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Full-scale study of pollutants removal in printing and dyeing wastewater based on anaerobic baffled reactor–A/O coupling process and microbial community analysis

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ABSTRACT

Improved anaerobic baffled reactor-anoxic/oxic (ABR–A/O) coupling process was used for printing and dyeing wastewater treatment in our full-scale study, which was able to reach the effluent standard stably, especial for the characteristic pollutants and aromatics removal. According to our results of microbial community analysis by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), the divisions of ABR enabled the functional microorganisms to distribute in different compartments and contributed to each compartment of performing its own functions with more efficiency. The first, second, and third compartments are major in macromolecular organics hydrolysis and sulfate reduction, while the main responsibilities of the fourth, fifth, and sixth compartments are aromatic compounds degradation and denitrification. Many kinds of aromatic anaerobic degradation microorganisms were found in our ABR, such as *Pseudomonas stutzeri*, *Dechloromonas aromatic*, *Pseudomonas putida*, and *Aromatoleum aromaticum*. Initially, abundances of bcrA genes climbed up and then dropped along with the sequential six compartments of ABR and it indicated that the highest aromatics degradation efficiency were obtained at the fifth compartment, rather than other compartments, which was probably caused by its moderate substrate concentration and biodegradability.

Keywords: Anaerobic baffled reactor (ABR); Printing and dyeing wastewater; Anaerobic hydrolysis; Aromatic compounds; Microbial community; Biodegradability

1. Introduction

The printing and dyeing wastewater is characterized by huge water yield and complex quality, with high chemical oxygen demand (COD) (but low (biochemical oxygen demand) BOD_5/COD) and high color. These wastewaters may also be hazardous due to the presence of metals, chlorides, or broken products of dyes [1,2], which is usually aromatic amine. The common method for printing and dyeing wastewater is still biological technology for its simplicity and cost-effectiveness [3].

However, the increasing standard of pollutant discharge and increasing modern dyestuffs (such as azo and indigo dye) and auxiliary used in the textile process give a new challenge for the biological treatment, which calls for the new reinforced biological treatment process.

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Anaerobic hydrolysis acidification process is very essential for printing and dyeing wastewater treatment since it degrades macromolecular pollutants, break chromophoric groups, and improve biodegradability [4,5], which reinforce the COD and nitrogen removal in the anoxic-aerobic process.

The anaerobic baffled reactor (ABR), which belongs to the third generation high-rate anaerobic reactor, earns highly appraisement for its high efficiency, outstanding working stability, and lower operating cost [6,7]. The special design of ABR which divides the whole system from one phase into the multi-phase process realizes microbial segregation in the different compartments and achieves effective separation of acid-forming microbes and methanogens. In recent year, ABR has been used widely in wastewater treatment [8,9].

In printing and dyeing wastewater, giving priority to aromatics of organic toxicants with low biodegradability, such as aromatic amines (an intermediate metabolite of azo dyes) was quite abundant, which requires more efficient anaerobic hydrolysis process like ABR [10,11].

Microorganisms perform a significant role in the biological treatment and the microbial community in ABR earns more attention [12]. The ABR provides a longitudinal distribution condition with gradient pollutants concentration for microorganism growth, which makes divisions in different compartments for the whole microbial community. However, the related functional bacterial communities and functional genes abundance in ABR for printing and dyeing wastewater treatment need to be further investigated and the biological degradation mechanism should be discussed, which would provide a theoretical basis for practical application and management.

The hazardous aromatic compounds which derived from the application of modern dyestuffs (such as azo and indigo dye) and auxiliary with environmental stability can be as cancerogenic and harm to human health, which is becoming the limiting factor in the treatment process of printing and dyeing wastewater.

Anaerobic pathways for the degradation of the aromatic compounds have been discovered years ago, such as *Thauera aromatica* and *Rhodopseudomonas palustris* [13]. The benzoyl-coenzyme A (benzoyl-CoA) is the key metabolite in lots of aromatic compounds degradation process [14] and the benzoyl-CoA reductase (BCR) is involved in anaerobic degradation pathways for several aromatic compounds in diverse bacteria. Therefore, the relative genes encoded BCR (like bcrA) were effectively used for anaerobic aromatics degradation bacteria detection [15].

