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# Performance and microbial diversity of an upflow anaerobic sludge blanket reactor in fluorescent whitening agent wastewater treatment

Wenjie Zhang[a,*], Luhan Wu[b], Yue Jin[c]

[a]*Guangxi Key Laboratory of Environmental Pollution Control Theory and Technology, Guilin University of Technology, Guilin 541004, China, Tel./Fax: +86 773 2536922; email: 2010053@glut.edu.cn*
[b]*Guangxi Collaborative Innovation Center for Water Pollution Control and Water Safety in Karst Area, Guilin University of Technology, Guilin 541004, China, email: 103375916@qq.com*
[c]*Guangxi Key Laboratory of New Energy and Building Energy Saving, College of Civil Engineering and Architecture, Guilin University of Technology, Guilin 541004, China, email: zhangwj_zhangwj@hotmail.com*

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**ABSTRACT**

Reducing the treatment cost of fluorescent whitening agent wastewater (FWW) is a challenge. In this study, an upflow anaerobic sludge blanket (UASB) reactor was used in the pretreatment process of FWW. The treatment performance was analyzed, and 16S rRNA analysis was employed to characterize the changes in microbial populations in the anaerobic sludge. The results show that an average chemical oxygen demand (COD) removal rate of 76.2% was achieved with an organic loading rate of 2.8–3.3 kg COD/m$^3$/d, and the average removal rate of aniline was 30%. Using an UASB reactor for pretreatment can reduce operation costs because fewer chemical regents are required. At the end of the experimental period, the bacterial diversity and dominant bacterial strains differed markedly. The dominant bacteria in the sludge were *Chlorobium* sp. (21.8%), *Desulfomicrobium* sp. (40.4%), and *Halothiobacillaceae bacterium* (26.6%). *Chlorobium* sp. might have been involved in the degradation of aniline in this study. Finally, this method was applied to a full-scale industrial plant, replacing the existing physicochemical treatment, which reduced the chemical usage by 40%–50% and operation costs by 20%–30%. These results indicate that the biological treatment using cultured bacteria is an effective and low-cost method that can be used to treat FWW.

*Keywords:* Anaerobic; Aniline; Pretreatment; Running cost

## 1. Introduction

Fluorescent whitening agent wastewater (FWW) production contains a large amount of benzene, benzene derivatives, and other chemical reaction intermediates [1]. Furthermore, a large amount of $H^+$ is generated during the production process. To neutralize the reaction system, alkaline chemicals such as NaOH or $Na_2CO_3$ are added; therefore, FWW is typically highly saline, which complicates treatment. Although the composition of FWW is related to various factors such as raw materials, production conditions, production processes, and operating conditions, it exhibits several characteristic physical and chemical properties, including a high concentration of organic matter, poor biodegradability, high salinity, deep color, and pungent odor.

Physicochemical methods combined with biological methods are often applied for FWW treatment [2,3]. FWW contains toxic biological substances, which inhibit microorganisms [4–7]; therefore, the direct use of biological processes is limited. In current treatment methods, the use of physicochemical methods as a pretreatment is recommended to reduce the concentration of biologically harmful substances in the FWW. Then, biological treatment is applied, followed by additional physicochemical methods for further purification to meet discharge standards [2].

* Corresponding author.

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However, this treatment process is expensive, and costs typically exceed the manufacturer's maximum tolerance. Therefore, reducing the cost is an important issue in FWW treatment.

A number of studies have shown that harmful substances can be effectively removed from wastewater by enriching cultured microorganisms in anaerobic reactors, including those with high salt and aniline concentrations [8–10]. For example, one study showed that, after cultivation, good electrochemical and degradation performances were maintained up to a salinity of 15,000 mg/L during biodegradation of a petroleum hydrocarbon mixture in microbial fuel cells [11]. Meanwhile, Sun et al. [12] observed that aniline loss in nitrate- and sulfate-amended microcosms and a phylotype with 92.7% sequence similarity to *Ignavibacterium album* was identified as the dominant aniline degrader. Olivares et al. [13] also indicated that cultured anaerobic sludge can transform aniline continuously, thus decreasing the cytotoxicity of the parent pollutant. Finally, Jiang et al. [14] noted that aniline could be completely degraded when initial concentrations were less than 750 mg/L.

