Analysis of Nitrification Efficiency and Microbial Community in a Membrane Bioreactor Fed with Low COD/N-Ratio Wastewater

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Abstract

In this study, an approach using influent COD/N ratio reduction was employed to improve process performance and nitrification efficiency in a membrane bioreactor (MBR). Besides sludge reduction, membrane fouling alleviation was observed during 330 d operation, which was attributed to the decreased production of soluble microbial products (SMP) and efficient carbon metabolism in the autotrophic nitrifying community. 454 high-throughput 16S rRNA gene pyrosequencing revealed that the diversity of microbial sequences was mainly determined by the feed characteristics, and that microbes could derive energy by switching to a more autotrophic metabolism to resist the environmental stress. The enrichment of nitrifiers in an MBR with a low COD/N-ratio demonstrated that this condition stimulated nitrification, and that the community distribution of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) resulted in faster nitrite uptake rates. Further, ammonia oxidation was the rate-limiting step during the full nitrification.

Introduction

Membrane bioreactor (MBR) technology is a reliable and promising process in wastewater treatment and reclamation owing to its distinctive advantages over conventional activated sludge (CAS) systems. Of particular significance is that the MBR systems avoid cell washout by retaining complete biomass, which favors the growth of autotrophic nitrifying bacteria and consequently increases the nitrification efficiency, as reported previously [1].

The nitrification pathway of ammonium removal in MBRs is a two-step reaction undertaken by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB): AOB oxidize ammonium to nitrite in the first step and then NOB oxidize nitrite to nitrate in the following step [2,3]. Nitrifiers (AOB and NOB) are autotrophic bacteria and could derive energy for growth solely from the oxidation of ammonium/nitrite. However, in conventional MBRs, nitrification is not a strictly independent pathway and carbon oxidation is inevitable during this autotrophic process, which results in a bloom of heterotrophs. Even in an anoxic/oxic MBR, a considerable fraction of the organic carbon is still oxidized aerobically due to endogenous respiration of biomass as well as the leakage of organic carbon to aerobic tanks caused by the high recirculation flow [4,5].

The unexpected heterotrophic metabolism under aerobic condition, on one hand, consumes a large quantity of influent organic carbon and oxygen. Huge amounts of waste activated sludge (WAS) are produced during this process and their microbial products have been verified as the active component causing membrane fouling in MBRs [6]. In addition, heterotrophs compete with nitrifying bacteria for oxygen and space [3,7] and the accumulation of heterotrophic waste also inhibits the activities of the Nitrosomonas and the Nitrobacter group [8]. In the presence of organic carbon, nitrifiers are usually outcompeted by heterotrophs, which eventually cause the nitrification efficiency to decrease [9,10]. Verhagen and Laanbroek [11] found that under such conditions the nitrifying bacteria were strongly reduced above the critical carbon-to-nitrogen ratios and the numbers of Nitrosomonas europaea decreased more than those of Nitrobacter winogradskyi.

In light of these findings, we explored a novel approach to improve the process performance and nitrification efficiency in an MBR. Since heterotrophs gain their energy primarily from organic carbon, it is possible to control heterotrophic metabolism by cutting down the external organic carbon supply or reducing the influent COD/N ratio [12,13]. We hypothesized that operation in a low organic loading mode would result in nitrification stimulation, and that the metabolism (e.g. proliferation) of activated sludge would be altered in this mode, which would consequently influence the operation of MBRs (e.g. membrane fouling). To the best of our knowledge, the information of the effect of influent COD/N ratio on MBR performance and microbial community, especially as applied to low strength municipal wastewater treatment, is very limited.

Therefore, the overarching goal of this study was to evaluate the process performance and nitrification efficiency of an MBR fed...
with low COD/N-ratio municipal wastewater. 454 high-throughput pyrosequencing was then used to analyze the resulting bacterial population by sequencing the bacterial 16S rRNA gene, allowing us to investigate the population dynamics of the nitrifiers and heterotrophs in MBRs fed with different COD/N-ratio wastewater.

