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Bacterial Community Structure of HA-A/A-MCO Sludge Reduction Process

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An advanced process combining sludge reduction and phosphorous and nitrogen removal is developed, for short, HA-A/A-MCO process. The bacterial community structure of the process is analyzed by PCR-DGGE fingerprinting. The results indicates that the microbial flora showed the distinction of highly diversity in anaerobic tank, anoxic tank and multistep continuous oxic tank of the HA-A/A-MCO process and each tank has its own diversified and stable dominant microorganisms, as in the center of the maintance of a good phosphorus removal performance and *leptospires sp.* undertakes the function of organic matter removal and helps to keep a good capability to remove organic matter. *Bacillus sp.* and *lampropedia sp.* and other so the co-activation of such preponderant bacterial community promotes the system to exert favourable and consistent function of phosphorous and nitrogen removal and sludge reduction. The results of species identification shows that *Acinetobacter sp.* lies bacterium can propagate in bacterial culture area, which will disappear because of predation of higher living organisms in protozoa growing area and metazoan growing area. Such predation can promote high effective sludge reduction of HA-A/A-MCO process.

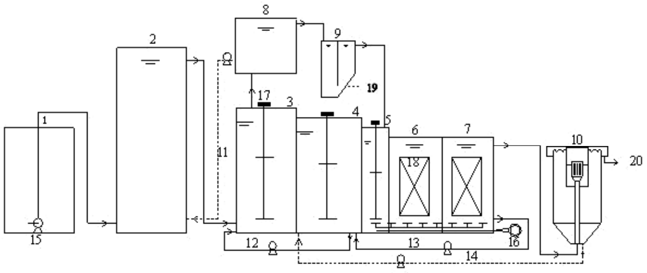
Key Words: PCR-DGGE fingerprinting, Phosphorous and nitrogen removal, Sludge reduction, Microbial predation.

INTRODUCTION

In order to explore the method of improving phosphorous and nitrogen removal in sludge reduction technologies[1], an advanced process combining excess sludge reduction and phosphorous and nitrogen removal is developed, for short, HA-A/A-MCO process. This study applies PCR-DGGE fingerprinting technique to analyze the bacterial community structure of phosphorous removal and denitrification system (anaerobic tank, anoxic tank, No.1 oxic tank, No.2 oxic tank and No.3 oxic tank). Meanwhile, the relation between bacterial community structure and treatment effect of the reactor will be discussed.

EXPERIMENTAL

HA-A/A-MCO is an advanced sludge reduction process which is developed by our research group, whose flow path is shown in Fig. 1.



1. Influent Tank; 2. Hydrolysis Acidification Tank; 3. Anaerobic Tank; 4. Anoxic Tank; 5, 6, 7. No.1 Oxic Tank, No.2 Oxic Tank and No.3 Oxic Tank of Multistep Continuous Oxic Tank, respectively; 8. Sidestream Sedimentation Tank; 9. Chemical Phosphorous Removal Tank; 10. Secondary Sedimentation Tank; 11. Phosphorus-release Sludge Return; 12. Denitration Liquor Return; 13. Nitration Liquor Return; 14. Excess Sludge Return;15.Flow Control Pump; 16.Air Compressor; 17. Stirrer; 18. Filler; 19. High Phosphorus Sludge; 20. Effluent

Fig. 1. Flow sketch map of HA-A/A-MCO process

It includes hydrolysis-acidification (HA) tank, anaerobic tank, anoxic tank, multistep continuous oxic tank, secondary sedimentation tank, sidestream sedimentation tank and chemical phosphorous removal tank. When the process operates steadily, influent flow is 20 L/h. Dissolved oxygen of each section of multistep continuous oxic tank is 0.5-1.5 mg/L, 0.5-1.5mg/L and 1.0-1.5 mg/L. Return ratio of excess sludge, nitration liquor, denitration liquor and anaerobic phosphorus release sludge is 40 %, 150 %, 100 % and 2 %, respectively. Sludge retention time (SRT) of the system is 60 d, mixed liquor suspended solids (MLSS) is 5100-5800 mg/L and sludge load is 0.18-0.21 kgCOD/kg MLSS.d

TABLE-1
MOST CLOSELY MATCHED SPECIES ABOUT SIX KINDS OF BACTERIUM'S 16SrDNA SEQUENCES IN GENBANK DATABASE

Band Number	Species	Proximal species of GenBank		Cs (%)
		Accession number	Classification	
1	ZZ1	ZN254561	Moraxellaceae; Acinetobacte	99.3
2	ZZ2	ZN254562	Spirochaetes; Spirochaeta	99.7
3	ZZ3	ZN254563	Bacillaceae; Bacillus	99.6
4	ZZ4	ZN254564	Oxalobacteraceae; Janthinobacterium	100.0
5	ZZ5	ZN254565	Actinobacteria; Microbacterium	98.6
6	ZZ6	ZN254566	Comamonadaceae; Lampropedia	97.0

Experimental water quality: Experimental wastewater is campus sewage of Chongqing University by adding amylum, glucose, milk powder, NH_4Cl, KH_2PO_4. Characteristics of the influent are as follows: ρ(COD) = 316-407 mg/L ρ(NH_3-N) = 30-40 mg/L; total nitrogen concentration ρ(TN) = 35-53 mg/L; ρ(TP) = 8-12 mg/L; pH = 7-8; temperature is 16-24 ℃.

Detection method: COD is analyzed by HACH-COD instrument, DO concentration is measured with an YSI oxygen meter, VFA is measured by distillation-titration method and other parameters were analyzed according to Ref. 2,3.

