Biomass and Bioenergy Vol. 5, No. 5, pp. 395–400, 1993
Printed in Great Britain.

0961-9534/93 $6.00 + 0.00

EFFECT OF NATURAL ZEOLITE SUPPORT ON THE KINETICS OF COW MANURE ANAEROBIC DIGESTION

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(Received 3 February 1993; revised received 15 June 1993; accepted 20 August 1993)

Abstract—The kinetics of anaerobic digestion of cow manure has been studied using a batch bioreactor with biomass immobilized on zeolite and a control digester. The apparent kinetic constant, K_o, and the mean rate of methane production decreased considerably with substrate concentration in the control digester showing a major inhibition process due to ammoniacal nitrogen accumulation. However, in the zeolite digester, the kinetic constant was virtually invariable in the COD range studied (1.4–9.8 $g\,l^{-1}$) due to the ionic exchange between the support and substrate. In addition, the yield coefficient was 5 times larger than the control digester for high substrate concentrations (9.8–10.3 g COD l^{-1}).

Keywords—Anaerobic digestion, cow manure, immobilization, kinetics, zeolite.

1. INTRODUCTION

Conventionally, anaerobic digestion of cow manure has utilized single pass unmixed reactors with hydraulic retention times (HRT) greater than 30 days.[1,2] Even by introducing mixing, as is the case in high rate digesters, it is still necessary to employ an HRT in the region of 20–30 days.[3,4] These long retention times necessitate large reactor volumes with the consequent additional capital and operating costs. The requirement for a long HRT may be attributed to the presence of complex organic components with a low biodegradation potential and also, possibly, due to the high concentration of ammonia.[5,6]

The toxicity of ammonia is strongly influenced by pH which determines the equilibrium concentration of free ammonia to the ammonium ion in solution. Values as low as 150 $mg\,l^{-1}$ NH_3–N have been reported as being toxic to anaerobic digestion at pH 8.[5] Under these conditions the proportion of free ammonia which is most toxic might be expected to be higher. Most digesters, however, work at around neutrality or under slightly acidic conditions where the equilibrium is biased towards the ammonium ion which has a far lower toxicity. Reports in the literature rarely distinguish between free ammonia and the ammonium ion in analytical methods and in the reporting of results. It is therefore not uncommon to find a wide range of values reported at which the digestion process is deemed to have been inhibited by ammonia/ammonium ion.[7,8] It is, however, well accepted that toxicity effects may be detrimental to the anaerobic process performance in both homogeneous and fixed film systems.[8] A discontinuous linear negative correlation has been shown between ammoniacal nitrogen concentration and methane production rate[9] in UASB bioreactors treating piggery wastewater. More specifically the anaerobic digestion of cow manure[6,10] has been found to be very difficult at ammoniacal nitrogen concentrations of 3 $g\,l^{-1}$; in both these cases the authors attributed these difficulties to ammonia toxicity rather than high volatile solids loadings. The effect is likely to be more pronounced on the methanogenic population in the digester as these are reported to have the greatest sensitivity,[11] *Methanobacterium formicum*, for example, was shown to be at least partially inhibited at an ammoniacal nitrogen concentration of 3.3 $g\,l^{-1}$. Not only do the variations in pH make it difficult to produce meaningful figures relating to toxicity but there is also a growing weight of evidence which suggests that the anaerobic consortium of bacteria can acclimatize to high ammonia concentrations.[12,13]

A combination of both of these may explain the wide range of toxicity values reported and even account for high values such as those encountered by Parking[7] and Speece[8] in completely mixed and biofilm reactors where ammoniacal nitrogen concentrations between 4000 and 14 000 mg l^{-1} have been observed.