Materials and Methods

Lab-scale MBR: Configuration and Operating Conditions

The lab-scale MBR (R0) consisted of a tank with an effective volume of 26 L (Figure S1 in Supporting Information). The influent came from a dynamic membrane separation (DMS) reactor (Figure 1). In our previous work, we have successfully decreased the COD/N ratio of raw wastewater through organic carbon recovery by the DMS reactor [14]. The characteristics of the wastewater are listed in Table 1. Two 40 cm × 30 cm flat sheet membrane modules (PVDF, 0.40 m, Kubota Corporation, Japan) were mounted vertically between two baffle plates located in the tank; the permeate flux (J) was set at 18–24 L/m² h. The operation conditions of R0 are summarized in Table 2.

Three phases with different hydraulic retention time (HRT) and sludge retention time (SRT) were performed to evaluate the reactor performance and to calculate the activated sludge yield coefficient (Y) under a low COD/N ratio. Over the 330 days' operation, sludge was periodically wasted from the tank to maintain a SRT of 45 d in Phase I, 91 d in Phase II and 182 d in Phase III, respectively. The dissolved oxygen (DO) concentration of the tank was in the range of 1–3 mg/L. Details of the MBR configuration and operation can be found in Supporting Information (Text S1 and Figure S1).

For a full understanding of the microbial population dynamics resulting from influent COD/N variation, bacterial compositions in five control reactors (R1−R5) were also evaluated in this study. The schematic diagram of the reactors can be found in Figure 1. R1 and R2 ran in parallel with R0, which were located in a landfill leachate. Membrane bioreactors (R1, R2, R4, and R5) were operated in a suction cycle of 10 min followed by 2 min relaxation to alleviate membrane biofouling and a chemical cleaning-in-place procedure (0.5% (v/w) NaClO solution, 2 h duration) was carried out if the trans-membrane pressure reached about 30 kPa during the operation. More information about the control reactors is available in our previous publications [6,15,16].

Calculation Procedures

The filtration resistance of R0 over time was calculated using the following equation according to the literature [17]:

$$r_t = r_0 + r_p + r_m = \text{TMP} / \mu J$$

where r is the total filtration resistance (m⁻¹), r_p is the cake layer resistance (m⁻¹), r_m is the pore-clogging resistance (m⁻¹), r_m is the intrinsic membrane resistance (m⁻¹), J is the permeate flux (m³/(m² s)), and TMP is the applied transmembrane pressure (Pa), and μ is the permeate viscosity (Pa s).

The sludge yield coefficient (Y, mgVSS/mgCOD) and decay coefficient (K_6, day⁻¹) in R0 were estimated from the material balance of substrate and biomass, according to the following equation:

$$\text{I/SRT} = Y N_{r2} K_p = Y^h e^{hT} N_{r2} K_p K_{4} e^{K_{4}T}$$

where N_r is sludge loading rate (kgCOD/(kgMLVSS-d)), T is the temperature (°C), and Y and h (or K_p and h) are the Arrhenius constant and exponent of Y (or K_p), respectively. Y and K_p values for conventional MBRs were determined according to the MBR book [18] and our previous studies.

Biokinetics of microbial activities in R0 inferred from ammonia, nitrate and acetate oxidation were estimated via extant respirometry. Specific oxygen uptake rates (SOUR_r) and specific ammonia uptake rates (SAUR_r) related to AOB, SOUR_r and specific nitrite uptake rates (SNUR_r) related to NOB, and SOUR_r related to heterotrophs at different temperatures were measured separately using batch assays [19]. Specific nitrification rate (SNR) was determined by the limiting rate of SAUR or SNUR. The detailed measurement and calculation procedures are shown in Supporting Information (Text S1 and Figure S1).

**Table 1. Characteristics of the raw, influent and treated wastewater (Unit: mg/L).**

|                     | Phase I     | Phase II    | Phase III   |
|---------------------|-------------|-------------|-------------|
| Raw wastewater COD  | 475.5 ± 175.4 | 452.0 ± 201.0 | 337.5 ± 103.5 |
| TN                  | 44.7 ± 10.5  | 41.5 ± 11.3  | 43.5 ± 7.3   |
| Influent wastewater COD | 66.5 ± 23.8  | 57.3 ± 18.8  | 75.2 ± 27.8  |
| TN                  | 203.2 ± 3.2  | 18.9 ± 2.3   | 26.7 ± 4.4   |
| NH₃-N               | 17.4 ± 5.2   | 16.2 ± 3.2   | 24.5 ± 4.9   |
| Treated wastewater COD | 14.3 ± 14.9  | 8.8 ± 5.6     | 17.0 ± 10.0  |
| TN                  | 198.3 ± 3.5  | 17.2 ± 5.0   | 23.6 ± 2.8   |
| NH₃-N               | n.d.        | n.d.         | n.d.         |

*Values are given as mean ± standard deviation and n = 18, 11, 13 for Phase I, Phase II and Phase III, respectively.

