Contribution of hydrodynamic characteristics on the performance of an aerobic biofilm conical fluidized bed

D. Zhou, X. T. Bi and S. Dong

ABSTRACT

The performance of a conical fluidized bed (TFB) bioreactor, including the biofilm thickness, microbial space density, microbial cell matrix and its efficiency for COD degradation at a bed expansion ratio of 14 to 90%, was studied and compared with a cylindrical fluidized bed (CFB) bioreactor. The hydrodynamic characteristics of the TFB, especially the internal-circulation of bioparticles associated with its unique tapered geometry of the bed, created a much more uniform axial distribution of the bioparticles, leading to the formation of thinner and more compacted biofilms in the TFB compared to that in the CFB. The thinner biofilm in the TFB tended to be stable and possessed more than 6 times of microbial population density compared to the CFB. As a result, thinner biofilms in the TFB contributed to a higher COD removal efficiency, which remained at over 95% at operated expansion ratios, about 15 to 25% higher than that in the CFB.

Key words | biofilm, conical fluidized bed, flow pattern

INTRODUCTION

Conical or tapered fluidized beds (TFBs) have been widely applied in various processes since 1960s, such as coating, drying and granulation of particulate materials (Ishii 1973; Dighe et al. 1981), biological processing (Levey et al. 1960) and immobilized wastewater treatment (Scott & Hancher 1976). For wastewater treatment, it was found that conical fluidized beds usually performed more efficiently in the pollutant removal ability than conventional cylindrical fluidized beds, CFBs (Wu & Huang 1995, 1996; Parthiban et al. 2007).

One of the most important aspects, which results in its superior performance over cylindrical fluidized beds, lies in the distinct hydrodynamic characteristics of the conical fluidized beds (Huang et al. 2000). At least four flow regimes have been identified in the conical beds, ranging from fixed, partially fluidized, and fully fluidized to turbulently fluidized beds (Peng & Fan 1997; Jing et al. 2000; Schaafsma et al. 2006). Moreover, particles in a conical fluidized bed circulated between the core and the annulus regions in an almost perfectly mixed state (Toyohara & Kawamura 1992; Zhou et al. 2009). Webster & Perona (1990) also showed that axial liquid mixing was enhanced in the tapered liquid-solids fluidized beds even for a small tapered angle (0.5°) in comparison with the cylindrical bed. Such hydrodynamic characteristics of TFBs resulted in following unique behaviors of biofilms as the reactor was employed for wastewater treatment: (1) weak drag force exerted on the bioparticles in the upper-part and then preventing the bioparticles from being washed out (Wu & Huang 1998); (2) the internal circulation enhanced bioparticle mixing in the TFBs; (3) different biofilm thickness, specific surface area and specific biomass density from the CFBs (Wu & Huang 1995, 1996).

Although the unique hydrodynamic characteristics and biofilm performances of TFBs had been widely studied respectively, systematic researches on the relationship of representative flow regimes of TFBs and biofilm behaviors are still quite limited in the literatures. This work intends to contribute a better understanding on how hydrodynamic behavior affects biofilm characteristics and indicate the biofilm performances of TFB from view of hydrodynamics, then guiding an appropriate running of this bioreactor.

The characteristics of TFB were directly compared to a conventional CFB, which was one of the most popular processes in biofilm wastewater treatment area by now.

**MATERIALS AND METHODS**

**Experimental set-up**

A conical column with a tapered angle of 8° and a cylindrical column with the same configuration were employed in this research, with the experimental set-up shown in Figure 2. Both units consist of 4 Plexiglas sections: a 200 mm high plenum chamber packed with 2.5 mm polyethylene beads for improving the fluid distribution; a distributor plate of 2% opening with 1.6 mm holes installed above the plenum chamber; a test section and an expanded section on the top. The test section is 435 mm high with a diameter of 89 mm at the bottom and 203 mm at the top for the conical bed, 762 mm tall and 102 mm in inner diameter for the cylindrical bed. A stainless steel screen was installed on the top of the distributor plate to prevent particles from falling into the plenum chamber. The initial packing volume of both beds was 1.6 ± 0.1 l, giving an initial packed bed height of 185 mm and 200 mm for conical and cylindrical beds, respectively.

