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Research Article

Effects of Inoculum Amount, Initial pH, and Nicotine Load on the Set-Up of Bioaugmented System with *Pseudomonas* Sp. HF-1 to Treat Tobacco Wastewater

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This study evaluated and optimized the influence of inoculum amount, initial pH, and nicotine load on the construction of *Pseudomonas* sp. HF-1 bioaugmented system for tobacco wastewater treatment. The results demonstrated that the optimum condition for the set-up of strain HF-1 bioaugmented system was 1.10 mg/g (dry weight of strain HF-1/dry weight of activated sludge) of inoculum amount, initial pH 7.0, and 250–1000 mg/L nicotine load. Higher than 1.10 mg/g could lead to noncolonization of strain HF-1 in activated sludge and failure of set-up of this bioaugmented system. Higher than pH 8.0 could restrain the colonization of strain HF-1 in activated sludge. Even though strain HF-1 colonizes in the activated sludge when pH was above 8.0, the removal of nicotine and total organic carbon (TOC) was suppressed due to low activities of bacteria in the activated sludge. Nicotine load did not show inhibition effect on set-up of bioaugmented system, but the ability of TOC removal was restrained when the nicotine load was above 1000 mg/L. This work could offer vital parameters for the set-up of bioaugmented system to treat tobacco wastewater in engineering.

1. Introduction

In China, tobacco industry plays an important role in national economy, which achieved 864.9 billion yuan of profits and taxes in 2012. China as the largest producer of cigarettes in the world produces 1.7 trillion cigarettes annually [1]. Sixty tons of tobacco wastewater would be generated to produce 1 ton of cigarettes [2]. Approximately 33 million tons of tobacco wastewater is discharged in China every year, which includes many toxic substances such as nicotine, aminobiphenyl, naphthylamine, and benzo(a)pyrene [3, 4]. Among which, nicotine can dissolve in water and diverse kinds of organic solvents [5]. It is responsible for carcinogenicity, teratogenesis, and mutagenesis. Since 1995, it has been listed in the Toxic Release Inventory Program of the Environmental Protection Agency [3]. Therefore, there is a major requirement to reduce nicotine content in tobacco wastewater.

So far, numerous efforts have been made to isolate nicotine-degrading microbes for tobacco waste or wastewater treatment [2, 6–8]. All these bacteria are expected to be effective to degrade nicotine within an environmental matrix, such as soil and wastewater. Bioaugmentation as the introduction of specific microorganisms is recognized as a promising and attractive means to improve the performance of bioreactors [9–11]. Definitely, in our previous studies, an efficient nicotine-degrading bacterium *Pseudomonas* sp. HF-1 was employed to construct the bioaugmented system to treat tobacco wastewater [12–14]. The strain HF-1 bioaugmented system showed high efficiency, with 100% nicotine degradation and more than 84% chemical oxygen demand removal within 12 h [15].

As a promising tobacco wastewater treatment, we also paid attention to study its mechanisms. According to Shao et al. [16], rapid elimination of nicotine oxidative stress by strain HF-1 is one of the main reasons for the excellent performance

of the bioaugmented system. As to the core process of bioaugmentation, colonization of strain HF-1 was regulated by the quorum sensing [17, 18]. As well known, the effect of quorum sensing mainly depends on the density of population. It suggested that inoculum played an important role in the construction of bioaugmented system. Additionally, Wang et al. [18] reported that pH also could affect quorum sensing signal secretion and existence and accordingly impact the character of sludge. It implied that pH also played an important role in its set-up. Besides, the initial pollutant concentration is also an important factor for the set-up of bioaugmentation system that concerned by various researchers [19–22]. Based on the analysis of its mechanisms, both inoculum and pH and pollutant load should be optimized for its application in engineering [23, 24].

Therefore, the objective of this work was to examine the favorite conditions for setting up of strain HF-1 bioaugmented system, including inoculum amount, initial pH, and nicotine load. The results present optimal conditions for the set-up of strain HF-1 bioaugmented system to treat tobacco wastewater in engineering, as well as reference to other bioaugmented system construction.

2. Materials and Methods

2.1. Microorganism, Tobacco Wastewater, and Reactor. *Pseudomonas* sp. HF-1, which is capable of utilizing nicotine as the sole source of carbon, nitrogen, and energy, was previously isolated [25]. The bacterial inoculum was prepared in sterilized 150 mL inorganic salt media (ISM) [containing (g/L): 0.2 K_2HPO_4, 0.8 KH_2PO_4, 0.2 $MgSO_4$, 0.1 $CaSO_4{\cdot}H_2O$, 0.0033 $NaMoO_4$, and 0.005 $FeSO_4{\cdot}H_2O$, pH7.0] with 1000 mg/L nicotine in 500 mL Erlenmeyer flasks and incubated at 30°C on an incubator shaker (130 rpm) for 10–12 h. The cells of strain HF-1 in the logarithmic growth phase were collected by centrifugation. The collections were washed three times with sterile water and then used for bioaugmented system inoculation ($OD_{600} \approx 1.0$).

