Journal of Agriculture and Environmental Sciences
December 2018, Vol. 7, No. 2, pp. 45-53
ISSN: 2334-2404 (Print), 2334-2412 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jaes.v7n2a5
URL: https://doi.org/10.15640/jaes.v7n2a5

# Study on Beer Wastewater as External Carbon Source in the $A^2/O$ Process for Enhancing Nitrogen and Phosphorus Removal

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**Abstract**

The study aimed to investigate the potential use of beer wastewater as external carbon sources to solve carbon deficiency problems and to estimate the removal efficiency of nitrogen and phosphorus in anaerobic-anoxic-oxic ($A^2/O$) process by laboratory experiments. It was shown that the removal efficiency substantially changed with addition of different dosage of external carbon (COD=0 $mg \cdot L^{-1}$, COD=30 $mg \cdot L^{-1}$, COD=50 $mg \cdot L^{-1}$, COD=70 $mg \cdot L^{-1}$, COD=90 $mg \cdot L^{-1}$ and COD=110 $mg \cdot L^{-1}$), and the removal efficiency of nitrogen was 57.98%, 68.57%, 82.06%, 66.9%, 54.32% and 40.56%, respectively. The removal efficiency of phosphorus was 76.98%, 75.41%, 73.33%, 72.07%, 70.86% and 69.36%, respectively. By comparison, COD=50 $mg \cdot L^{-1}$ was found to be the best dosage of external carbon, the efficiency of nitrogen removal was higher than that without external carbon source by 20.45%, and the efficiency of total phosphorus (TP) removal was just lower by 3.65% than that without external carbon source. The results show that beer wastewater can be an economical and effective external carbon source for enhancing nitrogen and phosphorus removal.

**Highlights**

► An $A^2/O$ system was used to treat domestic wastewater.
► Adding beer wastewater as external carbon sources to solve carbon deficiency problems and to estimate the removal efficiency of nitrogen and phosphorus.
► Addition of external carbon had a stronger impact on nitrogen removal than phosphorus removal.
► Appropriate addition concentration of beer wastewater was COD=50 $mg \cdot L^{-1}$.

**Keywords**: $A^2/O$, Beer wastewater, External carbon source, Nitrogen,and phosphorus removal;

**Nomenclature**

| | |
|---|---|
| $A^2/O$ | anaerobic-anoxic-aerobic |
| A/O | anaerobic-aerobic |
| COD | chemical oxygen demand ($mg \cdot L^{-1}$) |
| HRT | hydraulic retention time |
| SRT | sludge retention time |
| MLSS | mixed liquor suspended solid |
| VFA | volatile fatty acid |
| inf | influent |
| Ana | anaerobic zone |
| Ano | anoxic zone |
| Aero | aerobic zone |
| set | settling tank |
| eff | effluent |

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## 1. Introduction

The production of beer brewery uses a large volume of water and in turn discharges a large volume of wastewater which has the potential of causing considerable environmental problems (Zvauya, Parawira, & Mawadza, 1994). For each ton of beer produced, the amount of water consumed is about 10 tons~15 tons of water, while in China about 10 tons~30 tons of water is used for the production of one ton of beer (Xu, ZHONG, WU, & XIAO, 2000). Beer brewery is characterized by the following: (1) Brewery wastewater typically has a high chemical oxygen demand ($COD_{cr}$) about 1500 $mg·L^{-1}$~2500 $mg·L^{-1}$ from all organic components, (2) biochemical oxygen demand is about 700 $mg·L^{-1}$~1400 $mg·L^{-1}$, (3) and the value of pH range from 5.5 to 7.0, (4) brewery wastewater is characterized by the high biodegradability with no toxic harmful components which is widely used in biological treatment (Dai, Yang, Dong, Ke, & Wang, 2010). Because of the higher content of carbon which makes it feasible, brewery wastewater is considered as external carbon source.

