

**Comparative Study of Operation Performance and Microbial Community between
Chem-bioflocculation(CBF) Process and Chemically Enhanced Primary Treatment
(CEPT) Process**

Siqing Xia*, Feng Wang*, Yan Shi*, Yingfu Rao*, Dianhai Yang*

*State Key Laboratory of Pollution Control and Resource Reuse , School of Environmental

Science and Engineering ,Tongji University, Shanghai , 200092 , China

ABSTRACT

Chem-bioflocculation (CBF) process was a new municipal wastewater treatment process developed in recent years. The flocculant dosage was one of the most important factors in operation optimization of chem-bioflocculation(CBF) treatment process. Five levels of flocculant dosage (PAFC) from 30mg/L to 100mg/L were applied to compare operational performance between CBF process and chemically enhanced primary treatment (CEPT) process. Superior column loadings of COD, SCOD, SS, and NH₄⁺-N were obtained in CBF process. The column loading of TP was present as close level in two processes. The biodiversities of microbial communities, including Bacteria, Acitnomyces, Acidobacterium, and ammonia oxidizing bacteria (AOB), were analyzed by using PCR-DGGE method with group-specific primers at different flocculant dosage of polyaluminium ferric chloride (PAFC). The results indicated that CBF reactor contained special bacterial community structure other than CEPT reactor. The special distribution of bacterial population was even at different positions of two reactors. To quantify Bacteria cells, Real-Time PCR method was applied by using primers for 16SrDNA V3 regions. CBF reactor had a little less population size of Bacteria than traditional AS process, but great higher than that of CEPT reactor.

KEYWORDS

CBF process; CEPT process; PCR-DGGE; Real-Time PCR

INTRODUCTION

The combined chem-bioflocculation (CBF) process, which contained two reacting units, chem-bioflocculation reactor and nitrification tower filled with suspended plastic media, was suited for sewage treatment in south China cities with low energy consumption (Xia et al., 2002 and 2003). The COD, TP and SS could be effectively removed with relatively short HRT (30 mins) in chem-bioflocculation reactor by co-action from chemical and biological function. Some previous studies were reported focused on optimization of primary operational parameters (Zhang et al., 2005a and 2005b; Rao et al., 2005b) and microbiological function(Xia et al., 2005a, 2005b, and 2005c; Wang et al., 2004). The

automatic control strategy of CBF process also was discussed in some other studies (Rao et al., 2005a). CBF process has been applied in Zhuyuan wastewater treatment plant (1,700,000 m³/d) in Shanghai.

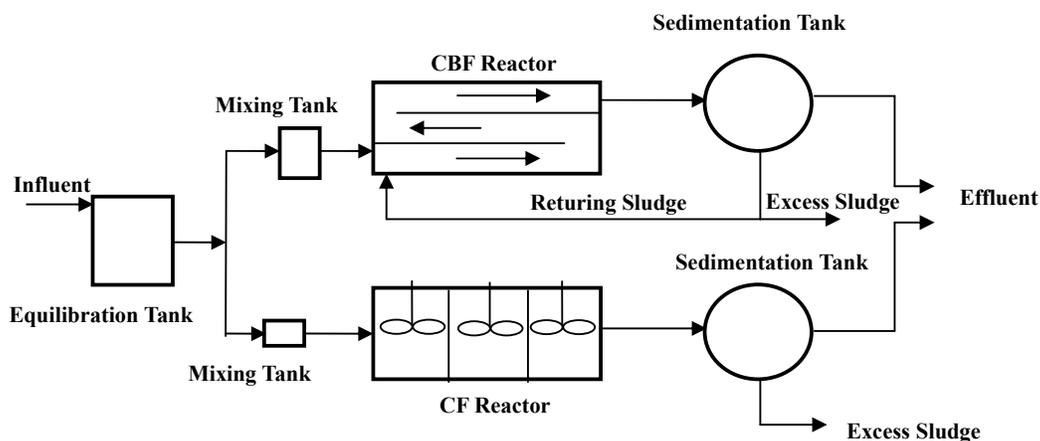
Compared with Chemically Enhanced Primary Treatment (CEPT) process, an obvious improvement was achieved in CBF process for performance of COD, SS and TP removal with the same chemical dosage. The PCR-DGGE and Real-Time PCR method were applied to investigate the community diversity and population density of several dominant bacterial groups in two processes respectively. The rich biodiversities and high density of bacteria were found in CBF reactor.