The tobacco wastewater used in this study was prepared as follows: tobacco waste (collected from China Tobacco Zhejiang Industrial Co., Ltd.) and distilled water was mixed at a ratio of 7 : 100 (g/mL). The pH of this wastewater was 5.2 ± 0.2; 2 M NaOH and 1 M HCl were used to adjust it to the desired value. In this wastewater, 1000 ± 116 mg/L nicotine and 6000 ± 400 mg/L total organic carbon (TOC) were contained.

Sequencing batch reactors (SBRs) with a working volume of 800 mL were established. Air was provided from the bottom of each reactor using a diffuser connected to an air pump, and the dissolved oxygen (DO) was maintained 3–5 mg/L. Activated sludge was inoculated into each reactor with a concentration of 3.63 ± 0.09 g SS/L. The activated sludge used as the endogenous seed culture was obtained from Qige wastewater treatment plant in Zhejiang Province, China. The reactors were operated on a 48 h cycle at 30 ± 2°C, and each cycle consisted of 5 min influent filling, 10 min effluent withdrawal, 30 min settling, and rest time aeration. The relative long HRT will be beneficial to the colonization of introduced strain HF-1. Effluent was discharged after settling at a volumetric exchange ratio of 50%. The sludge retention time (SRT) was about 4–6 days. Each experiment lasted for 20 days and clearly divided into two stages. The first one was the inoculated stage (stage a) including strain HF-1 inoculation from days 0 to 8 at a frequency of 48 h. The latter stage (stage b) started on the day 9 and terminated on the day 20 and no additional strain HF-1 was added to the reactors at that stage. According to our previous study, 12 d successive performance is enough to detect whether strain HF-1 colonizes in the activated sludge or not [15].

2.2. Effect of Inoculum Amount on Set-Up of the Bioaugmented System. In order to find the optimum inoculum amount for the set-up of strain HF-1 bioaugmented system, six SBRs were employed. Five of the reactors named I-1, I-2, I-3, I-4, and I-5 were inoculated with 0.55 ± 0.01, 1.10 ± 0.03, 1.66 ± 0.04, 2.21±0.05, and 2.76±0.07 mg/g (dry weight of strain HF-1/dry weight of activated sludge), respectively. The other reactor was not inoculated with strain HF-1 served as a control (CK). The initial pH of all reactors was 7.0.

2.3. Effect of Initial pH on Set-Up of the Bioaugmented System. To determine the effect of varied initial pH on the set-up of bioaugmented system, the pH of tobacco wastewater was adjusted to 5.0, 6.0, 7.0, 8.0, and 9.0, prior to inflow. The five reactors were, respectively, named as II-1, II-2, II-3, II-4, and II-5, which were inoculated with above optimized dose of inoculum as 1.10 ± 0.05 mg/g (dry weight of strain HF-1/dry weight of activated sludge). The other reactor without the inoculation of strain HF-1 was used as a control (CK) and the initial pH of the CK was 7.0.

2.4. Effect of Nicotine Load on Set-Up of the Bioaugmented System. The effect of nicotine was evaluated under above optimized conditions so as to find the most suitable and maximum nicotine load. Therefore, five reactors with 250, 500, 800, 1000, and 1200 mg/L of initial nicotine load were employed, named as III-1, III-2, III-3, III-4, and III-5 successively. The inoculation of these reactors was 1.00 ± 0.04 mg/g (dry weight of strain HF-1/dry weight of activated sludge). In this experiment, tobacco wastewater was diluted to 250 ± 20 mg/L of nicotine and then standard nicotine was added according to the needed.

2.5. Detection of Strain HF-1 in Activated Sludge. Total RNA from activated sludge on the 20th day was extracted using the Bioteke soil RNA isolation kit (Bioteke, Beijing, China) after washing by TENP buffer and then treated with DNase I (RNase-free) at a concentration of 1 U/μg for 30 min at 37°C to remove contaminating DNA. Reverse transcriptase PCR was used to detect the existence of strain HF-1 in all reactors. The primer sequences were as follows: forward primer *hsp* F, 5′-ACGGCTACAACTTCTACTACGGC-3′ (corresponding to positions 1105–1127) and reverse primer *hsp* R, 5′-CTTGATGGTTGGTTTCCTCCCT-3′ (positions 1775–1754). Reverse transcriptase PCR was performed in a final volume of 25 μL according to the instruction of

PrimeScript One Step RT-PCR Kit Ver.2 (TaKaRa, Dalian, China). The program was as follows: 30 min at 50°C, 2 min at 94°C, and then 35 cycles of 30 s at 94°C, 30 s at 60°C, and 2 min at 72°C.

2.6. Evaluation of Reactor Performance. Nicotine and TOC concentrations were the target pollutant indexes in this study. In order to evaluate the reactor performance, the concentrations of nicotine and TOC in the influent and effluent were measured every 2 days. The nicotine concentration was analyzed by WATERS high-performance liquid chromatography (HPLC) [15]. The TOC concentration was detected by a TOC analyzer (Shimadzu, Japan).