In order to investigate the practical application situation of ABR for printing and dyeing wastewater treatment, the full-scale study was conducted at a sewage plant located in an industrial park in Taihu Lake Basin (China). The printing and dyeing wastewater contributed 80% amount of the total water taken over wastewater.

In this study, the key objective was to investigate the performance of the ABR–A/O coupling process at full scale for printing and dyeing wastewater treatment; and to reveal the biological degradation mechanism of pollutants in this process, especial for aromatics anaerobic biological degradation in gradient compartments precisely, by analyzing structure and population size of the relative microbial communities, biological basis was provided for the ammoniation of anaerobic reactor and the denitrification mechanism of aerobic reactor.

2. Material and method

2.1. Full-scale process based on ABR in the sewage plant

The main part of the whole process is shown in Fig. 1. The key section is the biological process consisted of ABR with six compartments coupled in A/O process.

In order to strengthen anaerobic hydrolysis efficiency, especial for aromatic compounds degradation, the fourth, fifth, and sixth compartment (the last three compartments) was filled with combined filler (fixed at the supports). The ABR was designed to be two sets of reinforced concrete structure, with the size of $L \times b \times H$ = 48.0 × 30.0 × 7.5 m and effective depth of 7.0 m. Each set was divided into two groups of parallel operation, with HRT = 24.1 h. Main equipment: combined packing and support 2,160 m^3, eight sludge reflux pumps, four for use, and four for standby, model GW110-10-5.5, Q = 110 m^3/h, H = 10 m, n = 5.5 kW as shown in Table 1.

2.2. Operation condition

The designed scale is 2.0 × 10^4 m^3/d for the sewage plant, and the printing and dyeing wastewater occupy over 80% of the total taken over water yield as shown in Table 2.

2.3. Characterization and analysis of water quality

Characterization of water quality was conducted every day in stable operation. COD, ammonia–nitrogen (NH_3–N), total nitrogen (TN), total phosphorus (TP), and biochemical oxygen demand (BOD_5) were estimated according to

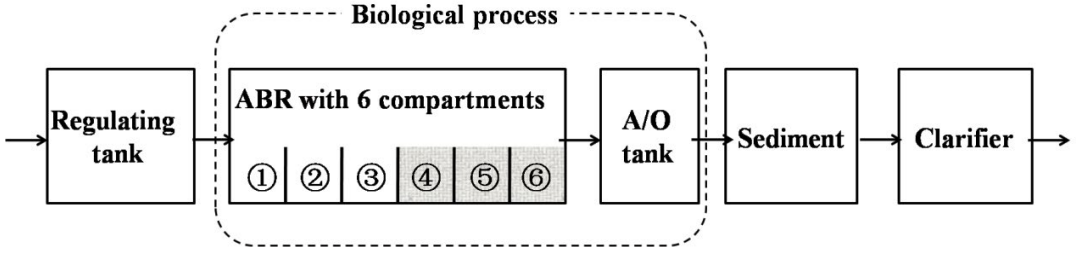


Fig. 1. Schematic diagram of full-scale ABR anaerobic hydrolysis–A/O coupling process. The gray in the fourth, fifth, and sixth compartments showed fillers addition.

Table 1
Main structures and specifications of the whole treatment process

Structure	Amount	Specifications
Regulating tank	1	40 m × 73.6 m × 4.0 m, effective volume is 8,800 m^3, HRT = 10.6 h
ABR	2	48 m × 30 m × 7.5 m, effective volume is 10,080 m^3, HRT = 24.1 h, combined filler, and its support is 2,160 m^3
A/O	2	48 m × 36 m × 6.0 m, effective volume is 9,504 m^3, HRT = 22.8 h (5.7 for anoxic, 17.1 for aerobic), DO < 0.5 mg/L for anoxic, 1–3 mg/L for aerobic, PAC was added in aerobic tanks
Sediment	2	Φ28.0 m × 5.5 m, effective height is 4.0 m, hydraulic surface loading is q = 0.72 $m^3/(m^2\ h)$, 50%–100% reflux, HRT = 3.0 h

the Standard Methods [16]. The specific organic matter (especial for aromatic pollutants) in the printing and dyeing wastewater was characterized by gas chromatography mass spectrometry (GC-MS; ZAB-HS, HP-5 for gas chromatographic column).

