

Size-dependent anaerobic digestion rates of flocculated activated sludge: Role of intrafloc mass transfer resistance

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Abstract

The anaerobic digestion rate for flocculated sludge has been considered to be lower than that of original sludge, particularly in the later stages of digestion; Chu et al. (2003) attributed this relatively slower rate to the increased mass transfer resistance for reactants through the large flocs after flocculation. This study confirmed that methane production was retarded by flocculation. The structure of the floc was identified with fluorescence in situ hybridization (FISH) and a confocal laser scanning microscope (CLSM) technique. To verify the mass transfer resistance induced by flocculation, microsensors were applied to assess the response of oxygen concentration distribution inside the flocs that are subjected to sudden changes in ambient oxygen levels. Response time for the electrode at a floc's center was five times greater than the response time in original sludge flocs. Although the effective diffusivity of oxygen in the floc increased by 2.3 times after flocculation, the increased size of the flocculated floc was the major contributor to the total mass transfer resistance to oxygen.

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1. Introduction

Sludge flocs are microreactors that adsorb and decompose pollutants. However, knowledge of the complex geometrical morphology and microbiological ecology inside flocs is far from comprehensive. This field has attracted considerable academic interest (Li and Ganczarczyk, 1989; Zartarian et al., 1994; Jorand et al., 1995; Sanin and Vesilind, 1996). Detailed knowledge of floc structure is required to better understand sludge processes (Chung and Lee, 2003; Chung et al., 2003). Zartarian et al. (1997) and Thill et al. (1998) employed the fluorescence in situ hybridization (FISH) and confocal laser scanning microscope (CLSM) techniques to analyze the interior structure of flocs. Chu and Lee (2004a,b) and Chu et al. (2004) applied

this technique to explore the interior structures of activated sludge flocs.

Microsensors have been employed to evaluate micro-environments in microbial communities (Revsbech and Jorgensen, 1986), particularly microbial communities in biofilms (Revsbech et al., 1989; Kuhl and Jorgensen, 1992; Schramm et al., 1996; Zhang and Bishop, 1994; Yu and Bishop, 1999, 2001; Bishop and Yu, 1999; Li and Bishop, 2002). Recently the microelectrode technique has also been widely applied to assess the micro-transport processes in an activated sludge floc (De Beer et al., 1998; Schramm et al., 1999a,b; Ploug and Jorgensen, 1999; Li and Bishop, 2004).

Anaerobic digestion is typically utilized as a stabilization technique to reduce the volume of waste activated sludge and produce bio-gas. Moreover, chemical flocculants are often utilized prior to sludge dewatering to enhance filtration rates. Although the dosage of polyelectrolyte in sludge is usually low, Dentel et al. (2000) demonstrated that the applied polyelectrolyte attached to and was carried out with the conditioned sludge at an amount up to 10 kg/ton dry solids (DS). Contradictory results have been reported regarding the effect of chemical conditioners on anaerobic digestion of sludge. Gossett et al. (1978) measured reduced

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methane production and the volatile solid destruction ratio of anaerobically digested wastewater sludge when organic coagulants were present. El-Mamouni et al. (1998) observed that the synthetic polyelectrolyte did not inhibit biomass activity, but enhanced the metabolite transfer rate and reduced the inhibitory effects of acetogenic and methanogenic bacteria in a UASB reactor. Chu et al. (2003) reported that the digestion rate of sludge flocculated with a cationic polymer was higher than for original sludge during the initial phase of digestion, attributable to the additional carbon source provided by the polymer. However, the digestion rate for flocculated sludge soon declined and became less productive than the original sludge during the later stage of digestion. Since flocculation considerably increased floc size, the authors speculated that the mass transfer resistance accounted for the reduced digestion rate in the flocculated sludge. However, further experimental evidence is required to confirm these speculative ideas.

By combining FISH/CLSM and a microsensor technique, this work demonstrated that the increase in total mass transfer resistance after polyelectrolyte flocculation was mainly attributable to the increased floc size.