2.7. Quantification of Strain HF-1 in Activated Sludge. Quantitative real-time PCR was carried out to quantify bacteria strain HF-1 in activated sludge. The 16S rRNA gene, amplified by the total bacterial primers, 338F (5′-CCTACGGGAGGC-AGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′), was used as a house keeping gene. The specific *hsp* gene of strain HF-1 was amplified by the following primer pair, *hsp* S (5′-ATACTGCCGACAACAACTAACC-3′) and *hsp* A (5′-CACTCCAGAAACGAA AAAACC-3′). Real-time PCR was performed in a 10 μL reaction mixture system containing 5 μL iQ SYBR Green Supermix (Bio-Rad Laboratories Inc., Hercules, CA), 0.2 μL each primer (10 μM), 1 μL template (approximately 5 ng), and 3.6 μL sterile H_2O. Real-time PCR conditions were as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 20 s, 58°C for 20 s, and 72°C for 20 s. At the end, melt curve analysis was performed by the addition of a final step, starting at 65°C and going to 95°C; the signal was monitored every 5 s with a 0.5°C temperature increment. The relative abundance of strain HF-1 was normalized by *hsp* gene expression against the 16S rRNA through $2^{-\Delta\Delta Ct}$ Method [26].

2.8. Statistical Analysis. All data were presented as mean ± SD value. One-way analysis of variance (ANOVA) was performed using SPSS software (version 19.0), and the levels of significance were tested at $P < 0.05$.

3. Results and Discussion

3.1. Effect of Inoculum Amount on the Set-Up of Bioaugmented SBRs. The initial amount of inoculum plays an important role in the bioaugmented system. Small inoculum may be insufficient to degrade the specific pollutant, while too large inoculum amount possibly destroys the ecological balance and consequently leads to poor degradation of the specific pollutant [19]. Therefore, appropriate inoculum amount is vital to the set-up of bioaugmented systems.

Reverse transcriptase PCR was used to detect the survival of strain HF-1 in activated sludge on day 20. As shown in Figure 1, bands approximately 700 bp in size were observed in samples from the reactors I-1 to I-2, while no band was detected in noninoculated reactor (CK) and inoculated reactors I-3, I-4, and I-5. It means that after stopping inoculation of strain HF-1 for 12 d, the key nicotine-degrading gene *hsp* in strain HF-1 still exists in the reactors I-1 and I-2.

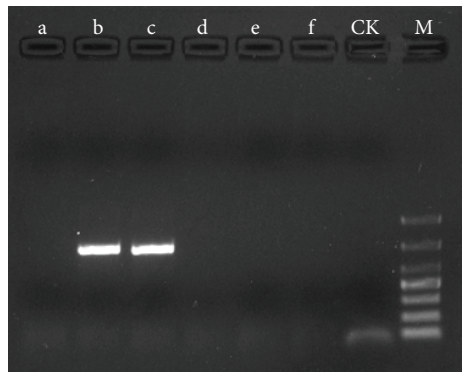

Figure 1: Reverse transcriptase PCR amplification of the partial *hsp* gene. Lane a: the noninoculated reactor on the 20th day; Lane b–f: inoculated reactors with 0.55 ± 0.01, 1.10 ± 0.03, 1.66 ± 0.04, 2.21 ± 0.05, and 2.76 ± 0.07 mg/g (dry weight of strain HF-1/dry weight of activated sludge) inoculum amount on 20th day, respectively; Lane CK: original activated sludge at 0 day; Lane M: DL 1000 marker.

It demonstrated that strain HF-1 successfully colonized in these reactors with low inoculum amount [27]. Thus, the appropriate inoculum amount for the colonization of strain HF-1 was 0.55±0.01 and 1.10±0.03 mg/g (dry weight of strain HF-1/dry weight of activated sludge).

Figure 2 shows the efficiency of pollutant removal in noninoculated reactor (CK) and inoculated reactors (I-1 to I-5). Compared to the noninoculated reactor with merely 20% of nicotine removal, inoculated reactors I-1 and I-2 showed high nicotine removal maintaining above 90%. Definitely, without strain HF-1 colonization, the reactors with larger inoculum amount (I-3, I-4 and I-5) did not show the same high efficiency to I-1 and I-2. Similarly, the TOC removal was between 12.44 and 34.88% in the noninoculated reactor, while reached 55.50% to 74.62% in the inoculated reactors I-1 and I-2 during the entire process. The TOC removal is below 50% in the reactors with larger inoculum amount (I-3, I-4 and I-5). It suggested that colonization of strain HF-1 in the activated sludge (I-1 and I-2) enhanced both nicotine degradation and TOC removal. Obviously, the nicotine and TOC removal in I-2 was more efficient than that in I-1. Taking the nicotine and TOC removal into account, the most suitable inoculum amount for the set-up strain HF-1 bioaugmented system belongs to 1.10 ± 0.03 mg/g.