One of the most commonly used processes is the Anaerobic- Anoxic-Oxic ($A^2/O$) system. In this system, ammonia is transformed into nitrite and nitrate during the process of nitrification in the aerobic tank, and then the supernatant which contains nitrite and nitrate flows into the anoxic tank for denitrification (Ma, Peng, & Wang, 2009; Zeng, Li, Yang, Wang, & Peng, 2011). In the meantime, phosphate is released coupled with excessive uptake by poly-P bacteria in the anaerobic tank and aerobic tank, respectively. To sum up, nitrogen and phosphorus removal can be achieved synchronously in $A^2/O$ process, which is widely applied in the large and medium-sized cities and towns in China. However, domestic wastewater has a lower concentration of organic matter. In order to maintain the desired effluent quality, there is certainly a need to find alternative carbon sources to retain a high efficiency of nitrogen and phosphorus removal(Fernández, Castro, Villasenor, & Rodríguez, 2011). At present, many researchers have paid attention to search those carbon sources with high efficiency and no by-products produced (Kampas et al., 2009; Soares et al., 2010) because ethanol was the main carbon source in beer wastewater, which had low molecular weight substrates that could be used readily by the microorganisms. In this study, brewery wastewater was used as an external carbon source in simulated domestic wastewater. The objectives of this study are as follows: (1) Investigating the effects of simultaneous nitrogen, $PO_4^{3}$-P and the concentration of COD removal in performance in $A^2/O$ process, (2) the optimized operational strategies in $A^2/O$ process were also discussed, such as the effect of dissolved oxygen (DO) concentration and the variation range of pH, (3) to achieve the highest efficiency of nitrogen and phosphorus removal of domestic wastewater at ambient volume of addition. Lastly, this study attempts to provide theoretical support and reference for the transformation of the sewage treatment facilities and the improvement of water quality.

## 2. Materials and methods

### 2.1 Experimental system and operation

The experimental system is shown in Fig 1. It consists of an influent tank, a laboratory-scale anaerobic-anoxic-oxic ($A^2/O$) reactor with a working volume of 52.15 L and a secondary settler which was vertical flow with a working volume of 26.1 L. The transparent plexiglass $A^2/O$ reactor was separated by baffles into 7 zones to accomplish anaerobic, anoxic and oxic reactions. The first zone was an anaerobic reactor for releasing phosphorus of inflow wastewater, and then the following zone was an anoxic reactor for denitrification of nitrite/nitrate recirculation from the last aerobic zone. The remaining five zones were conducted as separated aerobic zones for ammonization of organic nitrogen. The volume ratio of the anoxic zone to anaerobic to aerobic was 1:1:2. The flow rates of inflow, internal recycle and external recycle were controlled by peristaltic pumps. Stirrers were installed over the anaerobic and anoxic zones to maintain the biomass in suspension. The porous stone diffusers were installed over aerobic zones for aerating and for mixing the biomass in these zones. Trial operation conditions were as follows: The quantity of water intake was controlled at 4.3 $L·h^{-1}$, and the hydraulic retention time (HRT) was 12 h; the sludge retention time (SRT) in $A^2/O$ reactor was controlled at 15 days; the concentration of mixed liquor suspended solids (MLSS) was about 2800 $mg·L^{-1}$~3000 $mg·L^{-1}$; the ambient temperatures of all experiments were conducted at 26℃~28 ℃ by a heater and thermostat; andthe reflux ratio of sludge and internal recycle were controlled at 70% and 150%, respectively.

