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Application of micro-flocculation and sand filtration as advanced wastewater treatment technique

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ABSTRACT

To meet the strict wastewater discharge standard enacted from 2008 in China, a micro-flocculation and sand filtration process was developed and investigated as advanced wastewater treatment in a wastewater treatment plant. The average removal ratios of total phosphorus, suspended solids, and chemical oxygen demand (COD) from secondary effluents were 53.3, 58.8, and 31.1%, respectively. What's more, most of the endocrine disrupting compounds (EDCs) were removed during the process with an endocrine activity decreased from 76.59 ng/L to below the limit of quantification. Polymerase chain reaction–denaturing gradient gel electrophoresis shows that microbes existing in the sands could enhance the removal of COD and EDCs through biological degradation. This was also proved by fluorescence excitation–emission matrix spectroscopy study on wastewater before and after the micro-flocculation and sand filtration process. It is concluded that pollutants were removed through coagulation, adsorption, filtration, and biological ways during the process.

Keywords: Micro-flocculation; Sand filtration; Advanced wastewater treatment; Microbial community; EDCs

1. Introduction

During 2007 in China, the blue-green algae outbreak in Lake Taihu affected daily water supply and water safety in almost every city along the lake, and threatened China's developed east. Thereafter, discharge standard of effluents from wastewater treatment plants (WWTPs) around Lake Taihu are required to meet the first grade A standard instead of first grade B standard since 2008 as enacted by Chinese National Environmental Protection Agency (GB18918-2002).

For integrated removal of general organic substances and nutrients (nitrogen, phosphorus), anaerobic–anoxic–oxic (AAO) process has been widely used in the existing WWTP as secondary treatment [1]. Compared with chemical treatment methods, the AAO process shows economic and environmental advantages as there is no chemicals addition during the process [2]. However, previous researches have indicated that there is a conflict accompanied with the conventional AAO process. In this system, inadequate denitrification of the process could result in deterioration of phosphorus removal [3]. Hence, as outlined in Table 1, the residual concentration of suspended solids (SS) and total

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Table 1
Wastewater discharge standard (GB18918-2002) (mg/L)

Water quality index		First grade A standard	First grade B standard	Effluent from WWTP (A-A-O)*
COD		50	60	<50
BOD_5		10	20	<10
SS		10	20	15–20
Animal and vegetable oils		1	3	<1
Petroleum		1	3	<1
Anionic surfactant		0.5	1	<0.5
TN		15	20	<15
NH_3-N		5(8)	8(15)	<5(8)
TP	Built before 12/31/2005	1	1.5	1.0–1.5
	Built after 1/1/2006	0.5	1	
Color		30	30	<30
pH		6–9	6–9	6–9
Fecal *Escherichia coli*		10^3	10^4	10^3

*According to Fan et al. (2009) and Ma et al. (2009).

phosphorus (TP) in the traditional AAO reactor effluent usually exceed the maximum permissible level prescribed by the first grade A standard [1,2]. In order to obtain high treated water quality without retro-fitting the existing construction, advanced wastewater treatment of the secondary effluent is necessary. From this standpoint, various processes have been evaluated for advanced treatment of wastewater [4]. Among these, flocculation and sand filtration is a promising technology that has already been successfully used in drinking water purification [5]. Flocculation takes place in the sand filter and allows the filter to function at its most effective. After separating most flocs, the water is filtered as the final step to remove the remaining suspended particles and unsettled flocs. It is also occasionally used in the treatment of wastewater as a final polishing stage for the treated effluent. But few studies have been conducted on advanced treatment of wastewater using this process [6,7].