Based on previous studies, cultured microorganisms should improve the FWW biological treatment process. Because biological treatment is more economical than other processes [1], this could reduce FWW treatment costs. Anaerobic treatment can endure higher concentrations of organic matter and harmful biological substances [9]; therefore, it could replace physicochemical processes as the pretreatment process of FWW. To our knowledge, few studies have examined the treatment performance and microbial diversity of the anaerobic process during FWW treatment. Data on microbial diversity and population changes are critical to the construction and stable operation of anaerobic reactors, providing indispensable knowledge for engineering applications.

In this study, an upflow anaerobic sludge blanket (UASB) reactor was used for the pretreatment of FWW to reduce treatment costs. The treatment performance of the UASB reactor was investigated, and 16S rRNA analysis was employed to characterize the changes in microbial populations in the anaerobic sludge.

## 2. Materials and methods

### 2.1. Wastewater composition

The FWW used in this study was collected from a chemical plant mainly producing fluorescent whitening agents for paper manufacturing. Table 1 presents the water composition of the FWW from the production process. The FWW exhibited high salinity, high chemical oxygen demand (COD), and high $NH_4^+$–N. In addition, it contained aniline concentrations of 20–40 mg/L, which is harmful to microorganisms [15].

Table 1
Composition of FWW

| | |
|---|---|
| pH | 7.8–8.2 |
| Salinity (g/L) | 2.5–3 |
| COD (mg/L) | 500–1,100 |
| Aniline (mg/L) | 20–40 |
| TN (mg/L) | 264–300 |
| $NH_4^+$–N (mg/L) | 241–260 |
| TP (mg/L) | 0.1–1.7 |

### 2.2. Reactor

An UASB reactor equivalent to that described by Wenjie et al. [16] was used. The reactor was composed of plexiglass. The effective volume of the reactor was 9 L (height: 1.2 m and diameter: 8 cm). The water inlet was located at the bottom of the reactor, and the outlet was located at the top, and a gas–solid separator (GSS) was set on top of the reactor. The FWW was introduced through the inlet and a sludge layer. The treated FWW was separated by the GSS, and the separated sludge was returned to the sludge layer. The biogas was collected through the gas collector, where the pH was adjusted to 1. Temperature was maintained at 30°C. During the start-up period, 3 L of sludge from Qilidian wastewater treatment plant (Guilin, China) was inoculated, which contributed to one-third of the effective volume of the UASB reactor.

### 2.3. Measurement methods

Filtered dichromate-based COD (1 μm) was measured using the closed reflux colorimetric method [17]. Total nitrogen (TN) was determined using the persulfate method [18] and the ultraviolet spectrophotometric screening method for quantification of TN as $NO_3$–N (i.e., the oxidization product of persulfate digestion). Total phosphorus (TP) was measured according to Yue and Wenjie [19]. pH was measured using a pH meter (9010; Jenco, USA), and dissolved oxygen (DO) was measured using a DO meter (6010; Jenco, USA).

### 2.4. Microbial diversity

Sludge samples were collected at the start and end of the study. The samples were dewatered with a portable fast centrifuge. The sludge samples after dewatering were sealed in a centrifuge tube and stored at –20°C. In this study, the Power Soil DNA Isolation Kit (MOBIO, USA) was used to extract the DNA from the sludge samples. The purity of DNA extracted from the sludge samples was very high, but the content was low. After the extracted DNA passed the purity test, the DNA was amplified by polymerase chain reaction (PCR). Using the PCR technique, amplification of the target fragment was performed simultaneously with cleavage of the target DNA fragment. The primers used in this experiment were 968F and 1401R, and the amplified DNA fragment was the V6 region of bacterial 16S rDNA, with a sequence length of about 430 bp.

In this study, the target DNA fragment was isolated and purified by denaturing gradient gel electrophoresis (DGGE), and the DNA fragments of different microorganisms were fixed at different positions in the gel. After each step, each band was recovered and dissolved again for purification of the target DNA fragment. PCR products were sequenced by Shanghai Shenggong Co. (China). The sequenced gene was compared with the NCBI website, and DGGE imaging was analyzed using Quantity One software (Bio-Rad).