Potentially control could be exerted by the sequestering of ammonium ions from solutions using ion exchangers. Among the materials commonly used for this purpose is zeolite[14–17] which has shown removal rates of 0.05 g NH_4^+ g^{-1}. Used in isolation in a column of aqueous solutions removals near to 95% have been observed when operated in the downflow mode. The material has also been used as a selective exchanger of phosphorus and nitrogen compounds from municipal wastewaters.[18–19] In addition, zeolite has been found to be a successful support for the immobilization of microorganisms in anaerobic digestion.[20] The results obtained through previous research on the microbiology and biochemistry in anaerobic processes show the influences that different supports have on the immobilization of the microorganisms which carry out digestion.[21–25] Considering these two roles, the aim of this work was to carry out a comparative kinetic study on the anaerobic digestion of cow manure in two bioreactors: one with biomass supported on zeolite and the other with suspended biomass.

2. MATERIALS AND METHODS

2.1. *Experimental design*

Two glass flasks of one litre volume were used as digesters. The flasks were hermetically closed by rubber caps provided with two holes; one to introduce the fresh manure and the other for the biogas outlet. A lateral outlet was employed for mixed liquor sampling. The digesters were mixed by a magnetic stirrer at 350 rpm and were placed in a water bath at 35°C. A washing flask of 100 ml filled with a solution of 10% NaOH was used to bubble the biogas and remove the CO_2.

2.2. *Inoculum*

The digesters were inoculated with 200 ml of well digested cattle sludge (180 days digestion time) with a total solids (TS) concentration of 4.5% and volatile solids (VS) of 58.3% on a dry basis. Distilled water was used to bring the total volume to one litre.

2.3. *Support*

One digester was left as a control and the other received 20 g of zeolite (41% clinoptilolite, 40% modernite and 19% montmorillonite, calcite, quartz and vulcanic glass). The particle size was less than 2 mm and the elemental composition was: SiO_2: 67.9%, Al_2O_3: 11.9%, Fe_2O_3: 2.1%, CaO: 2.8%, MgO: 1.2%, Na_2O: 1.5%, K_2O: 1.1% and H_2O: 11.5%. This is a silicate with elongated structure, low fragility, high porosity and high specific surface area which makes it a particularly easy substance for anaerobic microorganisms to attach themselves to.[20] The amount of exchanger support material was 20 g l^{-1}. While larger amounts of support increased the biomass concentration and exchange effect, they also increased the apparent viscosity of the medium, thereby hindering mass transfer and decelerating the process.[25]

2.4. *Experimental procedure*

The experimental period began when the methane gas production of the mixture inoculum–water ceased. Five experimental runs were performed by adding different volumes of raw waste (20 ml, 40 ml, 80 ml, 120 ml and 160 ml) to the digesters. The fresh manure was prescreened in a 2 mm sieve. The characteristics of fresh manure used in the different runs are given in Table 1. After wastewater addition the cumulative methane production was measured. The duration of each experiment was the time interval for maximum gas production and COD removal from each load. During all of the experiments the reactors were run in a fill and draw mode with the volume of settled supernatant removed being equal to the volume of feed added; in all cases the settlement period was 2 h which was sufficient for separation and retention of the biomass.

2.5. *Analyses*

Total suspended solids (TSS), volatile suspended solids (VSS) and pH were determined in the mixed liquor at the beginning and the end of the experiment. Total COD (TCOD), alkalinity,

Table 1. Characteristics of fresh manure fed at different runs

Run	Load (ml)	TCOD ($g\,l^{-1}$)	Alkal. ($g\,l^{-1}$)	TVFA ($g\,l^{-1}$)	AMM. ($g\,l^{-1}$)	pH
1	20	52.1	9.0	4.2	1.9	8.1
2	40	41.2	7.7	2.9	2.1	8.3
3	80	45.4	7.4	7.3	1.9	8.1
4	120	50.1	7.9	8.0	2.2	7.9
5	160	47.3	7.2	2.6	2.0	7.8

Table 2. Characteristics of the supernatant at the beginning of the experiments in both digesters

	Control	Zeolite
TCOD ($g\,l^{-1}$)	1.2	1.2
Alkal. ($mg\,l^{-1}$)	2100	2250
TVFA ($mg\,l^{-1}$)	115	160
Ammoniacal-N ($mg\,l^{-1}$)	350	200
pH	8.2	8.3

total volatile fatty acids (TVFA), ammoniacal nitrogen and pH were analyzed in the feedings of each run and in the supernatant at the beginning of the experiment. The experiments were performed in duplicate and the results averaged.

