BIOPARTICULATE SOLUBILIZATION AND BIODEGRADATION IN SEMI-CONTINUOUS AEROBIC THERMOPHILIC DIGESTION

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Abstract. The effects of charge size, cycle time, and the presence of added N on the extent of solubilization and biodegradation of microbial solids were investigated during semi-continuous operation of an aerobic thermophilic digestion process. Use of a charge size of 50% of the bioreactor operating volume resulted in the removal of more than 59% of the total suspended solids concentration in the feed and showed enhanced performance compared with when a 25% charge size was used. The addition of a supplementary N source resulted in enhanced dissolved organic carbon (DOC) concentrations with the 50% charge size, but did not affect overall solids removal. With the 25% charge size, addition of a supplementary N source resulted in an overall improvement in process performance.

1. Introduction

Both microbial growth theory and most research results concerning microbial biodegradation processes are only directly relevant to soluble and/or miscible C energy substrates. This is in spite of the fact that particulate biodegradable substrates are widely encountered in wastewaters and waste sludges and in natural aquatic environments. Waste sludge biodegradation processes require solubilization of the particulate matter present prior to biodegradation of the released soluble lysis products. Despite the fact that the rate of solubilization is the limiting step in waste sludge biotreatment, relatively little research has been directed towards this aspect of such processes.

Recently, several West European governments have shown concern about the effectiveness of existing waste sludge treatment technologies, particularly with regard to the removal of pathogenic organisms and toxic chemicals. Sludge treatment under aerobic thermophilic conditions has been proposed as a means of improving current sludge treatment technology. It is intended that such processes be operated as pre-treatment processes in conjunction with installed conventional anaerobic mesophilic sludge treatment. Aerobic operation in the first stage is preferable when toxic compounds which are essentially recalcitrant under anaerobic conditions but which can be biodegraded under aerobic are present in waste sludges (McEvoy and Giger, 1986).

Several municipal aerobic thermophilic waste sludge treatment plants operate in the semi-continuous mode (Zwiefelhofer, 1985; Wolinski, 1985) as opposed to continuous flow mode described by most researchers (Smith *et al.*, 1975; Keller and Berninger, 1984; Kabrick and Jewell, 1982). The former have the advantage of a defined minimum

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retention time, a factor of critical importance with respect to the hygienization potential of the process.

Our understanding of the solubilization/biodegradation of microbial solids in aerobic thermophilic biotreatment systems remains scant. Hamer and Mason (1987) have proposed a model for the biodegradation of microbial solids in a semi-continuous system whereby growth of the aerobic thermophilic population occurs on the released soluble compounds contained in the cell cytoplasm following enzymatic degradation of a part of the feed microbe cell wall. The cytoplasm is under a high osmotic pressure such that lysis of the feed microbe cell wall easily causes release of the cell contents. Growth on this soluble substrate occurs simultaneously with the production of acetate when the system is run under O restricted conditions. As time progresses, the remainder of the cell wall fractions is hydrolyzed into utilisable soluble substrates whilst the accumulated acetate is used as an additional growth substrate. The effects of varying the charge size and cycle time (minimum particle residence time) on the performance of this process cycle have never been investigated in detail. Moreover, most treatment plants operate only on weekdays whereby on Mondays to Fridays a 24 hr cycle is normal, whereas charging the reactor between Friday and Monday allows a 3 day cycle time. Therefore, it was also of interest to examine the effects of cycle time on process performance.

The final variable investigated in these experiments was the question as to whether there was a requirement for addition of further nutrients. Whilst phosphate is most unlikely to be limiting in such processes, the availability of N could well be a problem. Nitrogen, when not present in excess would have to be obtained from organic-N components of the feed microbial cells. The differences in behavior of process systems in the presence of excess inorganic-N and under reduced availability of such sources were assessed.

