POPULATION DYNAMICS OF ATTACHED BIOFILM IN ANAEROBIC FLUIDIZED BED PILOT PLANT


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ABSTRACT

An innovative anaerobic fluidized bed (AFB) with extreme height (21 metres) was designed by the UCL engineers. Food processing wastewater with relatively low COD concentration less than 1,000 ppm was treated. During the start-up period of seven months, the applied organic loadings (2 to 16 kgCOD/m^3 - day) were increased stepwise with decreasing the HRT from 24 to 1.5 hours. Certain upflow velocities of 25 and 37 metres/hour were maintained to fluidize the ceramic particles up to 10 metres high. Population dynamics of the biofilm attached on particle medium was investigated in terms of increasing organic loading, cumulation of volatile fatty acids, SEM microscopic morphology and biogas production rate of BMP batch tests. About 0.09 kgVSS/kg medium of biofilm contributed to more than half amount of biomass while the suspended sludge was susceptible to hydraulic washing-out. Filamentous network and thrix-like anaerobic bacteria were grown initially in the deep holes or crevices of the medium. After this initial biofilm network formation, certain types of short rods and small cocci were embedded in the biofilm. High organic loading and high VFA concentration provided rapid growth of discrete rods which were washed out of the fluidized bed. Appropriate upflow velocity, HRT and organic loading were essential to this process stability.

KEYWORDS

population dynamics, attached biofilm, anaerobic fluidized bed, morphology, volatile fatty acids, food processing wastewater

INTRODUCTION

An upflow anaerobic sludge bed (UASB) packed with granular sludge and an anaerobic fluidized bed (AFB) packed with biofilm-attached particles were developed to enhance the biological capability for wastewater treatment during the last decades. Both processes provided extremely large amounts of biomass in a bioreactor to extend the sludge retention time (SRT) beyond 100 days. Meanwhile, the hydraulic retention time (HRT) could be shortened to less than 4 hours. However, sludge granulation in UASB and biofilm attachment in AFB were difficult for the low-strength wastewater due to hydraulic attrition, slow biomass growth rate and slow start-up procedure. So the rapid start-up technology of an anaerobic fluidized bed was very critical to the process feasibility for treatment of low-strength wastewater, such as food processing effluent. An innovative anaerobic fluidized bed with extreme height (21 metres) and 5 m^3 volume was designed and operated by the UCL engineers. The objectives of this study are to investigate the population dynamics of the biofilm attached on ceramic media in the pilot plant. Several approaches were employed to characterize the biofilm development,
such as increasing organic loads to enhance the biomass growth, analyzing volatile fatty acids to judge the predominant population of thrix-like bacteria (Wiegant & deMan, 1986; Suidan et al, 1990), SEM microscopy of biofilm to observe the microbial morphology shift due to different operating conditions (Gorris et al, 1988), and a series of BMP batch tests (Owen et al, 1979) to evaluate the bioactivity of different microbial growths existing in the AFB pilot plant.

MATERIALS AND METHODS

Anaerobic Fluidized Bed Pilot Plant

A fluidized-bed bioreactor with total volume of 5 m³ was constructed with a carbon steel column (φ0.5 m × 18 m height) and a gas-solid-liquid separator (φ1.5 m × 3 m height) at the top. A recirculating pump could be adjusted to provide the upflow superficial velocity (SV) at 25 m/hr or 37 m/hr and to fluidize the bioparticles with 30% of bed expansion. Twelve equally spaced sampling ports were installed along the reactor height to withdraw both the mixed liquid and the bioparticle samples for several analyses. The setup is depicted schematically in Fig. 1. The pilot was packed with 1,000 kg of fine ceramic particles which had average diameter of 0.5 mm and density of 1.7 g/cm³. At the beginning, 1.2 m³ of swine-digested sludge with 24.7 g/L VSS was inoculated into the pilot plant. The feeding wastewater was collected from a food processing factory which manufactured several kinds of food products, such as milk, meat, soft drink and soja etc. The influent concentration of these composite wastewater was about 600 - 1,000 mg/L of COD.

Water Analyses

The concentration of pH, COD, TOC, IC, SS, and VSS were analyzed according to the Standard Method (16th Ed., 1985). The volatile fatty acids (VFA) of acetic acid, propionic acid and butyric acid were analysed using a Shimadzu Gas Chromatograph-FID, packing with Chromosorb 101.