To cope with increasing water scarcity, the treated wastewater that meet the first grade A standard is partly reused for landscape irrigation. Concerning the ecological risk of the reused water, safety and reliability are of course the key requirements [8]. However, the growing usage of man-made chemicals has led to the release of endocrine disrupting compounds (EDCs), which can result in disruption of normal endocrine function with possible adverse health impacts [9]. Previous studies have definitely shown that the WWTPs are an important pollution source of EDCs released into the environment [6]. Thus, control of EDCs in wastewaters is urgent and necessary. As early as 1999, EDCs removal by activated sludge process was studied [10]. Taking the AAO process, for example, the primary removal mechanism of EDCs is the biodegradation [9]. With in-depth study, the fate and removal of various groups of EDCs by advanced wastewater treatment processes are also discussed, such as chlorination, ultraviolet photolysis, ozone treatment, activated carbon adsorption [8], and membrane filtration [11,12]. In the last decade, a lot of research has been dedicated to the various stages of wastewater treatment in terms of removal efficiency of EDCs in WWTPs [6].

For typical sand filtration, the removal of pollutants depends primarily on mechanical straining, sedimentation, impaction, interception, adhesion, and physical adsorption [13]. While slow sand filters rely on biological treatment processes of the thin biological layer on the surface, rather than physical filtration [7]. The foregoing studies on micro-flocculation and sand filtration were mainly focused on the operation performance and treatment efficiency [14]. However, limited studies have been undertaken to study the removal mechanisms of micro-flocculation and sand filtration involving involving biological processes, as limited studies have been undertaken [7].

Therefore, the objective of this study is to investigate the performance of micro-flocculation and sand filtration as advanced wastewater treatment option for secondary effluent, and its feasibility in satisfying the effluent discharge standard of conventional pollutants. Meanwhile, the ecological risk of this treatment process was assessed by examining EDCs and estrogenic activity in influents and effluents. In this work, mechanisms contributing to the removal of contaminants in micro-flocculation and sand filtration were explored simultaneously, to further define the role played by the biofilm on sand grains.

2. Materials and methods

2.1. Site description

The flow chart of the progress is presented in Fig. 1. The micro-flocculation and sand filtration (height: 4.3 m, total area: 1,008 m^2, treatment capacity: 10^4 m^3/d) was continuously fed with secondary effluent from an AAO system located at Kunshan city in China. Approximately 5–10 mg/L of polyaluminum chloride

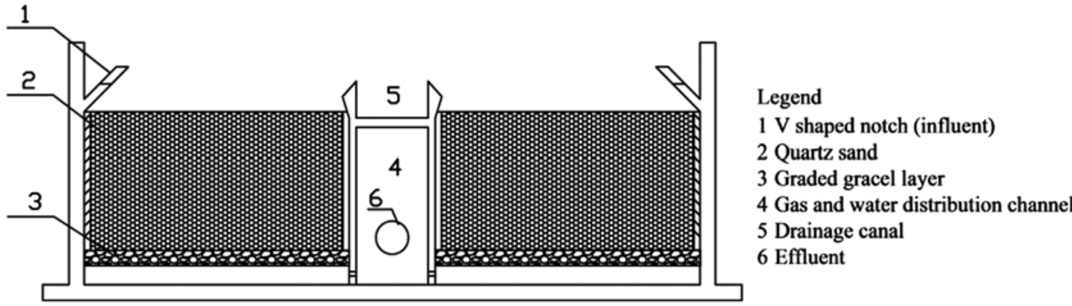

Fig. 1. Schematic representation of the micro-flocculation and sand filtration tank.

(PAC, $[Al_2(OH)_nCl_{6-n}{\cdot}xH_2O]_m$, $m \leqslant 10$, $n = 3$–5) was added as flocculant. Full-scale filter of 1.5-m sand media depth was used to study the process performance. The filter design and operation mostly relied on the data gained at lab scale in our previous studies. It was found that sand filtration with 1.0–2.0-mm effective sand size of uniform quartz sand media was most effective at the filtration rate of 5.4 m/h. The lower section of the filter contained 100 mm depth of coarse gravel (4–8 mm) as the graded gravel layer.

The system worked through alternation of filtration and backwash phases, whose duration could be adjusted by the control panel. The air–water washing filter was backwashed every 24–36 h, firstly, with an airflow of 15 L/(s m^2) for 2 min, then with an air/water flow for 4 min, and finally with a water flow of 6 L/(s m^2) for 4 min. For the air/water flow in the middle part, the intensity of airflow was 15 L/(s m^2) and that of water flow was 3 L/(s m^2).