2. Experimental

2.1. The samples

Waste-activated sludge was obtained from the wastewater treatment plant of the Neili Bread Plant of Presidential Enterprise Co., Taoyuan, Taiwan. The chemical oxygen demand (COD), suspended solids (SS) and turbidity data for the supernatant in the sludge, analyzed by adopting EPA Taiwan standard methods, were 0.0067 kg m^{-3} (COD), 0.0088 kg m^{-3} (SS) and 2.4 NTU (turbidity). The sludge sample was sludge sediment obtained after a 1 h settling period. The total solid content of the sludge was measured at 10.30 kg m^{-3} . The pH value of the original sludge was approximately 6.7.

Cationic polyelectrolyte T3052, obtained from Kai-Guan Inc., Taiwan, is a polyacrylamide with a molecular weight of 10^7 and surface charge of 2.27 meq/g. The sludge sample was first placed in a mixing unit, which was a 1-L baffled mixing chamber equipped with a paddle mixer. The mixer had a variable-speed motor directly connected to a torque meter. Flocculant solution (0.2% by weight) was then gradually poured into the mixing chamber while stirring first at 200 rpm for 300 s, and then at 50 rpm for a further 1200 s. The polyelectrolyte flocculation did not significantly alter the pH value of the sludge sample.

2.2. Digestion and test

The anaerobic digestion of sludge followed the procedure outlined by Chu et al. (2003). Eight ml of sludge (original or flocculated) was mixed with $1 \times 10^{-6} \text{ m}^3$

of anaerobe strains isolated by Chang et al. (1996) and $1 \times 10^{-6} \text{ m}^3$ of medium (with a composition of 0.35 g/L of K_2HPO_4 , 270 mg/L of KH_2PO_4 , 2.7 g/L of NH_4Cl , 100 mg/L of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 100 mg/L of $\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$, 20 mg/L of $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$, 12 g/L of yeast extract, and 1 mg/L of reszurin) and digested at 35 °C. Triplicate testing was performed for each sample to ensure data reproducibility.

A particle sizer (LS230, Coulter) measured floc size with a light scattering method. The mean floc size of the original sludge was 60 μm . Methane production was quantified with a gas chromatograph (Sigma 3B Perkin-Elmer) equipped with a packed column (200 \times 0.5 cm, 80/100 Porapak Q; Supacol, Bellefonte, PA) and a flame-ionization detector. Column temperature was maintained at 90 °C and the injector and detector were maintained at 55 °C. Nitrogen, with a flow rate of $3.33 \times 10^{-7} \text{ m}^3 \text{ s}^{-1}$, was employed as the carrying gas. An integrator (HP3396 Series II) was utilized to measure the methane concentration.

A confocal laser scanning microscope (CLSM) (OLYMPUS BX50) was employed to observe the internal structure of the sludge floc. The microscope was equipped with an image processor (OLYMPUS FV5 PSU) and an Argon laser source to stimulate fluorescence. A 10 \times objective and the software FLUOVIEW version 3.0 were utilized for imaging the sludge floc. Samples were scanned with the microscope at a fixed depth. A digitized image of the sludge floc was then obtained. Sludge samples for CLSM analysis were first fixed with 3% paraformaldehyde in phosphate buffer saline. The fixed sample was then embedded in agar that has low melting point (melting point of 75 °C and gelling point of 38 °C) for FISH. This research employed two different DNA probes, Eub 338 (nucleotide sequence 5'-GCTGCCTCCCGTAGGAGT-3' labeled by rhodamine and Arc 915 (nucleotide sequence 5'-GTGCTCCCCCGCCAATTCCT-3', labeled by tetra-chlorofluorescein, to identify, respectively, eubacteria and *Archaea*, the most common methanogenic bacteria in the sludge (Chu and Lee, 2004a). The stained samples were washed three times with hybridization buffer solution to remove excess probes.

