Nutrient removal performance of an anaerobic–anoxic–aerobic process as a function of influent C/P ratio

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Abstract: The laboratory scale anaerobic–anoxic–aerobic (A2O) process fed with synthetic brewage wastewater was designed to investigate the effects of changing feed C/P ratio on the performance of biological nutrient removal (BNR) processes. In the experiment, the influent chemical oxygen demand (COD) concentration was kept at approximately 300 mg L\(^{-1}\) while the total phosphorus concentration was varied to obtain the desired C/P ratio. Results showed that when the C/P ratio was lower than 32, phosphorus removal efficiency increased as C/P ratio increased linearly, while when the C/P ratio was higher than 32, the P removal efficiency was maintained at 90–98%, and effluent P concentration was lower than 0.5 mg L\(^{-1}\). However, regardless of the C/P ratio, excellent COD removal (90% or higher) and good total nitrogen removal (75–84%) were maintained throughout the experiments. It was also found that very good linear correlation was obtained between COD uptake per unit P released in the anaerobic zone and C/P ratio. In addition, the P content in the wasted activated sludge increased with the decrease in the C/P ratio. Based on the results, it was recommended that the wastewater C/P ratio and its effects be incorporated into BNR design and operational procedures, appropriate C/P ratios were used to achieve the effluent treatment goals.

Keywords: anaerobic–anoxic–aerobic (A2O) process; influent C/P ratio; biological nitrogen and phosphorus removal

INTRODUCTION

Phosphorus and nitrogen removal from wastewater is an effective approach for prevention of eutrophica-
tion in closed water systems, and biological nutrient removal (BNR) processes have proved to be the most economical means to remove nitrogen and phosphorus. But BNR processes are more complicated and sensitive to operation than conventional activated sludge. There are many factors which affect its performance, eg retention time, wastewater characteristics, temperature, etc. Wastewater characteristics have been thoroughly investigated and found to have a strong effect on the performance of BNR processes. Janssen2 stated that suitable composition of wastewater, ie biological oxygen demand (BOD): P and BOD:N ratios, were one of the prerequisites for BNR processes. Randall et al3 stated that all BNR processes must be either phosphorus limited or chemical oxygen demand (COD) limited, and they introduced the concept of the C/P ratio as a factor that affected BNR processes and noted that the C/P ratio of the influent wastewater entering the anaerobic zone governed the growth of poly-P bacteria (or polyphosphate-accumulating organisms (PAOs)) and consequently affected the phosphorus removal efficiency of the systems. Randall and Chapin4 stated if the C/P feeding ratio was lower, COD was limiting, the fraction of poly-P bacteria would be high, and this would lead to a high percentage of phosphorus in the wasted activated sludge (WAS). However, phosphorus removal would be limited, ie excess phosphorus would remain and be discharged into the effluent. If the C/P feeding ratio was higher, phosphorus was limiting, poly-P bacteria might not be the dominant species in the system, the excess COD would favor the growth of glycogen-accumulating organisms (GAOs), thereby reducing the fraction of PAOs in the activated sludge, and reduce the phosphorus content in

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the wasted activated sludge. Low effluent phosphorus concentrations would be observed under these conditions if secondary phosphorus release was insignificant. Andrew and David supported the fact that the C/P feeding ratio was found to be a key factor influencing an ‘internal energy-based’ competition between PAOs that mediate biological phosphorus removal and GAOs that did not. Both groups were previously known to use internally stored polyphosphate and glycogen, respectively, as energy sources for storing acetate as polyhydroxyalkanoates (PHA) in the anaerobic stage, and to use the reserved material for growth in the aerobic stage.

Though the anaerobic–anoxic–aerobic (A2O) process is well known as a simultaneous nitrogen and phosphorus removal process and is used widely throughout the world, many researchers mainly focus on the sequential batch reactor (SBR) and University of Cape Town (UCT) processes. To date, little research and technical effort has been made to investigate operational optimization and influence of different factors on the performance of the A2O process. Therefore, the objective of the study is to determine the influences of the C/P feeding ratio on the performance of biological nutrient removal in the A2O process. Variations of the P content in the WAS, anoxic P uptake and poly-β-hydroxybutyrate (PHB) production and utilization with the C/P feeding ratio were also investigated.