Scanning Electronic Microscopy

Anaerobic biofilm attached on medium and suspended sludge were sampled periodically from different heights of the fluidized bed. Preparation of SEM specimen included glutaraldehyde fixation, ethanol dehydration, critical-point drying, gold sputter coating. Morphological observation was done with JEOL JSM-35 a scanning electronic microscope.

Biochemical Methane Potential Test

BMP test of anaerobic biofilm and suspended sludge was conducted following the similar method of Owen's study (1979). A series of various wastewater or acetate concentrations were added into a series of serum bottles (100 mL) which were inoculated with certain amount of either bioparticles or suspended sludge which were withdrawn from the pilot plant at different operating periods. During the batch incubation at 35°C, the biogas production rate was measured in terms of duration time. The specific biogas production rate could be representative for the bioactivity of the existing biomass (mL Biogas/g VSS – day).

Fig. 1 Schematic of anaerobic fluidized bed pilot plant (volume:5m₃, height: 21m, diameter: 0.5 m): (1) feed pump, (2) feed flow meter, (3) distribution tank, (4) recirculation pump, (5) influent flow meter, (6) sampling ports, (7) biogas separator, (8) effluent tank.
RESULTS AND DISCUSSION

Volumetric Loadings and VFA Concentration Profiles in AFB Pilot Plant

During the start-up period, the food processing wastewater supplemented with 1,000 mg/L of acetic acid was fed into the AFB pilot plant. The feed flow rate was adjusted at HRT of 24 hours to maintain the volumetric loading at 1.7 kg COD/m³-day and the sludge loading at 0.21 kg COD/kg VSS-day. The supplemental acetic substrate enhanced the methanogenic growth and the COD removal efficiency up to 94.9%. With sufficient effluent recirculation the VFA concentration in the sampling port #3 (Fig. 2) and port #5 (Fig. 3) were as low as 15 mg/L and 10 mg/L respectively at Day 39. After 42 days of start-up operation, acetate substrate was cut off, and the HRT was decreased from 24 hours to 18, 12, 8, 6, 4 and 2.5 hours step by step. Therefore, the volumetric organic loadings increased stepwise up to 16 kg COD/m³-day. The limited COD removal efficiencies decreased stepwise from 87.5 to 57.8% due to insufficient biomass growth and retarded biodegradation of the VFA. Concentrations of acetic and propionic acid remained relatively high in both port #3 and port #5. (Fig. 2 & Fig. 3). Gorris (1988) demonstrated that the initial biofilm should be constructed with a strong network of methanotrix filaments, then the methanosarcina of discrete cocci would be entrapped by the filamentous network and certain structure of extra-cellular polymer (ECP). A dense network of various methanogenes could enhance the biofilm growth. Therefore, the volumetric loading was increased rapidly with shortening HRT of 1.5 hours at Day 124. Three days later (Day 127), the VFA concentration jumped up to 270 mg/L of acetic acid and 160 mg/L of propionic acid in the port #3 (Fig. 2). While the population dynamics changed significantly, suspended sludge was washed out drastically. Suidan et al. (1990) indicated that methanosarcina was the predominant population in the bioreactor with high concentration of acetic acid above 300 mg/L. Fig. 4 showed that the concentration profiles of acetic acid and propionic acid remained high along the heights of bioreactor. No significant reduction of TOC concentration profile was observed along the whole reactor associated with low concentration of inorganic carbon (IC). Incomplete biodegradation of the influent substrate reflected the overloaded (16 kg COD/m³-day) of this process. According to the existed biomass (55 kg VSS), the sludge loading increased rapidly from 0.76 to 1.1 kg COD/kg VSS-day that was too high to maintain the normal bioactivity. Therefore, the HRT was extended back to 6 hours and the loading was decreased to 3.8 kg COD/m³-day. At Day 130, the low volumetric loading decreased the sludge loading to 0.2 kgCOD/kgVSS-day. Better removal of TOC attained relatively low concentration about 50 mg/L below the port #5. However, a certain amount of suspended organic remained in the upper section (TOC:100 mg/L). Fig. 5 showed a significant reduction of acetic acid and propionic acid along the heights, less than 50 mg/L of VFA was observed in port #3 or port #5. The COD removal efficiency was improved at 82%. Complete biodegradation of the organic substrate reflected a relatively high concentration of IC, about 100 mg/L due to CO₂ production. Complete biodegradation of the organic substrate reflected a relatively high concentration of inorganic carbon (IC), about 100 mg/L due to CO₂ production.