The operation of the micro-flocculation and sand filtration chamber was controlled and determined by SCADA online-analyzers for operation duration, filtrated water turbidity, and head loss. Process parameters were recorded online, including flow, pH, and temperature.

During the treatment progress, the PAC dosing could enhance the floc growth and form micro-flocculation floc (diameter: 0.01–0.05 mm). These tiny flocs could be effectively entrapped and attached by/to the surface of filtering material and previously removed particles. Consequently, particles containing physico-chemical pollutants, even the smaller ones, could be strained and removed throughout the depth of the filter.

2.2. Sample collection and analytical methods

2.2.1. Physico-chemical parameters

Along with the experimental period, the quality of influent and effluent of micro-flocculation and sand filtration treatment plant were monitored for physico-chemical parameters. From August 2011 to December 2012, daily samples were collected and analyzed for TP, SS as well as chemical oxygen demand (COD). All samples were taken and immediately analyzed according to standard methods (APHA, 1995).

2.2.2. EDCs analysis

As mentioned above, EDCs here were also considered to be one type of the most important parameters for monitoring the performance of the micro-flocculation and sand filtration treatment plant. Therefore, influent and effluent samples were taken at bimonthly intervals for EDCs and estrogenic activity analysis. Concerning the high estrogenic activity and wide occurrence, mainly eight kinds of EDCs were selected as representative EDCs: octylphenol (OP), 4-n-nonylphenol (4-n-NP), bisphenol A (BPA), estrone (E1), estradiol (E2), estriol (E3), 17α-estradiol (17α-E2), and 17α-ethinylestradiol (EE2) [15].

The target EDCs in the samples were simultaneously determined. The effects of the treatment process on estrogenic activity were evaluated by examining estradiol equivalent (EEQ) concentrations in influents and effluents of the filter. All the methods for the analysis of EDCs and estrogenic activity were detailed in our previous work [16].

2.2.3. PCR-DGGE analysis

The polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) method has been gaining popularity as a technique to assess the microbial communities. It was used, in this study, to reveal the structure and composition of the microbial community in the samples collected from the process tank. In addition, some functional species that played an important role in the process may be identified [17]. For PCR–DGGE analysis, all the samples were taken after the backwash of the micro-flocculation and sand filtration treatment plant, which has been in operation for one and a half years.

During the PCR–DGGE analysis process, DNA from the reactor samples was isolated using the method described by Head et al. [18]. The product from DNA extraction was verified by electrophoresis in 0.8% agarose.

To amplify the V3 region of the bacterial 16S rRNA gene, the primers 341F-GC (5´-GC-clamp-CCTACGG-GAGGCAGCAG-3´) and 907R (5´-CCGTCAATTCCT TTGAGTTT-3´) were used with a 40-base-pair GC-clamp (CGCCCGCCGCGCGCGGCGGGCGGGGCGGG GGCACGGGGGC) attached to the 5´ end of the forward primer.

The procedures for PCR amplification and DGGE analysis were performed using similar methods to Zeng's [17]. DNA sequencing was carried out afterward with the ABI PRISM 3,730 automated sequencer (Applied Biosystems, Foster City, CA) by Shanghai Sangon Biological Technology Co. Ltd. These sequences were compared to other rRNA gene sequences of the Basic Local Alignment Search Tool server and expressed as percentage similarity. Sequences are deposited in GenBank database (http://www.ncbi.nlm.nih.gov) under accession numbers HQ891135 to HQ891140. CLUSTAL–X was used to compare multiple sequences [19]. After cluster analysis, phylogenetic trees were constructed using the unweighted pair-group method with arithmetic means (UPGMA) in the MEGA version 3.0 software package.

