A Study on Start-up Operation of Fixed-bed Biofilm Sequencing Batch Reactor (FbSBR) for Piggery Wastewater Treatment

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Abstract: The purpose of this work was to access the feasibility and stability of nitrogen and organic matters removal from piggery wastewater in Fixed-bed Biofilm Sequencing Batch Reactor (FbSBR) through the changes of hydraulic retention time (HRT) and substrate concentrations. At steady-state, 80 % total inorganic nitrogen was removed at its applied load of 0.16 kg N/m$^3$/d, while total COD$_c$ removal efficiency was 85 % when its load was at 0.8 kg COD/m$^3$/d. The temporary accumulation of nitrite in the effluent is mostly due to limitation of DO in the inner-layer of biofilm. In the cyclic work on day 61, the occurrence of ORP and DO breakpoints mostly matched to the breakpoint-like of nitrogenous compounds profiles, especially, of NO$\_x$ trendlines. It would be further utilized for online monitoring to determine operational modes during each steady-state period. The COD$_c$ profiles in cyclic work showed that most biodegradable organic compounds were consumed by facultative bacteria in anoxic phase and only non-biodegradable part was remained during rest of cycle time.

Keywords: piggery wastewater, biofilm, fixed-bed, sequencing batch reactor

Introduction

In general, piggery wastewater has considerable amount of organic matters and nitrogen. It has been widely used as a resource for providing essential plant nutrients [1]. However, its over-application possibly results in local soil pollution and eutrophication of water body. Due to that many countries are paying attention to the pollution coming from livestock farms, and have tighten legislation and discharging standards. Consequently, it is necessary to have a cost-effective treatment systems alternative to land application. Among them, biological treatment process is a convenient way to treat such wastewater [2,3]. The common nitrogen removal processes such as Anoxic/Oxic (A/O) and Anaerobic/Anoxic/Oxic (A$_2$/O) have a spatial sequence of anoxic zone (denitriﬁcation) and aerobic zone (nitrification). In conventional biological nitrogen removal systems, nitrifiers and denitrifiers are generally cultured as combined growth. To stabilize the growth environments for these bacteria, the phase separation technique using an inter-settler between the oxic and post-anoxic chambers or an integrated anaerobic/aerobic filter system has been applied [4,5]. Unlike these processes, sequencing batch reactor (SBR) can provide an anoxic and oxic conditions in a single tank in which nitrogen and organics can be removed simultaneously and effectively [6,7]. As the sequencing batch reactor has characteristics similar to the plug flow reactor, organic removal rate of SBR is higher than that of the completely mixed reactor [8]. Currently, much research has been conducted to develop such treatment processes by suspended culture [9-11]. However, it is known that nitrification efficiency in piggery wastewater treatment system is usually hard to be maximized due to the slow growth rate of nitrifiers and their sensitivity to operational conditions [12,13]. Thus, high concentration and stable activity of biomass in a bioreactor are most important conditions to remove nitrogen from piggery wastewater. In fact, it has been demonstrated that immobilized-cell and attached-growth (biofilm) biological systems have great advantages for the treatment of high-strength wastewater due to it allows higher biomass concentration per unit reactor [14,15].

According to the knowledge of biofilm reactor, high porosity of fixed media enables the creation of macro- and
Figure 1. Schematic diagram of lab-scale, Fixed-bed Biofilm Sequencing Batch Reactor (FbSBR) used in this study.

micro-environments within the system, so that the different bacteria involved can then grow and concentrate in zones within the reactor favorable to their metabolic activities. Due to those characteristics of biofilm nature, microorganisms in such system could be less sensitivity to inhibitors than suspension system. On the basis of analysis mentioned above, a sequencing biofilm batch reactor would be a good choice to treat piggery wastewater effectively. Thus, the aim of this work is to access the feasibility and stability of nitrogen and organic matters removal from piggery wastewater in Fixed-bed Biofilm Sequencing Batch Reactor (FbSBR). The performance of FbSBR operation was evaluated through the changes of HRT and substrate concentrations.