**Microbial Diversity Analysis**

DNA extraction and PCR amplification. Samples for pyrosequencing were obtained from oxic zones of the reactors (R0−R5) in August, 2011 and water temperature was about 27°C. R0 was operated in Phase I with SRT of 45 d and HRT of 4.6 h. All sludge samples were settled and concentrated onsite and immediately transported to the laboratory for further treatment. DNA extraction was processed using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) according to manufacturer’s protocols. The quantity and quality of the extracted DNA were assessed using a Nano-drop® ND-1000 spectrophotometer (Labtech International, UK). For genetic library construction, DNA from the 5 MBRs (R0, R1, R2, R4, and R5), and from R3, were each amplified by PCR using primer set 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 533R (5’-
TTACCGCGGCTGCTGGCAC-3') for the V1-V3 region of the 16S rRNA gene. The 20 μL PCR mixture contained 4 μL of 5×FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.4 μL of each primer (5 μM), 0.5 μL of DNA and 0.4 μL FastPfu Polymerase. The thermocycling steps were as follows: 95°C for 2 min, followed by 25 cycles at 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec and a final extension step at 72°C for 5 min. The fused forward primer includes a 10-nucleotide barcode inserted between the Life Sciences primer A and the 27F primer. The barcodes allowed

| Phase | J, L/(m²·h) | HRT, h | SRT, d | SAD, m³/(m²·h) | COD/N ratio Raw wastewater | COD/N ratio Influent wastewater |
|-------|-------------|--------|--------|----------------|---------------------------|-----------------------------|
| I     | 18          | 4.6    | 45     | 10.5          | 10.8±4.3                  | 3.3±1.0                     |
| II    | 24          | 3.4    | 91     | 14.3          | 11.3±5.3                  | 3.1±1.0                     |
| III   | 24          | 3.4    | 182    | 14.3          | 7.8±1.8                   | 3.0±0.6                     |

Figure 2. Variations in $r_f$, biomass concentration, OLR and COD/N ratio during $R_0$ operation. (The inverted open triangle indicates the time point of sludge sampling).

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Microbial Community in a Membrane Bioreactor

The image shows a heat map and a phylogenetic tree. The heat map displays relative abundance of different microbial communities across various conditions labeled as Zone 1 to Zone 7. The tree illustrates the evolutionary relationships between these communities.

Relative abundance is indicated by color intensity, with red representing higher abundance. The tree on the left side of the image is a dendrogram, likely indicating the phylogenetic relationships among the samples or conditions.

The labels Zone 1 to Zone 7 suggest different regions or stages of the bioreactor process, each with its own distinct microbial community composition.

The heat map's color gradient ranges from black (0) to red (0.31), indicating the relative abundance of the microbial populations.
sample multiplexing during pyrosequencing in a single 454 GS-FLX run.

**454 high-throughput 16S rRNA gene pyrosequencing.** After purification using the UNIQ-10 PCR Purification Kit (Sangon, Shanghai, China) and quantification using a TBS-380 (Turner BioSystems, Inc., USA), a mixture of amplicons was used for pyrosequencing on a Roche massively parallel 454 GS-FLX Titanium sequencer (Roche 454 Life Sciences, Branford, CT, USA) according to standard protocols [20]. To minimize the effects of random sequencing error, low-quality sequences were removed by eliminating those without an exact match to the forward primer, those without a recognizable reverse primer, those with length shorter than 150 bp, and those containing any ambiguous base calls (Ns) [21]. Barcodes and primers were then trimmed from the resulting sequences. Pyrosequencing produced 7818 (R0), 6629 (R1), 7429 (R2), 8265 (R3), 9854 (R4) and 7944 (R5) high-quality V1-V3 tags of the 16S rRNA-gene with an average length of 419 bp.