MATERIAL AND METHODS

Experimental set-up

A laboratory-scale activated sludge system was designed to investigate the effects of influent C/P ratio on the performance of an A2O reactor with an operating volume of 47.5L and a settler of 25L, as shown in Fig 1. The A2O reactor, made of Plexiglass, is separated into eight compartments, the initial two compartments are anaerobic stages, and then the following two compartments are anoxic stages, the last four are aerated, the volume ratio of the anaerobic/anoxic/aerobic stages is 1.25:1:2.75; all compartments are fully mixed. Stirrers provide mixing in each of the anaerobic and anoxic reactors to keep the biomass in suspension. Aeration is provided in each compartment of the aerobic zone by pressurized air passing through long stone diffusers. Every compartment has on-line sensors (dissolved oxygen concentration (DO), pH, redox potential (ORP), and temperature). The nitrate recirculation flow and sludge recycle flow are controlled by peristaltic pumps. The operational parameters for the A2O system are shown in Table 1.

Wastewater composition

The synthetic wastewater used throughout the studies was composed of brewage (3.2–3.4 mL L$^{-1}$), NH$_4$Cl (0.20 g L$^{-1}$), KH$_2$PO$_4$ (0.02–0.08 g L$^{-1}$), MgSO$_4$.7H$_2$O (0.05 g L$^{-1}$), NaHCO$_3$ (0.05–0.15 g L$^{-1}$), and CaCl$_2$.2H$_2$O (0.01 g L$^{-1}$). The main element of the synthetic wastewater: alcohol (31–47%); acetate acid (29–33%); propionate acid (3–6%); butyric acid (3.8–5%); slow biodegradable COD (5.9–20.9%); inert COD (3.1–12.3%), the proportion was that each component accounts for the total COD (TCOD). The feed COD and NH$_4$-N were kept at approximately 300 mg L$^{-1}$ and 50 mg L$^{-1}$, while the C/P ratio, that is influent TCOD/TP ratio, was varied between 18 and 62, C/P ratio was considered as an independent variable under four mean C/P feeding ratios of 55, 45, 34 and 23 to investigate the influences of C/P ratio on the performance of the A2O reactor.

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**Table 1. The operational parameters of the A2O system$^a$**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HRT (h)</th>
<th>SRT (d)$^b$</th>
<th>DO (mg O$_2$.L$^{-1}$)</th>
<th>T(°C)</th>
<th>Q$_H$(L.d$^{-1}$)</th>
<th>Q$_R$(L.d$^{-1}$)</th>
<th>Q$_{f}$(L.d$^{-1}$)</th>
<th>R</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td>9</td>
<td>10</td>
<td>2–3</td>
<td>20</td>
<td>127</td>
<td>404</td>
<td>64</td>
<td>2–4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$^a$Note: HRT — hydraulic retention time; SRT — sludge retention time; Q$_H$ — influent flow; Q$_R$ — internal recycling flow; R — internal recirculation ratio; Q$_{f}$ — return sludge flow; $r$ — return sludge ratio.

$^b$The clarifier volume was not used for the SRT determination.
Table 2. Run cycles of the whole experiments

<table>
<thead>
<tr>
<th>Run cycle</th>
<th>C/P ratio</th>
<th>Average C/P ratio</th>
<th>P concentration (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run I</td>
<td>18–25</td>
<td>23</td>
<td>12–16.7</td>
</tr>
<tr>
<td>Run II</td>
<td>30–36</td>
<td>34</td>
<td>8.3–10.0</td>
</tr>
<tr>
<td>Run III</td>
<td>41–51</td>
<td>45</td>
<td>5.9–7.3</td>
</tr>
<tr>
<td>Run IV</td>
<td>53–61</td>
<td>55</td>
<td>4.9–5.7</td>
</tr>
</tbody>
</table>

Note: COD = 300 ± 20 mg L\(^{-1}\), alkalinity = 300–400 mg L\(^{-1}\)

Reactors were operated for a period of more than two times mean cell residence time (MCRT) to obtain a steady state for each C/P ratio. The experimental reactor was seeded with sludge from the Wenchang sewage treatment plant (Harbin, China), samples were collected from each compartment of the reactor and measured at intervals of 1 day. The system was operated for 3 months before steady state was assumed and data collection started. The experimental work lasted 185 days. Table 2 shows the phases of the whole experiment procedures.

