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The performance of a combined nitritation–anammox reactor treating anaerobic digestion supernatant under various C/N ratios

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ABSTRACT
A combined nitritation–anammox reactor was developed to treat the digestion supernatant under various C/N ratios. Due to the difficulties for heterotroph to utilize the refractory organics, the reactor presented relatively stable performance with increasing supernatant addition. Nevertheless, the adverse effects of supernatant would accumulate during the long-term operation and thus weakened the activity and shock resistance of microbes, which further led to the gradual decrease of reactor performance after 92 days’ operation. Under this circumstance, supernatant with volatile fatty acids (VFAs) residuals was further introduced into the reactor to investigate the performance of combined nitritation–anammox process with VFA addition. With the appearance of VFAs, the nitrogen removal performance gradually restored and the reactor finally achieved stable and efficient performance with C/N ratio of 0.35. The VFA residuals within 150 mg/L in the supernatant served as the extra electron donors and stimulated the heterotrophic denitrification process, which was vital for the enhancement of reactor. The nitrogen removal rate and total nitrogen removal efficiency reached 0.49 kg N/(m³·day) and 88.8% after 140 days’ operation, respectively. The combined nitritation–anammox reactor was proved suitable to treat digestion supernatant.

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Introduction
In recent years, serious eutrophication which resulted from the discharge of excessive nitrogen with the accelerating industrialization and urbanization, has attracted considerable concerns in water environmental protection. The conventional technology for nitrogen removal, nitrification followed by denitrification process, required plenty of energy consumptions and organics addition (Münch et al., 1996; Ruiz et al., 2006). This technology would be especially costly and difficult in treating wastewater with high concentration of ammonia while relatively low C/N ratio. For these reasons, the anaerobic ammonia oxidation (anammox) based technologies have been considered as the promising alternatives for the traditional technology due to its being cost-saving and energy-efficient.

Among the anammox based technologies, the combined nitritation–anammox, which integrated the nitritation and anammox in one single reactor (Sliekers et al., 2002), presented remarkable advantages in operation (Joss et al., 2011) and was employed in more than 88% of full-scale application practically (Lackner et al., 2014). During this process, ammonia was partially oxidized to nitrite firstly, then the anammox would combine the formed nitrite and ammonia to dinitrogen gas with a small amount of nitrate production. In contrast to the traditional process, this autotrophic process did not require organic carbon addition, while the presentation of organics was reported to be

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inhibitory for the anammox process (Chamchoi et al., 2008; Tang et al., 2010).

However, in practical application, the raw wastewater treated by combined nitritation–anammox mostly contained a certain concentration of organics besides nitrogen compounds. Previous researches have focused on the influences of organic concentration and C/N ratio on the performance of anammox process, and synthetic wastewater was mostly engaged for experiment (Tang et al., 2013, 2014). Compared to the synthetic wastewater, the raw wastewater (anaerobic digestion supernatant was researched in this study) mostly contained refractory organics (Graja and Wilderer, 2001). In addition, in spite of the relatively low C/N ratio which was insufficient for denitrification process, the digestion supernatant still contained high concentration of organics due to the high level of ammonia. With the autotrophic combined nitritation–anammox process, these organics could be reserved while the ammonia was eliminated and the treated supernatant with organics residual could be recycled to the digester for further digestion, which possibly improved the digestion performance. Moreover, compared to the synthetic wastewater, the fluctuations in anaerobic digester might lead to variations in the compositions of supernatant (e.g., volatile fatty acids residuals), which might impact the subsequent nitrogen removal process significantly. However, the performance of combined nitritation–anammox process treating raw wastewater under various C/N ratios and the long-term effects of raw wastewater addition remained little understood.

In this study, a combined nitritation–anammox reactor was established by feeding synthetic wastewater and then operated under various C/N ratios with supernatant addition for 140 days. The performance of this autotrophic process under different C/N ratios and the long-term effects of supernatant addition were studied. Moreover, the volatile fatty acids (VFAs) containing supernatant which resulted from the fluctuations of anaerobic digester were added into the reactor for the investigation of reactor performance. The results revealed that the long-term addition of supernatant did alter the reactor performance significantly while the nitritation–anammox process still presented considerable capacity to accommodate the raw wastewater.