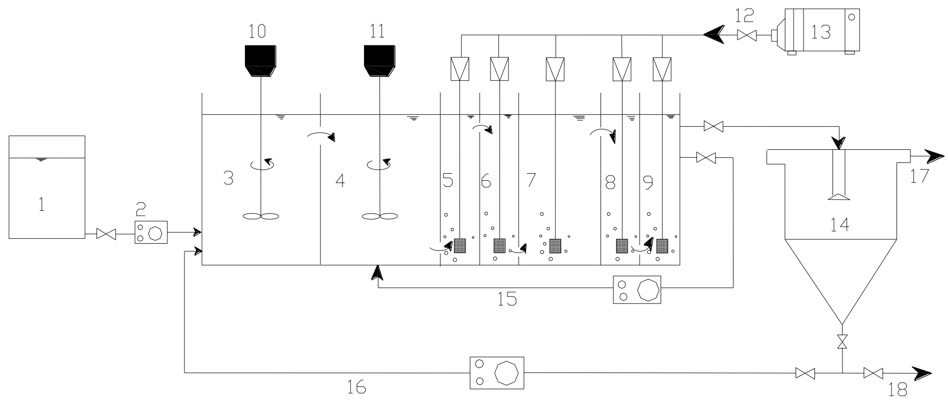


Fig 1 the schematic diagram of $A^2/O$ process

1: influent tank, 2: peristaltic pump, 3: anaerobic zone, 4: anoxic zone, 5~9: aerobic zone, 10: Stirrer, 11: Stirrer, 12: air flow meter, 13: air pressure, 14: settling tank, 15: internal recycle, 16: external recycle, 17: Effluent, 18: excess sludge.

To enhance nitrogen and phosphorus removal with external carbon source, beer wastewater (with concentration of 0 $mg \cdot L^{-1}$, 30 $mg \cdot L^{-1}$, 50 $mg \cdot L^{-1}$, 70 $mg \cdot L^{-1}$, 90 $mg \cdot L^{-1}$ and 110 $mg \cdot L^{-1}$ COD) was added to domestic wastewater. This mixed wastewater was considered as test water which was marked by test A ~ test E.

### 2.2 Sludge

The seed sludge was collected from the Wenchang wastewater treatment plant in Harbin (China), which is a typical nitrogen removal plant by anaerobic-aerobic (A/O) process treating municipal wastewater. At the beginning of incubation, sludge was put into a laboratory fermenter, and only tap water was added (to ensure no external carbon source existed) under the aeration condition for 7 days. The supernate was changed daily then domestic wastewater was added and the system was left for 45 days for acclimatization. Thereafter, the sludge was put into the reactor and allowed to operate for 5 days, and then the experiments began.

### 2.3 The property of wastewater

Simulated domestic wastewater fed into the reactor contained: glucose, $NH_4Cl$, $KH_2PO_4$ and different kinds of trace elements. The dosage of chemical reagents in simulated wastewater is shown in Table 1 (Banu, Uan, & Yeom, 2009). The pH of simulated domestic wastewater was controlled at 6.7~7.4 by adding $NaHCO_3$. The major characteristics of wastewater used in pilot tests are shown in Table 2. The simulated beer wastewater was made by diluting the beer with tap water. The average concentration of COD was controlled at 2100 $mg \cdot L^{-1}$.

Table 1 Dosage of chemical reagents in the simulated wastewater (Banu et al., 2009)

| Chemical reagents | Dosage($mg \cdot L^{-1}$) |
|---|---|
| glucose | 210 |
| $NH_4Cl$ | 200 |
| $NaHCO_3$ | 220 |
| $KH_2PO_4$ | 24~30 |
| $MgSO_4 \cdot 7H_2O$ | 5.6 |
| $FeCl_3 \cdot 6H_2O$ | 0.88 |
| $MnCl_2 \cdot 4H_2O$ | 0.19 |
| $ZnCl_2 \cdot 2H_2O$ | 0.0018 |
| $CuCl_2 \cdot 2H_2O$ | 0.022 |
| $CaCl_2 \cdot 2H_2O$ | 1.3 |

Table 2 Characteristics of wastewater used in pilot tests

| Contents | Range | Average |
|---|---|---|
| TN($mg \cdot L^{-1}$) | 38.73~43.16 | 40.95 |
| $PO_4^{3-}$- P ($mg \cdot L^{-1}$) | 4.25~6.73 | 5.49 |
| $NH_4^+$-N($mg \cdot L^{-1}$) | 26.81~38.27 | 32.54 |
| COD($mg \cdot L^{-1}$) | 210~244 | 227 |
| pH | 6.75~7.39 | 7.07 |

**2.4 Analytical methods**

$COD_{cr}$, total nitrogen (TN), ammonium ($NH_4^+$-N), $PO_4^{3-}$- P and MLSS were analyzed according to the description in the Standard Methods of (SEPA, 2002). DO, pH and temperature were monitored by using wTw-Multi-340i (Germany).

