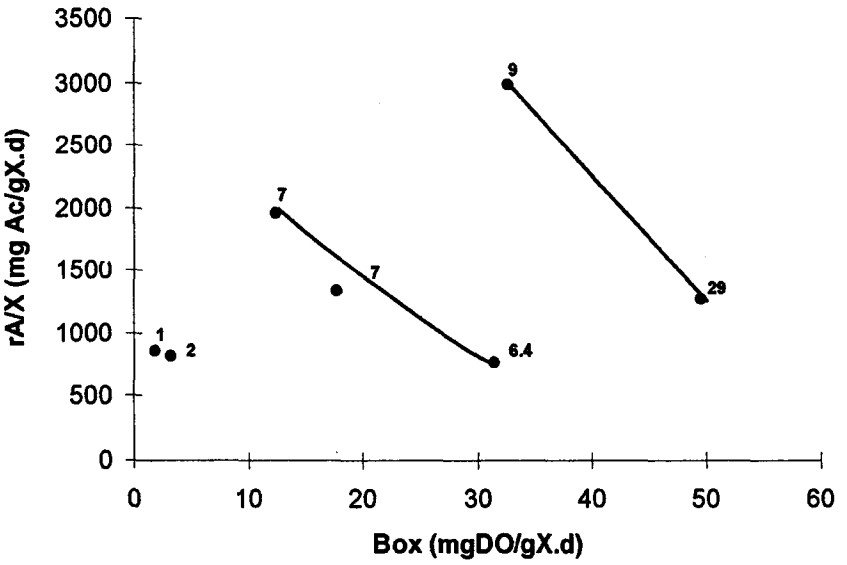

Fig. 8. Specific acetate consumption rate shows the negative effect of the B_{ox} at three different levels of B_x.

(gAc/L/d) is limited by the loading rate (gAmo/L/d), and the specific oxygen loading rate (B_{ox}).

The acetate uptake rate is negatively influenced by the level of specific oxygen loading rate (B_{ox} = mg O_2/g X/d). Figure 8 shows the specific acetate consumption rate as a function of both the B_{ox} and the B_x. It suggests that increasing B_{ox} adversely affects the acetate consumption rate. Three B_x levels can be distinguished here, one at the level 1–2 gAm/gX/d, another at around 7, and a third one of $B_x \geq 9$, suggesting that increasing specific acetamide loads need more oxygen.

CONCLUSIONS

These results show that *B. sphaericus* can degrade acetamide and does not need the participation of methanogens for it to use acetate as substrate for growth. At 6.4 mg oxygen/L, *B. sphaericus* exhibited a K_s for acetate of 37 mg/L, which makes it a better scavenger than methanogens that have K_s = 160–300 mg/L. Under these conditions, the coculture is possible, because *B. sphaericus* grows slowly with a μ_m = 0.10 per d; methanogens exhibit twice this value *(10)*.

Given that the hydrolysis of acetamide is intended as a first step for its methanization, the present study orients toward the coculture conditions. Results suggest that, in order to promote high acetamide hydrolysis and keep a low acetate consumption rate by *B. sphaericus*, the biomass loading rates (B_x) should be about 1 or 2 gAm/gX/d at low Am concentrations and low oxygen-loading rates (B_{ox} = 3 mgO_2/gAm/d). Under these conditions there would be enough acetate for the methanogens.

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