MATERIAL AND METHODS

Sampling

One pilot scale CBF process was constructed with a parallel CEPT process for comparison in a long term operation. The two processes (Figure 1) were fed with municipal sewage from Antin Town in Shanghai (50m³/d) and HRT in both reactors was about 30mins. The CBF reactor was divided into three channels, with decreasing aeration intensity. Coagulants were added to the mixing tank and flocculants were added to the first channel of the aeration tank. CEPT reactor also was divided into three sections, with mechanical mixing, and the same way of flocculant addition was applied in CEPT reactor. The optimized chemical dosage of Polyaluminum Ferric Chloride (PAFC) was studied in five operation steps. The monitored contamination included COD_{cr}, soluble COD (SCOD), SS, NH₄⁺-N and TP.

Figure 1 Construction of CBF and CEPT reactor



In order to study bacterial community in different locations of CBF and CEPT reactor, sludge samples, designated as CBF-1 to CBF-3, and CEPT-1 to CEPT-3, were collected from three channels of reactor, respectively. Traditional activated sludge, designated as TAS, was sampled from Quyang wastewater treatment plant in Shanghai, which adopted the traditional

activated sludge treatment process. The influents of both Quyang wastewater treatment plant and the CBF treatment process are municipal wastewater with similar water qualities.

Preparation of DNA templates and PCR conditions

10-ml MLSS was collected from the reactor and washed for three times by double distilled water. After centrifugation (12000×g , 4°C) for 5 min, dry weight of the deposit sludge was valuated via freeze drying. The dry sludge was used for DNA extraction based on the early method (Zhou et al., 1996).

The group-specific primers based on 16SrDNA were used for comparison of microbial community of Bacteria, Acitnomycetes , Acidobacterium and ammonia oxidizing bacteria(AOB) with PCR-DGGE method. The PCR mixture contained 1- μ l extracted DNA, 2.5mM MgCl₂, 1.25 U of Taq polymerase, 100 μ M of each dNTP and 0.25 μ M of each primer. Amplification condition was described in Table 1.

Table 1 Primer and Programs for PCR Amplification in DGGE Assay

Target	Primers	PCR condition ¹	References
First PCR Round			
<i>Bacteria</i>	P63f , P1378r	95□ 1min, 60□ 1min, 72□, 2min; 35cycles	(Muyzer et al., 1993)
<i>Acitnomycetes</i>	F243 , P1378r	95□ 1min, 63□ 1min, 72□, 2min; 35cycles	(Heuer et al., 1997)
<i>Acidobacterium</i>	31f , P1378r	95□ 1min, 53□ 1min, 72□, 2min; 35cycles	(Barns et al., 1999)
AOB	CTO189fAB/CT O189fC, CTO653r	95°C 1min, 63°C 1min, 72°C, 2min; 35cycles	(Kowalchuk et al. 1997)
Second PCR Round			
V3 Region of <i>Bacteria</i>	P338f-gc , P518r	95□ 1min, 60□ 1min, 72□, 2min; 35cycles	(Muyzer et al., 1993)

¹ Before each run of cycles, the temperature was held at 95°C for 10min and after each run the temperature was held at 72°C for 10 min

DGGE Assay

DGGE was performed with the Bio-Rad D gene system (Bio-Rad, Hercules, Calif.). PCR amplification products were loaded onto 8% (wt/vol) polyacrylamide gels in 1×TAE (20 mM Tris, 10 mM acetate, 0.5 mM EDTA [pH 7.4]). The polyacrylamide gels were made with denaturing gradients ranging from 45 to 60% (where 100% denaturant contains 7 M urea and 40% formamide) for acitnomycetes and acidobacterium, and 40 to 55% for bacteria and AOB.