Analytical methods
COD, mixed liquid suspended solid concentration (MLSS), alkalinity, \( \text{NH}_4^+\)-N, \( \text{NO}_2^-\)-N, \( \text{NO}_3^-\)-N, total nitrogen (TN) and \( \text{PO}_4^{3-}\)-P were measured according to standard methods (APHA). \(^9\) P content in the WAS was measured according to the method of Zhao Qingxiang. \(^10\) The DO and temperature were measured continuously using a WTW inoLab Oxi level 2 oxygen meter (inoLab, Germany). Continuous monitoring of pH and ORP were carried out using a WTW pH/Oxi340i on-line analyzer. PHB was measured using a rapid gas chromatographic method described by Smolders et al. \(^11\)

RESULTS AND DISCUSSION

Effect of C/P ratio on phosphorus removal efficiency
Figure 2 shows the variation of phosphorus concentration with C/P feeding ratio in the A\(^2\)O process. It can be observed from Fig 2 that when the C/P feeding ratio was lower than 32, phosphorus removal efficiency increased as C/P ratio increased linearly, P removal efficiency was as high as 98% at the C/P ratio of 32. When the C/P feeding ratio was higher than 32, the P removal efficiency was maintained at 90–98%, and effluent P concentration was lower than 0.5 mg L\(^{-1}\). It is apparent that the value of 32 for C/P ratio was the boundary between COD limiting and P limiting conditions in this system. The value complying with the limit permits was less than those reported by Tetreault et al. \(^12\)

It can be seen from Table 3 that average anaerobic P release, anoxic P and aerobic P uptake decreased with the increase of the C/P ratio, however, the P removal efficiency showed the opposite trend. Yagci et al. \(^13\) stated that at high C/P ratio, P is limiting, the existing organic carbon, mainly volatile fatty acids (VFA), was more than necessary to store all available P so that the net result is total P removal, whereas when P is not limiting, the P storage capacity is limiting and there was always a net P level in the effluent escaping the enhanced biological phosphorus removal (EBPR). As a result, average P removal efficiency increased with the increase of C/P ratio, in particular, when the C/P ratio was lower than the critical value of 32, P removal efficiency decreased dramatically. On the other hand, Filipe et al. \(^14\) noted that the presence of GAOs had important consequences for the phosphorus removal capability of EBPR systems, the competitive advantage of PAOs in EBPR systems was compromised because GAOs use the same substrate (VFAs) under the same conditions, the supply of VFAs to EBPR systems was limited, if a significant amount of GAOs accumulated, the percentage of VFAs available to PAOs was reduced, therefore decreasing the phosphorus removal capability of the systems. In this study, when C/P ratio is higher, available COD would not be completely utilized by the poly-P bacteria in the anaerobic zone, the excess COD would favor the growth of GAOs, which led to a reduction in the fraction of poly-P bacteria in the activated sludge, consequently, anaerobic P release,
The performance of an anaerobic–anoxic–aerobic process

Performance of an anaerobic–anoxic–aerobic process

The plot of average COD uptake per unit P release in the anaerobic zone versus C/P ratio is shown in Fig 5. It can be seen that anaerobic COD removed per unit P release has good positive linear correlation with the C/P ratio. Table 4 compares the variation of average anaerobic COD uptake per unit P release or P uptake with the C/P ratio in this study with the results reported by Punrattanasin. It can be seen that less COD removal was required in that system to release P in the anaerobic zone, however, the anaerobic COD uptake/system P uptake ratio of that system was greater than the results observed in this study. There are probably two reasons for the difference. One is that anaerobic conditions prevailed in the

anoxic and aerobic P uptake decreased as the C/P ratio increased. Table 3 shows that P uptake in the anoxic zone was also observed at each of C/P feeding ratio. Except the dilution, the ratio of anoxic P removal to anaerobic P release reached 60–70%, significantly reducing oxygen consumption.

Figure 3 shows the relationship of anaerobic P release with aerobic and anoxic P uptake. The correlation coefficient ($R^2$) between them reached 0.9914, and the average P uptake to P release is as high as 1.2, the value that was the theoretical ratio expected for an EBPR.