2.2.4. Excitation–emission matrix analysis

Fluorescence excitation–emission matrix (EEM) spectra of wastewater sample before and after the micro-flocculation and sand filtration process were recorded on a fluorescence spectrophotometer (model F-7,000, Hitachi, Japan). Three-dimensional spectra were obtained as previously described [20]. All contour maps were plotted using the same scale range of fluorescence intensities and number of contours.

3. Results and discussion

3.1. Performance of micro-flocculation and sand filtration

3.1.1. Removal of TP, SS, and COD

The main objectives of the filtration, including both SS removal and removal of TP, were confirmed using corresponding data obtained from the site treatment.

As shown in Figs. 2 and 3, for up to a year and a half, the influent ranges were about 0.3–2.12 mg/L for TP and 12–31 mg/L for SS, while the effluent concentrations were 0.01–0.69 mg/L and 5–12 mg/L, respectively. According to the first grade A standard of

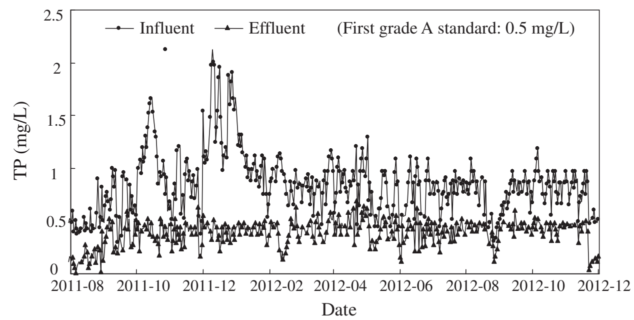

Fig. 2. The removal of TP during the micro-flocculation and sand filtration process.

"Discharge standard of pollutants for municipal WWTP" (GB18918-2002), the maximum permissible effluent concentration is 0.5 mg/L for TP and 10 mg/L for SS. Therefore, it can be said that the effluent of TP and SS can stably meet the new wastewater discharge standard. However, as displayed in Fig. 2, it was found that the average concentration of TP in effluents was more than 1 mg/L during October 2011 and January 2012, which is higher than the other months. This phenomenon may be attributed to the illegal discharge of the wastewater with high concentration of phosphorus. Consequently, the removal of TP was observed to be significantly available, since the start of the experiment, ranging from 15.4 to 98% (Avg. 53.3%).

Orthophosphate comprises approximately 90% of TP in biologically treated effluents [21]. The high removal of phosphorus is obtained upon the addition of flocculant PAC, simply described with the following reactions:

Primary reaction: $Al^{3+} + PO_4^{3-} = AlPO_4$

Side reaction: $Al^{3+} + 3H_2O = Al(OH)_3 + 3H^+$

The processes of bridge and entrapment of particles together with adsorption of phosphate ions on the particles surfaces are characteristic for these reactions also [22]. In this way, the phosphorus is removed by the formation of insoluble sediment of aluminum phosphate [$AlPO_4$] and aluminum hydroxide [$Al(OH)_3$], which also possess the combinability with SS.

The SS in the effluent of secondary biological treatment mainly comprise microbial residues and small organisms [14]. Differed from the typical flocculation sand filters, SS in the micro-flocculation and sand filtration tank were down to a concentration of 20 mg/L. Both the low concentration and the short detention times caused the particles to flocculate into micro-flocculation flocs with diameters only from 0.01 to 0.05 mm. The adsorption of the formed flocs and the entrapment of the filter media can both lead to the removal of SS. Besides, sand grains that are already contaminated with particulate solids may become

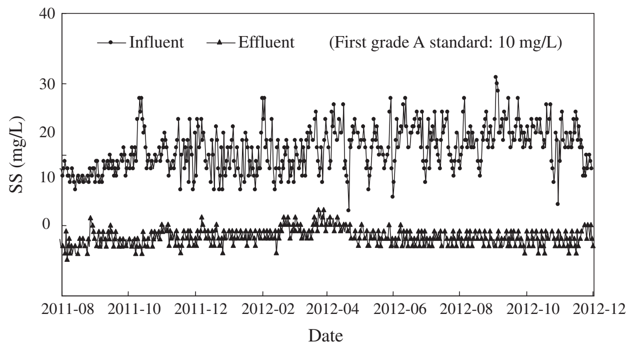

Fig. 3. The removal of SS during the micro-flocculation and sand filtration process.