**3. Results and Discussion**

**3.1 Nitrogen removal**

The effects of six various addition concentration (COD=0 $mg \cdot L^{-1}$, COD=30 $mg \cdot L^{-1}$, COD=50 $mg \cdot L^{-1}$, COD=70 $mg \cdot L^{-1}$, COD=90 $mg \cdot L^{-1}$ and COD=110 $mg \cdot L^{-1}$) of beer wastewater on nitrogen removal in the $A^2/O$ process are shown in Fig 2. After the addition of different concentration of beer wastewater, the evolution of TN in $A^2/O$ system did not change significantly (Fig 2). On the other hand, the removal efficiency of TN and $NH_4^+$-N changed remarkably (Fig 3 and Fig 4).

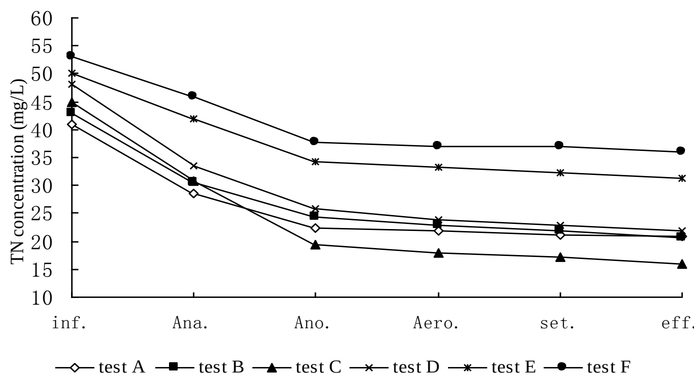


Fig 2 Evolution of TN in $A^2/O$ system at various dosage of external carbon
inf: influent, Ana: anaerobic zone, Ano: anoxic zone, Aero: aerobic zone, set: settling tank, eff: effluent

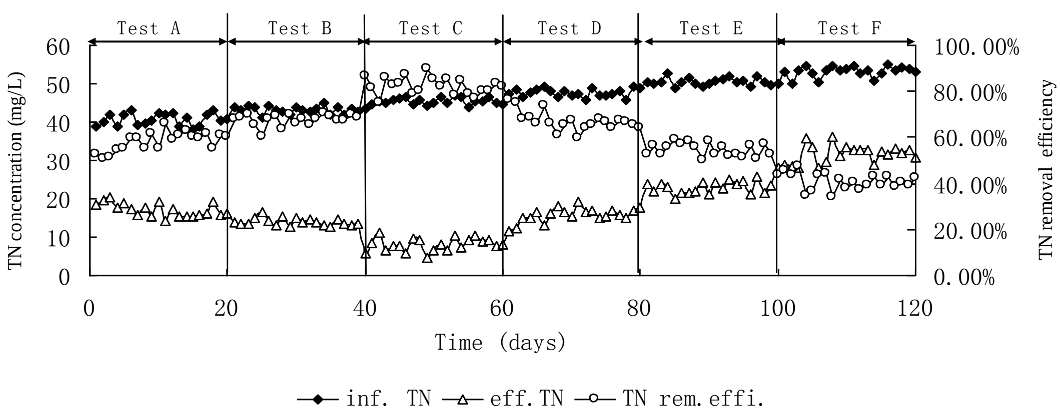


Fig 3 Variation of TN in $A^2/O$ system at various dosage of external carbon

As expected, TN removal efficiencies exhibited an incremental trend with the addition of external carbon (Naidoo, Urbain, & Buckley, 1998). When treating the domestic wastewater without the external carbon source addition (test A), the removal efficiency of TN was 57.98%. In test B, the content of TN removal efficiency increased to 68.57%, which was higher than test A by 10.59%. The efficiency of TN removal in test C was 82.06%, which was higher than test A by 24.08%. TN removal efficiency exhibited an incremental trend with the increase of beer wastewater concentration. The primary reason was that the increase of carbon content supported a great activity of microorganism which then increased the nitrate load supplied to the anoxic zones translating in an increased TN removal.