Effect of C/P ratio on COD removal efficiency

Regardless of the C/P ratio, COD removal of approximately 90% or higher was obtained for all experiments. The COD removal versus C/P ratio is shown in Fig 4. Greater than 79% COD was removed from solution in the anaerobic zone, while 6–11% COD removal was obtained in the anoxic zone. Very little COD was available in solution in the aerobic zone. It was also found that most of the readily biodegradable organic matter removed in the anaerobic zone was used to synthesize PHB, regenerating energy to complete the release of orthophosphate, then the combination of stored PHB providing internal carbon and slowly biodegradable organic matter formed by fermentation and hydrolysis were used to accomplish phosphorus uptake and denitrification in the anoxic zones, simultaneously, replenishing the glycogen utilized anaerobically and regenerating the intracellular polyphosphate pool.

Table 4. Comparison of anaerobic COD uptake per unit P uptake

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental results</th>
<th>Punrattanasin$^{15}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23</td>
<td>34</td>
</tr>
<tr>
<td>Anaerobic COD uptake/anaerobic P release</td>
<td>6.6</td>
<td>8.5</td>
</tr>
<tr>
<td>Anaerobic COD uptake/system P uptake</td>
<td>5.77</td>
<td>6.83</td>
</tr>
</tbody>
</table>

$^a$ Note: system P uptake does not include the P release or uptake in the secondary clarifier.

$^b$ Note: no units, as the value is the C/P ratio value.
Yong Ma et al.

second anoxic zone of that system with additional phosphorus release and PHA storage, whereas this system maintained anoxic environments with excess nitrate, thereby resulting in all of the phosphorus being released and PHA storage occurring in the anaerobic zone, instead of in both the anaerobic and anoxic zones as in that system. Second, the organic substrates of the wastewater in this system, mainly alcohol, had to be fermented to acetate before they were available for anaerobic storage, whereas in that system sodium acetate was added directly and it was readily available without fermentation, therefore it was used more efficiently. On the other hand, the influent COD in that system contained 400mg L$^{-1}$, much of which was colloidal, whereas in this study influent COD contained only 300mg L$^{-1}$, with lesser colloidal content, meaning that more substrate was available to the non-polyphosphate-accumulating organisms in that system than in this study.

**Effect of C/P ratio on nitrogen removal efficiency**

Figure 6 shows the nitrogen removal versus C/P feeding ratio in this system. Very good ammonium and total nitrogen removal efficiencies were obtained throughout all experiments, as shown in Fig 6. Ammonium and total nitrogen removal efficiencies were maintained at 90% or higher and 75–84%, respectively, the average effluent ammonium concentration and TN concentration were approximately lower than 1mg L$^{-1}$ and 15mg L$^{-1}$, respectively, which could comply with the discharge standards. It also found that effluent nitrate concentration was closely equal to effluent TN concentration, it was demonstrated that effluent N was mainly discharged in the form of nitrate nitrogen in the A$^2$O system.

It is well known that the anoxic environments would become anaerobic environments if nitrate was consumed completely in the anoxic zone, it was unavoidable for phosphorus release to occur and take up organic substrate at a slow rate, either due to a conversion reaction (hydrolysis, fermentation) or due to incoming readily degradable substrates not being taken up in the anaerobic zone. To improve the nitrogen removal efficiency, meanwhile, to enhance the function of anoxic P uptake in the anoxic zone so as to reduce the operation cost, the experiments maintained the nitrate concentration at the end of the anoxic zone at 1–3mg L$^{-1}$ by adjusting the internal recirculation flow rate according to the nitrate recirculation control strategies reported by Yuan et al.$^{16}$ Figures 7 and 8 show the variations of PO$_4^{3-}$–P and NO$_3^-$–N concentration in the anoxic zone versus C/P ratio as well as the variations of NO$_3^-$–N concentration at each compartment with C/P ratio, respectively. It can be observed that nitrate was available as an electron acceptor in the second anoxic reactor by PAOs for the oxidation of stored PHB to realize P uptake simultaneously. Maximum denitrification potential was utilized because enough nitrate was provided by controlling the internal recirculation ratio, the utilization of internal recirculation control strategies improved and optimized the denitrification and P uptake potential in the anaerobic zone of the A$^2$O system, average NO$_3^-$–N and PO$_4^{3-}$–P removal efficiencies were between 70% and 85% and 60% and 71% for four mean C/P ratios of 55,45,34,23 in the anoxic zone, respectively. As a result, the C/P ratio was observed to have only a slight effect on nitrogen removal in this study.
The relationship between anoxic PHB utilization and anoxic denitrification was also studied in the experiments. The results showed that anoxic PHB utilization did not correlate well with anoxic denitrification, but the average denitrification rate in the first anoxic zone increased as the C/P ratio increased, which was 1.43 mgNO₃⁻/N gMLSS⁻¹ h⁻¹, 1.54 mgNO₃⁻/N gMLSS⁻¹ h⁻¹, 1.60 mgNO₃⁻/N gMLSS⁻¹ h⁻¹ and 1.91 mgNO₃⁻/N gMLSS⁻¹ h⁻¹ for the C/P ratios of 23, 34, 45 and 55, respectively. However, some reduction of PHB was observed in the anoxic zone at each C/P ratio, as shown in Fig 9. Anoxic PHB utilization reached an average 20–39% at the four mean C/P ratios, it was possible PHB was utilized as an electron donor for denitrification simultaneously with the soluble/colloidal COD available in the anoxic zone. On the other hand, it was also found that PHB levels increased with the increase in the C/P ratio, this indicated that PHB production was controlled by the availability of COD rather than the utilization of energy from poly-P bonds. In addition, more COD was taken up anaerobically per unit of anaerobic PHB produced at lower C/P ratios, resulting in less PHB production. It was also possible that a lower rate of depolymerization of polyphosphate to obtain ATP for transporting and storing organic matter in the anaerobic zone occurred.