The analysis followed the recommendations of the Standard Methods.[26] Ammoniacal nitrogen determination was carried out by distillation of the samples previously buffered at pH 9.5 with a borate buffer solution and titration with NaOH of the distillates collected in excess sulfuric acid. Alkalinity measurement was done by a titration method, the end point being pH 4.5. TVFA values were obtained using a conventional distillation method of the sample previously acidified at pH 3.5 and subsequent titration of the distillate with NaOH using phenolphthalein solution as indicator.

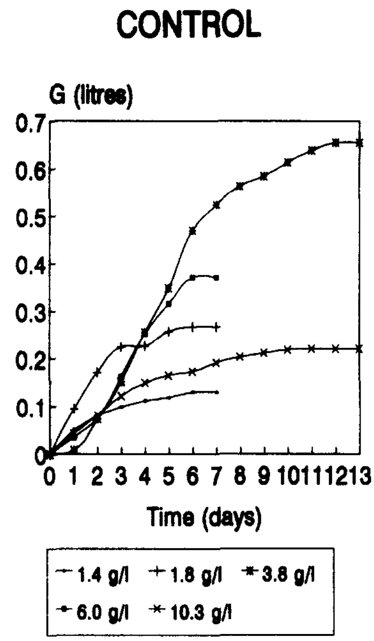

Fig. 1. Variation of the methane volume accumulated, G (l), with time (days) for the experiments with different initial COD levels ($g\,l^{-1}$) in the control digester.

3. RESULTS AND DISCUSSION

The concentration of TS and VSS in the mixed liquor before the experimental period was: $14.1\,g\,l^{-1}$ and $8.2\,g\,l^{-1}$ (control) and $34.0\,g\,l^{-1}$ and $8.3\,g\,l^{-1}$ (zeolite), respectively. The addition of zeolite resulted in an increase of the TSS, while VSS and pH remained practically constant (8.2–8.3).

The characteristics of the supernatant at the beginning of the experiments are summarized in Table 2. The values of this table show that alkalinity and pH in the zeolite digester increased while the ammoniacal nitrogen decreased due to cationic exchange. Zeolite sequesters ammonium ions and delivers calcium, magnesium and sodium.[27]

Figures 1 and 2 show the accumulated methane volumes (dry gas at standard temperature and pressure, expressed in litres) at different times (days) for the different feed volumes used in both the control and zeolite digesters. The variable concerned was the COD level, achieved by varying the volume of wastewater added to the digesters. The results show:

(a) For a given time, the methane volume produced decreased in the order: zeolite > control.
(b) The shape of the kinetic curves was very similar in both cases studied.

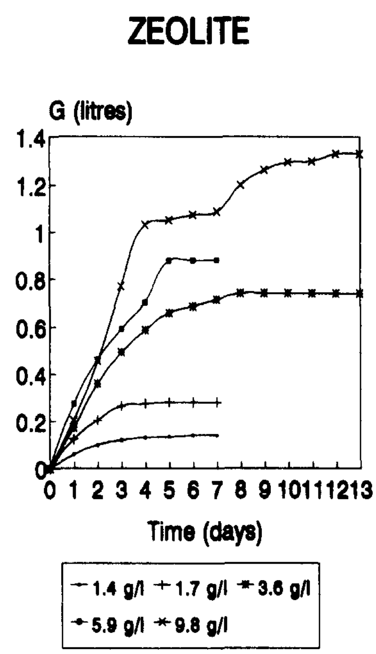

Fig. 2. Variation of the methane volume accumulated, G (l), with time (days) for the experiments with different initial COD levels ($g\,l^{-1}$) in the zeolite digester.