The electrophoresis was run for 16h at 60°C and 30 V. After the electrophoresis, the gels were soaked for 10 min in fixation buffer (10% ethanol, 0.5% acetic acid) and subsequently for 10 min in ethidium bromide gel stain (200ml 1×TAE, 25 µl ethidium bromide [10mg/mL]). The stained gel was immediately photographed on a UV transillumination table with a video camera module.

Quantification by Real-Time PCR

In real-time PCR experiment, SYBR Green method was used to detect the population size of bacteria in sludge samples. Real-Time PCR was performed in Rotor-Gene 3000 (Corbett Research, Australia). The 25-µl reaction mixture consisted of 2.5µl 10×PCR buff, 2.5 µl 10×SYB Green I, 2.0mmol MgSO₄, 10nmol each deoxynucleoside triphosphate, 10nmol each primer, and 1µl of template or standard 16SrDNA. Standard curve for Bacteria was constructed by using PCR product as standard 16SrDNA, amplified from DNA extracted from E. coli DH5 strain (stored in Lab). The primers and PCR condition were described in Table 2.

Table 2 Primer and Programs for PCR Amplification in DGGE Assay and Real-Time PCR

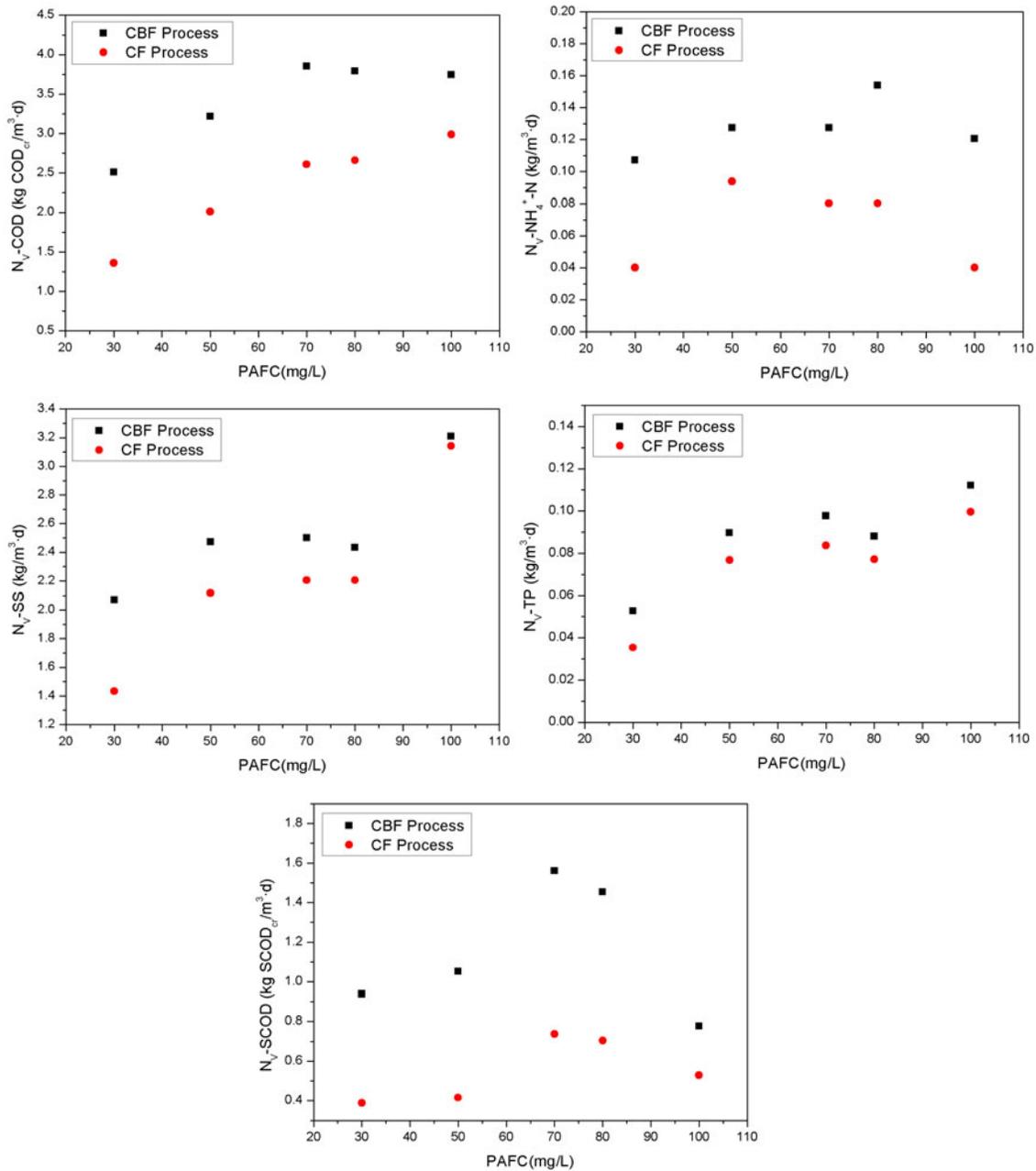
Target	Primers	PCR condition ¹	References
<i>Bacteria</i>	P338f, P518r	95□ 1min, 60□ 1min; 35cycles	(Muyzer <i>et al.</i> , 1993)