Furthermore, real-time PCR was used to estimate the dynamic changes of strain HF-1 in activated sludge. Gene *hsp* is one of the key of nicotine-degrading genes in strain HF-1. The more the copies of gene *hsp* detected, the more the amount of strain HF-1 [12]. As seen in Table 1, the target gene *hsp* was detected in all reactors at stage a. At stage b, the target gene *hsp* was only detected in I-1 and I-2, but not in I-3, I-4, and I-5. The results were consistent with the results from reverse transcriptase PCR analysis. It could be confirmed from real-time PCR analysis that strain HF-1 colonized in I-1 and I-2, but not in I-3, I-4, and I-5. Moreover, as shown in Table 1, the population of strain HF-1 in reactor I-2 was significantly higher than that in I-1 during the whole process ($P < 0.05$). It could be the reason why the

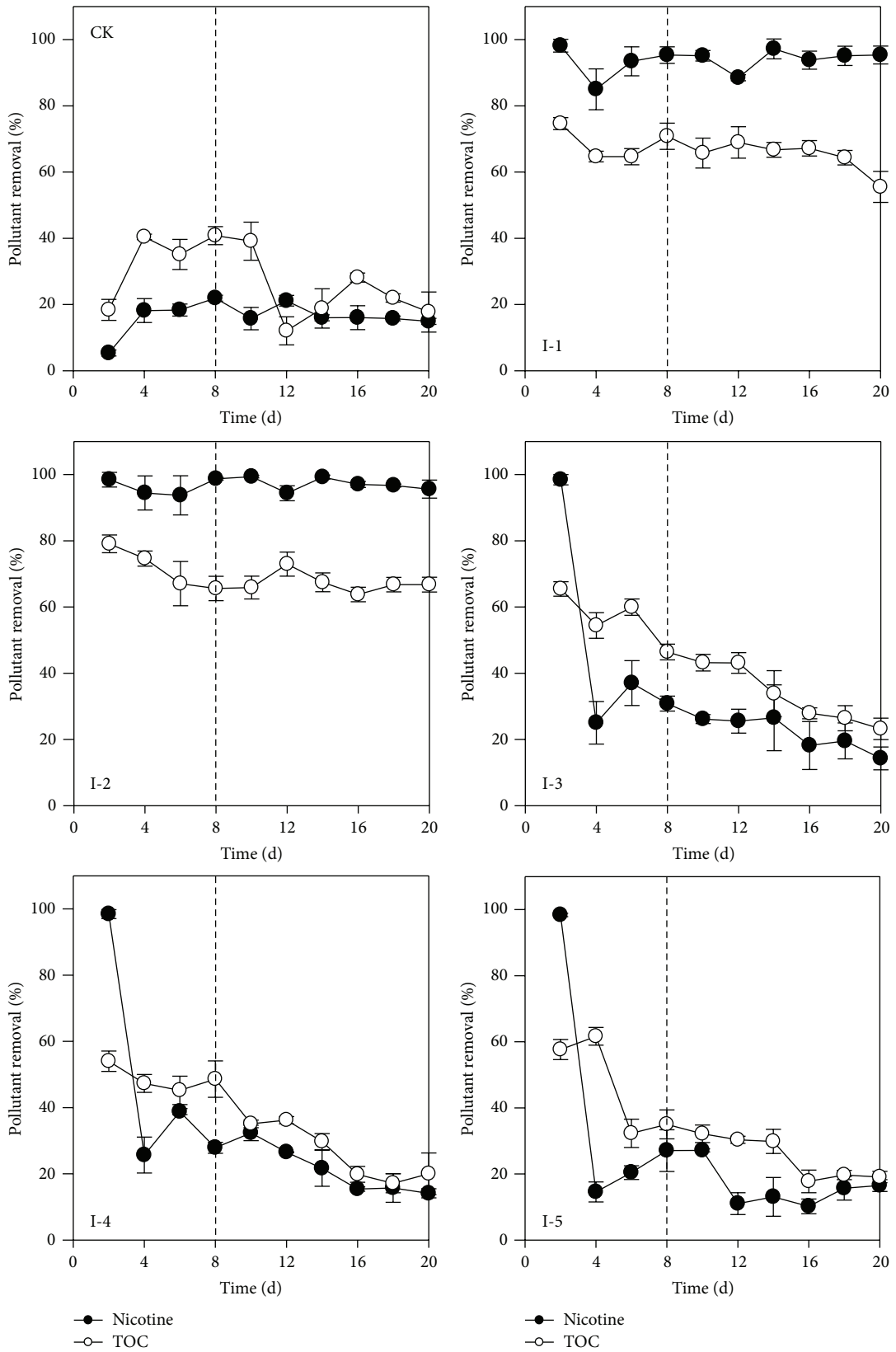

Figure 2: Effect of inoculum amount on pollutant removal in tobacco wastewater. The inoculum amount in reactor I-1 to I-5 was 0.55 ± 0.01, 1.10 ± 0.03, 1.66 ± 0.04, 2.21 ± 0.05, and 2.76 ± 0.07 mg/g (dry weight of strain HF-1/dry weight of activated sludge), respectively. CK: noninoculated reactor.