## 3. Results and discussion

### 3.1. Reactor start-up

The reactor start-up period lasted for 60 d (Table 2). In the reactor, FWW was diluted with tap water, and sugar was used as the cosubstrate for sludge cultivation. Both sugar and FWW contributed to the influent COD. To define the ratio of sugar and FWW in the influent, the FWW/sugar ratio was calculated as follows:

$$\text{FWW/sugar (\%)} = \frac{\text{FWW as COD (mg/L)}}{\text{FWW as COD (mg/L)} + \text{Sugar as COD (mg/L)}}$$

Following the cultivation period, the ratio of FWW (as mg COD/L) to sugar (as mg COD/L) was increased from 50% to 100%. The COD removal rate decreased as the influent COD concentration increased. On day 40–48, the average effluent COD decreased from 609 to 591 mg/L as the FWW/sugar ratio increased from 50% to 75%, and the average removal rate increased by 1.1%. Wenjie et al. [20] noted that trace elements accounted for the enhanced treatment performance of an UASB biofilm system. In the present study, increasing the FWW/sugar ratio from 50% to 75% increased the concentration of trace elements; therefore, an improvement in the removal rate was observed during this period. However, during days 49–60, when the FWW/sugar ratio was increased to 100%, the removal rate decreased significantly, indicating that the addition of a small amount of sugar resulted in the optimum performance. When the influent was completely changed to raw FWW, the average COD removal rate was only 40.3%; however, the treatment performance was stable, indicating that the start-up period of the UASB reactor was accomplished.

The effects of salinity were also considered, as shown in Table 2. As the influent salinity was increased from 0.5 to 3.0 g/L, which was the same concentration in the raw FWW, the COD removal rate was almost unchanged. These results indicated that the cultivated sludge could endure the salinity of the FWW with no adverse effects on microbial activity.

### 3.2. Reactor performance

At the end of the start-up period, FWW was diluted with tap water and introduced into the reactor as influent. The dilution ratio was calculated as follows:

$$\text{Dilution rate (\%)} = \frac{\text{Tap water volume (L)}}{\text{FWW volume (L)} + \text{Tap water volume (L)}}$$

At this stage, sugar was no longer used as a cosubstrate. The influent COD concentrations of the reactor were increased by gradually reducing the dilution ratio. Initially, the COD removal rate was only 10%–20% because no sugar had been added (Fig. 1). However, after 3 d, the COD removal rate increased rapidly to 50%–60%, indicating that the sludge cultivated during the start-up period rapidly adapt to change in water composition. When the influent concentration was further increased to 300–500 mg/L, the maximum COD removal rate of 80%–90% was achieved. With further increases of influent COD concentration, the COD removal rate decreased. For example, when the influent COD concentration was increased to 600–800 mg/L, the COD removal rate decreased to 40%–60% and the fluctuation interval was significantly increased compared with the previous stage. After this stage, raw undiluted FWW was introduced into the reactor. The raw FWW showed COD concentration fluctuations, with a maximum and minimum value of 1,100 and 500 mg/L, respectively (Table 1). At this stage, the COD removal rate tended to be stable, with an average removal rate of 50%. The experimental data indicated that FWW contained microbial activity-inhibiting substances; however, dilution could effectively reduce the effects of these substances.

The FWW contained an average aniline concentration of 30 mg/L (Fig. 2). After treatment, the average aniline concentration decreased to 20 mg/L, with an average removal rate of 30% in the reactor. A gradual reduction in dilution rate

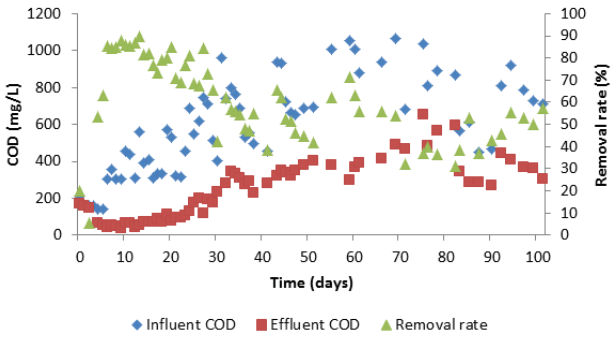


Fig. 1. COD removal performance during the study.