**Biodiversity analysis and phylogenetic classification.** Initially, sequences were analyzed by performing a BLAST search via the silva106 database at a uniform length of 150 bp and then clustered into operational taxonomic units (OTUs) by setting a 0.03 or 0.05 distance limit (equivalent to 97% or 95% similarity) using the MOTHUR program (http://www.mothur.org/wiki/Main_Page). From the cluster file, the rarefaction curves at z of 0.03, 0.05 and 0.10 were generated in MOTHUR for each sample. Taxonomic classification down to the phylum, class, order, and family and genus level was performed using MOTHUR via the silva106 database at a uniform sequence length of 400 bp with a set confidence threshold of 80%. Hierarchical cluster analysis was performed using the gplots package of R (http://www.r-project.org/) in Linux. The Chao linkage method was employed for distance calculation and the complete linkage method for cluster analysis in both coltree and rowtree of heatmap. MEGAN 4.0 software (http://ab.inf.uni-tuebingen.de/software/megan/) was then used to interactively explore the dataset. Each node is labeled by a taxon and the number of reads assigned to the taxon, and the size of a node (the pie chart) is scaled logarithmically to represent the number of assigned reads.

**Analytical Measurements**

Analytical measurements of chemical oxygen demand (COD), ammonium (NH₃-N) and total nitrogen (TN) in raw, influent and treated wastewater, total mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) in the system were performed according to the Standard Methods [22]. Protein was measured by the modified Lowry method using bovine serum albumin (BSA) protein as a standard [23]. Carbohydrate was measured according to the phenol-sulfuric acid method with glucose as a standard [24]. TOC and UV₂₅₄ of the filtrate of mixed liquor was quantified by a TOC analyzer (TOC-VCPN, Shimadzu, Japan) and 2802 UV/VIS spectrophotometer (Unico Inc., USA), respectively. Dissolved oxygen (DO) and temperature were monitored by using a DO meter HQ30d with probe LDO10103 (Hach Co., USA) online. Moreover, microscopic examination of the mixed liquor sample was performed according to the protocols [15] twice a week. Aquatic worms’ bloom was defined as the sharp decrease of biomass concentration with the presence of >1000 metazoa per L mixed liquor.

**Results and Discussion**

**Process Performance**

At the beginning of the experimental runs, a period of time intended for biomass acclimation (designated as start-up phase in
Phase I) was imposed on R0. The stable biomass concentration of 4.66±0.48 g MLSS/L, 7.09±0.67 g MLSS/L and 14.60±0.59 g MLSS/L was achieved in Phase I, Phase II and Phase III, respectively (Figure 2). Since a large fraction of influent organic carbon was recovered in the upstream process (Table 1), WAS production involved in the treatment of per ton wastewater was decreased by 60–80%. Moreover, with the feeding strategy adopted, in which a low organic loading rate (OLR) was applied to favor the growth of autotrophic microorganisms, it was inferred that a low COD/N ratio would cause a limiting supply of nutrients for microorganism growth, and could result in a low sludge yield. Relevant literature has documented that an autotrophic community could derive energy for growth from the oxidation of ammonium/nitrite [25], resulting in a thinner microcolony structure in bioreactors [7,13]. In view of this, we calculated the sludge yield coefficient (Y) and decay coefficient (Kd) to evaluate the carbon metabolism in R0. Y and Kd refer to microorganism growth and endogenous respiration. OriginPro 8 (OriginLab Corporation, USA) was applied to process the nonlinear curve fit and the results are shown in Figure S2.

In this study, the kinetic parameters were calculated as $Y = 0.362e^{-0.001T}$ mg VSS/mg COD and $K_d = 0.023e^{-0.006T}$ day$^{-1}$ using the operational data, and these constants at 20°C were lower than those of conventional MBRs (0.56–0.40 mg VSS/mg COD and 0.08–0.07 day$^{-1}$). This result indicated a limited rate of microorganism growth and biomass decay in a relatively autotrophic run [26]. Under such an oligotrophic environment, organic carbon was likely to be taken up by the starved community to derive energy for system sustainability rather than assimilated for microbial growth, and the significantly decreased $K_d$ is likely due to the lower aeration intensity [27,28]. In summary, we preliminarily concluded that the sludge reduction in R0 was a result of both source reduction (influent organic carbon) and process reduction (bio-metabolism pathways).