Table 1: Amount of target gene *hsp* in the inoculated reactors with different inoculum amount.

Stage	Reactor				
	I-1	I-2	I-3	I-4	I-5
	0.55 ± 0.01	1.10 ± 0.03	1.66 ± 0.04	2.21 ± 0.05	2.76 ± 0.07
a	241.69 ± 6.57^{a}	821.28 ± 9.65^{b}	298.21 ± 3.95^{c}	58.77 ± 1.22^{d}	1.26 ± 0.08^{e}
b	25.58 ± 0.52^{A}	40.76 ± 0.50^{B}	—	—	—

Stage a: from 0 d to 8th d with strain HF-1 inoculation; stage b: from 9th d to 20th d without strain HF-1 inoculation; the inoculum number of reactors I-1 to I-5 was 0.55 ± 0.01, 1.10 ± 0.03, 1.66 ± 0.04, 2.21 ± 0.05, and 2.76 ± 0.07 mg/g (dry weight of strain HF-1/dry weight of activated sludge), respectively.
Different lowercase letters in the same column indicate significant differences at stage a ($P < 0.05$); different uppercase letters in the same column indicate significant differences at stage b ($P < 0.05$).

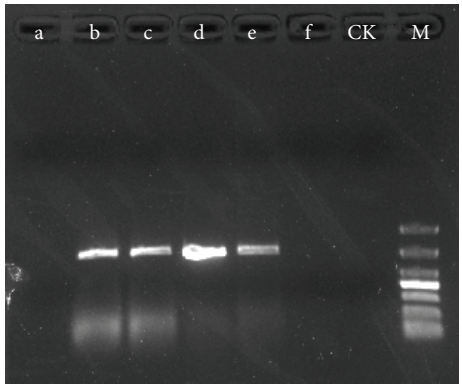

Figure 3: Reverse transcriptase PCR amplification of the partial *hsp* gene. Lane a: noninoculated reactor on the 20th day; Lane b–f: inoculated reactors with initial pH 5.0, 6.0, 7.0, 8.0, and 9.0 on the 20th day, respectively; Lane CK: original activated sludge at 0 day; Lane M: DL 1000 marker.

efficiency of pollutant removal in I-2 was more stable than that in I-1. According to the existing amount of strain HF-1 in the reactors, it could be confirmed that the most suitable inoculum amount for the growth and colonization of strain HF-1 was 1.10 ± 0.03 mg/g (dry weight of strain HF-1/dry weight of activated sludge).

3.2. Effect of Initial pH on the Set-Up of Bioaugmented SBRs. pH could significantly influence the physiology of microbes, such as optimal of enzyme activities, which finally influence the efficiency of biological treatment process [28]. In our previous study, it also showed that pH is beneficial to strain HF-1 colonization [18]. Thus, six reactors were constructed to study the effects of initial pH on the set-up of strain HF-1 bioaugmented SBRs to treat tobacco wastewater.

As shown in Figure 3, bands approximately 700 bp in size were observed in samples from reactors II-1 to II-4, while no band was detected in reactor II-5. It suggested that strain HF-1 can colonize in activated sludge under pH from 5.0 to 8.0. However, the colonization of strain HF-1 was completely restrained when the pH reached 9.0. Therefore, the initial pH could affect the set-up of strain HF-1 bioaugmented SBRs to treat tobacco wastewater.

As seen in Figure 4, the nicotine removal in all inoculated reactors improved, comparing with the 20% nicotine removal in noninoculated reactor (CK). The nicotine removal was up to 97.52, 97.42, and 98.60%, in reactors II-1, II-2, and II-3, respectively. Meanwhile the removal of nicotine was only 44.43 and 35.61% in reactors II-4 and II-5. The TOC removal was 40.19, 57.28, and 63.46% in reactors II-1, II-2, and II-3, respectively. Meanwhile the removal of TOC was only 28.29 and 27.73% in reactors II-4 and II-5. According to Ruan et al. [25], the growth and nicotine degradation of strain HF-1 in inorganic salt media were inhibited under alkali conditions. It could be the reason that the performance of pollutants removal in reactor II-4 was not as good as reactors II-1, II-2, and II-3. Alkali condition could inhibit the activity of indigenous microorganisms in activated sludge. Thus, low efficiency of pollutants removal under pH 9 was observed. It suggested that, compared to alkali condition, the strain HF-1 bioaugmented system could be set up under pH 5–7 more easily.

The survival of strain HF-1 was quantified by real-time PCR. As shown in Table 2, the copies of gene *hsp* at the stage b in reactor II-3 were significantly higher than that of other reactors ($P < 0.05$). The more copies of gene *hsp*, the higher activity of strain HF-1 in the bioaugmented system. Accordingly, the highest efficiency of nicotine degradation and TOC removal was observed in reactor II-3. In reactor II-4, the copies of gene *hsp* were significantly lower than others during whole process ($P < 0.05$). Thus, poor nicotine degradation and TOC removal in reactor II-4 were observed. No copy of gene *hsp* was detected in reactor II-5, which was the same as the reverse transcriptase PCR analysis (Figure 3). Thus, taking both the efficiency of pollutant removal and the activity of strain HF-1 into account, the most suitable pH was 7.0 for the set-up of strain HF-1 bioaugmented system. In addition, the initial alkali condition (pH 8-9) was not recommended in the engineering.