2.4. Microbial community analysis

The sludge samples (150 mg, deposit without drying) were collected. The total genomic DNA was extracted from each sample by using the FastDNA kit (Q-Biogene, MP Biomedicals, UK) as described in the manufacturers' instructions.

The PCR of 16s genes V3 region was performed for the total eubacteria investigation with the primers 338f (5-CCTACGGGAGGCAGCAG-3), with GC-clamp before DGGE) and 518r (5-ATTACCGCGGCTGCTGG-3), under the following conditions: 94°C/5 min denaturation step; 30 cycles of 94°C/30 s, 58°C/30 s, 72°C/45 s; and a final extension step at 72°C/10 min.

DGGE was carried out in a denaturing gradient gel electrophoresis system for the PCR products. Polyacrylamide gels (10% w/v) were 18 cm × 18 cm, thickness of 0.75 mm. The electrophoresis was conducted in 1 × TAE buffer at 60°C. The condition of each electrophoresis was 100 V, 12.5 h with denaturing gradients of 35%–65%. Gels were photographed using Kodak 1D Image Analysis Software after stained by ethidium bromide (EB).

Quantification of aromatic compounds degradation by microorganisms were analyzed by bcrA genes targeted real-time PCR. The primers BCR-1F (5-GTYGGMAC CGGCTACGGCCG-3) and BCR-2R (5-TTCTKVGCIACIC CDCCGG-3) were used.

The real-time PCR conditions for bcrA genes were: 10 min activation of the polymerase at 94°C, three cycles consisting of 30 s at 94°C, 30 s at 58°C, 1 min at 72°C, and then three cycles consisting of 30 s at 94°C, 30 s at 54°C, 1 min at 72°C, finally, the last cycle with 1 min at 94°C, 1 min at 50°C, 2 min at 72°C, and was repeated 30 times. While approximative quantification of the total eubacteria amount was performed by V3 region of 16 s genes, in order to act as the internal reference with the condition: 15 s at 95°C, 30 s at 60°C for 40 times of repetition. One last step from 60°C to 95°C with an increase of 0.2 deg/s was added to obtain a specific denaturation curve for all the real-time PCR assays above.

3. Results and discussion

3.1. Performance of the biological process based on ABR

Conventional wastewater quality parameters analyzed (daily, around the year) and obtained average results which are presented in Table 3.

After clarifier (secondary biological process) the results of the effluent were found much identical to the effluent standard of town sewage treatment plants and key industries major water pollutant discharge limits for Taihu Lake basin (DB32/1072–2007). The removal efficiencies of 92.25%, 92.13%, 89.21%, 78.53%, 92.18%, and 91.41% were obtained in this study for COD, BOD, NH_3–N, TN, TP, and color, respectively. These results demonstrated the high efficiency for various pollutants removal in the biological process based on ABR and the whole process can be considered very effective for the treatments of the printing and dyeing wastewater.

The ratio of BOD_5/COD is 0.28 for inflow and increased up to 0.36 in ABR effluent which is verified by high anaerobic hydrolysis capability of the ABR with improved biodegradability for the wastewater. Besides, NH_3–N and TN had a less removal through ABR since denitrify or anammox process might be occurring in the ABR compartment.

3.2. Characteristic pollutants analysis of the printing and dyeing wastewater

In printing and dyeing wastewater numerous organic toxicants with low biodegradability are found, which

Table 2
Inflow water quality and effluent standard

	pH	COD	TN	NH_3–N	TP	Chroma
Inflow	6–9	≤1,500	≤50	≤40	≤5.0	≤500
Effluent standard DB32/1072-2007	6–9	≤60	≤15	≤5	≤0.5	≤40

Table 3
Effluent quality for each section in the whole process

	COD (mg/L)	BOD (mg/L)	NH_3–N (mg/L)	TN (mg/L)	TP (mg/L)	Chroma
Adjusting tank	542 ± 95*	155 ± 20	30.6 ± 12.4	35.4 ± 3	3.2 ± 1	256 ± 64
ABR	367 ± 52	128 ± 15	26.3 ± 8.1	31.1 ± 3	2.85 ± 0.7	102 ± 32
A/O	81 ± 11	24.3 ± 4.4	3.7 ± 1.0	8.4 ± 1.6	1.37 ± 0.6	31 ± 8
Clarifier	42 ± 9	12.2 ± 2	3.3 ± 0.9	7.6 ± 1.2	0.25 ± 0.1	22 ± 8
Effluent standard	60	10	5	15	0.5	40

*All the data in Table 3 was averaged by daily monitored in 1 y.

were introduced by large dyestuff, auxiliary, and solvent application in the dyeing process. These organics and aromatics compounds were difficult to remove from this wastewater [17].