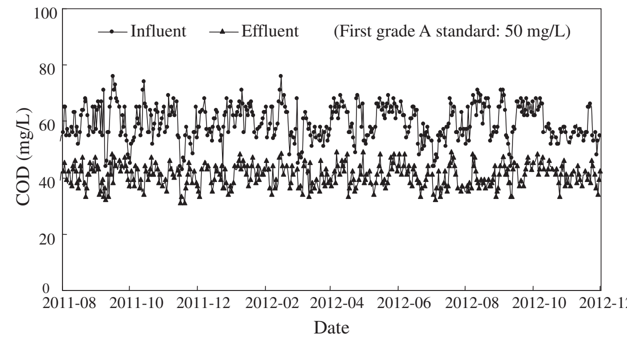

Fig. 4. The removal of COD during the micro-flocculation and sand filtration process.

more attractive. By adhering to the sand grains, the particulates lose surface charge and become attractive to additional particulates [23]. In this way, it takes most of the SS out and then generally filtered off through the filter. Therefore, during the entire study period, it was capable of removing 25–78.6% (Avg. 58.8%) of SS from wastewater after the treatment (Fig. 3).

The removal of COD was also observed to further evaluating the performance of the filter in removing conventional pollutants (Fig. 4). It was significantly stable, since the start of the experiment, ranging from 15.4 to 54.1% (Avg. 31.1%). Hence, it can also meet the new wastewater discharge standard well.

In wastewater, the major part of total COD is suspended COD followed by dissolved COD [24]. As being verified in the previous studies, the filtration treatment processes were efficient in the removal of the suspended COD, but the removal of the dissolved and colloidal COD remained limited under all experimental conditions [24]. However, by comparing the fluorescence EEM spectra before and after the micro-flocculation and sand filtration process (Fig. 5), it demonstrated that partially dissolved COD were removed during the process. According to the major regions divided by Chen et al. [25], the removed substances were mainly related to those for humic acid-like, fulvic acid-like as well as protein-like. The feasible mechanisms for the removal of these dissolved COD can be attributed to biological degradation, which will be discussed later.

3.1.2. Removal of EDCs

To estimate the ecological risk of this treatment process, the EDCs and estrogenic activity in influents and effluents were measured. Results were obtained from tests conducted on the micro-flocculation and sand filtration treatment of a secondary effluent. In the sewage treatment plant effluents, only E1, BPA, and 4-n-NP were regularly detected, while 17a–E2 was measured incidentally. In this study, the concentration of OP in the influent was relatively higher in comparison to the levels reported in other researches [26]. The concentrations of EDCs were proved to be

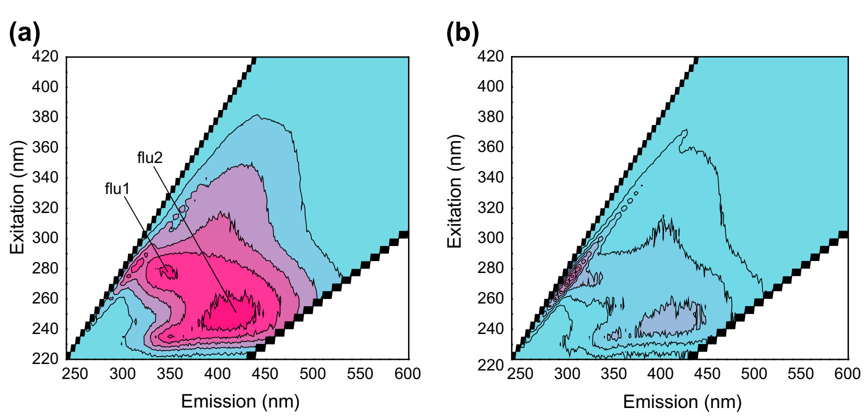

Fig. 5. EEM spectra before (a) and after (b) the micro-flocculation and sand filtration process.