1. Materials and methods

1.1. Reactor configuration and operation condition

An SBR (sequencing batch reactor) with a working volume of 5 L was used for the combined nitritation–anammox process. The reactor was made by polymethyl methacrylate with an inner diameter of 10 cm, as depicted in Fig. 1a. Sponges were used as the biomass carrier and the packing rate was 40% (V/V). The temperature was kept at 32 ± 1°C during the operation by a thermostat water jacket. The exchange volume ratio of the SBR was kept at 40%. Compressed air was supplied via a diffuser at the bottom of the reactor to keep the dissolved oxygen (DO) around 1.0–1.5 mg/L. The SBR was operated in the cycles consisted of 4 phases: feeding, reaction, settling and decanting. The duration of each phase lasted for 10, 420, 20 and 10 min throughout the experiment, respectively. The pH values ranged from 7.3 to 7.9 by adding NaHCO3 and 1 mol/L HCl.

1.2. Inoculum and influent condition

The reactor was seeded with 5000 mg anammox sludge (in wet weight) from an anammox reactor in the same laboratory and 6000 mg nitrification seed sludge (in wet weight) which was taken from the aeration tank of a municipal wastewater treatment plant in Beijing, China. With the oxygen supplement on the start-up of the combined nitritation–anammox process, the anammox sludge was prone to be inhibited by the oxygen since the biofilm was not formed and the anammox sludge presented flocculent at the start-up stage. Therefore, the amount of nitrification seed sludge was determined to be higher than the anammox sludge to some extent, which aimed to consume the oxygen and cover the anammox sludge and thus protect the anammox process.

During the start-up stage, the SBR was fed with synthetic wastewater: NH4HCO3 150–400 mg N/L, KH2PO4 15 mg P/L, CaCl2 300 mg/L, MgSO4·7H2O 200 mg/L, NaHCO3 400–800 mg/L, and trace element solutions I and II 1.25 mL/L (Molinuiev et al., 2009). After 47 days’ start-up stage, the raw supernatant from an anaerobic digester treating food waste and fruit/vegetable waste was added into the influent to supply 20–100% of the ammonia in the influent, while the total ammonia concentration in the influent was kept relatively stable around 400 mg/L (to achieve this, the supernatant was diluted and added into the influent to obtain specific C/N ratio and the shortage in ammonia was supplemented by
2. Results and discussions

The operation of the reactor could be divided into 6 phases according to the different dosages of supernatant: the start-up phase (P0), phases P1 to P5 in which various dosages of supernatant were added into the influent resulted in various C/N ratios (from 0.13 to 0.35, and the C/N ratio of raw supernatant is 0.35). The variations of the nitrogen compounds and organic matters of reactor through various phases are revealed in Fig. 2. For the comparative study between different phases, the last 7 days of each phase were considered as the steady status, thus the average values of the reactor performance in these days were determined as the representative. The results are summarized in Table 1.

1.3. Analytical methods

The influent and effluent were collected on a daily basis and were analyzed immediately for concentrations of ammonia, nitrite, nitrate and TOC according to the standard methods (APHA, 2005). The VFAs, volatile organic compounds (VOCs), heavy metals and soluble chemical oxygen demand (sCOD) of supernatant were determined according to APHA (2005) Standard Methods. The DO, temperature and pH were measured using Hach HQ30d (Hach Inc., Loveland, Colorado, USA).

1.3.1. Microbial community analysis

GeoChip 4.0 was engaged for the microbial community analysis in this study. GeoChip is a high-throughput metagenomic technology, and has been proven to be a powerful tool for functional profiling of microbial community (Liu et al., 2010; He et al., 2012). GeoChip 4.0 contained a variety of genes for major microbial functional genes, such as nitrogen cycling, carbon cycling, metal resistance, organic remediation and stress.

The sludge samples were collected after reactor start-up and at the end of the whole experiment. DNA extraction, purification, labeling and hybridization were subsequently carried out for GeoChip analysis as the methods described previously (Yang et al., 2013a; Lu et al., 2012). For the data processing, spots with signal to noise ratio (SNR) <2, thermophile >5, signal intensity <1000 were removed. Data normalization was based on logarithm transform, calculating relative abundance in each sample, then scaled up by average (Mean Ratio). Spots with more than 1/3 of the gene spots were considered positive. For the dissimilarity test of Adonis, Bray-Curtis distance was used to calculate the dissimilarity distance matrices from GeoChip data. The data analyses were conducted by the vegan package in R 2.15.0 (Zhang et al., 2013a) and on the website (http://ieg.ou.edu/).