A 10- μm diameter DO microelectrode (DO-10, Unisense, Denmark) was employed to evaluate the oxygen transport within both the original and flocculated flocs. The measurement of oxygen levels was based on diffusion of oxygen through a silicone membrane to an oxygen reducing cathode that is polarized against an internal Ag/AgCl anode. A micromanipulator was adopted to finely adjust the position of the electrode tip in the exact center of the floc, at a spatial resolution better than 20 μm . The stirring sensitivity of the sensor is less than 2%, and its response time (90%) is 1–3 s. The output of the sensor was recorded continuously by a picoammeter (PA2000, Unisense, Denmark) and a personal computer. A flow chamber was devised to physically keep the sludge floc in a stream of flowing water. Air aeration (210 $\mu\text{mol/L}$) was applied to

saturate the flowing water (stream 1) with oxygen. The incoming fluid was then switched to stream 2, which was de-oxygenized by applying nitrogen gas sparging. The DO level at the floc's center was recorded over time. After the DO level reached a low plateau value of about 80 $\mu\text{mol/L}$, the incoming stream was switched back to stream 1 and the DO level at the floc center increased again.

3. Results and discussion

3.1. Methane production

The amount of methane produced by digestion serves as a useful index to evaluate a sludge's biodegradability. Triplicate tests confirmed that the relative deviations of the methane production data were under 3%, as shown in Table 1.

The digestion rate of sludge flocculated at dosages greater than 15 g/kg DS declined over time. After 10 days of digestion, the methane production curves for the original and the flocculated sludges at dosages under 5 g/kg DS converged. For example, after 40 days of digestion, the amounts of methane produced for original and 5 g/kg flocculated sludge were 136 g- $\text{CH}_4/\text{kg DS}$ and 132 g- $\text{CH}_4/\text{kg DS}$, respectively; these values could be considered identical. The corresponding amounts of methane produced by 15 g/kg DS flocculated sludge and 40 g/kg DS flocculated sludge were 105 g- $\text{CH}_4/\text{kg DS}$ and 85 g- $\text{CH}_4/\text{kg DS}$, respectively; these figures corresponded to 23 and 38% reductions in methane yield from the original sludge, respectively. This difference is significant at a confidence level of 95%.

These experimental results for sludge digestion correlate with the conclusions obtained by Chu et al. (2003). That is, the flocculants enhanced the efficiency during the first stage of digestion (El-Mamouni et al., 1998), but inhibited digestion if the sludge was conditioned with a high dosage of cationic polymer (Gossett et al., 1978). This study, thus, showed that flocculants have different roles during different stages of digestion. Moreover, since the methane production rate for the flocculated sludge was higher than that of the original sludge, the flocculant had negligible toxicity to the microbial population (Chu et al., 2003). The retardation of the methane production by T-3052 flocculated sludge

observed over the later stages of digestion was not attributable to chemical toxicity effects.

3.2. Change in floc size

Table 1 lists the mean floc sizes in the original and flocculated sludges. The floc size of the original sludge flocculated with cationic polymer T3052 increased from 57 μm to 400 μm at a dosage of 40 g/kg DS, suggesting favorable flocculation.

The floc size decreased as digestion proceeded, implying a deterioration of the floc's structure. The size of the original sludge floc decreased only slightly, that is, from 58 to 51 μm following 40 days of anaerobic digestion. However, a dramatic reduction in the sizes of the sludge flocs was noticeable for the T-3052 flocculated samples. For example, the floc size of 40 g/kg DS flocculated sludge decreased by more than 55% during the first 10 days of digestion. The corresponding floc size of sludge flocculated with 15 g/kg DS also decreased from 190 to 135 μm . The floc size changed only slightly in subsequent digestion stages. On the 40th day of digestion, the T-3052 flocculated flocs remained three times larger than the original sludge flocs. The size reduction was less significant in cases in which a lower dosage of 5 g/kg DS was applied.