In order to investigate the organic toxicants reduction in the wastewater during coupled biological process, the organic compounds analyzed using GC-MS, especially for the aromatics which are particularly marked in Fig. 2.

After anaerobic hydrolysis by ABR, aromatics achieved considerable reduction and removal since both species and relative abundance reduced (notice that the scales of *Y*-axis were different in the three figures). The efficient biological degradation of aromatics indicated that there were a substantial quantity of microorganisms which can utilize aromatic compounds as carbon and energy sources, with favorable aromatic ring opening ability under anaerobic condition.

Particularly, the highest peak in the inflow (and ABR effluent, Fig. 2) is identified as N,N-dimethylacetamide, which is a kind of polar solvent and present in abundance. According to variation tendency of the highest peak (Fig. 2), the excellent removal of N,N-dimethylacetamide depends on the aerobic biological treatment, since it mainly occurred at the A/O process, but little in anaerobic ABR.

Moreover, the wastewater contains other inorganic characteristic pollutants, such as sulfate, chlorate, arsenic, etc. [18], which were removed efficiently by using improved ABR based coupling biological process (data not shown).

3.3. Microbial community analysis and pollutants biological removal mechanism

Both of the normal and characteristic pollutants (aromatics) in printing and dyeing wastewater can be removed effectively by using improved coupled base process on ABR during this study, however, the microorganisms play vital role in the biological treatment process.

As shown in Fig. 3, in ABR process from the first to sixth compartment, the pattern variation in band intensity included gradually rise and drop. The initially rise up of the band and then drop indicated that there was an abundance of different kinds of microorganism which varied along the serial process in ABR.

The pattern of former compartments has significant difference with the last three compartments as fillers were added. Moreover, the last three compartments showed higher biodiversity than the first three compartment. The stratified biofilm growing on the fillers can help enrich more kinds of microorganism. Furthermore, the increase in BOD_5/COD during the anaerobic hydrolysis process may also be responsible for the higher biodiversity in the last three compartments.

On the contrary, there were no difference found between anoxic and aerobic process for the homogeneously mixed sludge by internal reflux.

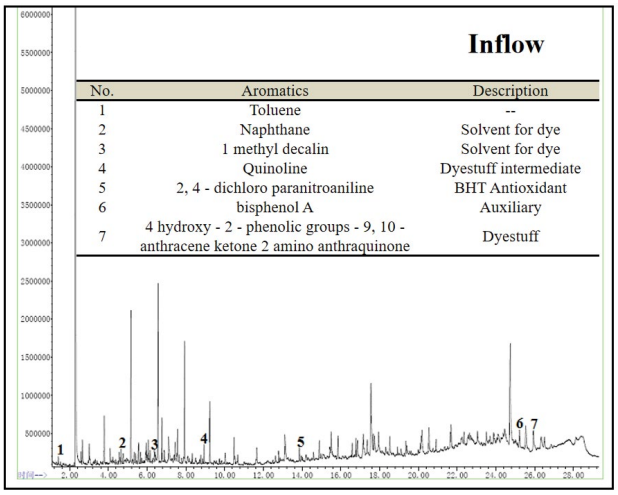
In Table 4, *Proteobacteria* and *Bacteroidetes* are dominated communities in our ABR reactor, and several kinds of functional bacteria (according to the closest species) are discovered.

Macromolecular organics hydrolysis bacteria: B8 and B9 showed closest phylogenetic relationship to *Bacteriodes helcogenes* and *Paludibacter propionicigenes* which were obligate anaerobic bacteria and fermentate the products resulting acetic acids and propionic acids (VFAs) separately [19,20] by macromolecular organics catabolism. B7 was identified as *Desulfatibacillum alkenivorans* which acts as a model organism for anaerobic alkane biodegradation, due to its versatile pathways involved in the oxidation of alkanes, organic acids, and *n*-saturated fatty acids coupled to sulfate reduction [21]. These (B7, B8, B9) bacteria could be observed only in first three compartment which perform macromolecular organics hydrolysis and extinct in the last three. It indicates that the first three compartment of ABR are accountable for macromolecular hydrolysis and BOD_5/COD increment which produced VFAs.