**Experimental**

**System Set-up and Operation**

The lab-scale reactor configuration was cylinder-shape and shown in Figures 1 and 2(a), which was a total volume of 21 L, working volume of 19 L and constructed from Plexiglas. The content of reactor consisted of biologically active zone with fixed-bed media (8.4 L) located on the middle part. Other rest volume (10.6 L) was divided into two parts in the bottom and top of reactor for placing mixer-impellers, air-diffuser, level-sensor and online measurement electrodes.

The filamentous supporting, rope-type, media was employed as fixed-bed for the formation of biofilm (Figure 2). It was manufactured by Hyosung BCPLUS of Seoul, Korea and constructed by Y-shaped nylon BCF (Bulky Continuous Filamentous) multi-filaments and nylon mono-filaments (Figure 2(c)) [16]. The characteristics of media are as follows: surface area per unit length of 1 m$^2$/m, width of 30 ~ 40 mm, and void fraction of 95 %. Since Y-shaped BCF causes microorganisms to retain more stable, the attached microorganisms are not easily affected by air flow and water circulation. In addition, previous studies for biological drinking water treatment showed that a system equipped with this rope-type media can improve the turbidity removal efficiency [17,18]. The 28 ropes (with 220 mm in length for each) were packed on the stainless steel frame and placed in the middle part of reactor, it results total surface area of media-matrix was 6.2 m$^2$.

Peristaltic pumps were used to feed wastewater directly into the FbSBR, as well as to decant the treated effluent. A recirculation was applied to improve mixing condition in the reactor, resulting in a recycle speed of 65 mL/min during the overall operating periods.

In this system, a cycle time was automatically monitored by electronic control-box. The operation modes during one cycle were described in Figure 3, it consist of feeding, anoxic, oxic, settling, and draw phases. HRT was varied by the change of feeding/drawing volume per one cycle as showed in Table 1.

Return activated sludge from municipal wastewater treatment plant was used for inoculation. Before continuous operation, the reactor was conducted in batch mode during one week for biomass stabilization and biofilm formation with synthetic wastewater. Initially, the biomass concentration was 5,500 mg MLSS/L, and synthetic wastewater was made by: Glucose, 100 mg COD/L; (NH$_4$)$_2$SO$_4$, 75 mg NH$_4^+$-N/L; Glutamate, 0.15 g/L; NaCl, 0.015 g/L; CaCl$_2$·2H$_2$O, 0.006 g/L; MgSO$_4$·7H$_2$O, 0.0051 g/L; and pH buffer solution (KH$_2$PO$_4$, 0.0021 g/L; K$_3$HPO$_4$, 0.009 g/L; Na$_2$CO$_3$, 0.09 g/L; NaHCO$_3$, 0.09 g/L).

**Feed Characteristics**

Raw wastewater was collected from influent of piggery
Table 1. Operating Times of One Cycle

<table>
<thead>
<tr>
<th>HRT (d)</th>
<th>Feed (h)</th>
<th>Anoxic (h)</th>
<th>Oxic (h)</th>
<th>Settle (h)</th>
<th>Draw (h)</th>
<th>Drawing volume /cycle (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.5</td>
<td>0.5</td>
<td>4.0</td>
<td>6.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1.00</td>
</tr>
<tr>
<td>4.25</td>
<td>0.5</td>
<td>4.0</td>
<td>6.5</td>
<td>0.5</td>
<td>0.5</td>
<td>2.235</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of Feeding Wastewater

<table>
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<tr>
<th>Items</th>
<th>Average value</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Unit</th>
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<td>pH</td>
<td>8.38</td>
<td>8.02</td>
<td>8.5</td>
<td>-</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>1243</td>
<td>830</td>
<td>1545</td>
<td>mgCaCO₃/L</td>
</tr>
<tr>
<td>SCOD₃</td>
<td>875</td>
<td>467</td>
<td>1400</td>
<td>mg/L</td>
</tr>
<tr>
<td>TCOD₃</td>
<td>1274</td>
<td>876</td>
<td>2000</td>
<td>mg/L</td>
</tr>
<tr>
<td>NH₄-N</td>
<td>255</td>
<td>130</td>
<td>350</td>
<td>mg/L</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>1.2</td>
<td>0</td>
<td>2.0</td>
<td>mg/L</td>
</tr>
</tbody>
</table>

Figure 3. Operation modes of the FbSBR during a cycle.

