

The Long-term Effect of Carbon Source on the Microbial Community in the EBPR System and Stoichiometry

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Abstract. In order to investigate the long-term effect of the characteristics and the evolvments of microbial community in the enhanced biological phosphorus removal(EBPR) under different carbon sources, three sequencing batch reactors were operated with switching acetate to propionate, glucose and domestic wastewater as the sole carbon substance. The results clearly show that there are very distinctive rates of phosphorus release or uptake per MLSS in each reactor. The microbial community of the sludge was analyzed using the polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) method during about 12 months of the operating phase. It was found that the number of major bands was decreased during the whole operating phase, indicating that the microbial community structure was getting simpler. The dominant bands of three reactors were excised and recovered to sequence it. The sequence, which was closely related to one of the putative PAOs group belong to beta-proteobacteria, was playing key role of P-removal in SBR1#. The sequence of SBR2# and SBR3# were related to some uncultured bacterium which were reported by Maszenan et al (2000) and McMahon et al (unpublished). Some stoichiometry parameters of SBR1# and SBR2# was confirmed at the end period of operating phase.

Keywords: Enhanced biological phosphorus removal, PCR-DGGE, polyphosphate accumulating organisms (PAOs) , microbial community, stoichiometry.

1. Introduction

Enhanced biological phosphorus removal(EBPR) ,operated with sequential anaerobic and aerobic periods, is considered as the efficiently suitable method for phosphorus removal. A group of bacteria known as polyphosphate-accumulating organisms (PAOs) are able to take up volatile fatty acids (VFA) and store them as polyhydroxyalkanoates(PHA), and this has attributed to release phosphorus in anaerobic phase. In the subsequent aerobic phase, PAOs use the stored PHA as energy sources for biomass growth, and take up orthophosphate into poly-p. Finally phosphorus is removed from the system through the wastage of excess sludge. This process involves the group of competitors known as glycogen accumulating organisms (GAOs) which compete with PAOs for anaerobic VFAs uptake in EBPR systems. Although PAOs would convert VFAs to PHAs, they do not perform anaerobic p release and aerobic p uptake transformations, hence they do not contribute to p removal and possibly causing instability or even failure of EBPR system.

Recently, in order to consider the proportion of the metabolic modeling of PAOs and GAOs, many studies has been focused on the effects of different carbon sources on the EBPR system [1], [2]. However, these researches were limited on the performance of reactors and used the synthetic water as the carbon substrate, meanwhile they were seldom focus on the microbial community dynamic changing. Compared with the synthetic water utilized domestic wastewater could better indicate the real conditions of full scale treatment. Thus, in this study, we used the switching between acetate and propionate, glucose, and domestic wastewater as the sole carbon source to determine the microbial community that are responsible for effects of the different carbon substrate in the EBPR process, we adapted the polymerase chain reactor(PCR)-

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denaturing gradient gel electrophoresis (DGGE) method that allow us to analyze the microbial composition and diversity of bacterium populations in the system without the need to isolate individual species. The purpose of this study is to characterize EBPR microbial communities under different carbon source conditions, and on the basis of the reactor operating results, to analyze the stoichiometry at the end period.

2. Materials and Methods

Three anaerobic-aerobic sequencing batch reactors(SBRs)with a working volume of 8L,called SBR1#, SBR2# and SBR3# respectively, were operated under similar conditions as described in Lu et al [3] and the typical 6 hour cycle was comprised of a 2.5 h anaerobic period, a 3 h aerobic period, and 30 minute settle/decent period. The activated sludge was taken from pilot plant which was treated with domestic wastewater. The SBR1# reactor was operated 350 days where the carbon source supplied to the feed was switched between acetate and propionate at a frequency of one to two sludge ages, while the SBR2# was supplied with glucose as a sole carbon source. The SBR3# was supplied with domestic wastewater from 1 days to 250 days, then fed with mixture wastewater which add acetate and phosphate into domestic wastewater to make sure the c/p rate in 20:1(COD:800mg/L,P:40 mg/L) until 350 days. The 2 L synthetic feed for SBR1# and SBR2# was composed of 0.3 L of solution A and 1.7 L of solution B. On the first 27 days, The mixed feed of solutions A and B contained 400mg COD/L and 20 mg P/L, and then increased to 800 COD/L and 40 mg P/L. Solution A contain (per litre) 3.41g acetate, 1.76ml propionic acid, 13.4, 2.68g glucose during the start-up period, then up to 6.83 g acetate, 3.56 ml propionic acid, 5.36 g glucose during the stable period. In addition, solution A also contain(per liter) 1.02g NH₄Cl, 0.01g peptone, 0.01g yeast extraction, 1.20g MgSO₄ • 7H₂O, 0.19g CaCl₂ 2H₂O, 7.94mg ATU, 4.00mL Trace elements liquid. Solution B contained 86.5mg K₂HPO₄ 3 H₂O and 52mg KH₂PO₄ during the start-up period, then up to 173mg K₂HPO₄ 3H₂O and 104mg KH₂PO₄ during the stable period.