Denitrifying bacteria: B1, B2, and B5 owned nitrate-reducing ability since they were identified as denitrifiers (*Pseudomonas denitrificans*, *Dechlorosoma suillum*, and *Dechloromonas aromatica*). Especially, B5 performs anaerobic aromatics degradation coupled with nitrate-reducing [22]. These kinds of denitrifiers were only found in the last three compartments. The combined fillers in the last three compartments provide stratified biofilm with more multiplicity of microorganisms which is responsible for the denitrifiers enrichment. Besides, the increasing organic carbon source (from previous anaerobic hydrolysis production) available for denitrifiers might be due to other reasons.

Aromatic compounds degradation bacteria: Anaerobic aromatics biodegradation has been documented under various conditions with nitrate, Fe(III), sulfate, or CO_2 as alternative electron acceptors [23]. The aromatics degradation bacteria were widely found in ABR reactor, especial in the fourth, fifth, and sixth compartment, such as B4 (highest enrichment in the fifth compartment), B5, B12 (with the greatest intensity in the whole DGGE) and B14, whose closest species were *Pseudomonas stutzeri* A1501 [23], *Dechloromonas aromatic* RCB, *Pseudomonas putida* KT2440

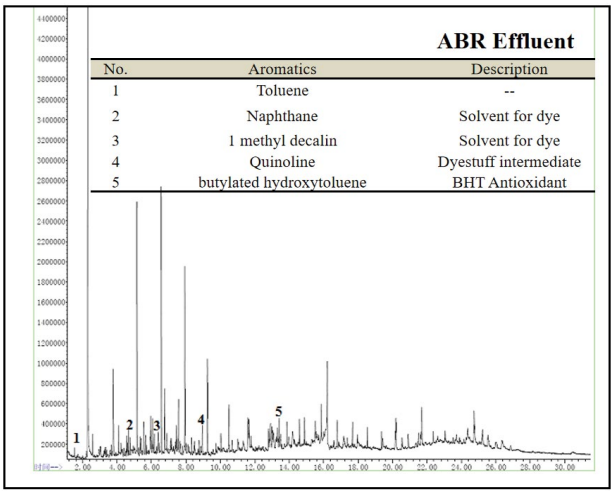
(a)



Inflow

No.	Aromatics	Description
1	Toluene	--
2	Naphthane	Solvent for dye
3	1 methyl decalin	Solvent for dye
4	Quinoline	Dyestuff intermediate
5	2, 4 - dichloro paranitroaniline	BHT Antioxidant
6	bisphenol A	Auxiliary
7	4 hydroxy - 2 - phenolic groups - 9, 10 - anthracene ketone 2 amino anthraquinone	Dyestuff

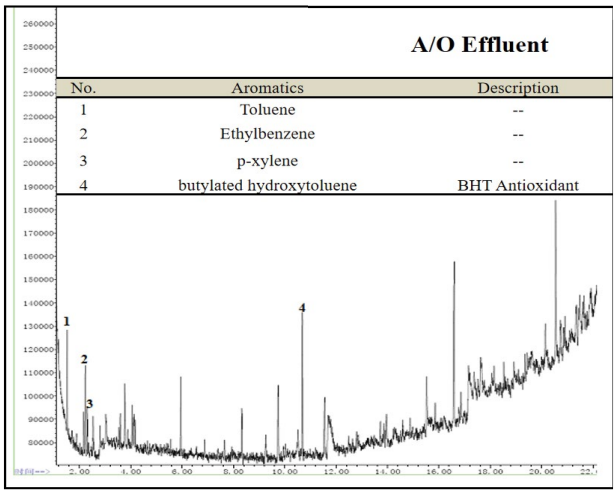
(b)



ABR Effluent

No.	Aromatics	Description
1	Toluene	--
2	Naphthane	Solvent for dye
3	1 methyl decalin	Solvent for dye
4	Quinoline	Dyestuff intermediate
5	butylated hydroxytoluene	BHT Antioxidant

(c)



A/O Effluent

No.	Aromatics	Description
1	Toluene	--
2	Ethylbenzene	--
3	p-xylene	--
4	butylated hydroxytoluene	BHT Antioxidant

Fig. 2. GC-MS spectrums for the stepwise biological processes. The peaks of aromatic compounds were specially marked and listed in the corresponding tables (the reliability is over 95% for all the listed aromatics).