![Fig. 2. Production of volatile fatty acids changed with organic loading and operating time at sampling port #3.](image1)

![Fig. 3. Production of volatile fatty acids changed with organic loading and operating time at sampling port #5.](image2)
Population Dynamics of Attached Biofilm and Suspended Growth

During the start-up period of seven months, bioparticles and suspended sludge were withdrawn periodically from two sampling ports of #3 (4 m high) and #5 (7 m high). Morphology of the attached biofilm and the suspended growth was observed by the scanning electronic microscopy (SEM). The inoculated sludge was taken from the digested swine in which spherical-shape and short rod anaerobes predominated with very dense population (photos 1 & 2). After one month of start-up, there were very few rod bacteria entrapped into the hole and crevice of the ceramic medium (photo 3). The rod bacterium was difficult to attach on the naked surface due to high liquid velocity of the fluidized bed. Gorris & Van Deursen (1986) also demonstrated that bacterium colony was entrapped and limited in the hole of sand medium during the start-up period. Gradually, there were quite different microbial morphology and biofilm structure observed between two ports with 3 metre interval in the pilot plant. Predominant filamentous bacteria (methanothrix-like) with crosslinkage network bloomed along the crevices of media which existed in the top section of the expanded bed (port #5, photo 4). After two months of start-up, the medium surface was almost covered by the filamentous network without naked surface (port #5, photo 5). A small amount of spherical and rod bacteria were entrapped into the network (photo 6) due to stepwise increased loading. The density-reduced bioparticle with thicker biofilm was fluidized upward to the port #5 (photo 7). In the middle section of the fluidized bed (#3 port), filamentous bacteria were still the predominant attached population during the first two months. However, population shift was observed after the substrate loading was increased significantly. The spherical and short rod bacteria occupied the hole mouth of medium (photos 8, 9 & 10). But several parts of naked surface with less biomass were still observed in port #3, while a large number of coccus, short rod and methanosarcina were presented in the suspended sludge (photos 11 & 12). During this period of high loading, volatile fatty acids were monitored in the reactor bottom due to rapid acidogenesis of the wastewater. Especially in the period of 2.5 month operation, the hydraulic retention time was shortened constantly, the concentrations of acetic acid and propionic acid were increased up to 250 ppm and 120 ppm respectively, then the pH value was dropped to 6.5 the condition was favorable for methanosarcina growth (Weigant, 1986). Therefore, apparent population dynamics was observed in these two ports (#3 & #5). Morphology changed more significantly in the bottom than in the top section of the fluidized bioparticles. On the other hand, there were different characteristics of colonial structure presented on the attached biofilm and in the suspended growth. Filamentous bacteria could be extended from the medium hole to the surface with dense network intermingled. This crosslinkage structure of filamentous bacteria was more feasible to biofilm growth without hydraulic sloughing. However, spherical and short rod bacteria could only be entrapped in the hole of medium. These discrete bacteria were more susceptible to hydraulic attrition without enough bridging structure, so they were almost presented in the suspended sludge instead of the attached growth. Microbial morphology and biomass growth were quite different between two sections of the fluidized bed. At the beginning of inoculation (Day 15), the attached biomass amounts in both port #3 and port #5 were 0.72 and 0.76 g - VSS/kg - medium with predominant population of short rod bacteria in the hole of medium. After 74 days of start-up,
the attached growth increased to 6.83 and 17.6 g–VSS/kg–medium in port #3 and port #5. When the fluidized bed was performed with the shortest HRT of 1.5 hours and the highest organic at 16 kg COD/m³–day, the attached biomass increased up to 23.13 and 90.37 g–VSS/kg–medium in two sections. Hence, the attached growth replicated 32 times in port #3, while more dramatic growth attained 120 times in port #5. Because the attached growth of port #5 bioparticles was developed well with filamentous network which entrapped more biomass and population of spherical and short rod bacteria. During the period of loading upswing, the difference of biomass attachment was more significant between these two ports. So the filamentous network was the critical factor for biofilm growth (Weigant, 1986).
Increasing of Microbial Activity Determined by BMP Batch Test