2.1. Start-up phase (P0)

The reactor showed the capacity of nitrogen removal on the inoculation immediately. Ammonia removal was observed and a small quantity of nitrate accumulation was found in the first 3 days. After 37 days’ operation, the ammonia in the influent increase to 389.5 mg/L, and the average concentrations of ammonia, nitrite and nitrate in the effluent were 20.5, 0.3 and 56.0 mg/L, respectively. The nitrogen loading rate (NLR) and corresponding nitrogen removal rate (NRR) were further increased to 0.50 and 0.41 kg N/(m³-day) on day 43. The reactor showed stable ammonia removal efficiency of 95% and total nitrogen (TN) removal efficiency of 82%, indicated that a quick start-up of combined nitrification–anammox process was achieved.

The rapid start-up of the combined nitrification–anammox process could be attributed to the highly activated inoculums and appropriate operation conditions. Compared to previous publications (Slikers et al., 2002; Zhang et al., 2013b), both the anammox and nitrification seed sludge were applied to inoculate the reactor, which resulted in the nitrogen removal at the beginning of inoculation. The start-up process was further accelerated by the subsequent operation which restricted the parameters (DO, pH and temperature) within the appropriate range (Chang et al., 2013) and thus promoted the growth of the microorganisms and the combination of anammox and nitrification processes, which resulted in rapid start-up of reactor.

2.2. Stable operation (P1, P2 and P3)

Achieving the successful start-up of the combined nitrification–anammox after 47 days’ operation, the raw digestion supernatant was gradually added into the influent within a series of dosages (contributed 20% to 100% of the ammonia in the influent from P1 to P5) to investigate the performance of combined nitrification–anammox reactor treating raw supernatant under various C/N ratios.

The operation was initiated with a small amount of raw supernatant addition in phase P1 (days 48–65). The addition of supernatant introduced various compounds companied with it mainly consisted of ammonia and organic carbons. For the comparative study between different phases, the ammonia in the influent was kept around 400 mg/L throughout the operation thus the corresponding NLR was steady around 0.5 kg N/(m³-day). Meanwhile, as the result of supernatant addition, the average TOC concentration in the influent varied accordingly, resulted in different C/N ratios in each phase (0.13–0.35 throughout the operation). In phase P1, the TOC in the influent reached 53.52 mg/L as the result of supernatant addition, which indicated the C/N ratio of 0.13. Nevertheless, in spite of the addition of supernatant in P1, the combined nitrification–anammox reactor presented stable and efficient nitrogen removal performance with NRR of 0.43 kg N/(m³-day) and TN removal efficiency of 83.69% (Table 1). Furthermore, the TOC in the effluent kept relatively stable compared to the influent, which indicated that there was no significant growth of heterotrophic bacteria. This could be attributed to the relatively low concentration of TOC and the organic matters in the supernatant mainly consisted of refractory ones (Graja and Wilderer, 2001). Under this circumstance, the
heterotrophic bacteria could hardly utilize these organics and out-compete in a stable autotrophic reactor (Wang et al., 2012), which resulted in the suppression of denitrification and the stability of combined nitritation–anammox process.

Subsequently, the addition of raw supernatant was increased in phase P2 (days 66–92) and the corresponding TOC and C/N ratio in the influent increased to 87.04 mg/L and 0.22, respectively (Table 1). The reactor presented decrease in nitrogen removal performance on the elevation of supernatant addition. With the operation conditions (e.g., ammonia in the influent, DO and pH) similar to previous phases, the TN removal efficiency decreased significantly while excess nitrate was produced, which indicated that the suppression of anammox process thus part of nitrite was converted by nitrite oxidation process. Nevertheless, along with the continuous operation, the NRR of reactor gradually restored and recovered the similar level in phase P1 after 15 days’ operation (Fig. 2). This indicated that the TOC from the supernatant of 87.04 mg/L and C/N ratio of 0.22 did not affect the stability of reactor performance significantly. In the preliminary period

![Fig. 2 – Performance of the combined nitritation-anammox reactor throughout the operation. (a) Profiles of the influent total nitrogen, effluent nitrogen compounds and total nitrogen removal efficiency. (b) Variations of the TOC in the influent and effluent.](image)