2. Materials and Methods

2.1. BIOREACTOR AND OPERATING CONDITIONS

A 1.5 L bioreactor (Bioengineering AG, Wald, CH) with full measurement instrumentation, i.e., pH, dissolved O concentration, temperature and impellar speed monitoring, was used for the experiments. Air (6 L hr^{-1}) was sparged into the bioreactor which was operated with an impellar speed of 900 rpm. The operating temperature was maintained constant at 60°C and the working volume was 1300 mL. The pH was not controlled.

2.2. AEROBIC THERMOPHILIC CULTURE AND FEED

Aerobic thermophilic microbes were obtained from an operating municipal waste sludge aerobic thermophilic digester (UTB, Umwelttechnik Buchs AG, Buchs/SG, CH). Pressed bakers yeast (at concentrations between 30 and 60 g L^{-1}) as the sole biodegradable C and N source was suspended in a nutrient solution containing KH_2PO_4 , 8 g L^{-1} ; K_2HPO_4 , 5.7 g L^{-1} ; ZnO , 3.26 mg L^{-1} ; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 43.2 mg L^{-1} ; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 16 mg L^{-1} ; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 1.36 mg L^{-1} ; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 3.8 mg L^{-1} ;

H_3BO_4 , 0.5 mg L^{-1} ; and MgCl_2 , 0.2 g L^{-1} . The stock solution was stored at 4°C for no longer than 5 days. Start-up was carried out by inoculating 500 mL aerobic thermophilic sludge into 800 mL of preheated (60°C) yeast suspension. The bioreactor was operated on a fill and draw cycle of either 25 or 50% bioreactor volume withdrawal of spent broth, followed by addition of the same volume of fresh yeast cell suspension. Yeast addition was carried out slowly so as not to cause a drop in temperature.

2.3. ANALYTICAL

5 mL samples were centrifuged at $30\,000 \times g$ and the supernatant removed. The solids were resuspended in water, poured into tared crucibles and left overnight at 105°C . They were reweighed after cooling in a dessicator.

After drying overnight, the crucibles used to determine dry weight were placed in an oven at 600°C for at least 5 hr, allowed to cool and then reweighed.

Appropriate dilutions of the supernatant were made and the DOC assayed directly on a TOCOR total organic C analyzer (Maihak AG, Hamburg, D). Inorganic C was removed by acidification and sparging with N.

$\text{NH}_4^+ - \text{N}$ was measured in the extracellular medium using an automated N analyzer (Skalar Analytical, Breda, NL).

All chemicals were of analytical grade and supplied by either Fluka (Buchs/SG, CH) or Merck (Darmstadt, D).

3. Results

The extent of solubilization and biodegradation of microbial cells in semi-continuous culture in which aerobic thermophilic bacteria are growing was investigated to examine the effects of charge size, bioreactor residence time and inorganic-N availability. Differences were obtained with respect to the amount of solids biodegraded at different residence times and inorganic-N source concentrations in the bioreactor feed. The results are shown in Figures 1 to 5. Figure 1 shows the amounts of total suspended solids in the feed and after 7 and 24 hr in the bioreactor. From the data for a 25% fill and draw volume and 1 day cycle time it can be seen that the amount of solubilization and biodegradation occurring between 7 and 24 hr is almost negligible, i.e., most effects occur during the first 7 hr in the bioreactor. Some increase in the amount of solids degraded between 24 and 72 hr can be seen from the data from 3 day cycle times. Significantly better biodegradation in terms of the percentage of total solids removed can be achieved with the larger fill and draw volume (50%), where the extent of total solids removal was consistently greater than 59% (of feed microbial cell concentration) irrespective of cycle time and the presence or absence of an inorganic-N in the feed (Figure 2). When a 25% charge size was used, the degradation efficiencies were lower and affected by the presence or absence of N in the feed. The volatile suspended solids were found to behave in the same manner as the total suspended solids with respect to the effects of charge size and N, but showed higher removal efficiencies at longer cycle times for the larger charge size (Figure 3). For both volatile and total suspended solids

Fig. 1. Total suspended solids in the feed (first bar), after 7 hr (middle bar) and after 24/72 hr as a function of cycle time, the presence (+ N) or absence (- N) of inorganic-N in the feed and charge size (25 and 50% of bioreactor operating volume).

