生物学的りん除去廃水処理プロセスにおいて生成したグラニュール汚泥の処理性能と微生物群集構造の経時変化

Temporal sludge performance and microbial community change of aerobic granular sludge from an EBPR wastewater treatment bioreactor

学籍番号　47106814
氏　名　欧　陽（OU, Yang）
指導教員　佐藤 弘泰 準教授

1 Introduction

Basing on self-immobilization reaction of activated sludge, granular sludge are small dense compact structured activated sludge flocs, which can settle very quickly and be easily separated in the secondary settler. There are four factors favor the formation of granules including increased organic loading rate, reduced settling time, extended starvation period and increased shear force.

Granulation is an attractive technology due to its two main advantages. First, granular sludge guarantees high biomass retention inside the reaction tank, which improves the efficiency of the wastewater treatment system in general. Secondly, granular sludge demonstrates high settling velocities facilitating efficient solid-liquid separation in the clarifier, which reduces the costs related to waste sludge dewatering and disposal.

Aimed to promote removal of Phosphorus (P) from wastewater without the need for chemical precipitant, EBPR is an activated sludge process recirculating sludge through anaerobic and aerobic conditions. Phosphorus is accumulated inside the cell of microorganism, and then be removed from the system by extract and dispose of excess sludge.

Researchers found that poly-P accumulated in excess sludge could be recovered by heating and adding precipitant with high recovery efficiency. These discoveries can offer a distinct advantage in recovering P from the liquid phase as “biophosphorite”.

The more dense the sludge are, the less cost would be need for heating and precipitating. Thus to make the P recover process more efficient, we need to minimize the amount of the sludge and concentrate it to the best. Thereupon, granular sludge’s two merits become useful in wastewater P recycling process. Thus for sustainable P removal and recovery, EBPR combine with AGS should be further studied.

2 Research Framework Structure

Thus the present study was conducted with following objectives:

1. Operation of Lab-scaled reactors and taking samples routinely, to monitor treatment behavior and sludge characteristics.
2. Analysis of the bacterial community of collected sludge samples using T-RFLP and Unidirectional Pyrosequencing methods
3. Exploration on the relationship between granular sludge treatment activity and bacterial populations

3 Material and Methods

3.1 Activated Sludge Reactors

The experiment has been done with sequencing batch reactors (SBR) which had a working volume of 10L and was fed with synthetic wastewater. The influent carbon concentration was around 120mgC/L and P was around 30.16mgPO_4/L. Each cycle was 4hr in total, consisting of an inflow and anaerobic phase (70min), an aerobic phase (changeable from130 to151 min), a settling phase (changeable from 30 to 9min) and effluent discharge (10min). Sludge retention time (SRT) was controlled at around 7days and Hydraulic retention time (HRT) was 8hr.

Dissolved oxygen (DO) concentration was controlled between 2.3 mg/L to 2.5 mg/L during the aerobic phase. Reactor was installed in 26^{o}C room temperature.

Granulation of sludge was achieved by gradually reducing of settling time and the reduced time was added to aerobic phase to extended starvation period. Thus only well-settling granules stayed inside the reactor,

while poor-settling biomass was washed out under these conditions.

3.2 Analytical Methods

Dissolved organic carbon (DOC) concentration in supernatant was measured with TOC analyzer (TOC-Vcsn, Shimadzu, Japan). Anions were analyzed by an ICS-3000 ion chromatograph with an AS12A column and an ASRS suppressor (Dionex). MLSS concentrations and SVI were analysed accordingto APHA. Dewaterability was monitored by the WCDS testing method which was developed by Dr Ning Li in our lab.

3.3 Bacterial community analysis

Activated sludge samples for bacterial community analyses were taken from the reactors during the aerobic phase and immediately 1:1 mixed with pure ethanol (0.75mL mixed liquor with 0.75mL pure ethanol) and stored at -80 ˚C after sonicated at amplitude of 30%(around 7W) for 15s. Then, PCR reaction was performed using barcoded universal primer pair 27f/519r which is targeted at a partial 16S r RNA gene for Pyrosequencing respectively.

And barcoded PCR products were submitted for unidirectional Pyrosequencing after purificated by QIAQuick PCR Purification kit and High Pure PCR clean Up Micro Kit.