**Table 1 – Performance of combined nitritation-anammox reactor during various phases.**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Inf. TN (mg/L)</th>
<th>Inf. TOC (mg/L)</th>
<th>Variation of TOC</th>
<th>C/N</th>
<th>NLR (kg N/(m³·day))</th>
<th>NRR (kg N/(m³·day))</th>
<th>TN removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>398.93</td>
<td>4.81</td>
<td>−0.97</td>
<td>0.01</td>
<td>0.50</td>
<td>0.39</td>
<td>77.88</td>
</tr>
<tr>
<td>P1</td>
<td>411.89</td>
<td>53.52</td>
<td>+2.50</td>
<td>0.13</td>
<td>0.52</td>
<td>0.43</td>
<td>83.69</td>
</tr>
<tr>
<td>P2</td>
<td>396.25</td>
<td>87.04</td>
<td>+8.39</td>
<td>0.22</td>
<td>0.50</td>
<td>0.43</td>
<td>85.10</td>
</tr>
<tr>
<td>P3</td>
<td>408.25</td>
<td>62.52</td>
<td>−6.94</td>
<td>0.15</td>
<td>0.52</td>
<td>0.42</td>
<td>88.85</td>
</tr>
<tr>
<td>P4</td>
<td>409.56</td>
<td>62.52</td>
<td>+23.83</td>
<td>0.22</td>
<td>0.51</td>
<td>0.35</td>
<td>68.62</td>
</tr>
<tr>
<td>P5</td>
<td>428.30</td>
<td>150.12</td>
<td>−57.80</td>
<td>0.35</td>
<td>0.55</td>
<td>0.49</td>
<td>88.85</td>
</tr>
</tbody>
</table>

* Positive number in the variation of TOC indicated the increase of TOC in the effluent compared to in the influent, and the negative number implied the TOC decreased after reactor treatment; NLR: nitrogen loading rate, NRR: nitrogen removal rate.
for supernatant addition, in spite of the elevated dosage of supernatant, the combined nitritation–anammox could still resist and gradually acclimated after a period of operation. Due to the fluctuations of reactor performance during P2 operation, the dosage of raw supernatant decreased in P3 (days 93–110), which resulted in the TOC and C/N ratio in the influent of 62.52 mg/L and 0.15, respectively (Table 1). Due to the reduction in supernatant addition, the reactor maintained stable and efficient nitrogen removal performance with NRR and TN removal efficiency of 0.42 g N/(m³·day) and 82.59%, respectively.

Throughout the operation from P1 to P3 with raw supernatant addition, the reactor showed relatively stable and efficient nitrogen removal performance with C/N ratio under 0.22. The combined nitritation–anammox revealed considerable resistance for supernatant addition. Besides, TOC removal could barely be detected even if mild increase in TOC could be occasionally monitored. The relative stability in TOC indicated the intensive suppression of heterotrophic bacteria due to the insufficiency of biodegradable organics. In contrast, during the metabolic process, the microorganisms would release extracellular polymeric substances or soluble microbial products (Xie et al., 2013; Ni et al., 2012), which resulted in the mild increase of TOC in the effluent.

### 2.3. Supernatant inhibition (P4)

In consideration of the stable performance along the operation during P1, P2 and P3 with supernatant addition, the dosage was elevated again in phase P4 (days 110–124) to similar level compared to phase P2 (the TOC and C/N ratio in the influent in P4 reached 89.74 mg/L and 0.22, respectively). Surprisingly, the reactor revealed dramatic reduction in nitrogen removal performance (Fig. 2). The NRR continued to decrease and was reduced to 0.35 kg N/(m³·day) on day 120, meanwhile the TN removal efficiency decreased to 68.62% (the NRR decreased by 16.9% and the TN removal efficiency decreased by 14.0% compared to in P3). Moreover, the TOC in the effluent continued to increase and further achieved 137.3 mg/L on day 124 (increased by 42.7 mg/L compared to in the influent).

The dramatic reduction in ammonia and TN removal performance indicated the decrease in nitritation and anammox activity. In addition, compared to the situation in P2, the effluent TOC in P4 continued to increase and exceeded the influent TOC. The TOC in the effluent finally achieved the highest level ever detected throughout the whole experiment at the end of P4, and reached 140 mg/L, which was 42.7 mg/L higher than that in the influent. The continuous increase of TOC in the effluent revealed the intensive hydrolysis of microorganisms, which further confirmed the existence of severe inhibition. Considering the similar influent condition in P2 and P4, the significant differences in reactor performance could be attributed to the long-term operation, during which the inhibition impacts of supernatant gradually accumulated and presented significantly when the reactor met shock elevation in supernatant addition. The adverse effects of supernatant firstly appeared at the beginning of P2 (18 days after initial supernatant addition on day 48), and the microorganisms presented considerable adaptive capacity at that time and the reactor restored to the effective operation rapidly. However, for phase P4, through 110 days’ operation in which 63 days are with supernatant addition, the adverse effects of supernatant gradually accumulated and further deteriorated the shock resistance and restorability of the combined nitritation–anammox process, which resulted in the dramatic decrease of reactor performance.