Sampling and Analysis

Both influent and effluent samples were measured two or three times a week. Samples were prepared by filtering through 0.45 µm of filter paper (GF/C-Whatman®). Ammonium was measured by selective electrode (Ammonia-Selective Electrode ORION®). Nitrite and nitrate were determined by UV spectrometric method. COD₃ was analyzed using a closed reflux method with HACH-kit. pH was examined by pH meter (inoLAB WTW). Oxidation reduction potential (ORP) was measured using a Beckman Φ 34 pH transmitter and a redox electrode Thermo Orion (Ag/AgCl reference system). DO was measured by YSI DO meter (Yellow Springs Instruments, USA). Conventional and other parameters of interest such as total suspended solid (TSS) and alkalinity were performed in accordance with the Standard Methods [19].

Calculation

The applied kg total inorganic nitrogen (TIN) and total chemical oxygen demand (TCOD) by the bioreactor m³/day, were calculated as the following equation (Eq. 1):

Applied loading rate (kg/m³/d) \( \frac{C_{\text{Influent}} \times Q}{V_{\text{biologically active zone}} \times 1000} \)  

(1)

The volumetric total inorganic nitrogen (TIN) and total chemical oxygen demand (TCOD) removal rates were calculated as follows (Eq. 2):

Volumetric removal rate (kg/m³/d) \( \frac{(C_{\text{Influent}} - C_{\text{Effluent}}) \times Q}{V_{\text{biologically active zone}} \times 1000} \)  

(2)

Where: C is substance concentration of COD or N (mg/L). Q is feeding rate (L/d). V is volume (L). The factor of 1000 is needed for the given units.

Results and Discussion

As mentioned in Experimental section, the reactor was inoculated by activated sludge and conducted by batch mode at the beginning of operation. After one week, large amount of sludge was adhered on the matrix of filamentous media and biofilm formation was observed and demonstrated in Figure 4.

During whole experiment, the reactor operated without pH control and with basic pH (about 8.4) in the influent. At the end of inoculation period, pH in the reactor decreased to 5.4 while initial pH was adjusted at about 8 (data not shown). This decrease of pH can be explained by nitrification occurring in the reactor, where ammonium is oxidized to nitrogen oxides and H⁺ ion is produced. After day 7, the reactor was operated as continuous mode and pH in effluent gradually increased up to neutral pH and then was mostly constant during the
Figure 4. Photographs of packed media (A) and biofilm formation (B) after one week inoculation.

Figure 5. Mixed liquor suspended solid (MLSS) profile in FbSBR.

The variation of MLSS concentration during whole experiment was shown in Figure 5. During batch mode operation for biofilm formation, suspended solid concentration rapidly decreased in first 4 days from 5500 to about 3000 mg/L. It demonstrated that the large surface and filamentous-type of BCPLUS media (around seven times higher than that of conventional media generally used in full-scale reactors [20]) has the advantage of retaining great amount of biomass on its matrix rapidly. During next 3 days, a little change of MLSS concentration was observed in the reactor. It seemed that after one week the maximum biomass-fixed capacity of media was achieved (approximately 7.7 g TS/m² media surface, TS = total solid). On day 7, suspended biomass was completely withdrawn from the reactor resulting MLSS concentration in reactor was negligible as showing in Figure 5. Consequently, microorganisms were only located on the matrix of media as biocatalyst in the reactor during the continuous operation phase. The operation with the formation of biofilm on the media not only that can enhance the quality of effluent in terms of suspended solid concentration, but also reduce sedimentation time in one cycle. In addition, the interferences in sensitivity of DO, pH, and ORP as well as level-sensor can be significantly avoided when they submerged in the reactor with very low amount of suspended solids. During next 17 days (until day 25), MLSS concentrations still remained as low level (10 ± 2 mg/L). In the following two weeks of experiment, it slowly increased up to about 70 mg/L. It might be due to both the accumulation of non-biodegradable particulates from influent and the suspended growth of bacteria in the reactor. After day 30, the increasing rate of MLSS concentration was likely to be higher than that of previous period and subsequently about 120 mg MLSS/L was observed by the end of experiment. Combining with results from the influent COD values in Figure 8, it seems that the increase of COD load brought about high growth rate of heterotrophic bacteria in the liquid phase of the reactor. This phenomenon was predicted in biofilm models that, in general, fast growing organisms are located at the outside, whereas the slow growing one can be found at the inside of biofilms [21]. In addition, it was experimentally verified in aerobic biofilms: fast growing heterotrophic organisms are located at the outside, whereas slower growing nitrifiers can be found at the inside [22,23].