Effect of C/P ratio on WAS production

Table 5 shows the variations of the biomass concentration at each C/P ratio. As shown in Table 5, large changes in the amount of P in the MLSS, from 10.4% for C/P ratio of 23 to 4.9% for C/P ratio of 55 could be observed. But the observed yield coefficients ($Y_{obs}$) were hardly affected by the C/P ratio. Mixed liquor volatile suspended solids (MLVSS) concentrations decreased as the C/P ratio increased. However, COD removals changed randomly as the C/P ratio changed, indicating that the accumulation of inorganic compounds was responsible for the MLSS concentration changes rather than MLVSS concentration, because the denitrification rate increased relative to the aerobic stabilization of COD as the C/P ratio increased, it is well known that anoxic stabilization resulted in less biomass production than aerobic stabilization. It seemed obvious that increased denitrification decreased the amount of biomass produced and, therefore, decreased the MLVSS concentrations as the C/P ratio increased.

Table 5 also shows that the MLVSS/MLSS increased as the C/P increased, and the MLSS concentration at the C/P ratio 55 was only 64% of that at the C/P ratio of 23. Because stored polyphosphate and cations, mainly potassium and magnesium, were measured as MLSS, it was expected that lower MLVSS/MLSS ratios would be observed as the amount of P increased in the sludge. McClinlock reported an MLVSS/MLSS ratio of approximately 70% in a system with high amounts of P in the sludge (>10% P/VSS), and a ratio of 80% or greater in a system with low amounts of P in the sludge (<4% P/VSS).

**CONCLUSIONS**

As discussed above, the C/P ratio of the influent wastewater had a substantial effect upon the performance of the BNR treatment system. Results showed that when the C/P feeding ratio was lower than 32, phosphorus removal efficiency increased as C/P ratio increased linearly, while when the C/P feeding ratio was higher than 32, the P removal efficiency was maintained at 90–98%, and effluent P concentration was lower than 0.5mg L⁻¹. However, regardless of the C/P ratio, excellent COD removal (90% or higher) and good total nitrogen removal (75–84%) were maintained throughout the experiments. It was also found that very good linear correlation was obtained between COD uptake per unit P released in the anaerobic zone and C/P ratio. In addition, the P content in the wasted activated sludge increased with the reduction of C/P ratio. On the other hand, the changes in the C/P ratios did not significantly affect the observed yield coefficient ($Y_{obs}$), but strongly affected the MLSS concentration. The MLSS concentration at the C/P of 55 was only 64% that in the system with a C/P ratio of 23.

This research provided the variations of COD, P, N removal efficiency and biomass production.
as a function of the C/P ratio. Once the C/P ratio of the wastewater has been characterized, a careful evaluation must be made to ensure high removal efficiency of phosphorus and nitrogen. It is recommended that the wastewater C/P ratio and its effects be incorporated into BNR design and operational procedures. Appropriate C/P ratios are needed to achieve the effluent treatment goals. To date, analysis of the metabolism and microorganisms involved in phosphorus release and uptake in the A2O process is still limited: a great deal of research and technical effort must been made in the near future.

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