However, with increasing the concentration of beer wastewater, there was a downtrend. In the test D, the TN removal efficiency was 66.9%, which was higher than test A by 8.92%. The efficiency of TN removal in test E and test F was 54.32% and 40.56%, which was lower than test A by 3.66% and 17.42% respectively. According to the data analysis, with the beer wastewater addition, the nitrogen removal improved remarkably.

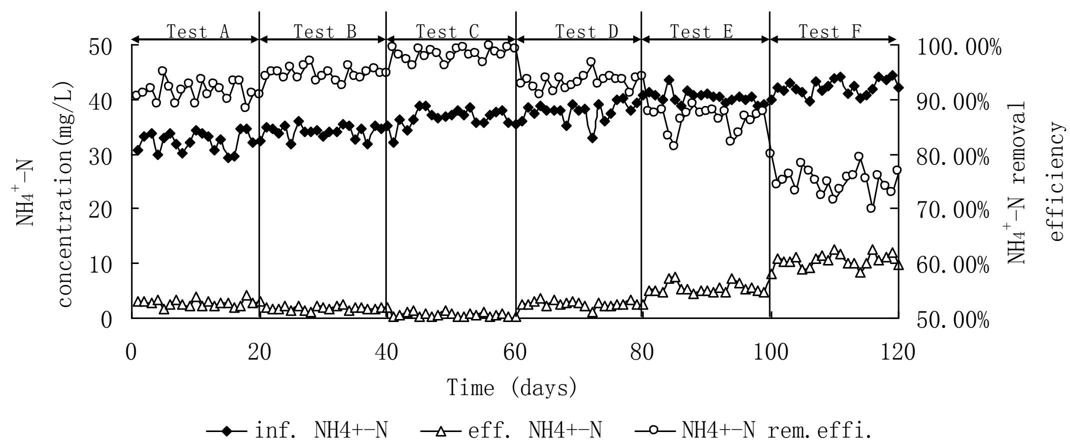


Fig 4 Variation of $NH_4^+$-N in $A^2/O$ system at various dosage of external carbon

Influent TN was mainly composed of $NH_4^+$-N (above 95%), and the overall $NH_4^+$-N removal efficiency was ranging (69.93% ~ 99.46%) in the $A^2/O$ system, as shown in Fig 3. When dosages of external carbon varied between COD=0 mg·L⁻¹ and COD=110 mg·L⁻¹, effluent $NH_4^+$-N concentration was changed from 26.81 mg·L⁻¹ to 38.27 mg·L⁻¹ during the entire experiment period. $NH_4^+$-N concentration was significantlyreduced because of the addition of beer wastewater (test A ~ test C), and the $NH_4^+$-N removal efficiency was 98.25% in test C. However, when the dosages of beer wastewater were above 70 mg·L⁻¹ of COD (test D ~ test F), the removal efficiency of $NH_4^+$-N decreased. According to (Zhang, Wang, et al., 2016), TN decreased from 25.33 mg/L to 14.12 mg/L. Also (Zhang, Yang, et al., 2016) observed that the nitrification performance was prominent, with the $NH_4^+$-N removal efficiency of 98% at all HRT tested (with the exception of HRT = 6 h), indicating a strong nitrifying activity in the BCO (biological contact oxidation) reactor. However, (Hu, Zhang, & Hou, 2018) indicated that when ethanol served as external carbon addition, the amount of $N_2O$ production in anoxic and anaerobic/anoxic experiments was 0.13 mg N/L and 0.06 mg N/L respectively, lower compared with acetate. The lower amount of $N_2O$ emission during denitrification process proved that ethanol is a potential alternative external carbon source for nitrogen removal. Consequently, the beer wastewater which provided sufficient nutrients for microorganisms was recommended as an effective and potential external carbon source for nitrogen removal. Can beer wastewater as a carbon source solve the problem of insufficient carbon source in the urban sewage treatment process and also provide a new way for brewery wastewater disposal? This study indicated that treatment of the mixed liquor of beer wastewater and domestic wastewater by activated sludge process can effectively enhance the nitrogen removal, but brewery wastewater contains some compositions (such as alcohol) which may affect the living environment of sludge after the excessive addition of brewery wastewater.