A gas bubbler was installed in the freeboard region above the bioparticle bed to introduce oxygen so that the aerobic condition in the entire reactor was maintained by recycling dissolved oxygen to the bottom of the reactor. The unit was operated under controlled temperatures. The fluidization medium used in this study is Kureha activated carbon spheres with an average diameter of 0.6 mm, a dry bulk density of 600 kg/m³, a wet density of 1320 kg/m³ and a surface area of 11001300 m²/g.

**Culture medium and feed water**

Synthetic wastewater was prepared with glucose (200 mg/l), NH₄Cl (75 mg/l) and KH₂PO₄ (18 mg/l). Trace metals and nutrients (Zhou et al. 2008) were also fed to supplement balanced feed for biofilm. The COD concentration of the prepared synthetic water was 220 ± 5 mg/l. The ingredients of culture medium for living cell count were beef extract (3 g/l), sodium chloride (5 g/l), peptone (10 g/l) and agar (20 g/l).

**Procedures and operating conditions**

Sampling: The bioparticles were sampled by a container with a cap that can be opened and closed freely at the sampling location without destruction of biofilms on both sampled and unsampled bioparticles. The number of bioparticles sampled from the system accounted for a negligible fraction of the total amount of bed particles.

Flow regimes: The flow patterns of both TFB and CFB at varied superficial velocities were observed carefully from the Plexiglas wall of the column. Superficial liquid velocity is the ratio of the inlet flowrate and cross-sectional area of the bottom of TFB and CFB. The expansion ratio is defined as the ratio of expanded volume over the initial volume of the fluidized bed.

Operating conditions: The inlet liquid flow rate and hydraulic retention time (HRT) of each run were controlled at 2 l/hour and 45 min, respectively. The bed expansion ratio was increased by adjusting the recycled liquid flow rate. The operating condition of each run in this research was given in Table 1. The other conditions were: temperature of 30°C, dissolved oxygen (DO) of 3–3.5 mg/l, and inlet pH of 6.5.

**Analytical methods**

DO and pH in the reactor were monitored by a VWR Model SP80PD analyzer. COD was determined by digesting the sample in a HACH COD digestion reagent vial for 2 h, followed by injecting the sample to a COD spectrophotometer (HACH DR/2000). Each sample solution for COD testing was filtered by a 0.22 μm cellulose millipore filter before testing.

Biofilm thickness was determined by an optical microscope equipped with a pre-calibrated graticule (Rabah 2003). 30–50 bioparticles were taken gently out of the reactor for the
determination of an average equivalent volume diameter, $d_v$ (μm),

$$d_v = \sqrt[3]{AB^2}$$  \hspace{1cm} (1)

Where A and B are the major and minor diameters of the bioparticles, respectively. The diameter of bare activated carbon carriers was measured after the biofilms were removed by stirring the mixture of bioparticles and 3 mm glass beads in the distilled water. The average biofilm thickness was estimated based on the difference between the average diameters of the bioparticles and bare carriers. The biofilm volume was then calculated by the difference of the average equivalent volume of bioparticles and the average volume of carriers. By considering the thickness of biofilm (around 20 μm) was negligibly small compared with the diameter of the carbon particle, the distribution of bioparticles was mainly determined by the size of bare activated carbon particles.

Microbial surface area density (CFU/μm²), $C_s$, is defined as the quantity of living cells at unit surface area of carriers,

$$C_s = N_T/(\pi n D^2)$$  \hspace{1cm} (2)

where $N_T$ is the total cell count, $n$ is the number of bioparticles and D is the average diameter of bare activated carbon. By assuming the carrier particles in the fluidized bed maintained at an initial quantity, then the variation of microbial cell quantity at unit surface area of the carriers could represent the variation of the total cell count of the bioreactor at various bed expansion ratios.