The microbial community samples were taken at the different period from the reactor to analyse the population dynamics. The 16SrDNA fragments were amplified by PCR using forward primer EUB341f (5'-CCTACGGGAGGCAGCG-3') with the GC clamp (5'-CGCCCGCCGCGCGGCGGGCGGGGCGG CACGGGGGG-3') and the reverse primer UNIV907r (5'-CCCCGTCAATTCCTTTGAGTTT-3'). Operation method of PCR amplification and DGGE are as reported by Ahn et al (2002) [4].

3. Results and Discussion

3.1 Reactor performance

The three SBRs (SBR1#, SBR2#, SBR3# reactors) achieved EBPR under different carbon sources. They were operated one year and the typical profiles of dissolved parameters in one cycle are shown in Figure1 (a), (b), (c). On day 1, the uptake of acetate and release of phosphate in the anaerobic phase was more significant in SBR1# than other reactors, and p removal rate of the SBR1# reactor was stable over 90% during the operating periods. The P concentration at the end of anaerobic period was higher when the reactor fed with acetate relative to utilize the propionate, and it was approximately 130mg PO₄-P/L with a acetate feed, as compared to around 60 mg PO₄-P/L with a propionate feed at end of period. According to Lu (2006) et al studied that it is likely to be more competitive to PAOs than GAOs under the carbon-switching strategy and used this method that the highly enriched Accumulibacter culture may be obtained [3].

Glucose, a typical substrate, was used in SBR2# reactor. Before the day of 150 d, the reactor had low p removal rate, meanwhile the P concentration at the end of anaerobic period was only approximately 33 mg PO₄-P/L. In the subsequent period, the effluent of p concentrate was decreased, the p removal rate kept increasing until day 350.The phosphorus transformation during a cycle of SBR3# is shown in Figure3. The p release and removal rate were lower than SBR1# and SBR2#. In the experiments day 250 to 350, the SBR3# reactor was operated in mixture carbon source which mixed with domestic wastewater and acetate to make sure the COD concentrate up to 200mg/L which was similar as other reactors. On day 350, the P concentrate increased to 76 mg/L at the end of anaerobic. However, the p release rate and p uptake rate were decreased due to the growth of the MLSS. As can be seen on this three figures, complete phosphorus removal was achieved in all the experiments.

The p release per MLSS rate under different carbon source conditions was shown in Figure1 (d). Anaerobic P release rate was found to be largest when acetate was the carbon source, and it was slightly decreased under the glucose as the sole carbon substrate competing with propionate.

