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Bioaugmentation strategy for the treatment of fungicide wastewater by two triazole-degrading strains

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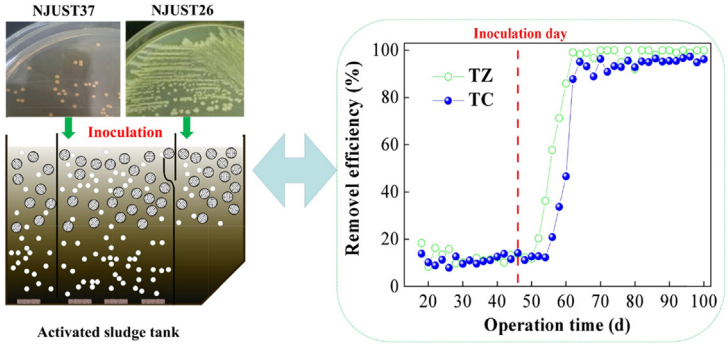
HIGHLIGHTS

- Bioaugmentation strategy was developed for the treatment of fungicide wastewater.
- Inoculation of NJUST26 and NJUST37 obviously enhanced TZ and TC removal.
- Stable bioaugmentation performance could be achieved during long-term operation.
- Both NJUST26 and NJUST37 lost their dominance during long-term operation.
- Bioaugmentation played a key role in formation of effective microbial community.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, bioaugmentation potential of 1H-1,2,4-triazole (TZ) degrading strain namely *Shinella* sp. NJUST26 and tricyclazole (TC) degrading strain namely *Sphingomonas* sp. NJUST37 was evaluated in a bench-scale activated sludge tank. The performance of the bioreactor demonstrated the feasibility of bioaugmentation by NJUST26 and NJUST37 in terms of almost complete TC and TZ removal, significant COD and TOC removal, as well as toxicity reduction. The effect of influent pH, hydraulic retention time (HRT) and triazole fungicide concentration was investigated in order to reach an optimal treatment performance. The bioaugmented process after the inoculation of NJUST26 and NJUST37 could maintain stable performance in terms of TZ, TC, COD and TOC removal from the wastewater. High-throughput sequencing analysis suggested that higher diversity and significant shifts in bacterial community structure were achieved after the inoculation of NJUST26 and NJUST37. However, both NJUST26 and NJUST37 lost their dominance during long-term operation, despite of the key role of the inoculation of NJUST26 and NJUST37 in the formation of effective microbial community.

1. Introduction

Triazole fungicides such as tricyclazole, propiconazole and tebuconazole, a class of systemic antifungal agents containing 1,2,4-triazole moiety, are widely used in both pre- and postharvest control of fungal diseases on fruit, vegetable, legume and grain crops [1]. Their fungicidal activity is exerted through inhibiting 14α-demethylase activity and thereby interfering with fungal cell-wall formation [1,2]. As a

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consequence of enzyme inhibition involved in the biosynthesis of steroid hormones, triazole fungicides can potentially produce endocrine-related side-effects on humans and wildlife [3,4]. Therefore, triazole fungicides often exhibit highly toxic, cancerigenic and teratogenic nature, showing a long half-life in the environment [5,6]. Industrial wastewater containing various triazole fungicides was produced due to the massive manufacture and extensive use of triazole fungicides, which should be properly treated in order to prevent the environmental risk.

Bioremediation, as is well known, plays a crucial role in the treatment of various organic pollutants because of its low consumption, high efficiency and ecological friendliness [7,8]. Nevertheless, the operation of conventional biological system for the treatment of wastewater containing triazole fungicides was rather difficult due to the high resistance and toxicity of triazole fungicides in the biological system. Stamatis et al. [9] indicated that triazole fungicides, such as cyproconazole, penconazole and tebuconazole, showed relatively low removal efficiency after secondary and tertiary treatment in municipal sewage treatment plant. Huete-Soto et al. [10] also found that tebuconazole and triadimenol could not be effectively removed in a biopurification system. These facts could be explained by the lack of specific functional species in the biological treatment system, which could tolerate the high toxicity or even possess the detoxification ability towards certain triazole fungicide. Thus, bioaugmentation through the inoculation of specific functional species could be considered as a promising alternative to solve the aforementioned problems [11,12].

However, strains capable of metabolizing triazoles fungicides were seldomly reported in the previous literatures. Sehnem et al. [13] isolated two tebuconazole degrading strains from soil contaminated by tebuconazole, namely *Enterobacter sakazakii* and *Serratia* sp. Two ipconazole degrading isolates, namely *Kitasatospora* sp. A1 and *Streptomyces* sp. D16, were isolated from paddy soil by Eizuka et al. [14]. *Pseudomonas putida*, which was isolated from tea rhizosphere, was found to be capable of degrading a triazole fungicide namely propiconazole [15]. These studies on biodegradation of triazole fungicides were mainly focused on the investigation of biodegradation performance and biodegradation pathway, whereas papers with technological orientation were in the minority. Very limited information is currently available on the engineering aspects in continuous treatment reactors [16,17]. Both the occurrence and the fate of triazole fungicides in the wastewater treatment system are largely unknown.

