Efficient recovery of carbon, nitrogen, and phosphorus from waste activated sludge

Yinguang Chen, Xiong Zheng, Leiyu Feng and Hong Yang

ABSTRACT

Carbon, nitrogen, and phosphorus need to be recovered to reduce the environmental impact of waste activated sludge (WAS). In this study the improved short-chain fatty acid (SCFA) production from WAS by the addition of kitchen waste to adjust the ratio of carbon to nitrogen (C/N), and the efficient recovery of nitrogen and phosphorus from the fermentation liquid were reported. Firstly, the optimum conditions for SCFA production were found to be pH 8, temperature 35°C, C/N ratio 21 mg-C/1 mg-N, and fermentation time 6 d, using the response surface methodology. After alkaline fermentation, the struvite precipitation method was applied to efficiently and simultaneously recover the released ammonia and phosphorus from the fermentation liquid. Finally, the fermentation liquid was used as the additional carbon source for biological nitrogen and phosphorus removal. It was observed that, compared with acetic acid, the use of fermentation liquid as carbon source showed greater removal efficiencies of total nitrogen and total phosphorus.

Key words | alkaline fermentation, biological nutrient removal, excess sludge, kitchen waste, nutrient recovery, short-chain fatty acids

INTRODUCTION

The activated sludge process is an economical and environmentally friendly treatment method for removing nutrients from wastewater. However, large amounts of waste activated sludge (WAS) are generated. For example, it was reported that nearly 10 million tons of dry WAS were annually produced in the EU (Appels et al. 2008). Thus, the treatment and disposal of WAS become a significant concern for researchers. WAS usually contains high contents of protein and carbohydrate, which can be reused to generate useful products (Feng et al. 2009; Jiang et al. 2009).

It is well known that biological nitrogen and phosphorus removal requires carbon source such as short-chain fatty acids (SCFAs) in the influent of wastewater treatment plants (WWTPs). However, the available SCFAs in municipal wastewater are usually not enough to ensure satisfied nutrient removal (Thomas et al. 2005). Therefore, in recent years, the SCFA production from anaerobic WAS fermentation has become an attractive research topic (Tong & Chen 2007). However, the efficiency of SCFA production is usually very low due to the lower carbon to nitrogen ratio (C/N), the poor sludge utilization by anaerobic microbes, and the quick consumption of SCFAs by methanogens. Meanwhile, there were significant amounts of ammonia and phosphorus released into the fermentation liquid (Tong & Chen 2009), and the direct use of this fermentation liquid as carbon source would increase the nitrogen and phosphorus loadings of WWTPs, which might be adverse to wastewater biological nutrient removal.

It was observed in our previous studies that an appropriate increase of the C/N ratio of the sludge fermentation system resulted in the enhancement of SCFA production (Feng et al. 2009). It is well known that kitchen waste is enriched in carbohydrate. Therefore, one main objective of this study was to improve the SCFA production from WAS fermentation by the addition of kitchen waste to adjust the C/N ratio.

Nitrogen and phosphorus in the fermentation liquid are valuable nutrient elements, which can be recovered as fertilizing minerals. Although many strategies can be used to achieve the recovery of ammonia or phosphorus, the method of struvite (MgNH₄PO₄·6H₂O; magnesium ammonium phosphate, MAP) formation has become a research focus in recent years, because NH₄⁺-N and soluble ortho-phosphorus (SOP) can be simultaneously recovered.
and the formed struvite is a slow-release fertilizer (Zhang & Chen 2009). Thus, the second purpose of this study was to recover the released nitrogen and phosphorus in the fermentation liquid by the struvite method.

After the recovery of nitrogen and phosphorus, the fermentation liquid was utilized as carbon source in wastewater biological nutrient removal, which was the third aim of this study. Its performance was compared with that of acetic acid, a commonly used carbon source in the literature. Also, the effect of fermentation liquid on the microbial community of biological nutrient removal was investigated.