Beside microbial morphology of biofilm development, the microbial activity of the existing biofilm was necessary to be evaluated by a series of biochemical methane potential test (BMP) which was developed by Owen et al (1979). Both biofilm-attached media and suspended sludge were withdrawn from the pilot plant during the different operating periods. Both wastewater and acetic acid were used as the substrates for acidegenes and methanogenes. A series of cumulative biogas (methane and carbon dioxide) production rates was measured and designated as the specific biogas production rate ($R$, $mL$ Biogas/g VSS – day) in these batch tests (Cheng, 1991). The higher gas production
rate was indicated with higher substrate concentration. So, the correlation between the specific biogas production rate \( (R) \) and the various COD concentrations \( (S) \) was modelled as the Monod equation, \( R = \frac{R_{\text{max}} \times S}{(K_s + S)} \). Non-linear regression and statistical analysis (Wen, in press) were employed to evaluate the biokinetic parameters \( (R_{\text{max}}, K_s) \). The observed data of various operating date were also translated to another special parameter of \( R_{500} \) which was equivalent to the specific biogas production rate with COD concentration of 500 g/L. Because the influent concentration of the pilot plant was about 500 g/L, so, the \( R_{500} \) values represented the similar bioactivity of the field microbes at various dates. Table 1 shows that \( R_{\text{max}}, K_s \) and \( R_{500} \) varied with the operating date and the HRT. During the first two months of start-up, the bioactivity of the attached growth increased significantly with the operating date and the HRT. When the HRT was shortened from 8 to 2.5 hours, and the various COD concentrations, the specific biogas production rate with COD concentration of 500 mg/L. Therefore, the HRT increased too rapidly with the shortened HRT from 8 to 2.5 hours, and incomplete biodegradation of raw wastewater resulted in increasing \( R_{\text{max}} \) and \( R_{500} \) at Day 117. Therefore, the HRT was extended back to 6 hours to restore the bioactivity of the existed bioreactor. \( R_{\text{max}} \) of 846 mL Biogas/g VSS – day was obtained as the highest value in this study. This \( R_{\text{max}} \) value was equivalent to 1.69 g COD/g VSS – day of substrate utilization rate that was greatly over the average bioactivity found in any reported data. So it was necessary to evaluate another bioactivity of methanogene that was recognized as the rate-limiting step of anaerobic fermentation. Table 2 shows the biokinetic parameters of acetic degradation with the attached growth and the suspended sludge. When the HRT was shortened to 2.5 hours, the high organic loading provided predominated growth of the suspended sludge with higher \( R_{\text{max}} \) of 400 mL Biogas/g VSS – day (Day 117) ... while the attached biofilm attained lower \( R_{\text{max}} \) of 255 mL Biogas/g VSS – day. Because volatile fatty acids (VFA) were accumulated in the fluidized bed with acetic acid above 200 mg/L and propionic acid above 100 mg/L (Fig. 4). The bulk solution of high VFA enhanced the bioactivity of suspended sludge with predominant population of short rod bacteria and \textit{methanosoreina} (photo 11 &12), the HRT was extended to 6 hours (Day 130). The VFA concentrations in the bioreactor were reduced below 50 mg/L of acetic acid and propionic acid (Fig. 5). Then the methanogenic activity of the suspended sludge decreased significantly to 131 mL Biogas/g VSS – day of \( R_{\text{max}} \). On the other hand, \( R_{\text{max}} \) of the attached biofilm increased to 339 mL Biogas/g VSS – day that was much higher than \( R_{\text{max}} \) of the suspended sludge. Therefore, the attached biomass grew up to 0.1 kg VSS/kg – media (Fig. 6) with the overall biomass of 28 Kg VSS that was also greater than VSS of the suspended sludge. Progress of the attached biomass on media exited in different heights of the pilot plant with two superficial velocities. When the recirculating flow rate was increased to 37 m/hr of upflow velocity, the bioparticles of port \#5 were fluidized upward to the position of port \#7, while the bioparticles of port \#4 were lifted up to port \#5 and port \#6 (Fig. 6). Meantimes, the suspended sludge with less density was washed out of the reactor, and the overall biomass of suspended sludge decreased drastically (Fig. 7). Figure 7 demonstrated an apparent contrary tendency with an interlacing point at Day 130. After this point, the low volumetric loading enhanced higher growth rate of the attached biofilm with more stability of the process performance (Gorris, 1988). According to these biokinetic parameters and the VFA concentration, it is clear that the biodegradation of wastewater needs enough HRT (about 6 hours) and higher biogas production rate of the attached biofilm in the fluidized bed.