Table 2
COD removal performance during the start-up period

| Time (d) | Influent COD (mg/L) | Salinity (g/L) | FWW/sugar ratio (%) | Effluent COD (mg/L) | Removal rate (%) |
|---|---|---|---|---|---|
| 1–10 | 300 | 0.5 | 50 | 47 | 85.0 |
| 11–15 | 400 | 1.0 | 50 | 54 | 86.8 |
| 16–20 | 600 | 1.5 | 50 | 156 | 72.9 |
| 21–29 | 800 | 2.0 | 50 | 260 | 64.5 |
| 30–39 | 1,600 | 2.5 | 50 | 609 | 58.9 |
| 40–48 | 1,600 | 3.0 | 75 | 591 | 60.0 |
| 49–60 | 1,600 | 3.0 | 100 | 978 | 40.3 |

led to a gradual increase in aniline concentration in the influent. When the influent aniline concentration was less than 10 mg/L, microbial activity in the reactor was not inhibited by aniline due to degradation of aniline in the reactor. However, as the dilution rate was further reduced, the influent aniline concentration exceeded the reactor's ability to degrade aniline. The aniline concentration in the reactor gradually increased and accumulated, inhibiting microbial activity in the reactor. As a result, the removal efficiency of the reactor was reduced, and the effluent COD concentration increased. Therefore, improving aniline degradation is critical to maintaining stable reactor operation. Previous results have indicated that both aniline degradation and the treatment performance of the reactor could be improved by adding cultured aniline-degrading bacteria [13,14].

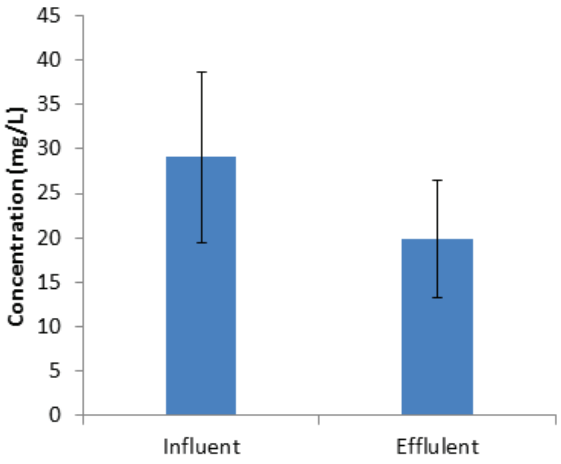


Fig. 2. Aniline removal performance during the study.

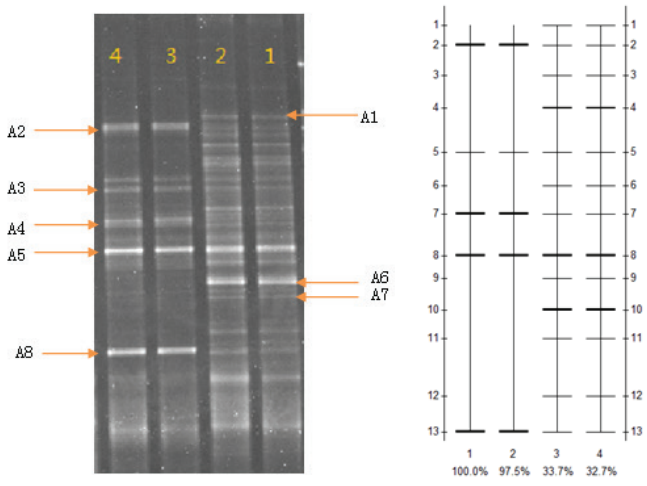


Fig. 3. DGGE bands and relative abundance.

### 3.3. Microbial diversity

Molecular biological experiments were performed with two replicates to ensure the accuracy of the analytical data. Fig. 3 presents the experimental results, where samples 1 and 2 were seed sludges at the start of the reactor, and samples 3 and 4 were sludge samples from the reactor at the end of the experiment. As shown by the DGGE results, there was good reproducibility among the repeated samples. At the end of the study, the strains in the sludge changed significantly compared with the inoculated sludge. In the 13 separated bands, A1, A3–A4, A6, and A9–A12 disappeared, while A2–A5, A7–A8, and A13 became dominant species in the reactor.

After sequencing, the obtained sequences were compared with the NCBI website (Table 3). The results of the phylogenetic analysis showed that each bacterium was of the same class as the corresponding branch of the phylogenetic tree. *Chlorobium* sp. has been reported in anaerobic benzene-degrading mixed cultures [21], which might have a functional enzyme for aniline degradation. Meanwhile, *Brevundimonas* sp., which can degrade complex organic matter, was found during the later phase of a composting period [22]. Uncultured *Desulfomicrobium* sp. was separated from an expanded granular sludge bed reactor during simultaneous biological removal of sulfur, nitrogen, and carbon [23]. Uncultured *Halothiobacillaceae bacterium* could include functional genes for sulfur oxidation [24]. The strains separated from the sludge at the end of the study were predominantly Proteobacteria, Firmicutes, and Chlorobi (Fig. 4). After acclimation, the microbial population structure became more concentrated. A3 and A7 belong to Alphaproteobacteria, A6 belong to Betaproteobacteria, A8 belong to Gammaproteobacteria, and A4 and A5 belong to Deltaproteobacteria.