**MATERIALS AND METHODS**

**Characteristics of WAS and kitchen waste**

WAS used in this study was obtained from the secondary sedimentation tank of a municipal WWTP, and then concentrated by settling at 4 °C for 24 h. The kitchen waste was collected from a dining hall of Tongji University, crushed by an electrical blender, and then filtered through a stainless steel sieve (US Mesh No. 10 with corresponding sieve openings of 2.0 mm). The main characteristics of WAS and kitchen waste are recorded in Table 1. It can be seen that the protein, carbohydrate, and lipid were the major organic matters in WAS and kitchen waste.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WAS</th>
<th>Kitchen waste</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SDb</td>
<td>Mean</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
<td>0.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Total solids (TS)</td>
<td>23.1</td>
<td>0.6</td>
<td>215.5</td>
</tr>
<tr>
<td>Volatile solids (VS)</td>
<td>13.4</td>
<td>0.5</td>
<td>190.3</td>
</tr>
<tr>
<td>Soluble chemical oxygen demand (COD)</td>
<td>0.8</td>
<td>0.1</td>
<td>110.5</td>
</tr>
<tr>
<td>Total COD</td>
<td>20.1</td>
<td>0.8</td>
<td>300.2</td>
</tr>
<tr>
<td>Total carbohydrate (as COD)</td>
<td>2.3</td>
<td>0.5</td>
<td>126.5</td>
</tr>
<tr>
<td>Total protein (as COD)</td>
<td>14.2</td>
<td>0.3</td>
<td>65.3</td>
</tr>
<tr>
<td>Lipid and oil (as COD)</td>
<td>0.18</td>
<td>0.1</td>
<td>41.5</td>
</tr>
<tr>
<td>Total nitrogen (TN)</td>
<td>2.6</td>
<td>0.2</td>
<td>7.8</td>
</tr>
</tbody>
</table>

*aAll values are expressed in g/L except pH.

*bSD: standard deviation.

**Optimization of the co-fermentation of WAS and kitchen waste for SCFA production using response surface methodology.**

It has been reported that pH, temperature, C/N ratio (mg-C/mg-N) and fermentation time were the key influencing factors of anaerobic digestion of organic waste (Feng et al. 2009). A series of fermentation reactors, with working volume of 5.0 L each one, were operated for the production of SCFAs. During the anaerobic fermentation, WAS and kitchen waste were taken as the feedstock. Also, the WAS was used as the inoculum for the start-up of the reactors, which were stirred at 80 rpm for mixing the fermentation substances. The pH values of these reactors were adjusted by adding 2 M NaOH or 2 M HCl. The temperatures investigated in this study were controlled by a water-bath and the operation time of these reactors was set at 1, 3, 5, and 7 d, respectively. During the fermentation process, the mixtures were withdrawn from the reactors for the analysis of the SCFA concentration.

Response surface methodology (RSM) is a powerful tool for evaluating the effects of multiple variables on the process performance. Thus, this method was used to obtain the optimum conditions for the SCFA production from co-fermentation of WAS and kitchen waste. The experiments were carried out based on the central composite design (CCD) by Design Expert (Version 8.0, Stat-Ease, Inc., USA) (Tong & Chen 2007), and the concentration of SCFAs was the dependent output variable. The experimental data obtained from the design were analyzed by the response surface regression procedure. According to the Design Expert software, the response of SCFA production during the co-fermentation of WAS and kitchen waste could be fitted by a predictive polynomial quadratic model (Equation (1)):

\[
Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1,j=1}^{4,3} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2
\]  

where \(Y\) represents the predicted SCFA concentration, \(X_i\) and \(X_j\) are the independent variables, and \(\beta_0, \beta_i, \beta_{ij}, \) and \(\beta_{ii}\) are the regression coefficients of the model.

For model validation, the same fermentation reactors were operated in the range of pH 6–10, C/N 14–26, temperature 20–50 °C and fermentation time 3–7 d, but the substrate with a different concentration was used. Then, the regression analysis of the experimental data and response surface was conducted.
Optimization of simultaneous recoveries of nitrogen and phosphorus in the fermentation liquid using response surface methodology

Previous studies pointed out that WAS fermentation led to the release of large amounts of ammonia-nitrogen (NH$_4^+$-N) and SOP in the fermentation liquid (Zhang & Chen 2009). It was reported that pH, Mg/N (mol/mol), and P/N (mol/mol) were the most important variables when NH$_4^+$-N and SOP were simultaneously recovered in the formation of struvite (Tong & Chen 2009; Zhang & Chen 2009). RSM was also adopted to determine the optimum conditions of simultaneous recoveries of the released nitrogen and phosphorus in the fermentation liquid of WAS and kitchen waste.