¹ Before each run of cycles, the temperature was held at 95°C for 10min and after each run the temperature was held at 72°C for 2 min

RESULTS AND DISCUSSION

With the same PAFC dosage, higher volume loading was achieved for COD_{Cr}, SCOD and SS in CBF reactor (Figure 2). Volume loading of NH₄⁺-N was also improved; however the value was low in both reactors. One reason for the close performance for NH₄⁺-N removal might be the low ammonia concentration in influent (average 15.8mg/L during the whole operation). The increase of performance reflected the microbial function happened in CBF reactor on the based on chemical flocculent function. The phosphate was possibly removed mainly by chemical flocculent because CBF and CEPT reactor had the similar volume loading for TP removal. The difference between two processes showed a gradual decrease for volume loading of SS, COD_{Cr} and SCOD with the increase of PAFC dosage, which might result from inhibition of PAFC on microbial function. The optimized dosage of PAFC was close to 70mg/L (9.7 mg/L for Al₂O₃ calculated) in this study when sludge retuning ratio was 33%, polyacrylamide (PAM) dosage was 0.5mg/L, and aeration intensity in three channels was 6.0m³/h, 3.5 m³/h, and 0.8 m³/h respectively.

Figure 2 Column loadings for primary contaminations in CBF and CEPT reactors

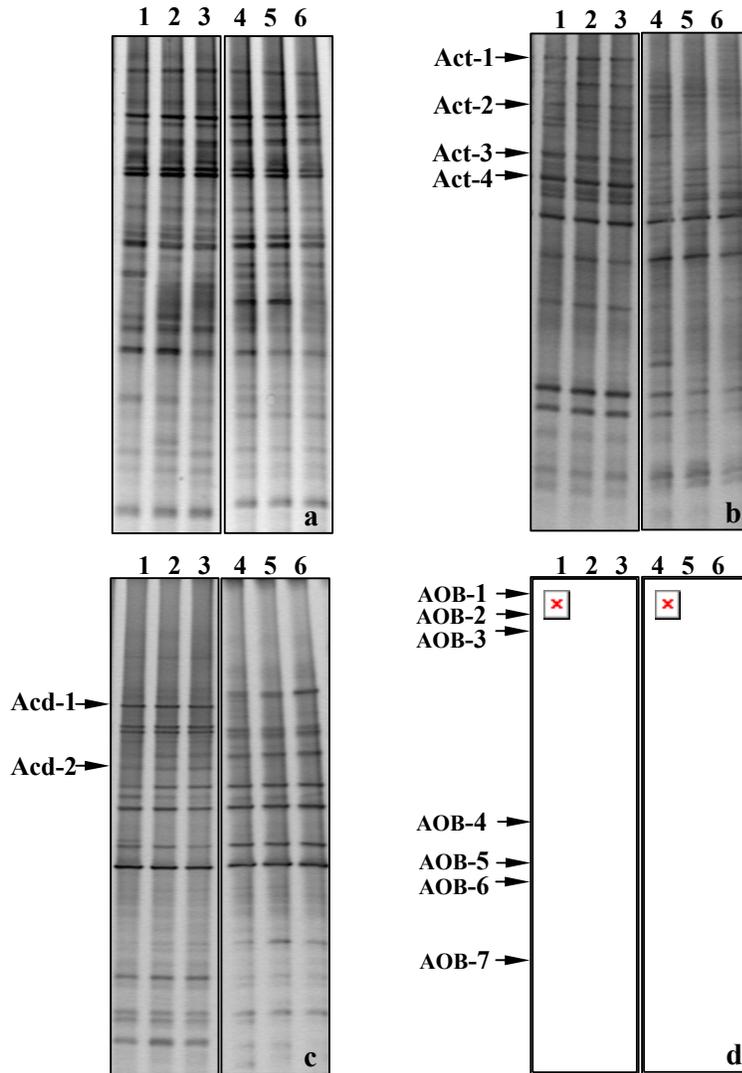