The large floc size correlates with the reduced digestion efficiency for the T3052-flocculated sludge. Mass transfer resistance is therefore, proposed to explain the size-dependent digestion efficiency, as shown in Table 1. During anaerobic digestion, the sludge is first hydrolyzed into small molecules, such as acetic acids or other long-chain organic acids (acidogenic stage). These long-chain compounds are then decomposed to form methane (methanogenic stage). The large floc size of T3052-flocculated sludge prohibited efficient species' movement within the floc, and hindered the subsequent acidogenic and methanogenic stages that produce methane.

3.3. Floc imaging

Fig. 1 shows two CLSM images from the original- and T3052-flocculated sludge flocs (15 g/kg DS). The staining of bacteria highlighted the position of the solid phase. A loose interior was confirmed in the CLSM image of the original sample. The floc contained both small and large

Table 1
Change in methane production and floc size during anaerobic digestion

| Time (d) | Original sludge | | 5 g/kg DS | | 15 g/kg DS | | 40 g/kg DS | |
|----------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | CH_4 (g/kg DS) | d_f (μm) | CH_4 (g/kg DS) | d_f (μm) | CH_4 (g/kg DS) | d_f (μm) | CH_4 (g/kg DS) | d_f (μm) |
| 0 | 0 | 60 | 0 | 140 | 0 | 190 | 0 | 400 |
| 6 | 41 | 60 | 60 | 118 | 48 | 143 | 46 | 232 |
| 12 | 83 | 59 | 77 | 116 | 69 | 120 | 55 | 181 |
| 20 | 115 | 57 | 98 | 100 | 81 | 100 | 69 | 180 |
| 30 | 131 | 57 | 120 | 98 | 85 | 101 | 77 | 172 |
| 60 | 144 | 54 | 137 | 94 | 100 | 96 | 80 | 160 |

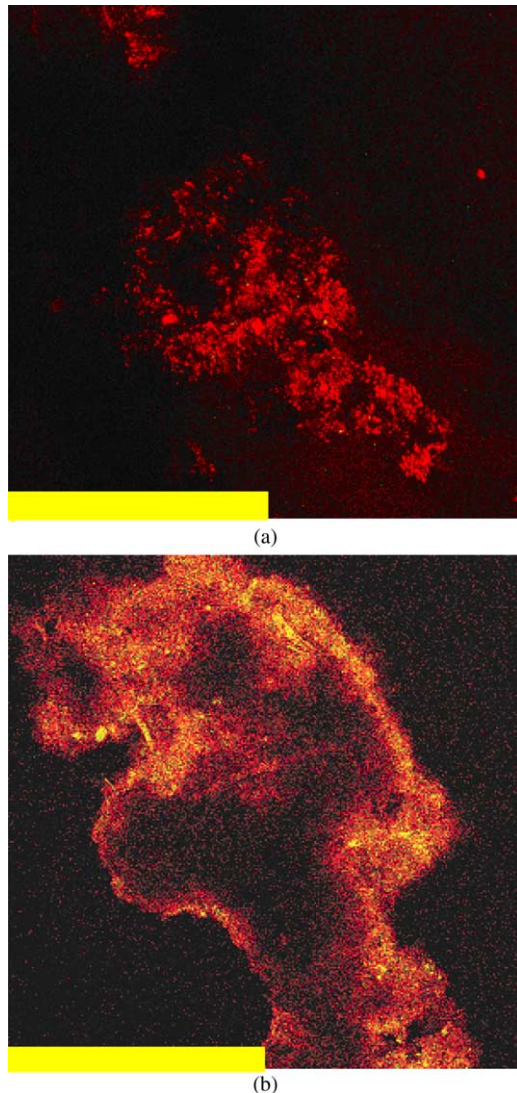


Fig. 1. Images from confocal laser scanning microscope. (a) Original sludge floc, (b) T3052-flocculated sludge floc.

voids. The interiors of the flocculated flocs had minimal amounts of fluorescence light, likely a result of the light extinction effect deep in the floc interior.