Microbial volume density (CFU/μm³), $C_v$, is defined as the number of cells per unit of biofilm volume,

$$C_v = N_T/\left\{1/6n\pi [(D+d_v)^3 - D^3]\right\}$$  \hspace{1cm} (3)

The estimation of living cell quantities was carried out using the standard plate count method (APHA 2005). 80–100 bioparticles were sampled from different levels of the reactor and then put into a 250 ml flask containing 100 ml sterilized distilled water and several glass beads, shaken over night on a shaker to dislodge living microbial cells into the bulk water. Then, to improve the measurement accurate, the sample was

![Figure 2 | Schematic diagram of experimental set-up.](image)

**Table 1 | Operating conditions of conical and cylindrical fluidized beds**

<table>
<thead>
<tr>
<th>Conical fluidized bed</th>
<th>Cylindrical fluidized bed</th>
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<tr>
<td>Initial bed height mm</td>
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diluted to 300–3,000 CFU per ml, of which 0.1 ml was then plated out and cultivated at 37°C for 48 h. It should be noted that the standard plate count method had limitations (Pinhasi et al. 1997) on accurate cell counting as certain proportions of unculturable cells were not accounted in the results. However, since the same constitution of feed water and the environmental condition of the reactor were controlled throughout the research, the quantity of living cells estimated by the standard plate count method is comparable by assuming that the proportion of unculturable cells changed very little in different runs. Each plate count test was conducted in triplicate.

Scanning electron microscopy (SEM)

Bioparticle samples were plunge-frozen at atmospheric pressure into sub-cooled liquid nitrogen using an Emitech K1250X cryo-prep unit. Once frozen, the samples were drawn into an evacuated transfer chamber. Bioparticles were deposited directly onto a cryo-stub or strips of Whatman filter paper glued to a copper cryo-stub, or kept in a high pressure freezing hat filled with water to keep the samples fully hydrated. Just before plunge freezing, a paper tissue was used to wick moisture away from the particles. Samples were then sublimed at a temperature of −90°C and transferred to the cold stage of a Hitachi S4700 FESEM at −148°C. If necessary, further sublimation was carried out during the visual inspection of the sample under SEM.

RESULTS AND DISCUSSION

Hydrodynamic characteristics

The behaviour of bioparticles in both the conical and cylindrical fluidized beds at several different bed expansion ratios were studied by means of flow-pattern observation and bioparticles distribution analysis. The bioparticles were suspended and remained at a relatively static state at low bed expansion ratios (<20%, in both of the conical and the cylindrical fluidized beds). In the cylindrical fluidized bed (CFB), the fluid exerted stronger drag force on the particles with the increase in bed expansion ratio, leading to an enhanced turbulent movement of bioparticles. However, the axial size segregation of the bioparticles was still observable even at a bed expansion ratio as high as 90%, as shown in Figure 3. Segregation of bioparticles in the cylindrical fluidized bed was also found by Hidalgo & Garcia-Encina (2002), where the smaller bioparticles were even started to be washed out from the cylindrical fluidized bed at a bed expansion ratio of 80%.

Although axial segregation of bioparticles also existed in the conical fluidized bed (TFB) at a low expansion ratio of 14%, the axial mixing and distribution were improved significantly with a slight increase in the bed expansion (see Figure 3). The uniform particles distribution in the TFB was also reported by Shi et al. (1984) and Littman (1964) in a liquid-solid tapered fluidized bed and a gas-solid tapered fluidized bed, respectively. Such an improvement was considered to be a result of the elevated natural internal circulation of particles in TFBs. Downward flow of bioparticles at the near wall region and a slight hump at the center of the fluidized bed upper bed surface were observed in the TFB reactor at each expansion ratio, indicating the circulation of bioparticles between the core and the annulus regions of the reactor. Such a flow regime belonged to the “fully fluidized-bed regime” or “transition regime” defined by Peng & Fan (1997), as shown in Figure 1. The corresponding flow regimes at different bed expansion ratios are indicated in Figure 4.

Such uniform distribution of bioparticles in the TFB should contribute more uniform pollutant removal efficiency in the entire reactor.