4 Results and Discussion

4.1 Run1:Confirmation of operation manner

Run1 was successfully operated for nearly 3 months which confirmed the possibility of getting EBPR AGS by shortening settling time, as shown in Fig.1.

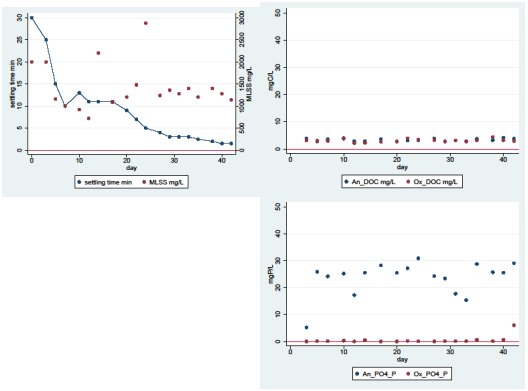

Fig. 1 Time course profiles of granulation and treatment performance of Run1 along the whole experimental period: settling time shortening and MLSS (a); DOC and PO4-P after the anaerobic and aerobic phase (c) and (d).

4.2 Run2

As shown in Fig 2, granules started to appear on the 11th day and stable granule treatment was observed from day 31 to day 69 when most biomass was in granular form. During good EBPR period before day 53th, TOC removal efficiencies remained higher than 98%, while around 90% of the influent P was removed.

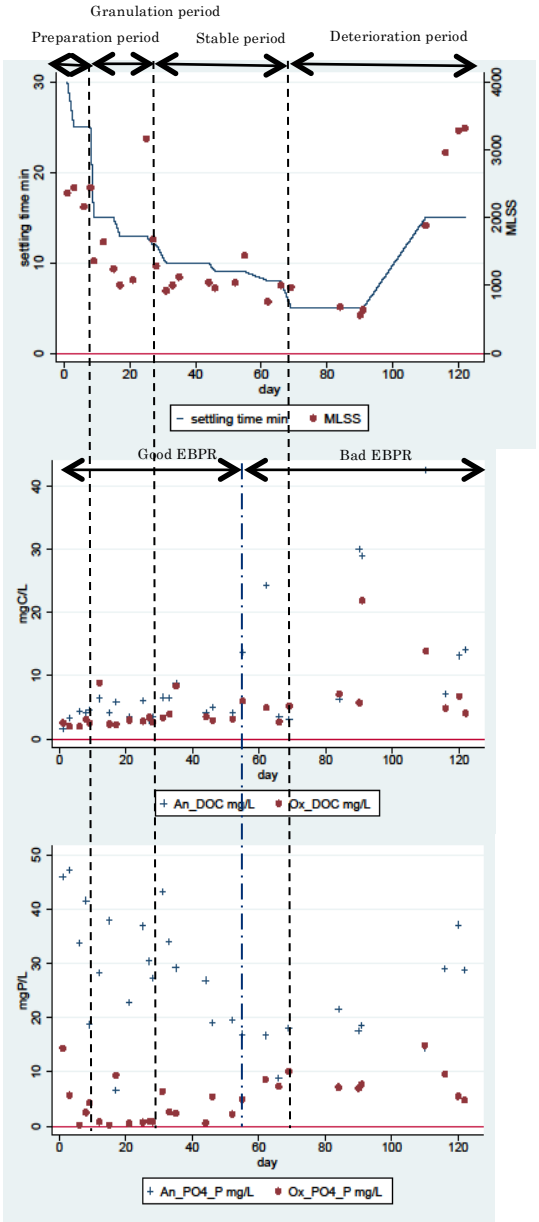

Fig. 2 Time course profiles of granulation and treatment performance of Run2: settling time shortening and MLSS; DOC and PO_4-P after the anaerobic and aerobic phase.

As shown in Fig 3, 24 Major OTUs were selected to present the main change in microbial community of Run2. The three lines in the heatmap is stand for day 11, day63, and day69 which are the changing point as shown in Fig2. The major OTU shows close relationship and shifted accordingly.