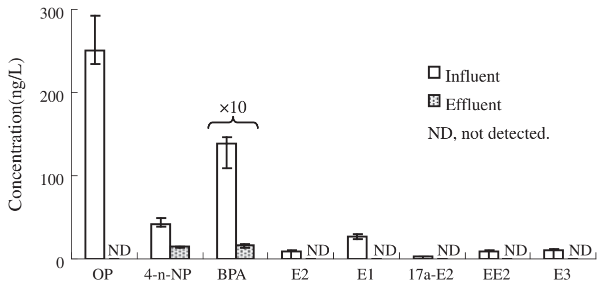

Fig. 6. Removal of EDCs during micro-flocculation and sand filtration.

further markedly altered after the micro-flocculation and sand filtration (Fig. 6). Except 4-n-NP (65.78%) and BPA (88.49%), all the detected EDCs were almost removed into below the limit of quantification. EEQ concentrations were about 76.59 ng/L in the influent and not detected in the effluent, indicating that the treatment process was sufficient to reduce the estrogenic activity of wastewater.

Biodegradation and/or adsorption to solids were generally presumed to be the main mechanism accounting for the removal of EDCs in the WWTPs [9]. According to the literature [27], within the filter, transport mechanisms and active movement bring EDCs in contact with the agglomerated particles, with the sand grains, and with the biofilm on sand grains.

Retention of EDCs in the filter system is partially due to adsorption and straining. The rejection efficiencies of EDCs strongly depend on EDCs' physico-chemical properties. For these nonpolar and hydrophobic estrogens, the log Kow values indicate that a considerable amount of them should appreciably adsorb onto particulates and be easily removed during the treatment. Thus, it also meets the conclusion that, suspended particulate matter contribute significantly to the total load of EDCs in wastewater [26].

Adsorption onto flocs plays a significant role in the removal of EDCs, but adsorption itself does not result in complete mineralization [27]. According to Liu et al. [10], the EDCs were initially absorbed at a very fast speed, then being biodegraded within a few hours. Among these target EDCs, 4-n-NP retains a relatively low removal efficiency level in the effluent as it is quite resistant to biodegradation. The data indicate that BPA is generally removed with a considerable removal rate and compounds except 4-n-NP are with high biodegradability. And the feasible role that microbial activities play in the removal of EDCs will be discussed in later part of this paper.

Overall, micro-flocculation and sand filtration showed a good ability for the removal of EDCs and estrogenic activity. Whereas, a large amount of ambiguity still persist on the occurred EDCs removal process mechanism to draw the quantitative conclusions. As a result, it is necessary to look further on the removal mechanisms to improve the existing treatment effectiveness and to develop new treatment strategies to remove EDCs from wastewater.

3.2. Distribution of microbial community structure

According to Delahaye et al. [13], the filtration process involves both biological and physical mechanisms. As time progresses, microbiological growth occurs, mainly consisting of bacteria which form a fixed microbial ecosystem (biofilm) on the sand of filters.

The functionality and efficiency of WWTP are partially determined by the structure of the microbial community and the distribution of microbial species in the process. The structure and components of a microbial community are influenced by selecting pressure during the treatment process, which in turn determines the overall functionality of the plant [19].

The components and use of these micro-organisms in micro-flocculation and sand filtration would be interesting to evaluate, by introducing the analytical approach PCR–DGGE to the treatment process.

The homology of sequences for the six representative bands (A–F) removed from the DGGE profile of the samples were most closely to those in the V3 region of 16S rDNA from uncultured bacterium (Accession # AB518132), uncultured bacterium (Accession # FJ230910), uncultured bacterium (Accession # FJ201237), uncultured bacterium (Accession # FJ201204), *Flavobacterium sp. a306* (Accession # EU434366), uncultured *Flavobacterium sp.* (Accession # GQ249374), uncultured *Methylophilaceae* bacterium (Accession # EF662764), and uncultured *Methylovorus sp.* (Accession # GQ420969), respectively. Phylogenetic analysis further proved their close relations (Fig. 7).