The DGGE patterns for *Bacteria*, *Actinomycetes*, *Acidobacterium* and AOB in two reactors were described in Figure 3. As shown, two processes both had special community structure. Some obvious bands were uniquely found in CEPT reactor, such as Band Act 1-3 (Figure 3b), Act 1-2 (Figure 3c), and AOB 1-8 (Figure 3d), indicating that CBF process had selectivity for some specific bacterial species. For the two reactors was fed with the same influent, so they both had identical continued inoculation from influent. The bacterial compositions in CEPT process might be result from selection of flocculent dosage (70mg/L Polymeric AlCl₃, FeCl₃, PAFC) because no obvious favorable condition, such as aeration, was designed in

CEPT process. While in CBF process, decreasing aeration intensity also provided certain growth condition for bacterial population except same flocculent dosage. The different growth micro-environment led to varied community structure in two reactors. In each reactor, almost all identified bands were found in each channel or section, reflecting great similarity in *Bacteria* community structure at different position. The high fluidness of sludge might cause such even distribution.

Figure 3 DGGE analysis of sludge sample. (a) *Bacteria*; (b) *Acitnomycetes*; (c) *Acidobacterium*; (d) AOB

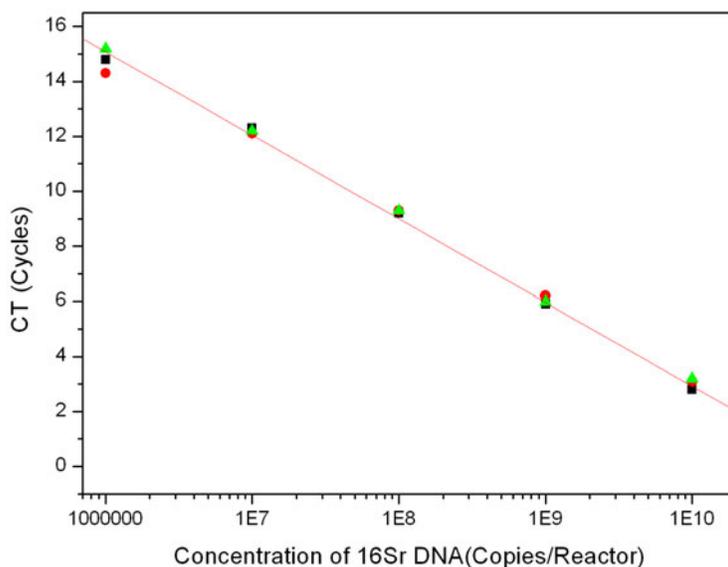
Lane 1: CBF-1; Lane 2: CBF-2; Lane 3: CBF-3; Lane 4: CEPT-1; Lane 5: CEPT-2; Lane 6: CEPT-3