Biofilm thickness

The thickness of biofilm in the TFB was more sensitive to the flow regimes than that in the CFB, resulting from the different flow behaviors of bioparticles in the two reactors. The superficial liquid velocity at the bottom of TFB was greater than that in the CFB at the same expansion ratio, and could be
even greater than the terminal settling velocity of particles as reported by Shi et al. (1984). Furthermore, because of the internal circulation in the TFB, all bioparticles visited the bottom of the TFB frequently to expose them to high shear stresses induced from the high superficial liquid velocity. With the increase of the bed expansion ratio, the abrasion resulted from the shear at the bottom became more intensified. Compared with the TFB, superficial liquid velocity of the CFB was almost uniform along the axial direction, and bioparticles experienced a relatively less shearing as the bed expansion ratio increased. The difference in biofilm thickness between TFB and CFB in Figure 4 appears to be more significant at higher bed expansions, because the internal particle circulation and particle abrasion by shearing in the near entrance region became more important at higher superficial liquid velocities. TFB tended to possess much thinner biofilm than CFB at high expansion ratios.

**Microbial quantity**

Not only biofilm thickness, but also the microbial surface area density, Cs, and microbial volume density, Cv, behaved quite differently with the increase of bed expansion ratio in TFBs and CFBs, as shown in Figures 5(a) and (b), respectively. The estimated Cs and Cv only decreased slightly as the bed expansion ratio increased to less than 80% in the CFB. The microbial cells were detached, as also visually observed, as the bed expansion ratio further increased from 80 to 90%, due to the intense abrasion by the shearing. Figure 6(b) further shows that the thinner biofilm was less populated with microbial cells in the CFB reactor. It should be noted that both of the estimated Cs and Cv in this paper refer to the performance of living cells determined by means of the standard plate count method.

In the TFB, Figure 4 shows that the biofilm thickness decreased dramatically from 31.4 to 4.3 μm as the bed expansion ratio increased. The difference in biofilm thickness between TFB and CFB in Figure 4 appears to be more significant at higher bed expansions, because the internal particle circulation and particle abrasion by shearing in the near entrance region became more important at higher superficial liquid velocities. TFB tended to possess much thinner biofilm than CFB at high expansion ratios.
expansion ratio increased from 14 to 90%. However, the estimated \( C_s \), which represents the total quantity in the bioreactor when multiplied by the total surface areas of the number of carrier particles, decreased only slightly from 178 to 132 CFU/\( \mu \text{m}^2 \) although the biofilm became much thinner. Meanwhile, the estimated \( C_v \) of the biofilm increased significantly from about 10 to 60 CFU/\( \mu \text{m}^3 \), as shown in Figure 5(a), which is opposite to the decrease in the CFB shown in Figure 5(b).

Figure 6(a) further shows that \( C_s \) decreased only slightly with the decrease in the biofilm thickness at thicknesses greater than 7.8 \( \mu \text{m} \). At biofilm thickness less than 7.8 \( \mu \text{m} \), the cell population decreased sharply as the biofilm further decreased. \( C_v \) in the biofilm of the CFB increased steadily with the decrease in biofilm thickness, opposite to the gradual decrease in the TFB in Figure 6(b). One should also note that the total microbial quantity estimated by \( C_s \) value in TFB was as high as 6 times of that in the cylindrical fluidized bed.

Above results showed that although biofilm were abraded significantly with the increasing of shear force, the total quantity of microbial maintained relative stable in the TFB compare to the CFB due to the thinner biofilm in the TFB was much more densely.

The scanning electron microscopy (SEM) images (Figure 7) of both thin biofilms (collected from the run of 90% bed expansion ratio) and thick biofilms (collected from the run of 14% bed expansion ratio) confirmed the results of the measured microbial surface area density and microbial volume density further more. Thin biofilms with a thickness of only 4.6 \( \mu \text{m} \) in Figure 7(a) was densely populated by microorganisms and dense extracellular polymers, revealing that the thin biofilm could still remain as a dense array of microorganisms in the TFB. The thick biofilm in Figure 7(b), with a thickness of 56 \( \mu \text{m} \), contained many large water channels and voids, and microorganisms populated much more sparsely than the thin biofilm in Figure 7(a). These images also provided supporting evidence to the previous observations and model predictions (Rabah et al. 2005) that thinner biofilm tended to have higher biomass density and lower porosity.