Among these bacterial species, uncultured bacterium (Accession # AB518132, FJ201237, and FJ201204) is known to be capable of nitrification even at cold temperature [28]. The DNA sequence of band A in the PCR–DGGE profile was found highly comparable to these three species and they were also phylogenetically related. Therefore, it is speculated that the species capable of nitrification may have become dominant in the micro-flocculation and sand filtration process.

Besides, it has been reported that *Flavobacterium sp. a306* and uncultured bacterium (Accession # FJ230910) show the characteristic of antibiotic resistance even under high level of antibiotic pressures [29]. As a consequence, it is possible that the micro-flocculation and sand filtration process can remove antibiotic to a certain degree, which needs to be further discussed.

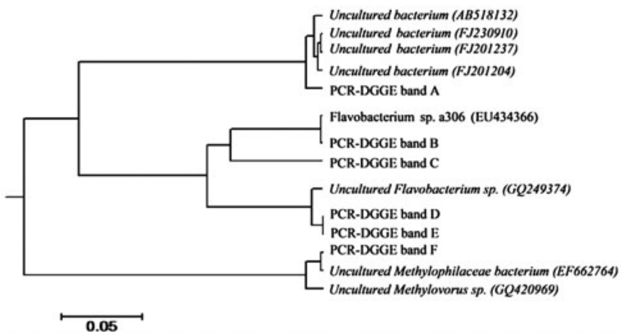

Fig. 7. Phylogenetic analysis of six 16S rDNA bands obtained from the micro-flocculation and sand filtration treatment plant. Phylogenetic tree determined by UPGMA analysis of the sequences of PCR-amplified V3 region in 16S rDNA. GenBank accession numbers are shown in brackets.

3.3. Mechanism analysis on biological removal of organic compounds

It is well known that many living micro-organisms have a flocculent growth habit and produce extracellular polymers which enhance biosorption of particles [30]. As indicated by Elmitwalli et al. [23], the removal of colloidal COD depends on biological processes, bioconversion, and/or biosorption. For dissolved COD, although humic acid and fulvic acid concentrations in wastewater effluent appear to be variable, it may represent an important sorption source for organic matters due to their polyphenolic and aromatic structure. This characteristic of these compounds further promote the removal of dissolved COD. Therefore, the feasible mechanisms for the removal of the dissolved COD can be attributable to biological degradation, hydrolysis of biodegradable particulate and conversion of dissolved organic matter.

At the same time, the existence of species capable of nitrification in the biofilm during the process is also responsible for the biodegradation removal of EDCs, as indicated by Auriol et al. [27]. Besides, dissolved organic matter is capable of consuming •OH and offers protective sorption sites for the EDCs; so that, the removal of dissolved COD also had been verified to further promote the removal of EDCs [26].

Upon the whole, the structure of the microbial community and the distribution of microbial species in the process were investigated, getting the conclusions that the high removal efficiency achieved by micro-flocculation and sand filtration is partly attributed to biological processes in the layer of slime material that accumulates above the sand surface. However, more detailed investigations need to be carried out to draw the quantitative conclusions in order to provide more information that is valuable.

4. Conclusions

(1) Micro-flocculation and sand filtration proved quite effective for the removal of TP, SS, and COD from the secondary effluents as well as the removal of EDCs. The excellent effluent quality could magnificently meet the strict wastewater discharge standard newly enacted in China. Consequently, micro-flocculation and sand filtration should be a promising advanced treatment technology for the current WWTP effluent polishing techniques.

(2) Our results revealed significant information in the activity and diversity of microbial community during the micro-flocculation and sand filtration treatment progress. Microbes, which are indicated to exist in the layer of slime material accumulated above the sand surface of the filtration tank, can enhance the removal performance of the process significantly. With the analysis of microbial community, this research gave rise to new perspectives on a probable way to remove EDCs and antibiotic from WWTP effluents.

Acknowledgements

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