[24], and *Aromatoleum aromaticum* EbN1 [25]. These bacteria were found extensively in the last three compartments and all of them owned special pathways for the catabolism of central aromatic intermediates, especially for the central benzoyl-CoA pathway [26].

Sulfur and chlorate-reducing bacteria: B7 and B13 were identified as *Desulfatibacillum alkenivorans* and *Desulfurivibrio alkaliphilus*, having ability to perform anaerobic respiratory metabolism, taking thiosulfate, and elemental sulfur (B13) or sulfate (B7) as the electron acceptors [27,28]. It is noticeable that the sulfate reduction mainly occurred at the first three compartments since B7 prefer enriching in these compartments. B2 and B5 (as denitrifiers) also take part in per-chlorate reduction. The presence of such bacteria also validates the sulfur and chlorate pollution in the printing and dyeing wastewater.

Other functional bacteria: B6 (*Tannerella forsythia*) and B10 (*Pseudomonas fluorescens*) which only discovered in the compartments with fillers (fourth, fifth, and sixth), observed no special pollutants removal abilities, however, they were involved in biofilms formation on abiotic surface by their extra-cytoplasmic protein interactions with the surface [29]. B3 (*Microbacterium testaceum*) showed *N*-acyl homoserine lactone-degrading activity [30] and predicted the capability of signal molecule transfer and response in biofilm formation.

In the A/O process, *Alphaproteobacteria* took the domain part. B15 and B16 were identified as denitrifying and nitrifying bacteria (Table 4), respectively. B17 whose closest species was *Parvibaculum lavamentivorans* able to ω-oxygenate the commercial surfactant linear alkylbenzenesulfonate (LAS) [31]. Especially, B18 was identified as *Novosphingobium* sp. PP1Y which has ability to grow a wide range of aromatic compounds and using the aromatic fraction as the sole carbon and energy source [32]. It performs aromatics degradation under aerobic condition with the assisted ABR process, further removes aromatics in the wastewater.

In general, many functional microbial communities were found which were responsible for various corresponding pollutants removal in printing and dyeing wastewater. Importantly, ABR, through the separation of biological

Table 4
Closest species and their classification for the representive bands in DGGE

No.	Closest species*	Classification
B1	*Pseudomonas denitrificans* ATCC 13867	*Gammaproteobacteria*
B2	*Dechlorosoma suillum* PS	*Betaproteobacteria*
B3	*Microbacterium testaceum* StLB037	*Actinobacteria*
B4	*Pseudomonas stutzeri* A1501	*Gammaproteobacteria*
B5	*Dechloromonas aromatica* RCB	*Betaproteobacteria*
B6	*Tannerella forsythia* ATCC 43037	*Bacteroidetes*
B7	*Desulfatibacillum alkenivorans* AK-01	*Deltaproteobacteria*
B8	*Paludibacter propionicigenes* WB4	*Bacteroidetes*
B9	*Bacteroides helcogenes* P 36-108	*Bacteroidetes*
B10	*Pseudomonas fluorescens* WCS365	*Gammaproteobacteria*
B11	*Shewanella* sp. ANA-3	*Gammaproteobacteria*
B12	*Pseudomonas putida* KT2440	*Gammaproteobacteria*
B13	*Desulfurivibrio alkaliphilus* AHT2	*Deltaproteobacteria*
B14	*Aromatoleum aromaticum* EbN1	*Betaproteobacteria*
B15	*Hyphomicrobium denitrificans* ATCC 51888	*Alphaproteobacteria*
B16	*Nitrosomonas eutropha* C91	*Betaproteobacteria*
B17	*Parvibaculum lavamentivorans* DS-1	*Alphaproteobacteria*
B18	*Novosphingobium* sp. PP1Y	*Alphaproteobacteria*

*Similarities ≥ 97% for all the closest species.

phase, maked the acidogenic fermentation bacteria, denitrifying bacteria, and aromatic compound degradation bacteria distributed in each cell along the way and show different advantages. Each compartment in ABR performs its own functions efficiently. For example, the first three compartments are responsible for macromolecular organics hydrolysis and sulfate reduction, while the last three compartments performs aromatic compounds degradation