Fig. 2. The amount of total suspended solids biodegraded as a percentage of the concentration in the feed after 7 hr (left hand bar, where data available) and after 24/72 hr as a function of cycle time, the presence (+ N) or absence (- N) of inorganic-N in the feed and charge size (25 and 50% of bioreactor operating volume).

a significant increase in the amount biodegraded could be measured after 72 hr compared to the amount biodegraded after 7 hr where data are available.

The results obtained from DOC analysis are shown in Figure 4. The data for a 25% charge size show some consistency while that for 50% charge size shows very different

Fig. 3. The amount of volatile suspended solids biodegraded as a percentage of the concentration in the feed after 7 hr (left hand bar, where data available) and after 24/72 hr as a function of cycle time, the presence (+ N) or absence (- N) of inorganic-N in the feed and charge size (25 and 50% of bioreactor operating volume).

Fig. 4. Dissolved organic carbon concentration in the feed (first bar), after 7 hr (middle bar) and after 24/74 hr as a function of cycle time, the presence (+ N) or absence (- N) of inorganic-N in the feed and charge size (25 and 50% of bioreactor operating volume).

results in the presence of and in the absence of added inorganic-N. Under conditions of N excess with a 50% bioreactor volume charge size, the DOC concentration at the end of the charge period (24 or 72 hr) is only slightly higher than the influent DOC, and there is some suggestion as to an actual reduction in DOC during the biodegradation

process. Very much higher concentrations of DOC could be found in the bioreactor at both 1 day and 3 day cycle times in the absence of added N. When 25% bioreactor volume charge size was used, the DOC remained constant between 7 and 24/72 hr in the absence of added N whilst slight increases in the levels of DOC were found when N was added to the feed.

The data for inorganic-N (Figure 5) indicate that for the smaller charge size, inorganic-N accumulates in the absence of an added inorganic-N source, such that the

Fig. 5. NH_4^+-N in the feed (first bar), after 7 hr (middle bar) and after 24/72 hr as a function of cycle time, the presence (+ N) or absence (- N) of inorganic-N in the feed and charge size (25 and 50% of bioreactor operating volume).

concentrations of NH_4^+-N found at the end of the 24 hr period are nearly as high as those found when NH_4^+-N is included in the feed. Use of the 50% bioreactor volume charge size resulted in a much lower accumulation of NH_4^+-N in the bioreactor when N was omitted from the feed compared with when N was present. It should be noted that some loss of NH_4^+-N was undoubtedly occurring by stripping from the bioreactor due to non-optimal outlet gas condenser operation and thus resulted in an odor of ammonia around the bioreactor.

The pH in the bioreactor was not controlled and the variation in pH under different sets of conditions can be seen in Figures 6 and 7. The initial values are not important since they will depend on the circumstances prevailing during the time preceding the start of operation. The pH values for a charge size of 50% of the bioreactor volume (Figure 7) clearly show that the greatest degree of variation occurs with a 1 day cycle time both with and without added N. The effect of fresh feed addition on the pH can be clearly seen in this figure.

In the presence of N, operation during the weekend (3 days) allows a significant rise in the pH value, whilst in the absence of N the value at the end of the experiment is little different from that at the start.

Fig. 6. Variation in pH as a function of the cycle time and of the presence (+N) or absence (-N) of inorganic-N in the feed for a charge size of 25% of the bioreactor operating volume.

Fig. 7. Variation in pH as a function of the cycle time and of the presence (+N) or absence (-N) of inorganic-N in the feed using a charge size of 50% of the bioreactor operating volume.

With a charge size of 25% of the bioreactor volume (Figure 6), less effects on pH can be seen and a gradual rise in pH occurred in all cases, with the exception of 1 day cycle times in the absence of N where the pH ended each 24 hr only slightly lower than at the start of the cycle.