### 3.2 Denitrifying phosphorus removal

$PO_4^{3-}$- P evolution and TP variation in $A^2/O$ system at various dosages of external carbon is shown in Fig 5 and Fig 6 respectively.

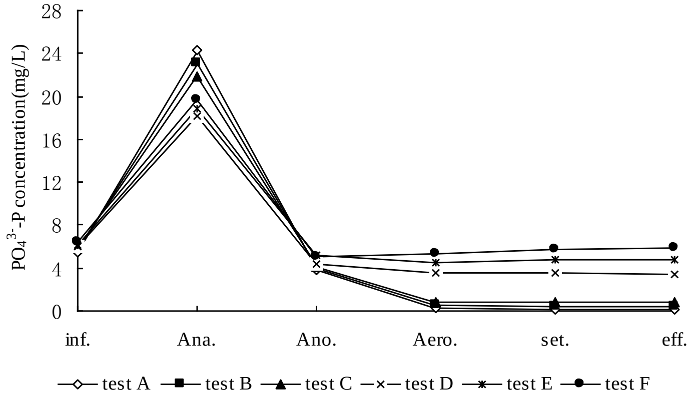


Fig 5 Evolution of $PO_4^{3-}$- P in $A^2/O$ system at various dosage of external carbon
inf: influent, Ana: anaerobic zone, Ano: anoxic zone, Aero: aerobic zone, set: settling tank, eff: effluent

Because there were both ordinary phosphorus accumulating bacteria and denitrifying phosphorus removing bacteria in activated sludge, the phosphorus release and the denitrifying dephosphatation reactions occurred simultaneously. In the anaerobic zone, the content of readily biodegradable was high. The ordinary phosphorus accumulating bacteria absorbed volatile fatty acids (VFAs), and transformed it into PHB; meanwhile, they release phosphorus in cells to obtain energy. The phosphorus release rate was much higher than the rate of denitrifying phosphorus uptake. Therefore, the concentration of $PO_4^{3-}$-P in the anaerobic zone increased gradually. In the anoxic zone, denitrifying phosphorus removing bacteria absorbed phosphorus by $NO_3^-$-N as electron acceptor with the decreased VFAs concentration in mixed liquor, which led the concentration gradual decrease of $PO_4^{3-}$-P. From (Zhang, Wang, et al., 2016) observation, $PO_4^{3-}$-P dropped from 36.79mg/L to 0.54mg/L in the anoxic zone ($A_1$ to $A_4$), with the denitrifying phosphorus removal efficiency of 97.83%.