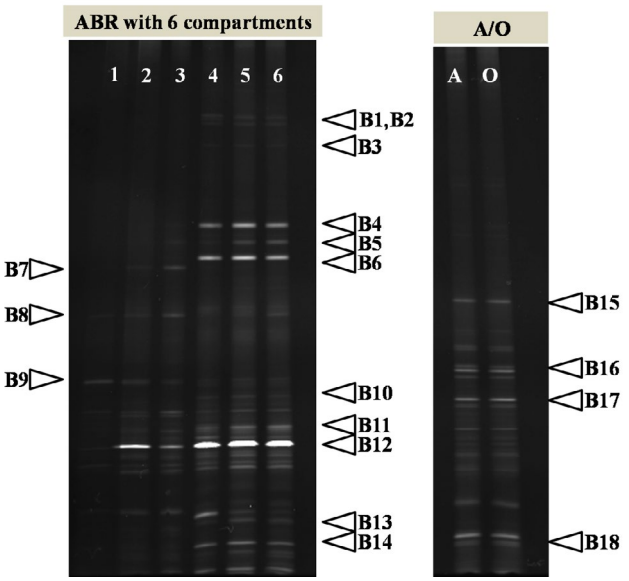


Fig. 3. DGGE profile for the biological process, includes six samples in each compartment of ABR, and two samples in the A/O process.

(rings opening), chlorate reduction, and denitrification. This maked the system more efficient in removal, more resistant to impact load, and more stable in operation.

3.4. Functional genes quantification for aromatics degradation in ABR

Detoxification is an important part in printing and dyeing wastewater treatment [33]. However, the aromatic compounds were performs toxicity of refractory organic pollutants in printing and dyeing wastewater (Fig. 2).

The bcrA gene is involved in anaerobic degradation for many kinds of aromatic compounds among the various bacteria, which is effectively used for anaerobic degradation bacteria detection [15].

In order to better understand the aromatic compounds anaerobic degradation process by microorganism in ABR. The quantification of bcrA genes is obtained for each compartment by real-time PCR.

Fig. 4a shows the bcrA gene abundance involved in per ng genomic DNA, which was converted to absolute abundance in environmental samples according to DNA concentration and sample mass. However, the trend of bcrA along the serial compartments were very similar since a little difference in DNA extracted concentration among the six samples.

The results of fifth compartment showed that the highest abundance of bcrA genes with 4.5×10^5 copies/mg sludge and the sixth compartment followed. Moreover, the last three compartments has higher values as compare to the first three. It is proven that the aromatics anaerobic degradation mainly occur in the last three compartments, especial for the fifth one.

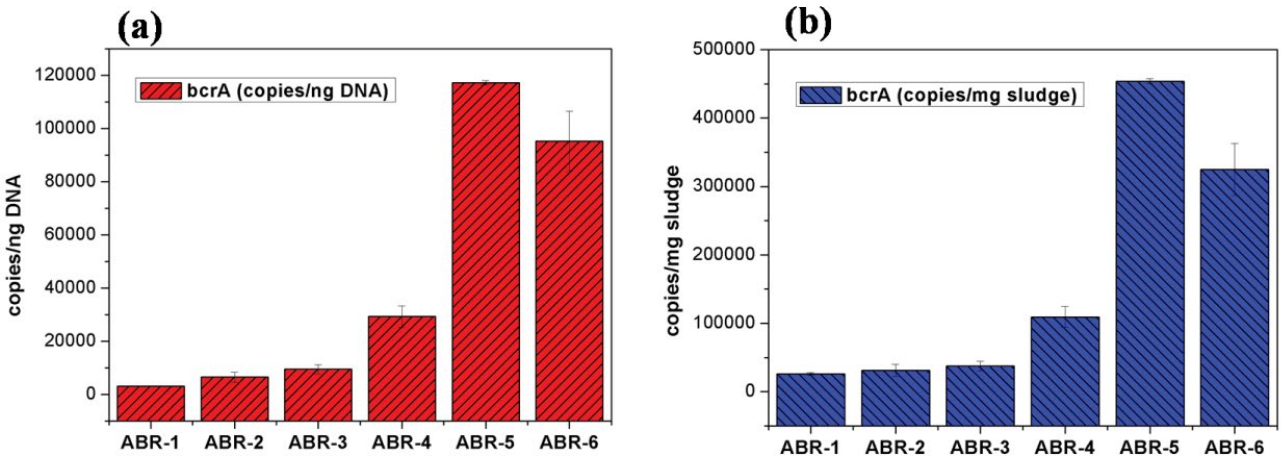


Fig. 4. Real-time PCR results for bcrA genes quantification (a) relative abundance with per genomic DNA and (b) absolute abundance with per milligram sludge.