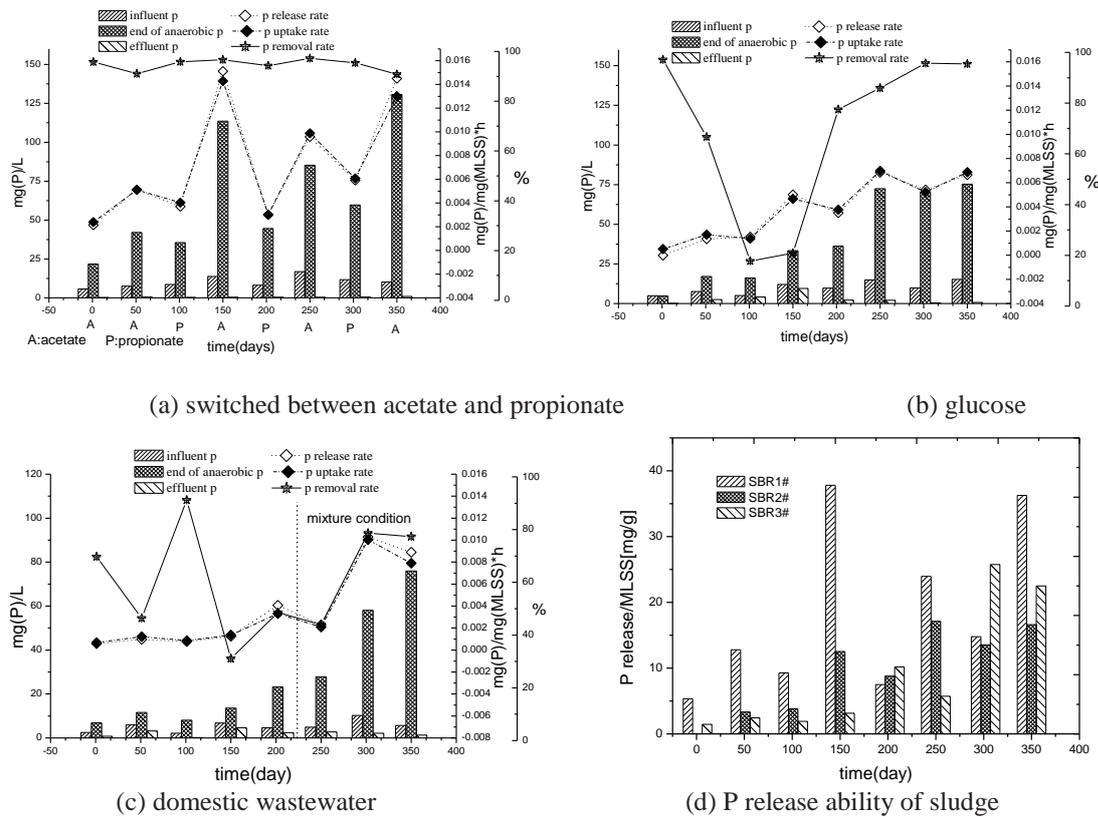


Fig. 1: Phosphorus removal performance using different carbon sources

3.2 PCR-DGGE

Microbial communities of the three reactors in the operating period were investigated by the DGGE (Fig 2), and the population dynamics was analyzed by the Quantity one software. On day 1, the sample of the seed sludge had less bands which were detected by the software. A common trend was apparent during the day 1 to 57: the quantity of the bands had increased more obviously in all the three reactors, especially in the domestic wastewater condition (SBR3#). Accompany with the stable p removal rate, the diversity of the whole community became simple and clearly, although some bands appeared and intensified (such as the band a, f, g, h, i, k, l, m, n, o, p), and some bands disappeared and weakened (such as the band b, c, d, e, j). It can also be concluded that the microbial community participating EBPR can be changed depending on the long-term effecting of different carbon source. On day 286, while the p removal ability of three reactor were steady operated last two month, the band a, k, f, g, h, o and p were increased intensity and getting more clearly. It can be considered that these bands separately represent the dominant species in these three reactors, and combined with the result of EBPR operating these bands should be playing key roles of p removal in the present EBPR process.

3.3 Sequencing of DGGE bands

DNAs recovered from these selected bands were sequenced and compared to GenBank. The bands a, f, g, h, p, were successfully sequenced. DNA recovered from band a, which was considered to play key roles in SBR1# reactor, was most similar to Rhodocyclus group, beta Proteobacterium which were reported by Hesselmann (1999) et al and Crocetti et al(2000) [5], [6]. These bacteria belong to β -Proteobacteria. DNA sequence of Band h was found to be related to uncultured bacterium (AB231448), which detected from EBPR system by Kondo et al(unpublished). Band g belong to Tetrasohaera australiensis, a typical GAO bacterium was reported by Maszenan et al(2000) [7]. Band f was similar as AF527588.1 (McMahon et al unpublished). The phylogenetic tree was made by the neighbor-joining algorithm using the Clustal w program which was shown in Fig 3.