The results of microbial quantity and SEM images showed that thinner biofilm, achieved in the TFB under stronger liquid shearing and the internal solids circulation, contained much higher population and denser matrix of microbial cells than that in the CFB. As high hydrodynamic shear force was widely reported to promote the formation of denser and thinner compact biofilm (Vieria et al. 1993; Kwok et al. 1998; Liu & Tay 2002), the relatively higher shearing at the bottom of the TFB is thus considered to be responsible for the formation of such a dense and thin compact biofilm in the TFB. Since the cell population was only slightly influenced by increasing the bed expansion ratio, the TFB possesses a great capacity in anti-shock loading.

**Biological degradation efficiency**

The difference in biofilm performance between the TFB and the CFB, including biofilm distribution, thickness and microbial population, resulted in different pollutant removal efficiencies for the two types of reactors. Chemical oxygen demand (COD) removal efficiency was especially used to compare the biological degradation performance of the TFB and the CFB. As shown in Figure 8, COD removal efficiency maintained more than 95% in the TFB due to the fact that the microbial quantity remained stable and at high levels, although the biofilm thickness varied from 31.4 to 4.3 \( \mu \text{m} \) as the bed expansion ratio increased from 14 to 90%. However, COD removal efficiencies in the CFB were generally 15 to 25% lower than that in the TFB, mostly due to the low microbial quantity, poor axial distribution of the biofilm and lower nutrient transfer rate across the thicker biofilm (Jewell 1990). Moreover, the removal efficiencies of COD
expansion ratios from 14 to 90%, which was 15–25% higher than that in the CFB. Due to its stable microbial population and high pollutant removal efficiencies, conical fluidized bed bioreactors have potential advantages of being high efficiency and strong shock resistant.

**CONCLUSIONS**

Bioparticles in the tapered fluidized bed (TFB) flowed upward at the center and downward near the wall, forming an internal circulation of bioparticles, while bioparticles were always suspended in the cylindrical fluidized bed (CFB) even at a bed expansion ratio of as high as 90%. Such a difference in the hydrodynamic characteristic of the two reactors gave rise to the differences in biofilm behaviour and reactor performance of the TFB and the conventional CFB. Bioparticles tended to distribute uniformly in the entire TFB rather than axially segregated in the CFB. Most of the bioparticles visited frequently the bottom region of the TFB where stronger shear stress was exerted on the biofilm, leading to formation of thin and compacted biofilms in the TFB. However, the thin biofilm in the TFB was only influenced slightly by the increase in the bed expansion ratio, and was attached by a dense matrix of microorganisms and extracellular polymers. Thus the TFB performed much more stable than CFB at a bed expansion ratio of as high as 90%. Such a difference in the hydrodynamic characteristic of the two reactors gave rise to the differences in biofilm behaviour and reactor performance of the TFB and the conventional CFB. Bioparticles tended to distribute uniformly in the entire TFB rather than axially segregated in the CFB. Most of the bioparticles visited frequently the bottom region of the TFB where stronger shear stress was exerted on the biofilm, leading to formation of thin and compacted biofilms in the TFB. However, the thin biofilm in the TFB was only influenced slightly by the increase in the bed expansion ratio, and was attached by a dense matrix of microorganisms and extracellular polymers. Thus the TFB performed much more stable than CFB at a wide operation range expansion ratio from 14% to 90%. Generally, the population of microbial cells in the TFB was 6 times as high as that in the CFB at bed expansion ratios studied in this work.

Above characteristics of the biofilm in the conical fluidized bed showed high COD removal efficiencies at bed expansion ratios from 14 to 90%, which was 15–25% higher than that in the CFB. Due to its stable microbial population and high pollutant removal efficiencies, conical fluidized bed bioreactors have potential advantages of being high efficiency and strong shock resistant.

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