3.3. Effect of Nicotine Load on the Set-Up of Bioaugmented SBRs. The target pollutant load is another critical factor that influences the success set-up of bioaugmented system. The hazardous substance can induce the toxicity on microbes and consequently influence the efficiency of pollutant removal [24]. Thus, the effect from nicotine on the set-up of strain HF-1 bioaugmented system was evaluated.

As seen in Figure 5, the bands approximately 700 bp in size were observed in all inoculated reactors. Strain HF-1 survived in all inoculated reactors after another 12 days of wastewater treatment. It suggested that 250–1200 mg/L nicotine load did not affect the colonization of strain HF-1 in activated sludge.

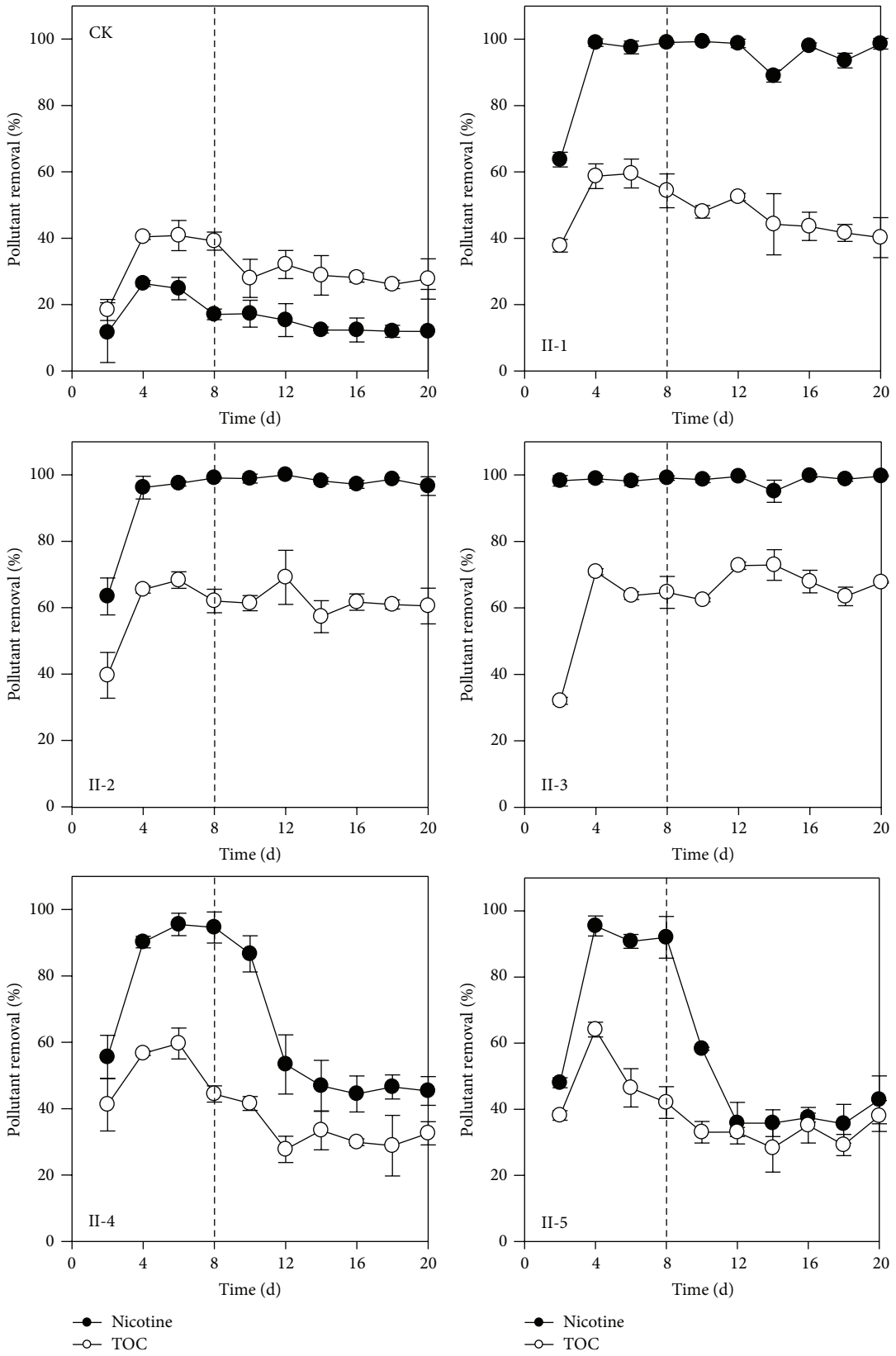

Figure 4: Effect of initial pH on pollutant removal in tobacco wastewater. The initial pH in reactor II-1 to II-5 was 5.0, 6.0, 7.0, 8.0, and 9.0, respectively. CK: noninoculated reactor.