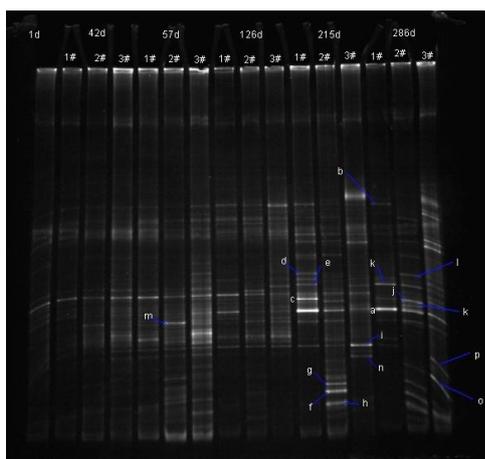


Fig. 2: Change of bacterial commu

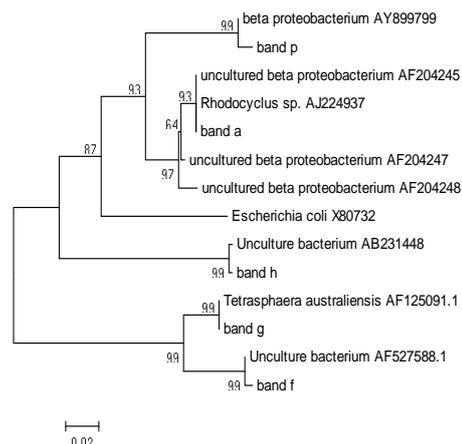


Fig. 3: Neighbor-joining tree of DGGE band monitored by DGGE

3.4 Stoichiometry of the different carbon source

Publication	Carbon source	PHB/V FA	PHV/V FA	PH ₂ MV/VFA	PHA/V FA	Gly/V FA
	C-mol/C-mol	C-mol/C-mol	C-mol/C-mol	C-mol/C-mol	C-mol/C-mol	C-mol/C-mol
Smolders et al 1994	Acetate	1.10	0.12	N/A	1.22	N/A
Filipe et al 2001	Acetate	1.15	0.15	N/A	1.3	0.53
Hesselmann et al 2000	Acetate	1.11	0.29	N/A	1.4	0.6
Lu et al 2006	Acetate	1.18	0.07	N/A	1.26	0.46
This study	Acetate	1.156	0.145	N/A	1.30	0.467
Oehmen et al 2005	Propionate	0.04	0.55	0.65	1.23	0.32
This study	Propionate	0	0.578	0.681	1.26	0.3
This study	Glucose	0	0.27	0	0.27	0.15

Appendix 1. Comparison of anaerobic carbon transformations with literature studies

The experimental results from this study with different carbon source are compared with the model stoichiometric from other studies, as outlined in Appendix 1. Upon comparison with literature results [8]-[11], the PHB/VFA ratio obtained in our acetate study was slightly below the Lu et al (2006) reported, which had the similar operated conditions. When glucose was the sole carbon source, PHV was the primary PHA fractions produced, while PHB and PH₂MV were not produced. Overall the stoichiometries of this study is fitting the metabolism predicted very well when fed with acetate and propionate, reported by Oehmen et al (2005) [12], however those studies had not identified and analyzed the group of the dominant bacterium.

Less studies was concerned that the PHA/Glucose rate when fed with the glucose as the carbon source for a long term operating conditions, and identified the microbial community of EBPR.

4. Conclusions

The EBPR system working with switching acetate to propionate, glucose, municipal wastewater as the carbon source presented the best P-removal capacity for almost 12 month, while the sludge activity from each reactor showed various abilities of the phosphorus release or uptake per MLSS, and the microbial community structure was changed depending on the different carbon source conditions in a long term. The result of DGGE showed that the microbial community became simple and clear in all the three reactors, and the determination of phylogenetic affiliation demonstrate that the key bacterium group contained in the three reactors were *Rhodocyclus* group and uncultured bacteriums. The results from this study confirmed the PHA and Glycogen stoichiometries under long term effect of the different carbon sources conditions.

5. References

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