The variations of the total filtration resistance with operation time in the three phases of R0 are also shown in Figure 2. After a successful acclimation in the start-up phase, the reactor operation was gradually stabilized with a relatively low rate of increase of $r_t$. The operation cycles in Phases I and II of R0 lasted for about 100 and 60 days, respectively, relatively longer than that in our previous study [15,16]. Even if the low liquor temperature (5–15°C) caused membrane filtration to deteriorate by the Phase III, the chemical cleaning cycle was still about 30 day. To clarify the mechanism of alleviation of membrane fouling in R0, the average content of TOC, protein, carbohydrate and UV254, representing dissolved organic matter (DOM) in the supernatant of the mixed liquor, was quantified as 9.40±2.91 mg/L, 11.49±1.11 mg/L, 10.11±3.09 mg/L and 0.090±0.003, respectively ($n = 7$). The DOM level was relatively lower than that of conventional MBRs [6,15,16], which presumably mitigated cake layer (or gel layer) formation and pore clogging during membrane filtration. To explain our result, we hypothesize a combined metabolic synergy, i.e., in addition to the decreased production of soluble microbial products (SMP) mentioned above, it is likely that there was an

![Figure 5. AOB and NOB sequences from R0–R5 assigned to NCBI taxonomies using BLAST and MEGAN.](doi:10.1371/journal.pone.0063059.g005)
efficient food web (carbon metabolism) in the autotrophic nitrifying community, which ensured maximum heterotrophic utilization of SMP produced by nitrifiers and prevented significant accumulation of nitrifier waste materials, as reported by Kindaichi et al. [9]. In future, further attempts will be made to clarify the principle of SMP production and degradation during autotrophic nitrification in an MBR, and its impact on fouling and membrane filtration.

**Biokinetics of Nitrifiers and Heterotrophs**

Table 1 summarizes the average characteristics of the influent and treated wastewater in R0. Ammonium could not be detected in the treated water during the experimental operation. In three phases, ammonium was predominately oxidized to nitrate (99.8±0.1%, n=42) rather than nitrite (0.2±0.1%, n=42). The negligible nitrite accumulation (<0.04 mg NO2⁻/N/(gVSS-h)) during the full-scale nitrification decreased the risk of NO2⁻ reduction or its chemical decomposition, which subsequently reduced the transient and stabilized N2O and NO emissions, as documented previously [2,19].

**Table 1.** Comparison of influent and treated wastewater in R0

| Parameter | COD (mg/L) | N (mg/L) | COD/N ratio |
|-----------|------------|----------|-------------|
| Influent   | 1000       | 250      | 6.49        |
| Treated    | 400        | 100      | 2.00        |

**SOUR₅, SOUR₄ and SOUR₃ of the biomass in R₅ were calculated as 3.91, 1.71 and 4.64 mgO₂/(gVSS-h) (R² = 0.9953, 0.9994 and 0.9963) at 19.8°C, and 3.47, 2.96 and 3.89 mgO₂/(gVSS-h) (R² = 0.9922, 0.9960 and 0.9896) at 23.7°C, respectively.** The biokinetics of nitrite to nitrate oxidation were significantly (p=0.05) higher than the biokinetics of ammonium to nitrite oxidation, illustrating that ammonia oxidation limited the full-nitrification. SNR was then determined as SAUR. It has been reported that with the SRT prolonged, biomass rennovation became slower and enzymatic activity (e.g., SNR) decreased due to competition from the biomass derived from the limiting supply of nutrients [29], while the SNR in R₅ was calculated as 18.9e⁻⁰.⁰⁰⁹⁷ mgN/(gVSS-d) (R² = 0.8370), even higher than that of the conventional MBRs with shorter SRTs [26,29]. Thus it is possible that the decrease in the influent COD/N ratio inhibited the metabolism of heterotrophs, thereby favoring the nitrifiers in competing for oxygen and scarce substrate (SOUR₅ > SOUR₄ > SOUR₃) [11], especially under prolonged SRT conditions.

Further investigation using 454 high-throughput 16S rRNA gene pyrosequencing was performed to compare the microbial diversity and composition (heterotrophs and nitrifiers) of bioreactors fed with different COD/N-ratio wastewater.