To conduct the experiments, 500 mL of the fermentation mixtures was fed into a glass beaker, which was mechanically stirred at 200 rpm. Then, the pH was adjusted using 2 M NaOH or 2 M HCl. In the fermentation mixtures, the molar concentration of the released NH$_4^+$-N was observed to be greater than that of the released SOP. Thus, in order to simultaneously recover the released nitrogen and phosphorus by the struvite formation (the theoretical molar ratio was 1:1), a certain amount of KH$_2$PO$_4$ solution (140 g/L) and MgCl$_2$·6H$_2$O solution (320 g/L) should be added. According to our previous publication, the reaction time of 5 min was enough for the struvite formation (Tong & Chen 2007), and the residual concentrations of NH$_4^+$-N and SOP in the fermentation liquid were analyzed.

Application of the fermentation liquid to enhance biological nutrient removal

Since the main components of the fermentation liquid were SCFAs (mainly acetic and propionic acids), protein, and carbohydrate, it can be used as the carbon source for biological wastewater nitrogen and phosphorus removal in sequencing batch reactors (SBRs). Two SBRs (SBR-1 and SBR-2), with working volume of 4 L each, were operated on three 8 h cycles (1.5 h anaerobic, 1 h aerobic, 1 h anoxic, 30 min aerobic, 1 h anoxic and 20 min aerobic period, followed by 1 h settling, 5 min decanting and 95 min idle phase) per day. These SBRs were maintained at 21 ± 1 °C, and the dissolved oxygen concentration was about 6 mg/L in the aerobic phase. The mixed liquor suspended solids and sludge retention time were controlled at approximately 3,000 mg/L and 22 d, respectively.

The synthetic wastewater was prepared from the concentrated feed and phosphorus stock solution. The concentrated feed consisted of (g/L): 25.88 peptone, 4.24 yeast extract, 33.94 MgCl$_2$·6H$_2$O, 19.09 MgSO$_4$·7H$_2$O and 8.91 CaCl$_2$·2H$_2$O. The phosphorus stock solution contained (g/L): 23.47 KH$_2$PO$_4$ and 17.60 K$_2$HPO$_4$. Both SBRs were initially cultured with acetic acid, and the C/N ratio was controlled at 20. It was observed that the effluent nitrogen and phosphorus concentrations in these SBRs became relatively stable after almost 80 d of operation, and then the fermentation liquid was used to replace acetic acid in SBR-2 (SBR-1 was still cultured with acetic acid). After the stable operation, the nutrient removal efficiency using the fermentation liquid as carbon source was compared with that using acetic acid.

Analytical methods

Fluorescence in situ hybridization (FISH) analysis was adopted to investigate the microbial communities in the SBRs according to the previous method (Ji & Chen 2010; Zhu & Chen 2011). The measurements of SCFAs, protein, carbohydrate, lipid, COD, TS, VS, NH$_4^+$-N, TN, SOP, and total phosphorus (TP) were described in our previous publications (Feng et al. 2009; Zhang et al. 2010).

Statistical analysis

All tests were performed in triplicate; analysis of variance was used to test the significance of results and $p < 0.05$ was considered to be statistically significant unless otherwise stated.

RESULTS AND DISCUSSION

Co-fermentation of WAS and kitchen waste under alkaline conditions for highly efficient carbon recovery

Usually the co-fermentation of WAS and kitchen waste has been conducted under acidic or neutral conditions (Chen & Wu 2010). Our previous publication pointed out that WAS fermentation under alkaline conditions, especially at pH 10, can significantly enhance the production of SCFAs (Yuan et al. 2006). However, whether the alkaline fermentation can obviously benefit the co-fermentation of WAS and kitchen waste is still unknown. Thus, the co-fermentation of WAS and kitchen waste for the efficient production of SCFAs was investigated at different fermentation pHs (ranging from 6.0 to 10.0). NaOH was usually used to adjust the fermentation pH in the literature, but the higher cost of the
alkali reagent would limit the wide application of the alkaline fermentation. It was reported that calcium hydroxide (Ca(OH)₂) could be used as an alternative to NaOH during anaerobic WAS fermentation (Li et al. 2014). Therefore, in this study Ca(OH)₂ was chosen to adjust the fermentation pHs for conducting the alkaline co-fermentation of WAS and kitchen waste for SCFA production.