The DGGE images were analyzed using Quantity One software, which can analyze the band pattern and obtain the

Table 3
Homology search results for 16S rRNA gene sequences of the main bacterial members in the sludge

| Band no. | Relatives (%) | GenBank no. | Strains |
|---|---|---|---|
| A1 | 99 | JF818055.1 | Uncultured *Alkaliphilus* sp. |
| A2 | 99 | EF153291.1 | *Chlorobium* sp. |
| A3 | 100 | EU368189.1 | *Brevundimonas* sp. |
| A4 | 100 | HQ049491.1 | Uncultured *Desulfomicrobium* sp. |
| A5 | 99 | HQ049491.1 | Uncultured *Desulfomicrobium* sp. |
| A6 | 100 | HQ088556.1 | Uncultured *Thiobacillus* sp. |
| A7 | 99 | EF188561.1 | Uncultured Alphaproteobacteria |
| A8 | 99 | HQ087445.1 | Uncultured *Halothiobacillaceae bacterium* |

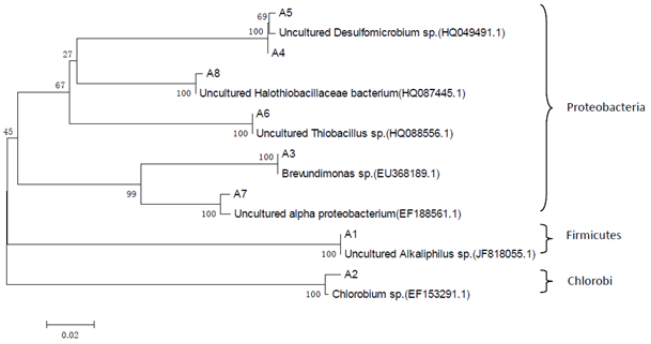


Fig. 4. Phylogenetic tree based on 16S rDNA sequence comparisons.

relative concentration of each microorganism from the brightness of the band to obtain the proportions of different bacteria and structure of the bacterial community. Biodiversity was calculated according to the Shannon–Wiener index. The microbial population structure of A1–A8 was obtained from the analysis using Quantity One software (Fig. 5).

A5 and A6 prevailed in the sludge during the start-up period, accounting for 19.8% and 27.7% of the community, respectively (Fig. 5). Meanwhile, A1–A4 and A7 were present in smaller proportions. In addition, there were a large number of other low-brightness bands, which were classified as "other", which accounted for 31.3% of the total bacteria. The biodiversity index of the sludge from the start-up period was 2.21. Proteobacteria accounted for 61.7% of the total bacteria, of which A5 and A6 constituted the largest proportion, belonging to the most dominant bacteria.

The structure of bacteria was significantly altered at the end of the study. A2, A3, A4, A5, and A8 accounted for 21.8%, 6.7%, 19.2%, 21.2%, and 26.6% of the community, respectively, while only 4.4% was classified as "other". A1, A6, and A7 disappeared at the end of the study, indicating that they could not adapt to the FWW treatment. A2 increased from 1.8% to 21.8%, A4 increased from 2.0% to 19.2%, and A8 increased from 5.5% to 26.6%, indicating that these three bacteria were able to adapt to the FWW, and prevailed in the reactor. There was a slight increase in A5, which was also a dominant strain in the reactor. At the phylum level, Proteobacteria accounted for 73.8% and Chlorobi accounted for 21.8% of the total. The biodiversity index decreased from 2.21 to 1.65 at the end of the study. Similarly, the diversity index decreased significantly, which is consistent with the results in Fig. 3. These results indicate that the cultivated sludge could adapt to the FWW treatment.

Next, the appropriate dilution rate and organic loading rate (OLR) were determined (Fig. 6). The COD removal rate decreased steadily with increasing FWW dilution rate, and reached a maximum of 85.1% at a dilution rate of less than 25%. Thereafter, the COD removal rate gradually decreased to 80% with a decreasing dilution rate of 45%–55%, and rapidly decreased when the dilution rate was further decreased to 35%–45%. At a dilution rate of over 85%, the average COD removal rate was only 41.3%. The results indicate that the optimal dilution rate in terms of COD is 45%–55%.