The concentration of aromatic compounds would decrease gradually along with the serial compartments in ABR, theoretically. However, beyond its expectation, the abundance of bcrA genes did not decrease accordingly, but first raised up to fifth compartment and then drop (Fig. 4). Moreover, results showed that the abundance of bcrA in last three compartments were much higher due to the stratified biofilm on fillers (discussed above, section 3.3 (Microbial community analysis and pollutants biological removal mechanism)), there are several reasons were probably responsible for the peculiar variation tendencies of bcrA in ABR.

Under anaerobic conditions, the macromolecular aromatics was first degraded into small aromatics, such as azo-reductases cleave azo dyes into the corresponding aromatic amines, and then aromatic rings were opened. Initial hours (shorter hydraulic retention time (HRT) in frontal compartments), most of aromatics in ABR were in the cleavage period, but in the following hours (longer HRT in latter compartments) they were into the ring opening period, which induces more bcrA genes enrichment in the latter compartments. In addition, the total HRT is 24.1 h in the whole ABR reactor during this study which provided adequate cleavage and ring opening time for aromatics.

Besides, as toxic organic, the higher concentration of aromatics would restrain the growth and activity of microorganisms, even if the microorganism is able to degrade aromatics. Therefore, the lower abundance of bcrA genes was obtained in the frontal compartments.

Furthermore, one of the most advantages of ABR used in this study is high-efficiency and stepwise anaerobic hydrolysis, which increases BOD_5/COD gradually along the serial compartments. It is well-known that the co-metabolism effect based on other micro-molecule organics is significant for aromatic degradation by microorganisms. It indicated that more anaerobic hydrolysis products with high bioavailability probably lead higher removal efficiency of aromatic, and also the highest BOD_5/COD (0.37, during the whole ABR process) was obtained at the effluent of the fourth compartment, which coincided with the highest bcrA abundance in the fifth compartment.

In summary, the best unit for anaerobic aromatic rings opening and degradation is the fifth compartment in ABR due to the most suitable condition provided, including moderate substrate concentration and BOD_5/COD ratio. The bcrA or other these kinds of functional genes can be used to reflect the number of aromatics at a certain extent since the quantification of total aromatics in wastewater is difficult.

According to microbial community and functional genes abundance analysis in each compartment in ABR, it confirms that the six-compartment ABR in this study, due to its special structure and hydraulic condition, provides better distribution for different functional microbial communities, reasonably, in different compartments, which effectively helps various pollutants removal. However, the microbial community analysis is based on PCR-DGGE in our study, which is a simple and fast method, but without precise microbial information (compare to high throughout sequencing). DGGE was combined to real-time PCR to reveal the microbial mechanisms in this paper, and further study should be done in order to provide better understanding on the mechanisms.

4. Conclusion

- The addition of filler in ABR (fourth, fifth, and sixth compartment) improves its efficiency. The whole ABR based biological process can reach to the effluent standard which is stable for long period and demonstrated its availability for printing and dyeing wastewater treatment, especially for the characteristic pollutants, that is, aromatics compounds.
- The functional microorganisms distributed in different compartments of ABR and each performs its own functions effectively. According to microbial community analysis, the first three compartments are major in macromolecular organics hydrolysis and sulfate reduction, while the last three compartments performs aromatic compounds degradation (rings opening), chlorate reduction, and denitrification.
- Many kinds of aromatic anaerobic degradation microorganism were found in ABR, such as *Pseudomonas stutzeri*,

Dechloromonas aromatic. The abundances of the bcrA genes first raise up to fifth compartment and then drop along with the serial and indicated that the highest aromatics degradation efficiency was obtained at the fifth compartment, probably caused by its moderate substrate concentration and BOD_5/COD ratio.

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