Table 2: Amount of target gene *hsp* in the inoculated reactors with different initial pH.

Stage	Reactor				
	II-1	II-2	II-3	II-4	II-5
	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0
a	492.28 ± 72.36^{a}	873.61 ± 92.95^{b}	1085.79 ± 15.02^{c}	10.716 ± 3.83^{d}	4.30 ± 0.30^{d}
b	32.95 ± 8.49^{A}	30.65 ± 4.49^{A}	47.28 ± 3.33^{A}	5.71 ± 0.61^{B}	—

Stage a: from 0 d to 8th d with strain HF-1 inoculation; stage b: from 9th d to 20th d without strain HF-1 inoculation; the initial pH of reactors II-1 to II-5 was 5.0, 6.0, 7.0, 8.0, and 9.0, respectively.
Different lowercase letters in the same column indicate significant differences at stage a ($P < 0.05$); different uppercase letters in the same column indicate significant differences at stage b ($P < 0.05$).

Table 3: Amount of target gene *hsp* in the inoculated reactors with different nicotine load.

Stage	Reactor				
	III-1	III-2	III-3	III-4	III-5
	250 mg/L	500 mg/L	800 mg/L	1000 mg/L	1200 mg/L
a	133.38 ± 28.9^{a}	261.83 ± 39.63^{a}	847.25 ± 28.97^{b}	1072.64 ± 27.15^{b}	1442.08 ± 15.22^{c}
b	19.56 ± 1.76^{A}	20.55 ± 2.41^{A}	36.65 ± 3.71^{B}	55.73 ± 2.46^{C}	15.73 ± 1.01^{A}

Stage a: from 0 d to 8th d with strain HF-1 inoculation; stage b: from 9th d to 20th d without strain HF-1 inoculation; the nicotine load of reactors III-1 to III-5 was 250, 500, 800, 1000, and 1200, respectively.
Different lowercase letters in the same column indicate significant differences at stage a ($P < 0.05$); different uppercase letters in the same column indicate significant differences at stage b ($P < 0.05$).

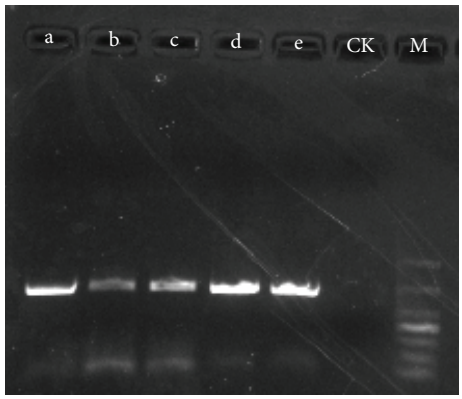

Figure 5: Reverse transcriptase PCR amplification of the partial *hsp* gene. Lane a–e: inoculated reactors with 250, 500, 800, 1000, and 1200 mg/L nicotine on the 20th day, respectively; Lane CK: original activated sludge at 0 day; Lane M: DL 1000 marker.

As shown in Figure 6, the introduction of strain HF-1 can completely degrade nicotine in all setting concentrations of nicotine. However, with increasing of nicotine, the TOC removal at the day 20 was 74.44%, 73.85%, 68.77%, 63.91%, and 38.13% in turn. The increasing of nicotine load results in decreasing of TOC removal, especially under 1200 mg/L of nicotine load. According to previous study, nicotine can induce oxidative stress in activated sludge [16, 29]. Therefore, the poor performance of TOC removal in the reactor with 1200 mg/L nicotine load could be attributed to the toxicity from nicotine on indigenous bacteria in activated sludge. Though nicotine load does not affect colonization of strain HF-1 in activated sludge, it should be controlled below 1200 mg/L for achieving high efficiency of pollutant removal in this system.

Real-time PCR analysis showed that, with the increasing load of nicotine, the amount of strain HF-1 increased at stage a (Table 3). Similarly, the growth of strain HF-1 increased with increasing of nicotine but decreased sharply when nicotine was over 1200 mg/L in pure culture [25]. After another 12 days of operation, the number of strain HF-1 in the reactor III-4 was significantly higher than others. As mentioned above, the removal of TOC with 250–1000 mg/L nicotine load was 97.75, 195.65, 285.76, and 322.9 mg/d, respectively. Meanwhile only 259.92 mg/d of TOC was removed under 1200 mg/L of nicotine load. Thus, taking the survival of strain HF-1 and the removal of TOC, nicotine load should be controlled below 1200 mg/L when the strain HF-1 bioaugmented system was set up. Additionally, in order to treat high concentration of contaminant, wastewater load is always stepwise increased for microbial adaption. Definitely, a long time should be paid for the set-up of reactor [30]. In this study, the strain HF-1 bioaugmented system could be constructed with good performance under 1000 mg/L of nicotine load. Operation with stepwise increase of nicotine load is not necessary. Thus, from the perspective of nicotine, there was no influence to the set-up of strain HF-1 bioaugmented system, which will be beneficial to its application in engineering.