3.4. DO response

Fig. 2 shows the DO responses for the original- and the T3052-flocculated flocs (15 g/kg DS). Time scales were moved to meet the origin to demonstrate the difference in the DO responses. The assessment of original floc (100 μm) verified that the response time for DO to decline from high to low levels was roughly 2 s, close to the response time of the electrode (1–3 s). The response time for the electrode at the floc's center increased to 10 s. A finite, but small, mass transfer resistance was thereby identified for the original sludge floc.

For the flocculated floc with a diameter of 300 μm , the response times for both the DO-increasing and -decreasing

stages were 12–20 s, about 20–100% longer than the response times for the original floc. Since the flocculated floc had a diameter three times that of the original floc, the response time of the flocculated floc should be nine times that of the original floc, if molecular diffusion were the sole controlling factor with a constant diffusivity. The not-so-long response time noted for the flocculated floc demonstrated that the diffusivities for original and flocculated flocs might be different.

Assume that the oxygen transfer in a floc can be modeled as a process that occurs in a porous sphere of radius r_f and an effective diffusivity of D . Then the concentration evolution at the center of the sphere, if the surrounding oxygen concentration was suddenly changed from C_0 to C_1 at $t=0$, can be stated as

$$\ln \left[\frac{1}{2} \frac{C_0 - C}{C_0 - C_1} \right] = -\frac{\pi^2 D}{r_f^2} t$$

Fig. 3 shows the model fitting results for the original floc and for the flocculated floc, with the best-fit slopes identified as -0.286 and -0.215 , respectively. The effective diffusivities of oxygen in the floc interior are estimated at 2.9×10^{-10} and 1.97×10^{-9} m^2/s , respectively. That is, the effective diffusivity for the flocculated floc was determined to be close to the value proposed by Li and Bishop (2004), i.e. about 20% lower than that in water (2.24×10^{-9} m^2/s). Conversely, the diffusivity of oxygen in the original floc was much lower than in the flocculated floc, only about 13% of the level of oxygen in pure water. Chu et al. (2004b) recently demonstrated that internal pores of flocculated flocs are considerably larger than those in original flocs. In this work, the effective diffusivity of the original floc increased by 2.3 times after flocculation. This experimental finding correlates with the conclusions obtained by Chu and Lee (2004b).

The mean floc sizes in anaerobic digestion were 60 μm for the original flocs and 400 μm for the 40 g/kg DS flocculated flocs, as shown in Table 1. With a higher diffusivity, the flocculated flocs still exhibited a higher overall mass transport resistance ($\propto r_f^2/D$) than the original flocs, a result of the larger size of the original flocs. This experimental observation supports the speculations made by Chu et al. (2003) that mass transfer resistance hinders the anaerobic digestion process in well-flocculated flocs. The oxygen in the original flocs easily penetrated the floc, owing to its small floc size that, thereby, presented negligible intrafloc mass transfer resistance. During anaerobic digestion, large hydrolyzed molecules diffused into the floc and were further degraded by embedded bacteria. These large molecules required more time to transfer within floc than did oxygen.

Another rationale explaining the identified delayed methane production could be the coverage of bacteria surfaces by a polymer network, as proposed by Chatellier et al. (2001). Further work is needed to quantitatively