Tricyclazole (TC), which is quite persistent in the soil-water system, is a typical antifungal agent used to control rice blast disease [18]. 1H-1,2,4-triazole (TZ) was often used as the parent compound in the synthesis of various triazole fungicides such as TC. Therefore, wastewater from the sites manufacturing triazole fungicides often contains both high strength TZ and triazole fungicides such as TC, which were rather recalcitrant in conventional biological treatment system such as activated sludge tank. For example, the system treating triazole fungicides containing wastewater, which is located in Changzhou Fengdeng Environmental Technology Service Co. Ltd (Jiangsu Province, China), is consisted of air flotation, microelectrolysis, Fenton, coagulation, sedimentation, upflow anaerobic sludge blanket (UASB) and powdered activated carbon treatment tank (PACT). The influent of UASB contained 85–150 mg L^{-1} TZ and 50–60 mg L^{-1} TC, while the effluent of PACT still contained 50–90 mg L^{-1} TZ and 15–30 mg L^{-1} TC. Therefore, further disposal by activated carbon adsorption was used in order to achieve environmentally acceptable discharge, leading to the increase of treatment cost. In order to develop an effective bioaugmentation strategy for the treatment of wastewater from the sites manufacturing triazole fungicides, two strains named as *Shinella* sp. NJUST26 and *Sphingomonas* sp. NJUST37, which could respectively utilize TZ and TC as their sole carbon and nitrogen source, were isolated in our previous study [19].

In this study, both NJUST26 and NJUST37 were inoculated into an activated sludge tank treating synthetic wastewater containing both TZ and TC. The objectives of this study were (1) to evaluate the feasibility of this bioaugmentation strategy based on the inoculation of these two specific functional species for the treatment of triazole fungicide wastewater, (2) to investigate the effect of various operational parameters on TC and TZ removal, and (3) to assess the succession of microbial community structure after the inoculation of these two specific functional species.

2. Materials and methods

2.1. Substrate and inocula

The activated sludge used as the inocula was taken from a full-scale PACT tank located in Changzhou Fengdeng Environmental Technology Service Co. Ltd, which was operated for the treatment of fungicide producing wastewater containing both TZ and TC. TZ degrading strain namely *Shinella* sp. NJUST26 were isolated from soil contaminated by TZ [19]. TC degrading strain namely *Sphingomonas* sp. NJUST37 was isolated from the activated sludge taken from a bioreactor treating fungicide producing wastewater. Before the inoculation of NJUST26 and NJUST37, these two strains were cultivated separately in LB medium at 30 °C and 170 rpm until the bacteria grew into the logarithmic phase (about 36 h after inoculation). Then the bacteria were harvested by centrifugation at 6000 × *g* for 5 min, and then the deposition was resuspended and washed thrice with 0.9% NaCl solution for further use.

The synthetic wastewater was prepared as follows: KH_2PO_4 0.76 g L^{-1}, $Na_2HPO_4{\cdot}12H_2O$ 3.06 g L^{-1}, NH_4Cl 0.1 g L^{-1}, $MgSO_4{\cdot}7H_2O$ 0.2 g L^{-1}, $CaCl_2$ 0.05 g L^{-1}, SL-4 10 mL L^{-1}, TZ and TC at desired concentrations. The trace element solution SL-4 was prepared according to Shen et al. [20].

2.2. Bioreactor operation

A bench-scale activated sludge tank with working volume of 35L (Fig. 1) was designed to verify the bioaugmentation strategy for enhancing TC and TZ removal from wastewater. Fine air bubbles for aeration were supplied by means of air bubble diffusers placed at the bottom of the reactor to keep dissolved oxygen (DO) concentration higher than 5.0 mg L^{-1}. For the immobilization the microbes, polyhedral hollow ball with diameter of 25 mm, density of 144.5 kg/m^3 and specific surface area of 500 m^2/m^3, were added into bioreactor and occupied 20% of reactor volume. The bioreactor was installed in a temperature controller chamber to keep operation temperature at 30 ± 2 °C. Before the start-up of the bench-scale bioreactor, the seed activated sludge was added into the bioreactor at the mixed liquor suspended solids (MLSS) concentration of 3.5 g L^{-1}.

The operation period was divided into six phases and the operational condition for each phase were summarized in Table 1. During the start-up of the bioreactor (phase I), synthetic wastewater containing 100 mg L^{-1} TZ and 30 mg L^{-1} TC was pumped into the reactor with hydraulic retention time (HRT) controlled at 4 d. After 45 days' acclimatization, the performance of the activated sludge tank in terms of TZ and TC removal was found to reach steady status. In phase II, in order to enhance TZ and TC removal, both NJUST26 and NJUST37 were inoculated into the reactor on day 46 at respective dosage of 2.5 g biomass (dry weight), leading to inoculation ratio of 2% for both NJUST26 and NJUST37, as compared to the initial MLSS concentration. 100 days later, the effect of influent pH was investigated through the adjustment of the ratio of KH_2PO_4 and $Na_2HPO_4{\cdot}12H_2O$ in the influent (phase III). In phase IV, the effect of TZ and TC concentration on reactor performance was studied. In phase V, the influent flow rate was gradually reduced to evaluate the system performance at different HRTs. In period VI, the long-term performance of the activated sludge tank was evaluated. For each operational condition, the activated sludge tank was run for at least 10 days to reach a stable performance. At least 10