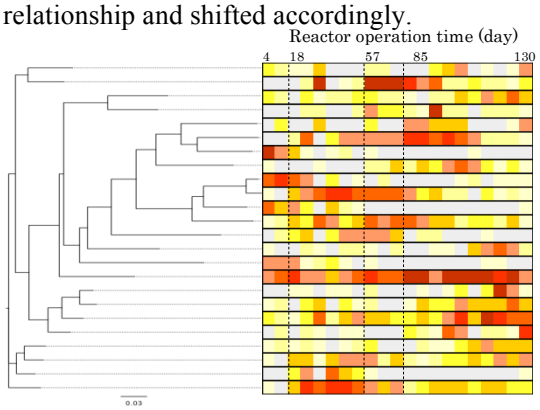

Fig. 3 Partial tree and heatmap distribution of major OTUs of Run 2

Table.1 Major OTUs and their taxonomy identity in tree order for Run 2

OTU	Custom Name
1239	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhod
720	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhod
1399	Root;Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Ch
1533	Root;Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriale
3535	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chiti
3680	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chiti
4144	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chiti
1619	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales
3563	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales
4644	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Cyto
3926	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales
2557	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales
2342	Root;Bacteria
2443	Root;Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales
541	Root;Bacteria;Chloroflexi
2803	Root;Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Thi
4052	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burk
82	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burk
2408	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales
1799	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Co
2980	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhod
4750	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhod
3729	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhod
4	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhod

The OTUs abundant between day 11 to day 69 and disappear latter should stand for species related with granulation, such as OTU4614 (*Cytophagaceae* sp.) and OTU4 (*Thauera* sp.). And similarly, the OTUS abundant before day 63 and disappear latter should stand for species related with EBPR. OTU720 (*Propionivibrio* sp.) and OTU2557 (*Sphingobacteriales*, unclassified sp.) for example, is thought to be related with EBPR performance.

OTUs those were little before day 63 and 69 but become dominant in the latter days, such as OTU2433 (*Novosphingobium* sp.), OTU2408 (*Burkholderiales*, unclassified sp.)and OTU 1799 (*Hydrogenophaga* sp.)should be species compete with EBPR AGS and have bad effect.

4.3 Run3

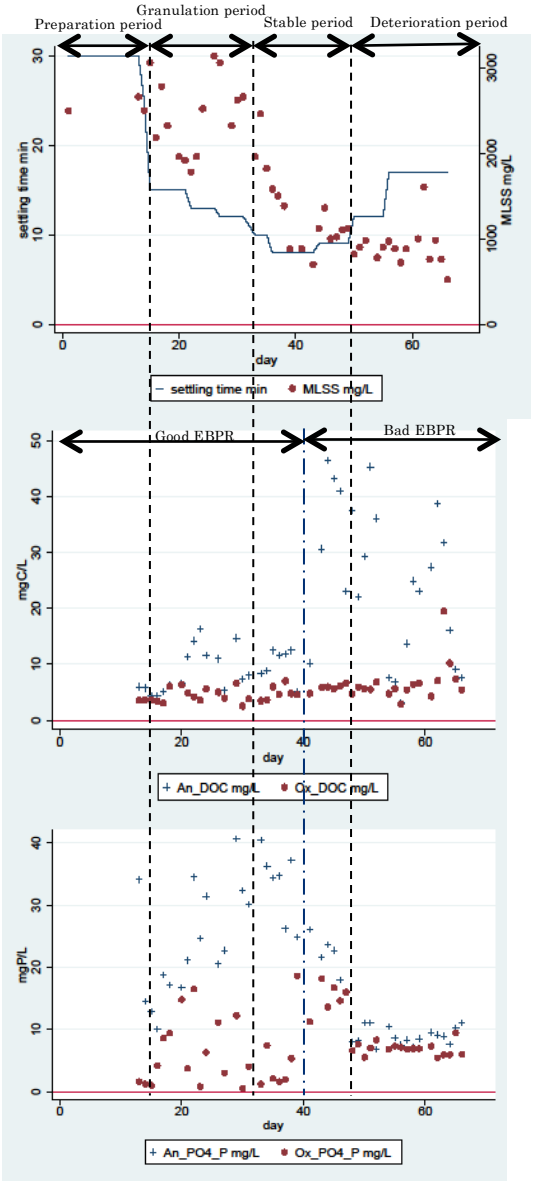

Fig. 4 Time course profiles of granulation and

treatment performance of Run3: settling time shortening and MLSS; DOC and PO_4-P after the anaerobic and aerobic phase

As shown in above Fig 4, granules started to appear on the 14th day and stable granule treatment was observed from day 34 to day 49 when most biomass was in granular form. During good EBPR period before day 41th, the TOC removal efficiencies remained, in general, higher than 95%, while P removal fluctuated.