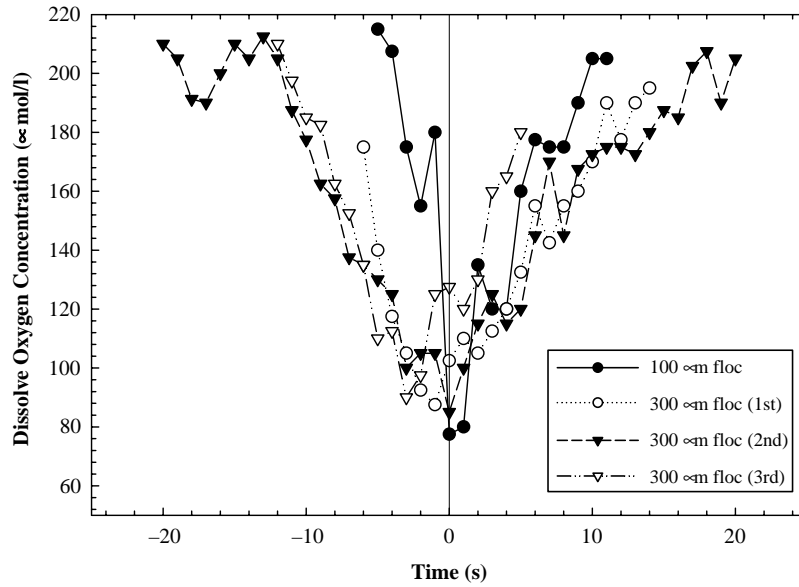


Fig. 2. Dissolved oxygen responses of original and T3052-flocculated sludge flocs.

estimate this effect in the intrafloc transport/reaction processes.

4. Conclusion

This work examined the possible role of mass transfer of species in the anaerobic digestion rate of flocculated sludge. Batch digestion tests were conducted with original and flocculated sludge, and the changes in the interior structure of flocculated sludge flocs were evaluated with fluorescence in situ hybridization (FISH) and confocal laser scanning microscope (CLSM) techniques. The ease of oxygen

transport within flocs was assessed with a microelectrode technique.

Flocculation markedly increased floc size. Correlating with the observations by Chu et al. (2003), the production of methane by 15 g/kg DS flocculated sludge and 40 g/kg DS flocculated sludge was reduced by 23 and 38%, respectively. The large floc size correlated with the reduced digestion efficiency for the T3052-flocculated sludge. The CLSM images demonstrated that the interior of the flocculated flocs had minimal fluorescent light. Based on the microelectrode technique it was estimated that the diffusivity of oxygen in well-flocculated flocs was 2.3 times greater than that in original flocs. However, since the floc

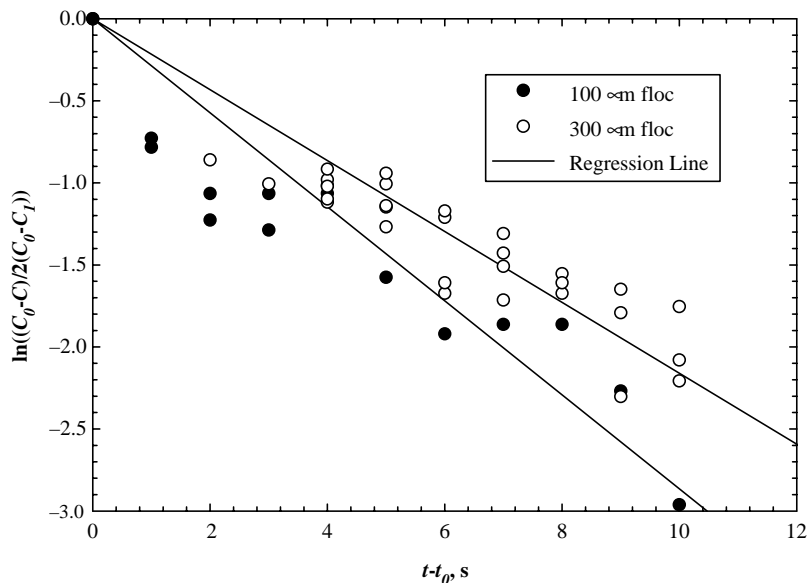


Fig. 3. Model fitting to determine the slopes of regression lines.

size of the flocculated sludge was five times that of the original floc during the anaerobic digestion test, the former still had greater total mass transfer resistance than the original floc.

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