RESULTS AND DISCUSSION

Bacterial community diversity analysis of HA-A/A-MCO system: We can find from Fig. 2 that activated sludge PCR-DGGE finger-print analysis of anaerobic tank, anoxic tank, No. 1 oxic tank, No. 2 oxic tank and No. 3 oxic tank all obtains many amplification bands. Each band stands for a kind of species. So, bacterial community presents diversity in each reaction tank of HA-A/A-MCO process which is provided with the function of simultaneous phosphorous and nitrogen removal and sludge reduction.

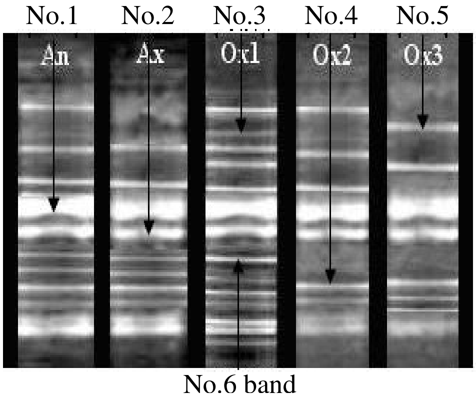


Fig. 2. Microbial communities DGGE fingerprints of HA-A/A-MCO reactor

Sequencing results analysis: No. 1 to No. 6 bands from five representational sludge sample are sequenced by Yingjun Biotechnology Co., Ltd. The sequencing result is submitted to NBCI to obtain the serial number and then submit it to Genbank. Retrieve with Blast and compare with homology of known sequence from GenBank/EMBL/DDBJ. Select complete sequence with higher homology and contrast with ClustalX (1.81 version) software, make cladogram with PHYLIP software, adopt Neighbor-Joining methods to construct phylogenetic tree and then, determine preliminarily the species. The results are shown in Table-1.

No. 1 and No. 2 band can be detected in every reaction tank and their luminance is more powerful than other's. This indicates that corresponding the two kinds of species (ZZ1 and ZZ2) occupies larger proportion in the activated sludge. We submitted it to sequences of the two kinds of species (ZZ1 and ZZ2) to Genbank and conduct Blast comparison and the results showed that the proximal species with No.1 band is *Acinetobacter sp.* LUH 1469 (FJ860877), their homologies is high up to 99.3 %. Therefrom, determine preliminarily bacterial strain ZZ1 be *Acinetobacter sp.*. Rhee G Y[4] extracted *Acinetobacter sp.* from biological phosphorus removal system and tested its phosphorus removal ability. The testing result indicates this kind of species is provided with the ability of releasing phosphate under anaerobic condition and it has the ability of uptake phosphorus under the aeration condition. So, he identifies that *Acinetobacter sp.* is phosphate accumulating organisms. Thus, we infer that bacterial strain ZZ1 may be facultative phosphate accumulating bacteria, which can widely distribute in anaerobic tank, anoxic tank and aeration tank of the system. ZZ1 species can insure favourable phosphorus removal effect of HA-A/A-MCO process.

The proximal species with No. 2 band is *Spirochaeta sp.* (M71240), their homology is 96 %. So, we can determine preliminarily bacterial strain ZZ2 be *Spirochaeta sp.*. This kind of species are facultative bacteria for the most part, they can utilize carbohydrate (such as glucose, acetic acid, *etc.*)[4] and their zymolytes are quite extensive, so they can exist in anaerobic tank, anoxic tank and aeration tank of the system and undertake the function of organic matters removal.

The proximal species with No. 3 band is *Bacillus firmus* (AJ491843), their homology is up to 99.6 %. We determine preliminarily bacterial strain ZZ3 be *Bacillus firmus sp.*. We consider that the technological conditions of No. 1 oxic tank are appropriate for propagation of ZZ3 *Bacillus firmus sp.* and bacterial strain ZZ3 disappears in No. 2 oxic tank and No. 3 oxic tank because of being preyed by protozoa and metazoan. The proximal species with No. 4 band is *Janthinobacterium sp.* (AQ717380), their homology is up to 100 %. Therefore, we determine preliminarily bacterial strain ZZ4 be *Janthinobacterium sp.*.

The proximal species with No. 5 band is *Microbacterium sp.* (AM403723), their homology is up to 100 %. So, we determine preliminarily bacterial strain ZZ5 be *Microbacterium sp.*. This species generally is taken for oxic bacteria or anaerobic bacteria. According to DGGE finger-print in Fig. 2, it is known that this kind of bacteria can survive in No. 3 oxic tank through resisting predation of highlevel microbe.

The proximal species with No. 6 band is *Lampropedia hyalina* (AB089485), their homology is up to 97 %. We can determine preliminarily bacterial strain ZZ6 be *Lampropedia hyalina sp.*. According to DGGE finger-print in Fig. 2, it is known that luminance of No. 6 band in Ox2 and Ox3 sludge sample is the weakest, that is, bacterial strain ZZ6 is the least in the activated sludge of No. 2 oxic tank and No. 3 oxic tank. The main reasons for is predation of highlevel microbe in No. 2 oxic tank and No. 3 oxic tank.

Conclusion

The microbial flora showed the distinction of highly diversity in anaerobic tank, anoxic tank and multistep continuous oxic tank of the HA-A/A-MCO process and each tank has its own diversified and stable dominant microorganisms, as so the co-activation of such preponderant bacterial community promotes the system to exert favourable and consistent function of phosphorous and nitrogen removal and sludge reduction. The results of species identification shows that *Acinetobacter sp.* lies in the center of the maintance of a good phosphorus removal performance and *leptospires sp.* undertakes the function of organic matter removal and helps to keep a good capability to remove organic matter. *Bacillus sp.* and *lampropedia sp.* and other bacterium can propagate in bacterial culture area, which will disappear because of predation of higher living organisms in protozoa growing area and metazoan growing area. Such predation can promote high effective sludge reduction of HA-A/A-MCO process.

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