4. Discussion

One of the key factors controlling the rate of microbial growth is the availability of nutrients. When a pool of soluble nutrients is available, both metabolism and growth can occur optimally provided that no nutrient is present at either growth limiting or inhibitory concentrations. When microbes are presented with organic-N, this must first be converted into a readily assimilable form. The thermophilic process microbes in the system described here are faced with such a problem. Clearly, when N is provided as ammonia, the need to release N from proteins, RNA and DNA, and from feed microbe cell wall organic polymers is not growth restricting. However, when excess soluble inorganic-N is unavailable, the growth and metabolic efficiency of the thermophilic process microbes is reduced. Egli and Quayle (1986) showed that for N-sufficient growth of a methylotrophic yeast, a C to N ratio of 12 : 1 was necessary. For the bacterium *Klebsiella pneumoniae*, Egli and Fiechter (1981) showed that this ratio was 8.75 : 1. If it is assumed that the empirical formula for microbial biomass on an ash and moisture free basis is: $\text{CH}_{1.8}\text{O}_{0.43}\text{N}_{0.23}$ (Hamer and Harrison, 1979) and a typical C content for microbial biomass to be 47% then this corresponds to a N content in biomass of 12.6%. Thus the theoretical C to N ratio in microbial biomass is 4.35 : 1, a ratio which suggests sufficient N availability when microbial cells are used as a substrate for growth of thermophilic process microbes assuming the N requirement of such microbes is not excessively high. From the results presented here it is apparent that in the aerobic thermophilic digestion process, one of the major rate limiting steps could be the release of N from complex polymeric particles. Eastman and Ferguson (1981) discussed the fact that hydrolysis of particulate material constituted the rate limiting step in anaerobic digestion processes. In aerobic thermophilic digestion processes where soluble N resources are limited, the further hydrolysis of N bound in organic polymers may further limit the overall rate of digestion.

The system investigated here was a semi-continuous process such that the effect of reduced N availability in the feed was alleviated by accumulation which took place as a result of only limited removal of the bioreactor contents at the end of each cycle time. The effect of removing a large proportion of the bioreactor contents (50% as opposed to 25% of the bioreactor volume) on N accumulation can also be clearly seen (Figure 5).

The advantages of operating with larger charge sizes can be related to the amount of readily utilizable C and energy substrates in the system. Given that lytic products from lysis of the feed microbial cells will be readily utilized, the remaining cell wall debris is likely to be effectively only slowly biodegradable. Since, with a 50% bioreactor volume charge size more of the readily utilizable soluble lytic product will be present a higher degree of biodegradation is possible. In addition, use of the larger charge size prevents high level accumulation of slowly biodegradable material.

The variations in pH also demonstrate advantages for the process as a result of a five day (Monday to Friday) operation. During the one day cycle time a progressive decrease in pH occurs, it is only due to the fact that 3-day (weekend) cycle time allows a regeneration of the pH to higher values that a collapse of effective operation as a result of low pH conditions, causing inhibition, is prevented.

Hamer and Mason (1987) presented a model for semi-continuous operation of the aerobic thermophilic process. This clearly showed that variation in thermophilic process microbe activity and thus in the rates of substrate utilization could be measured as a function of time. The system investigated had a repeated three day cycle time. In systems with a 1 day cycle time, these variations in process microbe activity are unlikely to have any significance since the bacterial activity should remain consistently high due to repeated substrate addition. When the one day cycling is interrupted by a 3 day cycle (Friday–Monday) the effects of process thermophilic microbe inactivation may reduce process efficiency.

5. Conclusions

Semi-continuous operation of the aerobic thermophilic process for the biodegradation of microbial solids results in high substrate removal. Addition of inorganic-N results in enhanced removal of solids especially under conditions where low charge sizes are used. Operation with 50% bioreactor volume charge size results in slightly enhanced performance over 25% bioreactor volume charge size. The effects of 3 day cycle time (weekends) are effectively only seen in pH effects and offer only advantages in this respect for overall process efficiency.

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