Figure 6 describes the variations of nitrogenous compounds in both influent and effluent of FbSBR system. As showed in these results, the nitrification process was observed at very beginning of this experiment. It was demonstrated by the decrease of ammonium and production of NO₂⁻-N concentrations during inoculation period. About 80 % of ammonium nitrogen was nitrified at day 6 (Figure 7). Nitrite nitrogen (NO₂⁻-N) build-up was observed during first 4 days but it was not detected after next two days. While nitrate nitrogen (NO₃⁻-N) concentration increased continuously during whole batch phase and then on day 6 it reached level of about 72 mg/L which is likely equivalent to NH₄⁺-N concentration in synthetic wastewater at starting time. It shows that nitrifiers (ammonium oxidizing bacteria, AOB; nitrite ox-
dizing bacteria, NOB) were well acclimatized in the reactor.

On day 8, the continuous operation mode was started and the reactor was fed by diluted piggery wastewater (as mentioned in Experimental section). Between day 8 and 17, the reactor was run at 9.5 days of HRT. Interestingly, very high nitrification efficiency was immediately achieved, it was demonstrated by very low effluent ammonium concentration and, additionally, the trendline of nitrate is similar to that of ammonium in the influent (Figures 6 and 7). It indicates that nitrifiers were not shocked by composition of diluted piggery wastewater. The nitrification efficiencies were constantly about 99.5% in next one week while HRT was decreased by 2.2 times (from 9.5 to 4.25 days) on day 17, it results total inorganic nitrogen (TIN) applied loading rate was increased from about 0.04 to 0.07 kgN/m$^3$/d. This data shows that nitrification activity of nitrifiers in biofilm was not affected by decreasing HRT. In addition, the effluent nitrite concentrations had been almost at background level, therefore, it can be said that complete oxidation of ammonium to nitrate had been occurred during first 16 days of the continuous phase.

On day 24, new series of raw piggery wastewater was collected and characterized by higher concentration of ammonium as well as COD (about two times) in comparison with previous raw wastewater that caused TIN load was increased up to around 0.16 kgN/m$^3$/d during rest operation time of this study (Figure 7). After two days feeding with new raw wastewater, NH$_4^+$-N concentration in the effluent increased up to about 50 mg/L, however, it then gradually decreased and was lowered to around 2 mg/L on day 37 resulting over 99 % nitrification efficiencies were observed (Figures 6 and 7). It means that ammonium oxidation activity of biofilm could be followed up rapidly with the two times increase of TIN load. In the case of NO$_3^-$-N, nitrate concentration was suddenly decreased to about 5 mg/L after one week feeding with new raw wastewater and, subsequently, had been being about 15 mg/L during the following week. But, in contrast, nitrite was accumulated and 100 mg NO$_2^-$-N/L was observed in the effluent on day 30. Because nitrification efficiencies were not changed during mentioned periods (Figure 7), it can be, therefore, thought that incomplete nitrification (ammonium is only oxidized to nitrite) occurred in the reactor. Theoretically, biological nitrification is carried out in the sequential oxidation of ammonium via nitrite to nitrate by AOB and NOB, respectively. It has been also known that nitrite oxidizers are generally inhibited by the presence of free ammonia (0.1 to 10 mg N/L), dissolved oxygen limitation, and nitrous acid (HNO$_2$, 0.22 to 0.8 mg N/L) [24,25]. Due to difference between the half oxygen saturation coefficients of ammonium oxidation and nitrite oxidation (0.16 and 0.54 mg/L, respectively) [26], therefore low dissolved oxygen could limit nitrite oxidation. Considering the results obtained between day 27 and 41, the incomplete nitrification was mostly due to the limitation of dissolved oxygen in biofilm. Because the increase of TCOD load during this period that brought about rapidly (Figure 8) growth rate of heterotrophic bacteria in outer-layer of biofilm that can decrease DO concentration in inner-layer of biofilm where autotrophic nitrifiers would be located.
pH was shown at neutral (data not shown), it can be assumed that the average HNO₂ concentration was low, it therefore concluded that HNO₂ would not be corresponded for the accumulation of nitrite. During last 20 days, nitrite was not further accumulated whereas nitrate concentration in the effluent was gradually increased and constantly about 65 mg/L. It would be due to equilibrium of the numbers or activities between the AOB and NOB were reestablished in the reactor system.