Real-Time PCR Assay for *Bacteria* 16SrDNA Copies

The primers for V3 region also were applied for determination of *Bacteria* cells density in Real-Time PCR experiment. Standard template, amplified from 16Sr DNA, was diluted into 10^6 to 10^{10} copies/ μL to obtain the stand curve. Correspondingly, in amplification prepared for standard curve, the amount of copies in each reactor mixture was set at 10^6 to 10^{10} copies/reaction. The result of optimized amplification was described in Figure 4. Target product was obtained in all reactors and the standard curve had R^2 value more than 0.99, implying it was qualified for determination of cells density.

Figure 4 Quantization data for Cycling and Standard Curve



Bacteria population sizes in CBF, CEPT reactor, and traditional AS process were described in Table 3. As shown, *Bacteria* population size in CBF was 20% less than that in traditional AS process, suggesting that the microbial function in CBF reactor might be close to or a little weaker than traditional AS, but was far higher than that in CEPT reactor. Relatively favorable living conditions such as efficient DO and substrate might be the major reason for such difference between three processes, because no aeration was set in CEPT reactor. Return sludge also helped to keep the biomass concentration in CBF and AS processes. Another potential reason for the gap in cells density might be the special floc composition, such as relatively high chemical composition other than biological composition in sludge of CBF and CEPT processes. Though CBF and CEPT reactors had similar high biodiversities; however, great distance in biomass level might suggest certain difference in microbial function. The less *Bacteria* population density in CEPT might imply the weaker biological degradation ability than that in CBF reactor.

Table 3 Quantities of *Bacteria* Population in Sludge

Target cells	<i>Bacteria</i> 16S rDNA	
	Copies/L	Cells/L ¹
CBF-1	7.8×10^{12}	2.2×10^{12}
CBF-2	7.9×10^{12}	2.2×10^{12}
CBF-3	8.0×10^{12}	2.2×10^{12}
CEPT-1	2.6×10^{10}	7.2×10^9
CEPT-2	2.8×10^{10}	7.2×10^9
CEPT-3	3.6×10^{10}	1.0×10^{10}
Traditional AS	1.0×10^{13}	2.7×10^{12}

¹ The average 16SrDNA copies per genome in bacterial cells were assumed to be 3.6copies based on the average 16SrDNA copies found in cultured Bacteria (Klappenbach *et al.*, 2001). Three measures were carried out at each samples.

CONCLUSION

In this study, co-action of microbial and chemical function was investigated by comparing removal performance of primary contaminations in CBF and CEPT processes. Superior performance was found in CBF reactor when flocculant dosage varied in the range from 30mg/L to 100mg/L. Optimized dosage for PAFC was found close to 70mg/L. The phosphorus composition was mainly removed by chemical flocculation process. Higher volume loadings of COD, SCOD, and SS in CBF process indicated biological function followed by chemical action. Although superior volume loading of NH_4^+ -N was also found, the better removal performance for NH_4^+ -N in CBF reactor was not confirmed for the Low NH_4^+ -N concentration in influent.

In order to investigate characters of bacterial community, community structure and population size were detected by PCR-DGGE and Real-Time PCR method, respectively. Almost all identified bands were found in each sample in each reactor, reflecting great similarity in bacterial community structure at different position in two reactors. Two reactors provided different living microenvironment for bacterial population. CBF process could supply superior growth condition than CEPT process, resulting in special community structure other than CEPT reactor. The bacterial community in CBF reactor was consisted by certain special bacterial compositions other than that in CEPT reactor, in which bacterial community might be derived from influent. Cells density also showed great difference in two processes. It was revealed that traditional AS and CBF process had close cells density, while cells density contained in CBF process was greatly higher than CEPT process. Even distribution of biomass also found at different positions in two reactors.