First, the experimental and predicted responses of co-fermentation of WAS and kitchen waste for the production of SCFAs based on CCD tests were conducted, and a quadratic model suggested by Design Expert was obtained to describe the production of SCFAs during the co-fermentation of WAS and kitchen waste. Moreover, in the range of pH 6–10, C/N 14–26, temperature 20–50 °C and time 3–7 d, a series of experiments were conducted to test the developed model. Figure 1(a) shows the comparison between the predicted and experimental results, and the determination coefficient (R²) was observed to be greater than 0.98, suggesting that the developed quadratic model could be used to predict the co-fermentation of WAS and kitchen waste for SCFA production.

According to the ‘point optimization’ tool of Design Expert, the optimum conditions of the co-fermentation of WAS and kitchen waste were found to be pH 8, C/N ratio 21, temperature 35 °C, and fermentation time 6 d. Using this alkaline fermentation condition, the SCFA concentration could achieve 556.3 mg COD/g VS. In the literature, the maximum SCFA yield from the mixture of kitchen waste and sludge was 392.6 mg COD/g VS at pH 6.99 (Chen & Wu 2009). These results showed that the co-fermentation of WAS and kitchen waste under the alkaline conditions could produce much more SCFAs than that under the neutral condition. According to our previous studies, the reasons for the higher production of SCFAs might be due to the improvement of substrate hydrolysis and the inhibition of methanogenesis under the alkaline pH (Yuan et al. 2006; Chen et al. 2007; Zhang et al. 2010). On the other hand, the kitchen waste usually contained much oil, and this oil was also reported to inhibit the activity of methanogens under anaerobic conditions (Lalman & Bagley 2001).

**Optimizing conditions for simultaneous recoveries of nitrogen and phosphorus by struvite precipitation**

When the high production of SCFAs from the alkaline co-fermentation of WAS and kitchen waste was achieved under the optimum condition, large amounts of ammonia and phosphorus were simultaneously released. Before using this alkaline fermentation liquid, the released nitrogen and phosphorus should be removed. Our previous studies have shown that the struvite precipitation method could be used to simultaneously recover the released ammonia and phosphorus in the fermentation liquid of sole WAS fermentation (Tong & Chen 2009; Zhang & Chen 2009). In this study, the optimum condition for simultaneous recoveries of nitrogen and phosphorus in the fermentation liquid of co-fermentation of WAS and kitchen waste was investigated through the RSM and regression analysis.

The CCD tests were performed randomly to minimize the unpredicted effects on the responses, and the Design-Expert software showed that among all polynomial models the quadratic model was the most suitable one for the simulation of the NH₄⁺-N and SOP concentrations. In the range of P/N 0.8–1.4, Mg/N 1.0–2.0, and pH 9.5–10.5, a series of experiments were conducted to validate the developed model of recoveries of NH₄⁺-N and SOP, and the

![Figure 1](https://iwaponline.com/wst/article-pdf/68/4/916/440021/916.pdf)

*Figure 1* | Predicted versus experimental data of SCFAs (a) and NH₄⁺-N and SOP recovery efficiencies (b) from co-fermentation of WAS and kitchen waste.
comparisons between the predicted and experimental values are shown in Figure 1(b). It can be observed that the predicted data were very close to the experimental data, indicating that the model used in this study can be used for the predictions of NH4\(^+\)-N and SOP recoveries.

Furthermore, the surface and contour plots of RSM were used, because holding the third factor at a fixed level and using a function of two factors at a time is useful for understanding the internal interaction effects of two factors. It can be seen from Figure 2(a) that the increase of Mg/N ratio at a low pH level could not achieve an obvious SOP reduction, while an obvious SOP decrease can be found at high pHs. Figure 2(b) illustrates that the SOP recovery efficiency was enhanced under the condition of lower P/N and higher pH. Also, it can be observed in Figure 2(c) that the SOP recovery efficiency was increased under the lower P/N and higher Mg/N condition. Since a slight rise in pH would cause a significant decrease in phosphorus content at a fixed ratio of Mg/N or P/N, pH was the critical influencing factor that affected the SOP concentration. The interactive effects of the variables on NH4\(^+\)-N are illustrated in Figures 2(d)–2(f). It can be seen that pH 10 was beneficial to the nitrogen recovery, and the NH4\(^+\)-N concentration was not significantly influenced by the Mg/N ratio, when both pH and P/N were controlled at constant levels.