**Taxonomic Complexity of the Bacterial Community**

Six 16S rRNA gene libraries were constructed from pyrosequencing of R₀, R₁, R₂, R₃, R₄ and R₅ communities with 7818, 6629, 7429, 8265, 9854 and 7944 high-quality reads (average length of 419 bp). The number of sequences was comparable to our previous study [20]. The MOTHUR program was first used to assign these sequence tags into different phylogenetic bacterial (99.8%) clusters using the MEGAN 4.0 software (Figure 5). The ratio of spectrum distribution of phyla *Protobacteria*, *Chloroflexi* and *Nitrospirae* in Z2, Z₄ and Z₅ in Figure 3. Autotrophic bacteria in phyla *Protobacteria* and *Nitrospirae* may be depleted in R₅ under such a copiotrophic environment. Although the influent COD/N ratio was also about 10 in R₅, its community structure was quite different from the other MBRs (Figure 3). Race has reported that not only the quantity but also the source of the organic carbon affected the make-up of the heterotroph community as well as AOB in mixed cultures [3]. The phyla *Chloroflexi* (9.5%), *Firmicutes* (4.4%) and *Planctomycetes* (10.5%) referring to polysaccharide degradation [32], anacrobic fermentation [21] and sulfated polymeric carbon utilization in the marine environment [33] were enriched in the R₅ community (Figure 4), suggesting a versatile bio-metabolism of the inert component in the leachate.

Specific comparison of nitrifier communities was conducted using the MEGAN 4.0 software (Figure 5). The ratio of spectrum color in each pie represents the ratio of the relative abundance of reads assigned to the corresponding family, genus or species in R₀...
which indicated a deterioration of nitrification. In R5, the influent AOB and 41 OTUs of NOB were detected at a 3% distance, were outcompeted by the heterotrophs and thus only 31 OTUs of Nitrospira spp.-related NOB was likely due to the propensity of Nitrobacter spp., respectively. These results are consistent with previous literature than those in the CAS (R3) system (Figure S3). Under a high organic substrate concentration (R4), the nitrifiers with low growth rate and poor growth yield. With the influent COD/N ratio decreased from 10.0 (R1 and R2) to 3.0 (R0), the population of AOB and NOB was increased by 4.7% and 189.3%. The community distribution of AOB (155 OTUs) and NOB (353 OTUs) in R0 resulted in faster nitrite uptake rates and a rate-limiting step of ammonia oxidation during the nitrification. Under a high organic substrate concentration (R4), the nitrifiers were outcompeted by the heterotrophs and thus only 31 OTUs of AOB and 41 OTUs of NOB were detected at a 3% distance, which indicated a deterioration of nitrification. In R3, the influent organic carbon contained a large fraction of complex protein-like substances (data not shown), and Nitrospira referring to AOB were highly enriched while NOB species (Nitropira) decreased. This result is consistent with a previous report that the protein-like organic substrate facilitates the growth of AOB [3].

Conclusions

Sludge reduction and membrane fouling alleviation were induced by the decrease of influent COD/N-ratio. The reduced SMP production and efficient carbon metabolism in the autotrophic nitrifying community facilitated membrane fouling mitigation.

References

1. Hocaoglu SM, Insel G, Colakog EU, Orhon D (2011) Effect of sludge age on simultaneous nitrification and denitrification in membrane bioreactor. Bioresource Technology 102: 6655-6672.
2. Ahn JH, Kwan T, Chandran K (2011) Comparison of Partial and Full Nitrification Processes Applied for Treating High-Strength Nitrogen Wastewaters: Microbial Ecology through Nitrous Oxide Production. Environmental Science & Technology 45: 2734-2740.
3. Racz I, Datta T, Goel R (2010) Effect of organic carbon on ammonia oxidizing bacteria in a mixed culture. Bioresource Technology 101: 6434-6460.
4. Virdis B, Rabary K, Yuan ZG, Keller J (2008) Microbial fuel cells for simultaneous carbon and nitrogen removal. Water Research 42: 3013-3024.
5. Yuan Z, Keller J, Lant P (2003) Optimization and control of nitrogen removal activated sludge processes: a review of recent developments. In: Agathos, S.N., Reineke, W. (Eds.), Biotechnology for the Environment: Wastewater Treatment and Modeling. Waste Gas Handling, vol. 3C, 187–227.
6. Wang ZW, Wu ZC (2009) Distribution and transformation of molecular weight of organic matters in membrane bioreactor and conventional activated sludge process. Chemical Engineering Journal 150: 396-402.
7. Basson JP, Kleerebezem R, Rosado AS, van Loosdrecht MCM, Dezotti M (2012) Effect of Different Operational Conditions on Biofilm Development, Nitrification, and Nitrifying Microbial Population in a Moving-Bed Biofilm Reactor. Environmental Science & Technology 46: 1546-1553.
8. Li F, Chen JH, Deng CH (2006) The kinetics of crossflow dynamic membrane bioreactor. Water SA 32: 199-204.
9. Kindaichi T, Ito T, Okabe S (2004) Ecophysiological interaction between nitrifying bacteria and heterotrophic bacteria in autotrophic nitrifying biofilms as determined by microautoradiography- fluorescence in situ hybridization. Applied and Environmental Microbiology 70: 1641-1650.
10. Lee LY, Ong SL, Ng WJ (2004) Biofilm morphology and nitrification activities: recovery of nitrifying biofilm particles covered with heterotrophic outgrowth. Bioresource Technology 95: 209-214.