Acknowledgement

This work was supported by New Century Excellent Talent Scholarship of China (NCET-05-0387) and Doctorial Unit Scholarship of China(20050247016).

REFERENCES

- [1] Barns, S. M., Takala, S. L., Kuske, C. R., 1999. Wide distribution and diversity of members of the bacterial kingdom acidobacterium in the environment. *Appl. Environ. Microbiol.* 65, 1731-1737.
- [2] Heuer, H., Krske, M., Baker, P., Smalla, K., Wellington, E. M. H., 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl. Environ. Microbiol.* 63, 3233-3241.
- [3] Klappenbach JA, Saxman PR, Cole JR, Schmidt TM. 2001. rrndb: the ribosomal RNA operon copy number database. *Nucleic Acids Res* 29(1):181-184
- [4] Kowalchuk, G. A., Stephen, J. R., Boer, W., Prosser, J. I., Embley, T. M., Woldendorp, J. W., 1997. Analysis of ammonia-oxidizing bacteria of the β subdivision of the class proteobacteria in coastal sand dunes by DGGE and sequencing of PCR-amplified 16S Ribosomal DNA fragments. *Appl. Environ. Microbiol.* 63, 1489-1497.
- [5] Muyzer, G., Ellen, C. D. W., Andre, G. U., 1993. Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA. *Appl. Environ. Microbiol.* 59, 695-700.
- [6] Rao YF, Xia SQ, Yang DH, Yu S. (2005a) Automatic control of wastewater treatment process. *Industry Control and Application (China)* 24(4):34-40
- [7] Rao YF, Xia SQ, Yang DH, Zhang ZB. (2005b) A comparative study between chemical-biological flocculation and chemical flocculation processes. *Industrial Water & Wastewater (China)* 36(2):8-10
- [8] Wang F, Fu YG, Xia SQ, Yang DH (2004) Characteristics of municipal sewage Chem-Bioflocculation treatment process by using PCR-DGGE technology. *Environmental Science* 25(6): 74-79 (In Chinese)
- [9] Xia SQ, Shi Y, Fu YG, Ma XM (2005a) DGGE analysis of 16S rDNA of ammonia-oxidizing bacteria in chemical-biological flocculation and chemical coagulation systems. *Appl Microbiol Biotechnol.* 2005 Jun 28:1-7 [Epub ahead of print]
- [10] Xia SQ, Wang F, Fu YG, Yang DH, Ma XM (2005b) Biodiversity analysis of microbial community in the chem-bioflocculation treatment process. *Biotechnol Bioeng.* 89(6): 656-9
- [11] Xia SQ, Wu ZP, Yang DH, Gao TY(2003) Chemical and Biological Flocculation Process to Treat Shanghai Municipal Sewage. *Journal of Tongji University* 31(9): 1098-1101(In Chinese)

- [12] Xia SQ, Yang DH, Gao TY (2002) Experimental study on chemical and biological flocculation process for treating municipal sewage. *Shanghai Environmental Sciences* 22:16-18. (In Chinese)
- [13] Xia SQ, Yang DH, Xu B, Zhao JF (2005c) Chemical and biological flocculation process to treat municipal sewage and analysis of biological function. *Journal of Environmental Sciences* 17(1): 163-167
- [14] Zhang ZB, Xia SQ, Zhao JF, Rao YF. (2005a) Chemical-biological flocculation/suspended carrier bed process for low strength wastewater treatment. *China Water & Wastewater(China)* 21(2):44-46
- [15] Zhang ZB, Xia SQ, Zhao JF, Yang DH, Rao YF. (2005b) Research on the chemical-biological flocculation process for treating municipal wastewater. *Industrial Water Treatment (China)* 25(7):53-56
- [16] Zhou, J. Z.; Mary, A. B.; James, M. T. (1996) DNA Recovery from Soils of Diverse Composition *Appl. Environ. Microbiol.*, 62, 316–322.