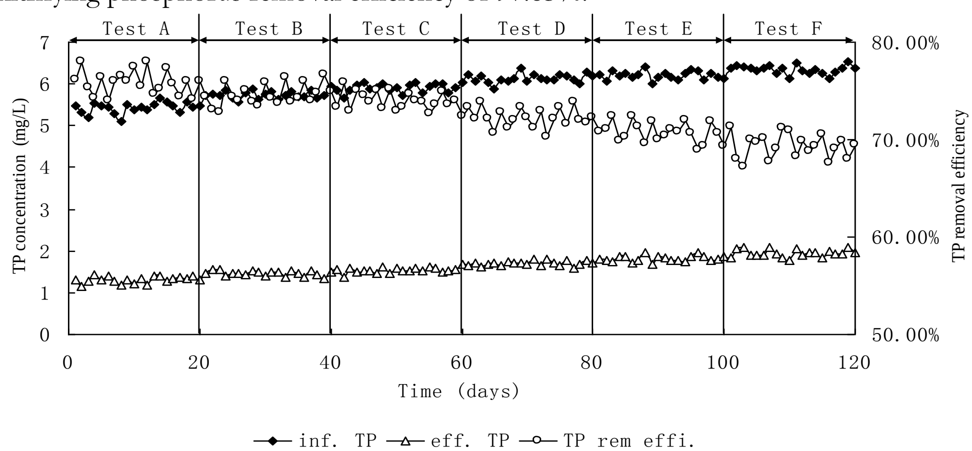


Fig 6 Variation of TP in $A^2/O$ system at various dosage of external carbon

Under various dosage of external carbon (test A ~ test F), the removal efficiency of TP measured in the system were 76.98%, 75.41%, 73.33%, 72.07%, 70.86%,and 69.36%, respectively. The results showed that removal efficiency of TP in $A^2/O$ system decreased with an increase in the dosage of beer wastewater and effluent TP concentration increased remarkably. Especially in test E and F, the concentration of TP decreased and then rose again. The phenomenon was mainly attributed to the fewer electron acceptors in the mixed liquor. Due to lack of electron acceptors, denitrifying phosphorus removing bacteria cannot continue oxidative metabolism and appeared ineffective internal phosphorus release.

### 3.3 COD removal

The evolution of COD in $A^2/O$ process at various addition concentration of beer wastewater is depicted in Fig 7 and Fig 8.

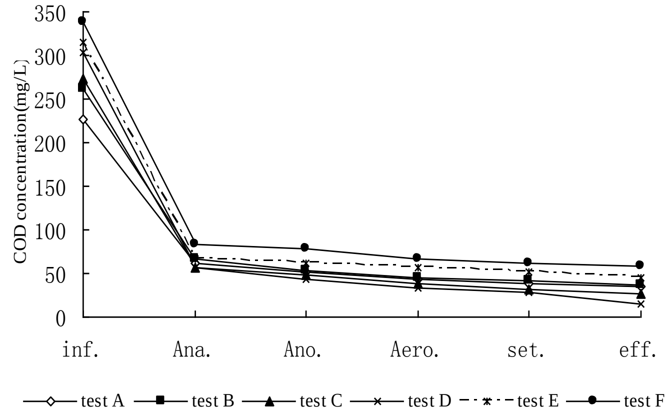


Fig 7 Evolution of COD in A²/O system at various dosage of external carbon
inf: influent, Ana: anaerobic zone, Ano: anoxic zone, Aero: aerobic zone, set: settling tank, eff: effluent

(Zhao et al., 2016) reported that the average influent COD was around 246.94 mg/L, dropped to 97.32 mg/L at the beginning of the anaerobic phase, and then was degraded to 52.63 mg/L at the end of the anaerobic phase. In this study, the COD concentration significantly decreased in the anaerobic zone but slightly decreased in the anoxic zone and almost remained unchanged in the aerobic zones. The reasons for these observations may be due to the following explanations(Kuba, Van Loosdrecht, & Heijnen, 1996): (1) COD concentration decreased due to dilution by the sludge reflux in anaerobic and the nitrification liquid reflux in anoxic zone, (2) biodegradable organic compounds were primarily consumed by PAOs released phosphate in the anaerobic zone, and (3) small amounts of residual organic matter were utilized by denitrification in anoxic zone. Fig 4 showed that under different dosages of external carbon source, approximately 55%~76% of COD was utilized in the anaerobic zone, values which are not far from those observed by (Du et al., 2018), which were higher about 80% in systems with acetate, propionate, acetate and propionate mixed (1:1) as carbon sources. However, in this study 19%~26% of COD was consumed in the anoxic zone with quite low COD available in the aerobic zone.