When the OLR was less than 2.8 kg COD/m$^3$/d, the COD removal rate decreased from 85.1% to 71.9% with increasing

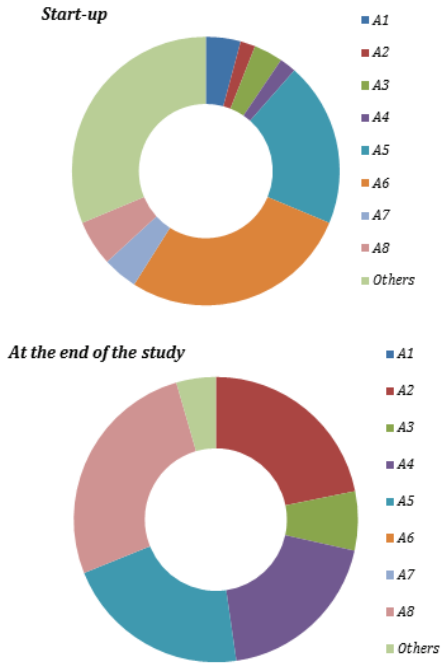


Fig. 5. Taxonomic classification of the microbial communities at the species level at the start and end of the experimental period.

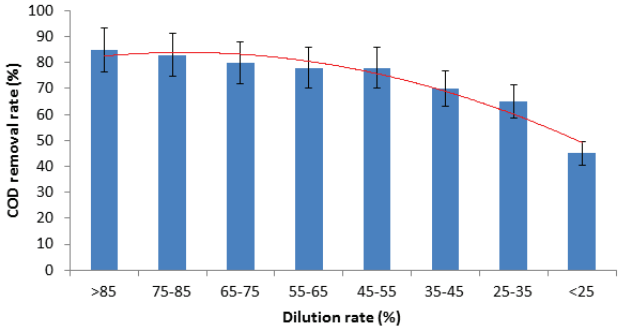


Fig. 6. Relationship between the dilution ratio and COD removal rate.

volume load from 1 to 2.8 kg COD/m$^3$/d and dilution rates from 25% to 55%. When the OLR was 2.8–3.3 kg COD/m$^3$/d, the average COD removal rate was 76.2% with a dilution rate of 55%. When the OLR was greater than 3.3 kg COD/m$^3$/d, the COD removal rate fluctuated between 54.2% and 69.0%, and the average COD removal rate was 65.5%. These results indicate that the optimum OLR for treating FWW was 2.8–3.3 kg COD/m$^3$/d in this study.

Finally, the experimental results were applied to a full-scale FWW treatment plant in Shandong, China. The FWW treatment plant included physicochemical pretreatment process, aerobic biological treatment process, and post-physicochemical treatment process, where an anaerobic biological treatment process was built to replace the physicochemical pretreatment process. After the modification, the treatment efficacy of anaerobic biological treatment process is 50%–60%, which is lower than that of lab-scale test. The

poor mix condition might be the main reason for the lower treatment efficacy, which is often reported in full-scale case [17]. However, the treatment efficacy of anaerobic biological treatment process is higher than that of the original physicochemical pretreatment process, which was only 30%–40%. The treatment efficacy of aerobic biological treatment process was improved by 20%–25%. Thereafter, the plant's chemical usage of pretreatment and post-physicochemical treatment process decreased by approximately 40%–50%. Wastewater treatment costs were reduced by approximately 20%–30%. This suggests that using an anaerobic process as a pretreatment can effectively reduce the running costs of FWW treatment. Further improvement of the treatment performance of anaerobic and aerobic processes will be the focus of future research.

## 4. Conclusions

This study evaluated the performance of an anaerobic process for FWW pretreatment. The cultivation period was accomplished in 60 d using an UASB reactor for FWW treatment. The COD removal rate reached 80%–90% at an influent concentration of 300–500 mg/L. The COD removal rate was 40%–60% using raw FWW. The treatment performance was maintained with an influent aniline concentration of less than 10 mg/L. The microbial strains separated from the sludge during the study were predominated by Proteobacteria, Firmicutes, and Chlorobi. Application of this treatment process to a manufacturing plant in China resulted in reduced chemical usage and lower operation costs, indicating that this method can be used to optimize FWW treatment. Improving aniline degradation performance remains an important issue for FWW treatment that requires further research.

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