4. Conclusions

Inoculum amount plays an extremely important role in the set-up of strain HF-1 bioaugmented system, and the most suitable inoculum amount for strain HF-1 colonization was 1.10 mg/g. Furthermore, initial pH was another critical factor that influences the set-up of strain HF-1 bioaugmented system. The optimal pH is 7.0 for its set-up. Additionally, nicotine load had nearly no inhibition on the set-up of strain HF-1 bioaugmented system. The bioaugmented reactor

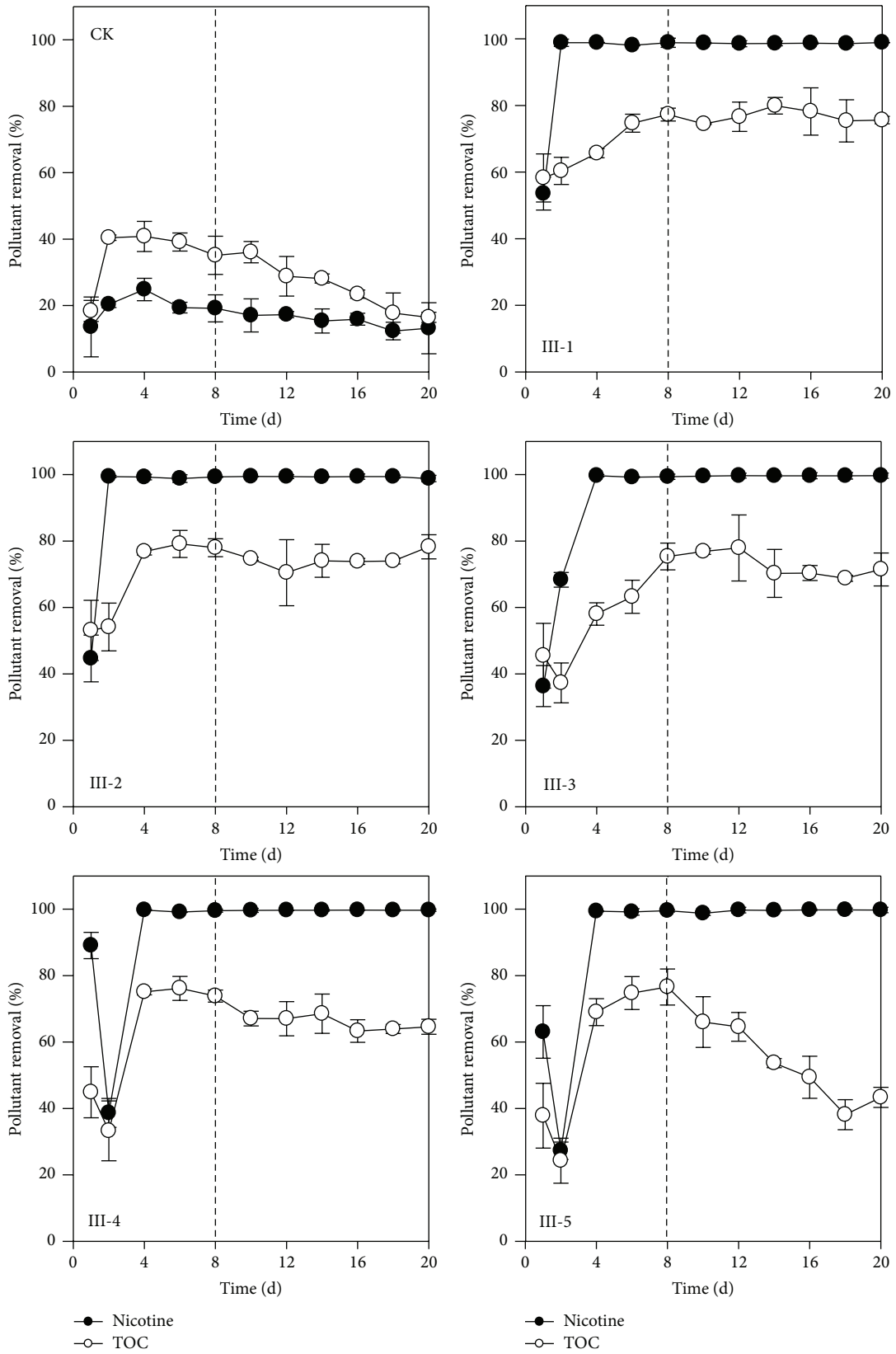

Figure 6: Effect of nicotine load on pollutant removal in tobacco wastewater. The nicotine load in reactor III-1 to III-5 was 250, 500, 800, 1000, and 1200 mg/L, respectively. CK: noninoculated reactor.

was set up successfully under 250 to 1200 mg/L of nicotine. However, the efficiency of TOC removal decreased when nicotine was above 1000 mg/L.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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