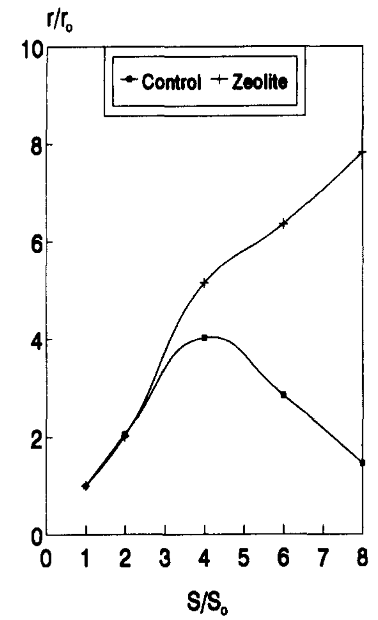

Fig. 3. Variation of the mean rate of gas production (r/r_0 as a function of the substrate concentration (S/S_0).

The mean rates of gas production (r) referring to the digester volume, were calculated after a digestion time of 7 days. In Fig. 3, S_0 denotes the lowest substrate concentration used in each experiment and r_0 the corresponding mean rate. The plot of r/r_0 versus S/S_0 (Fig. 3) shows a maximum which is well defined in the control digester, after which the relative rate decreased gradually. This phenomenon suggests the occurrence of an inhibition process at concentrations slightly higher than those investigated. On the other hand, the mean rate of gas production increases gradually with the substrate concentration in the zeolite digester. The mean rate relationship between the zeolite digester and the control digester increased as the loads increased showing the resistance of the zeolite digester to the inhibitory effect.

In order to characterize each experiment kinetically and thus facilitate comparisons, the following equation was used:[25]

$$G = G_m[1 - \exp(-K_o t)] \tag{1}$$

where: G (l) is the volume of methane gas accumulated at a given time t (days); G_m (l) is the maximum volume accumulated at an infinite digestion time and is the product of the initial substrate concentration and the yield coefficient of methane; K_o (days^{-1}) is an apparent kinetic constant that includes the biomass concentration ($K_o = KX$). The fact that the biomass concentration in the digesters (X) remains virtually constant (8.2–8.3 g VSS l^{-1}) facilitates interpretation of the results. In fact, on comparing the apparent kinetic constants it is seen that all of them are multiplied by the same factor (X).

Equation (1) coincides with that proposed by Roediger in Edeline (1980)[28] to relate the volume of gas in a batch anaerobic digestion process. According to this equation, methane production conforms to a first-order kinetic model.[29,30]

As can be seen in Figs 1 and 2, curves were obtained coinciding with the predictions of eqn. (1). Thus, G was zero at $t = 0$, and the rate of gas production became zero at $t = \infty$. Also, the slopes of the curves decreased with increasing time. On the other hand, taking Napierian logarithms in eqn. (1), and ordering the terms, the following is obtained:

$$Ln[G_m/(G_m - G)] = K_o t \tag{2}$$

indicating that $Ln[G_m/(G_m - G)]$ versus t should give a straight line of slope equal to K_o with intercept zero. As an example, Fig. 4 shows part of the experimental data for both digesters. The value of G_m has been considered to be equal to the volume of methane accumulated at the end of each experiment. Representation of the experimental data as indicated, eqn. (2), gives straight lines with the intercept practically at zero. Thus, it is possible to fit the experimental data to the proposed model. Once it had been

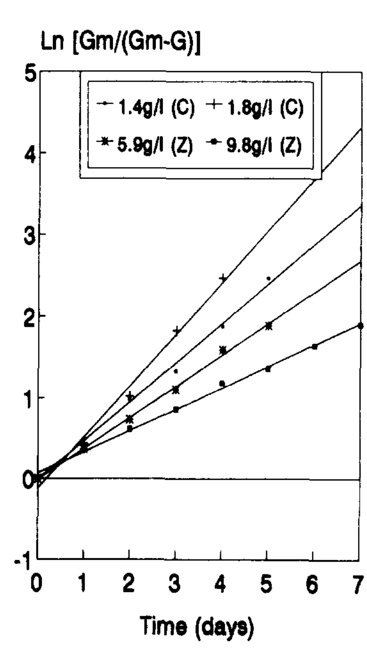

Fig. 4. Representation of the $Ln[G_m/(G_m - G)]$ values versus time (days) for both digesters. (C): control; (Z): zeolite.