According to our previous studies and the experimental data of this study, it can be found that the optimum conditions of P/N, Mg/N, and pH were 1.26, 1.93, and 10, respectively. It is well-known that the theoretical molar ratio of Mg:N:P in the struvite should be 1:1:1 (Tong & Chen 2007). However, the observed experimental molar ratio was 1.93:1:1.26 in this study. The higher Mg/N and Mg/P ratios might be due to some soluble organics such as protein combining with soluble Mg\(^{2+}\) and the formation of insoluble Mg\(_3\)(PO\(_4\))\(_2\). In the literature, some reported molar ratios were also not in accordance with the theoretical ones. For example, the observed Mg/P was between 1.3 and 1.8 (Jaffer et al. 2002; Tong & Chen 2007), and P/N was between 1.1 and 1.67 (Doyle & Parsons 2002; Tong & Chen 2007).

**Implementations of the recovered carbon source to enhance biological nutrient removal**

Our previous studies showed that the fermentation liquid from sole WAS fermentation can be used as carbon source for enhanced biological phosphorus removal or biological nitrogen and phosphorus removal (Tong & Chen 2007; Ji & Chen 2010). However, it has never been reported whether the fermentation liquid of alkaline co-fermentation
of WAS and kitchen waste can improve wastewater nitrogen and phosphorus removal. The TP and TN removal efficiencies of the acetic acid SBR were 77 and 71%, respectively. When the fermentation liquid of alkaline co-fermentation of WAS and kitchen waste was adopted as the carbon source for biological nutrient removal, the TP and TN removal efficiencies were increased to 92 and 85%, respectively. Obviously, compared with acetic acid, the fermentation liquid exhibited higher removal efficiencies of wastewater nitrogen and phosphorus at the same COD, indicating that this fermentation liquid was an excellent carbon source for biological nutrient removal.

The reasons for the fermentation liquid-induced higher nutrient removal efficiency were further explored and discussed. Compared with sole acetic acid, much propionic acid was present in the fermentation liquid of alkaline co-fermentation of WAS and kitchen waste. It was reported that the higher fraction of propionic acid in the influent increased the contents of poly-3-hydroxyvalerate and poly-3-hydroxy-2-methyvalerate in activated sludge (Li et al. 2008), which benefited wastewater nitrogen and phosphorus removal. Our previous studies also showed that, compared with acetic acid, using the fermentation liquid as carbon source resulted in a higher denitrifying phosphorus removal (Ji & Chen 2010). Since these two SBRs displayed a similar ammonia oxidation efficiency, the higher denitrifying phosphorus removal caused by the fermentation liquid might result in the greater TN removal. Therefore, the presence of propionic acid in the fermentation liquid might be one reason for the increased biological nitrogen and phosphorus removal efficiencies. Also, the FISH technique was used to determine the microbial community in these two SBRs, and it was found that the relative abundances of phosphorus-accumulating organisms (PAOs) and glycogen-accumulating organisms in the SBR fed with the fermentation liquid were 56 and 3%, while those in the SBR fed with acetic acid were 35 and 9%. These results indicated that using the fermentation liquid as the carbon source benefited the abundance of PAOs, which was consistent with the higher TP removal efficiency in the SBR.

CONCLUSIONS

This study introduced a new method for greatly recovering carbon, nitrogen, and phosphorus from WAS and kitchen waste. It was found that the co-fermentation of WAS and kitchen waste under alkaline conditions can achieve efficient carbon recovery, and the optimum conditions were pH 8, temperature 35 °C, C/N ratio 21, and fermentation time 6 d according to the RSM analysis. After the anaerobic fermentation, the RSM optimization study indicated that the released ammonia and phosphorus in the fermentation liquid could be simultaneously recovered by struvite precipitation (P/N 1.26, Mg/N 1.93, and pH 10). It was observed that using the resulting fermentation liquid as the carbon source for biological nutrient removal greatly improved the removal efficiencies of TN and TP, especially wastewater phosphorus removal. The main reason for the higher nutrient removal efficiency was that the presence of propionic acid in the fermentation liquid benefited the abundance and activity of PAOs, which caused the satisfied phosphorus removal.

ACKNOWLEDGEMENTS

This work was supported by the National Hi-Tech Research and Development Program of China (863) (2011AA060903), the National Science Foundation of China (51178324 and 51278354), and the Foundation of State Key Laboratory of Microbial Metabolism (Shanghai Jiao Tong University) (MMLKF12-11).

REFERENCES


Ji, Z. & Chen, Y. 2010 Using sludge fermentation liquid to improve wastewater short-cut nitrification-denitrification and


First received 18 October 2012; accepted in revised form 11 April 2013