Supporting Information

Figure S1 Schematic of R0 fed with low COD/N-ratio municipal wastewater.
Figure S2 Nonlinear curve fit of Y and Kd, and analysis of variance (ANOVA).
Figure S3 Rarefaction curves of OTUs defined by 3%, 5% and 10% distances in R0–R5 sludge samples.
Table S1 Substrate composition for SOURs.

Author Contributions

Conceived and designed the experiments; JXM ZWW CWZ. Performed the experiments; JXM SML. Analyzed the data; JXM QYW ZWW. Contributed reagents/materials/analysis tools; JXM ZCW CWZ ZWW. Wrote the paper: JXM ZWW CWZ.
21. Lu L, Xing D, Ren N (2012) Pyrosequencing reveals highly diverse microbial communities in microbial electrolysis cells involved in enhanced H$_2$ production from waste activated sludge, Water Research 46: 2425–2434.
22. APHA (2012) Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
23. Hartree EF (1972) Determination of protein: a modification of the Lowry method that gives linear photometric response. Analytical biochemistry 48: 422–427.
24. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350–356.
25. He Z, Kun JJ, Wang YB, Huang YL, Mansfeld F, et al. (2009) Electricity Production Coupled to Ammonium in a Microbial Fuel Cell. Environmental Science & Technology 43: 3391–3397.
26. Han SS, Bae TH, Jang GG, Tak TM (2005) Influence of sludge retention time on membrane fouling and bioactivities in membrane bioreactor system. Process Biochemistry 40: 2393–2400.
27. Ramdani A, Dold P, Deleris S, Lamarre D, Gadbois A, et al. (2010) Biodegradation of the endogenous residue of activated sludge. Water research 44: 2179–2188.
28. Keskes S, Hmaied F, Gannoun H, Bouallagui H, Godon JJ, et al. (2012) Performance of a submerged membrane bioreactor for the aerobic treatment of abattoir wastewater. Bioresource Technology 103: 28–34.
29. Huang ZH, Gedalanga PB, Avagathanagul P, Olson BH (2010) Influence of physicochemical and operational parameters on Nitrobacter and Nitrospira communities in an aerobic activated sludge bioreactor. Water Research 44: 4351–4358.
30. Ma JX, Wang ZW, Yang Y, Mei XJ, Wu ZG (2013) Correlating microbial community structure and composition with aeration intensity in submerged membrane bioreactors by 454 high-throughput pyrosequencing. Water Research 47: 859–869.
31. Ye L, Shao MF, Zhang T, Tong AHY, Lok S (2011) Analysis of the bacterial community in a laboratory-scale nitrification reactor and a wastewater treatment plant by 454-pyrosequencing. Water Research 45: 4390–4396.
32. Kragelund C, Levantesi C, Borger A, Thelen K, Eikelboom D, et al. (2007) Identity, abundance and ecophysiology of filamentous Chloroflexi species present in activated sludge treatment plants. FEMS Microbiology Ecology 59: 671–682.
33. Bengtsson MM, Ovreas L (2010) Planctomycetes dominate biofilms on surfaces of the kelp Laminaria hyperborea. BMC Microbiology 10: 261.