The adverse effects could be resulted from the various compounds companied with supernatant. Besides the highly concentrated ammonia and organic carbons, heavy metal (Cu = 2.58 mg/L) and toxic organics (CCl₄ = 40.68 μg/L, p-isopropyltoluene = 124.19 μg/L) in trace amount were also detected in the supernatant, which might mainly result from the residual of pesticides in fruit/vegetable waste. These compounds were proved to be inhibitory for the nitritation–anammox process potentially in previous research (Jin et al., 2012; Yang et al., 2013b). Therefore, for the treatment of raw supernatant by combined nitritation–anammox process, the long-term impacts of inhibitors in trace amounts (e.g., heavy metals and toxic organics) should be also taken into consideration more than the nitrogen and carbon compounds. The adverse effects of supernatant tended to accumulate during the long-term operation and gradually reduce the shock resistance of microorganisms. This was also in accordance with the research conducted by Tang et al. (2011, 2014) who suggested adding the anammox granules periodically to resist the long-term adverse effects caused by antibiotics and high-strength organics.

### 2.4. VFAs addition (P5)

Due to the fluctuations in the performance of anaerobic digestion, VFAs might gradually accumulate and present in the supernatant. Compared to the TOC detected in phases P1 to P4 which mainly consisted of refractory carbons, VFAs were highly biodegradable and tended to impact the combined nitritation–anammox process differently. For this reason, the performance of combined nitritation–anammox reactor with supernatant addition, which contained VFA residuals, was further investigated.

The addition of raw supernatant was continued to be maintained in P5. Different from the previous phases, the supernatant in P5 was derived from the anaerobic digester during fluctuation period thus VFAs around 300 mg/L could be detected. The supernatant was diluted 2 times in P5 to serve as the influent directly (150 mg/L VFAs in the influent), thus the supernatant contributed 100% of ammonia in the influent and the C/N ratio was identical to that of the original supernatant. In addition, due to the introduction of VFAs, the TOC and C/N ratios in the influent in P5 were increased to 150.12 mg/L and 0.35, respectively. Unexpectedly, rather than suppression, the reactor revealed excellent performance for nitrogen removal with VFA addition (Fig. 2). The NRR and TN removal efficiency gradually recovered from the depression in P4 and continued to increase along the operation. The NRR and TN removal efficiency was increased to 0.49 kg N/(m³·day) and 88.85% at the end of P5 on day 140, the highest values obtained during the whole experiment. Notably, different from the previous phases, considerable elimination of TOC was observed during the phase P5, which indicated the existence of intensive heterotrophic denitrification with VFAs addition.
Because of the shortage in sufficient biodegradable organics from P0 to P4, the reactor didn’t reveal considerable denitrification activity and the heterotrophic denitrification biomass in nitrification seed sludge could hardly out-compete the anammox bacteria. Nevertheless, due to the introduction of biodegradable organics (VFAs) in P5, denitrification process gradually recovered and coexisted with nitrification and anammox processes. Under this circumstance, this reactor was developed into a nitritation–anammox–denitrification system and Chen et al. (2009) named it SNAD (simultaneous partial nitrification, anammox and denitrification) process. For the detailed analysis of the nitrogen removal performance in phase P5, model calculation based on stoichiometric equations was carried out to figure out the nitrogen removal contributed by anammox or denitrification approaches. The procedures for model calculation were performed essentially as described previously (Wang et al., 2010). In the model calculation, nitrification, anammox and denitrification were assumed as the major reactions involved in nitrogen transformation. Based on the stoichiometric equations of these reactions and the variations of ammonia, nitrite, nitrate and COD, the quantity of nitrogen consumed by ammonia oxidation bacteria, nitrite oxidation bacteria, anammox and denitrification could be determined. As the results showed in Fig. 3, in spite of the utilization of partial TOC (mainly VFAs) in the last phase, only 14.4% of TN was removed through denitrification process due to the limited biodegradable organics, while the majority of TN removal was attributed to the anammox approach. The combined nitritation–anammox was still the predominant reaction for the nitrogen removal.