### Table 1: Biokinetic Parameter of Wastewater Degradation with Attached Biofilm

<table>
<thead>
<tr>
<th>Date (Days)</th>
<th>H.R.T. (Hours)</th>
<th>( R_{\text{max}} ) mL Biogas/g VSS day</th>
<th>( K_s ) mg/L</th>
<th>( R_{500} ) mL Biogas/g VSS day</th>
<th>( R^2 )</th>
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</thead>
<tbody>
<tr>
<td>34</td>
<td>24</td>
<td>72</td>
<td>48</td>
<td>0.97</td>
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<tr>
<td>62</td>
<td>8</td>
<td>353</td>
<td>395</td>
<td>197</td>
<td>0.92</td>
</tr>
<tr>
<td>117</td>
<td>2.5</td>
<td>246</td>
<td>511</td>
<td>122</td>
<td>0.99</td>
</tr>
<tr>
<td>130</td>
<td>6</td>
<td>846</td>
<td>2192</td>
<td>157</td>
<td>0.93</td>
</tr>
<tr>
<td>160</td>
<td>4</td>
<td>76</td>
<td>247</td>
<td>51</td>
<td>0.92</td>
</tr>
</tbody>
</table>

\( R = \frac{R_{\text{max}} \times S}{(K_s + S)} \)

### Table 2: Biokinetic Parameter of Acetic Degradation with Attached Biofilm and Suspended Sludge

<table>
<thead>
<tr>
<th>Date (Days)</th>
<th>H.R.T. (Hours)</th>
<th>( R_{\text{max}} ) mL Biogas/g VSS day</th>
<th>( K_s ) mg/L</th>
<th>( R^2 )</th>
</tr>
</thead>
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<tr>
<td>117</td>
<td>2.5</td>
<td>Attached</td>
<td>225</td>
<td>235</td>
</tr>
<tr>
<td>130</td>
<td>6.0</td>
<td>Biofilm</td>
<td>339</td>
<td>1611</td>
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<table>
<thead>
<tr>
<th>Date (Days)</th>
<th>H.R.T. (Hours)</th>
<th>( R_{\text{max}} ) mL Biogas/g VSS day</th>
<th>( K_s ) mg/L</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>117</td>
<td>2.5</td>
<td>Suspended</td>
<td>400</td>
<td>1200</td>
</tr>
<tr>
<td>130</td>
<td>6.0</td>
<td>Sludge</td>
<td>131</td>
<td>9531</td>
</tr>
</tbody>
</table>

\( R = \frac{R_{\text{max}} \times S}{(K_s + S)} \)
During the start-up period of an anaerobic fluidized bed pilot plant, only a small amount of thrix-like microbes could be entrapped into hole and crevices of the ceramic medium, while large parts of cocci and rods were suspended along the high reactor (21 metres). Low organic loading should be applied to enhance the predominant filamentous growths which were embedded and bloomed along the medium crevices. Too rapid increasing of organic loading above 10 kg COD/m³·day would attain significant accumulation of volatile fatty acids. Concentration of acetic acid more than 200 mg/L promoted predominant growth of cocci and rods which would be washed out of the fluidized bed at the superficial velocity of 37 metre/hour. Appropriate hydraulic retention time (6 hours) and upflow velocity (25 m/h) were performed to enhance the predominant filamentous network covering the media surface. The attached biofilm increased up to 23.13 and 90.37 gVSS/kg medium in the sampling port #3 (4 - m high) and port #5 (7 - m high) after four months of start-up. After this initial biofilm network formation, certain types of short plump rods and small cocci were embedded in the biofilm. A reasonable methanogenic activity of 399 mL Biogas/gVSS - day (Rmax : 0.67 gCOD/gVSS - day) was obtained for the attached biofilm. Comparative stability of the process performance would be expected.

ACKNOWLEDGEMENT

Financial and engineering support by the Union Chemical Laboratories, Industrial Technology Research Institute is gratefully appreciated.

REFERENCES