Figure 8 shows the trends of COD_t during whole operation of FbSBR, it seems that almost biodegradable organic compounds in the influent were oxidized resulting removal efficiency of about 80 % at the end of this experiment while TCOD_t load was calculated by around 0.8 kg/m³/d. It can be assumed that the remaining COD_t in the effluent is corresponded for non-biodegradable organic portion in piggery wastewater.

Cyclic study

To complete understanding insight of the reactor performance in FbSBR, the cyclic study was carried out on day 61 (last two hours of immediately previous cycle were also monitored, indicated by minus values on horizontal axis). The online analysis data were presented in Figure 9.

Considering the variations of physical parameters, there was a little change of pH (about 1.0 unit) while DO and ORP profiles changed following the nature of each phase. Deeply, however, it shows that there is a break-

point on ORP trendline during anoxic phase (between minute 45 and hour 1) and on DO trendline during oxic phase (between hour 9.5 and 10), (except the sudden changes due to alternating operation phase). Interestingly, those breakpoints mostly match to the breakpoints-like of nitrogen compounds profiles, especially, of NOₓ trendlines. With ORP, its breakpoint was occurred when there was no further decrease of nitrate concentration after first 1 h of anoxic phase. Taking together with the constant reduction of COD concentration, reasonably, it can be said that ORP breakpoint is mostly caused by the reduction rate of NOₓ concentration (the same phenomenon was obtained on cyclic study on day 31 when portion of NO₂⁻-N is predominant, data not shown). With DO, the breakpoint was observed when nitrite concentration started to decrease and higher increasing rate of nitrate was detected. It also should note that ammonium oxidation efficiency was nearly 100 % at such point and there was no considerable change of COD in last 3.5 h of oxic phase. Consequently, it can be sure that oxygen consumption rate in the bioreactor was remarkably decreased during last 2 h of oxic phase. Thus, DO concentration rapidly increased in comparison with previous three hours and was 4.5 mg/L by the end of oxic phase. From COD_t trendline, it shows that almost biodegradable organic compounds were consumed by facultative bacteria in anoxic phase and only non-biodegradable part remained during rest of cycle time. During oxic phase, ammonium concentration constantly decreased while nitrate and nitrite were produced. The accumulation of nitrite during anoxic phase might be due to incomplete denitrification, while the evident of nitrite during oxic phase can be caused by higher oxygen saturation coefficients nitrite oxidation when limitation of oxygen diffusion in biofilm would be occurred. The consumption of alkalinity for nitrification was evidently observed during oxic phase.

Conclusion

High amount of biomass (approximately 7.7 g TS/m² media surface) can be fixed on the matrix of media rapidly and simultaneous nitrogen and organic can be removed on that. The FbSBR was regarded as an effective and compact system as it showed acceptable volumetric removal rates of total inorganic nitrogen (0.12 kg N/m³/d) and organic (0.66 kg COD/m³/d). The occurrence of incomplete nitrification is mostly due to limitation of DO in inner-layer of biofilm. For further study, the effects of DO will be investigated to remove nitrogen via nitrite instead of nitrate. It is suitable and cost-effective method for treating low C/N piggery wastewater in this study. The occurrence of ORP and DO breakpoints
would be utilized for online monitoring to determine operational modes during each steady-state period. Molecular analysis techniques would be used to characterize bacteria population in both suspension biomass and biofilm. Specifically, the distribution of the ammonia oxidizers, the nitrite oxidizers, and the heterotrophic bacteria in the fixed-bed biofilm should be examined under different operating conditions. These findings could help us to further understand the behavior of the FbSBR and, hence, to propose a solution for optimizing the performance of this system.

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References