Besides the nitrogen removed by denitrification, the nitritation–anammox process contributed 85.6% of the TN removal, meaning the NRR of 0.42 kg N/(m³·day), which was still 20% higher than in P4. However, the TOC and C/N ratio in P5 revealed significant increase compared to P4. Moreover, the inhibition caused by supernatant would be further accumulated through the continuous operation. For these reasons, the recovery of reactor performance in P5 rather than inhibition indicated that the VFA addition might promote the combine nitritation–anammox process besides the denitrification within appropriate concentration range (150 mg/L in this study). VFAs were reported to be the substrate of anammox bacteria and served as the electron donors for nitrate reduction during the dissimilatory nitrate reduction to ammonia (DNRA) process (Kartal et al., 2007). It is reported by previous researches that the addition of VFAs could enhanced the nitrogen removal performance of anammox process and improve the TN removal efficiency under appropriate conditions (Winkler et al., 2012a,b). Therefore, the VFAs remained in the supernatant (within 150 mg/L) might serve as the extra electron donors for anammox bacteria through DNRA process thus stimulating the anammox reaction, which resulted in the promotion of the combined nitritation–anammox reactor and enhanced the performance for nitrogen removal.

Throughout the operation, the combined nitritation–anammox reactor received the increasing dosage of raw supernatant while kept relatively stable. For the comparative study between different phases, the ammonia in the influent was kept around 400 mg/L thus the supernatant was still diluted 2 times in the last phase (the average ammonia concentration of raw supernatant was 813 ± 59 mg/L). Under this circumstance, the C/N ratio in the influent was the same to the raw supernatant. The reactor still revealed efficient nitrogen removal performance. In addition, with VFA residuals in the supernatant, the reactor could gradually restore from the suppression of supernatant and further obtained the highest NRR throughout the whole experiment. These indicated that the combined nitritation–anammox was suitable for the treatment of supernatant. For further research to treat the supernatant without dilution, merely the exchange volume ratio should be reduced thus the SBR could receive the supernatant directly (for the treatment of supernatant which was diluted 2 times, the exchange volume ratio was 40% in this study, and it was equivalent to the situation that SBR received supernatant directly by reducing the exchange volume ratio to 20%).

2.5. Microbial community analysis

GeoChip 4.0 was engaged to present an overview of the microbial community structure of the combined nitritation–anammox reactor treating complex supernatant, and figure out the influences by supernatant addition. Detrended correspondence analysis (DCA) was conducted to examine whether the structure of microbial community changed significantly by supernatant addition. As shown in Fig. 4, samples were grouped by phases and samples from different phases were well separated from each other. In addition, the dissimilarity tests also showed that microbial community structures were significantly different between P0 and P5 (p < 0.05). The DCA and dissimilarity results revealed that the long-term addition of digestion supernatant altered the microbial community structure of this reactor significantly.

Subsequently, Shannon and Simpson Indices were calculated to indicate the functional gene diversity, while the gene number was presented to figure out the variation in gene abundance (Table 2). The results showed that the gene diversity and gene abundance were both decreased significantly in P5 compared to in P0 (p < 0.05) even after the restoration in phase 5, which indicated that the supernatant shaped the whole microbial community mainly by suppression.

3. Conclusions

A combined nitritation–anammox reactor was established to treat the digestion supernatant under various C/N ratios. The supernatant which mainly consisted of refractory organics suppressed the growth of heterotroph which thus resulted in relative stability of combined nitritation–anammox process. Nevertheless, the adverse effects of supernatant would gradually accumulate during long-term operation and led to the decrease of reactor performance. The VFA residuals within 150 mg/L in the supernatant served as the extra electron donors and stimulated the heterotrophic denitrification process, which thus restored and enhanced the nitritation–anammox reactor rather than inhibition. The combined nitritation–anammox reactor was proved to be suitable to treat the anaerobic digestion supernatant.
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**Table 2 – Comparison of detected genes and diversity indices by GeoChip 4.0.**

<table>
<thead>
<tr>
<th></th>
<th>Phase P0</th>
<th>Phase P5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total numbers of genes detected</td>
<td>22911</td>
<td>19910</td>
</tr>
<tr>
<td>Shannon index</td>
<td>9.94</td>
<td>9.78</td>
</tr>
<tr>
<td>Simpson index</td>
<td>20638</td>
<td>17588</td>
</tr>
</tbody>
</table>

**Fig. 3 – Model calculation of the combined nitritation-anammox reactor based on stoichiometric equations.**

**Fig. 4 – Detrended correspondence analysis (DCA) of GeoChip data.** The values of Axes 1 and 2 represent the percentage of total variations that can be attributed to the corresponding axis.

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