Table 3. K_0 values ($days^{-1}$) with 95% confidence limits for each digester and experiment

Load (ml)	Control	Zeolite
20	0.49 ± 0.09	0.57 ± 0.05
40	0.44 ± 0.10	0.61 ± 0.08
80	0.07 ± 0.08	0.34 ± 0.07
120	0.09 ± 0.04	0.37 ± 0.09
160	0.02 ± 0.06	0.25 ± 0.07

checked qualitatively that the experimental data conformed to the proposed model, the parameter K_0 was calculated analytically using a nonlinear regression program.[31] Table 3 lists the K_0 values with 95% confidence limits obtained for each digester and feed volume.

From the results it can be seen that the apparent kinetic constant for the control digester decreased considerably with the substrate concentration, which reveals the occurrence of a major inhibition effect due to ammoniacal nitrogen accumulation. However, in the zeolite digester the inhibitory effect is considerably diminished due to the ionic exchange between the support and substrate. This behaviour is demonstrated by the slight variation of the kinetic constant that remained virtually invariable in the range of 20–120 ml feed volume. The ratio between the kinetic parameters of the zeolite digester and the control digester increased gradually with the substrate concentration to a maximum value of 12 times for the maximum substrate concentration studied. This behaviour is according to the exchange of ammonium ion (between the support and the medium) that occurs in the digester with zeolite, which determines a virtually constant and low concentration of ammoniacal nitrogen in the effluents of the reactor (Table 4). In contrast to this, the control digester shows an increase in the ammoniacal concentration from 0.45 to 1.30 $g\,l^{-1}$ when the feed volume increases from 20 to 160 ml.

Table 4 lists the initial and final COD values, ammoniacal nitrogen concentration in the effluents, and maximum methane volume for the digesters used.

The yield coefficient of methane, Y_p, was determined from the maximum methane volume produced and the initial and final COD, which were known in each case (Table 4). This coefficient was virtually constant for the zeolite reactor in the range of CODs studied and its average value was 0.32 l CH_4 STP g^{-1} COD. In contrast to this behaviour, the control digester showed a constant value for this coefficient only in the range 20–120 ml of feed volume (0.325 l CH_4 STP g^{-1} COD); for a feed volume of 160 ml, the coefficient decreased to a value of 0.06 l CH_4 STP g^{-1} COD, 5 times lower than for the zeolite digester, showing a major inhibition process in methane production.

4. CONCLUSION

Natural zeolite containing clinoptilolite (41%, see Section 2.3) have ion exchange properties showing high selectivity for ammonium ion. Because of the properties of these materials as ionic exchangers and adsorbers they neutralize biological media by ionic exchange, and can trap cells increasing their viability. The property of ionic exchange is very useful in anaerobic wastewater treatment of cow manure, because of the high amounts of ammoniacal nitrogen and therefore of ammonia ions generated by bacterial metabolism during the anaerobic treatment.

As is shown in the experimental work developed, the combined effect of ionic exchange and biofilm fixing significantly improved the kinetic constants, mean rate of methane production and yield coefficient of zeolite-supported digester in comparison with the control digester.

Table 4. Initial and final COD values ($g\,l^{-1}$), ammoniacal nitrogen concentration in the effluents ($g\,l^{-1}$) and maximum methane volume, G_m (l), obtained in the digesters used

Load	Control digester				Zeolite digester			
(ml)	I. COD	F. COD	G_m	Amm.	I. COD	F. COD	G_m	Amm.
20	1.40	0.50	0.130	0.45	1.40	0.40	0.139	0.35
40	1.80	1.10	0.267	0.60	1.70	0.85	0.281	0.35
80	3.80	1.95	0.656	0.70	3.60	1.90	0.741	0.30
120	6.00	5.20	0.370	1.00	5.90	4.60	0.883	0.35
160	10.30	6.60	0.220	1.30	9.80	6.20	1.330	0.35

Acknowledgements—The authors want to acknowledge the support of the Alexander Von Humboldt Foundation and Program of Scientific Cooperation with Iberoamerica (Spanish Foreign Ministry) to develop this work.

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