As shown in Fig.5, 30 Major OTUs were selected automatically to present the main change in microbial community of Run3. The three lines in the heatmap is stand for day 14, day33, and day49 which are the changing point as shown in Fig 4. The major OTU shows close relationship and shifted accordingly.

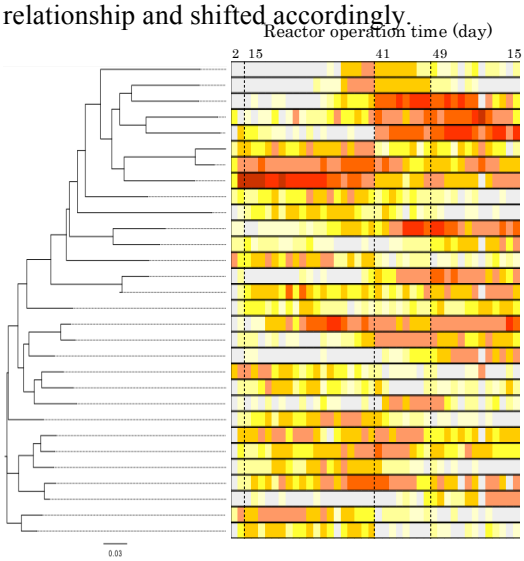

Fig.5 Partial tree and heatmap distribution of dominant OTUs of Run 3

The OTUs abundant between day 14 to day 49 and disappear latter should stand for species related with granulation, such as OTU4750 (*Zoogloea* sp.), OTU454 (*Aquiflexum* sp.) and OTU4 (*Thauera* sp.).

And similarly, the OTUS abundant before day 41 and disappear latter should stand for species related with EBPR. OTU1921,OTU3758 (*Propionivibrio* sp.)and OTU316 (*Cytophagaceae* sp.) for example, is thought to be related with EBPR performance.

OTUs those were little before day 41 and 49 but become dominant in the latter days, such as OTU1211, OTU2803 (*Thiothrix* sp.), OTU 1399 (*Rheinheimera* sp.), OTU555,OTU1239 (*Dechloromonas* sp.) and OTU 2200 (*Cloacibacteriu*.sp.) should be species compete with EBPR AGS

The taxonomy identities of the major OTUs are as shown in Table 2.

Table.2 Major OTUs and their taxonomy identity in tree order for Run 3

OTU	Custom name
3661	Root;Bacteria;TM7;TM7_genera_incertae_sedis
4430	Root;Bacteria;TM7;TM7_genera_incertae_sedis
1211	Root;Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichac
2803	Root;Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichac
1399	Root;Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Chromatia
2980	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocycla
4750	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocycla
4	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocycla
1921	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocycla
3759	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocycla
555	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocycla
1239	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocycla
2715	Root;Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales;Rhodocycla
1799	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamona
1987	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamona
2310	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamona
82	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderi
2408	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales
2872	Root;Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales
1323	Root;Bacteria;Proteobacteria;Betaproteobacteria
863	Root;Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphin
1203	Root;Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis
454	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Cyclobacte
3535	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinopha
413	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinopha
2029	Root;Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales
3997	Root;Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriacea
2200	Root;Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriacea
316	Root;Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae
2952	Root;Bacteria

5 Conclusions

In the present study, three different granulation EBPR runs was monitored. In all of these runs granular sludge treatment and solid-liquid separation behavior of is to some extent satisfying, showing that granular EBPR sludge can be achieved by shortening settle time.

Bacteria species, such as *Propionivibrio* sp., *Cytophagaceae* sp., *Thauera* sp.,and *Zoogloea* sp., were thought to have positive effect on EBPR AGS performance. Bacteria species, such as *Thiothrix* sp., *Rheinheimera* sp., *Dechloromonas* sp., *Novosphingobium* sp., *Hydrogenophaga* sp.,and *Cloacibacteriu* sp., were thought to have negative effect on EBPR AGS performance.

Further investigation is needed to clarify why and how bacteria under these taxonomies affect granulation in different ways.