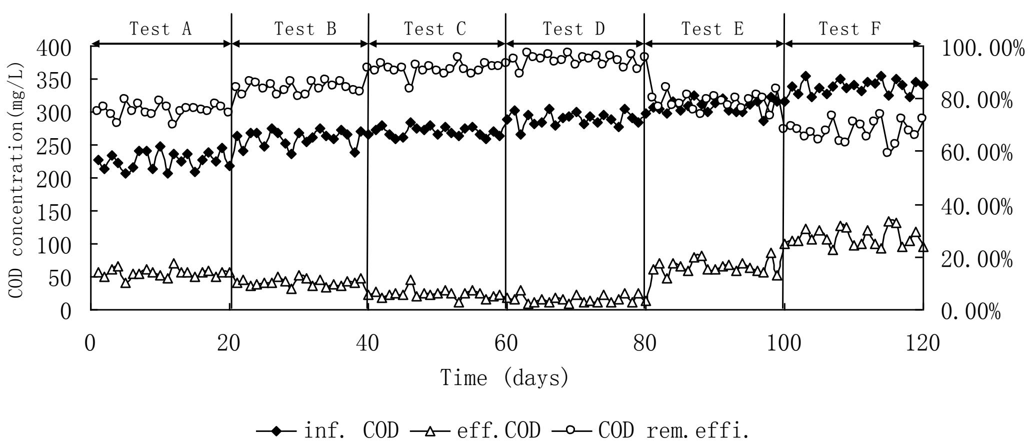


Fig 8Variation of COD in A²/O system at various dosage of external carbon

Figure 8 showed the variation of COD removal efficiency with beer wastewater as external carbon source in the A²/O process within a certain range; the COD removal efficiency increased with the increase of the content of brewery wastewater. However, it would decrease with excessive addition of brewery wastewater. The COD value of effluent and the COD removal efficiency are shown in table 3. During this period, the denitrification rate of nitrate-N in the denitrifying pool was increasing. This result was mainly due to enhancing proportion of available carbon source through increasing concentration of beer wastewater. The result indicated that the COD concentration of brewery wastewater waslower than 70 $mg\cdot L^{-1}$, and the optimum addition COD concentration was 50 $mg\cdot L^{-1}$. (Zhang, Yang, et al., 2016) reported that the COD removal efficiency (>80%) was stable at all the HRTs. The effluent COD was less than 50 mg/L, indicating the high-efficiency utilization of carbon sources.

Table 3 The COD value of effluent and the COD removal efficiency

| Tests | COD concentration of effluent ($mg\cdot L^{-1}$) | COD removal efficiency (%) |
|---|---|---|
| Test A | 227 | 74.58 |
| Test B | 261 | 85.99 |
| Test C | 273 | 90.25 |
| Test D | 303 | 94.95 |
| Test E | 315 | 85.80 |
| Test F | 338 | 82.79 |

### 3.4 Law of changes in pH

pH was quite important in the treatment process of domestic wastewater. pH was an important factor for maintaining dominant microflora which would direct the living environment of the microorganisms in the sludge. Only in the appropriate pH conditions, the microorganisms were able to remove nitrogen and phosphorus in wastewater. It is apparent in table 4 that influent pH was controlled at about 7.0 and the effluent pH was relatively stable at various addition concentration of beer wastewater (from test A ~ test F). The parallel batch tests showed that with the dosages of beer wastewater increasing, influent pH value would decrease and sewage acidity would affect the metabolism of the microorganisms within the reactor. Therefore, we should consider the proportion of beer wastewater dosing in practical engineering applications

**Table 4 Variations of pH in $A^2/O$ system**

| Tests | influent | anaerobic zone | anoxic zone | Aerobic zone | settling tank | effluent |
|---|---|---|---|---|---|---|
| Test A | 7.02 | 7.18 | 7.24 | 7.24 | 7.23 | 7.18 |
| Test B | 7.15 | 7.10 | 7.10 | 7.16 | 7.15 | 7.21 |
| Test C | 6.94 | 7.04 | 7.17 | 7.03 | 7.18 | 7.06 |
| Test D | 7.19 | 6.92 | 6.93 | 6.85 | 6. 89 | 6.91 |
| Test E | 7.01 | 6.91 | 7.01 | 6.97 | 6.99 | 6.89 |
| Test F | 6.79 | 6.87 | 6.83 | 6.71 | 6.72 | 6.77 |

## 4. Conclusions

(1) By adding beer wastewater as an external carbon source to the low C/N value municipal for increasing the number of available carbon source. This method could significantly improve the efficiency of nitrogen and phosphorus removal with the high utilization rate and no by-products generated in $A^2/O$ system;

(2) The composition of beer wastewater was complex. Essentially the process involves lactic acid fermentation as well as alcoholic fermentation which led to beer wastewater acidity. The experimental data showed that an appropriate proportion of beer wastewater dosing would not affect the normal operation of the reactor.

(3) The appropriate addition concentration of beer wastewater was COD=50 $mg\cdot L^{-1}$. In the actual operation, there is a need of controlling the dosage of beer wastewater to satisfy the different nitrogen and phosphorus removal.

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