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Enhanced pyridine biodegradation under anoxic condition: The key role of nitrate as the electron acceptor

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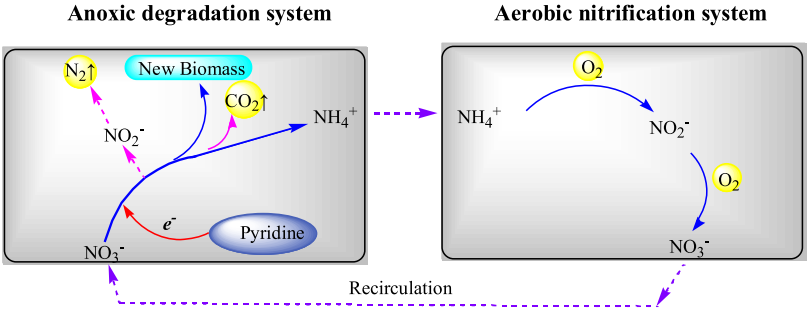
HIGHLIGHTS

- Pyridine biodegradation was significantly enhanced in the presence of nitrate.
- The integrated predenitrification process was effective for pyridine removal.
- High recirculation rate was suggested for the operation of ABR–MBBR system.
- *Paracoccus*, *Thiobacillus* and *Paludibacter* were dominant in the anoxic system.

GRAPHICAL ABSTRACT



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ABSTRACT

Enhanced biodegradation of pyridine under anoxic condition was elucidated in this study through both batch and continuous experiments. The results of the batch experiment indicated that pyridine biodegradation was significantly enhanced in the anoxic condition, probably due to the presence of the electron acceptor, i.e., NO_3^-. Pyridine could be mineralized in the anoxic condition, accompanied by the release of ammonia. Pyridine could be degraded optimally at neutral to slightly alkaline pHs. High concentrations of nitrate or pyridine had inhibitory effects on the anoxic degradation of pyridine. The combined anoxic–aerobic process consisted of an anaerobic baffled reactor (ABR) and a moving-bed biofilm reactor (MBBR) was operated to investigate the performance of pyridine biodegradation and total-N (TN) removal in this integrated system. The NH_4^+ released from pyridine biodegradation in ABR was nitrified completely into NO_3^- in MBBR, which was then recirculated into ABR, serving as the electron acceptor for pyridine biodegradation. With the effluent recirculation rates increased from 0% to 400%, both pyridine biodegradation and TN removal were improved, probably due to the high availability of nitrate and the mitigation of the toxic effect over the biomass at relatively high recirculation rates. In addition, high-throughput sequencing analysis demonstrated that *Paracoccus*, *Thiobacillus* and *Paludibacter* were the dominant species in the anoxic degradation system.

1. Introduction

Pyridine and its derivatives, one class of the most important nitrogenous heterocyclic compounds (NHCs), occur widely in the environment as a result of coal gasification, oil shale retorting,

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pesticide use, and manufacture of dyes, explosives, pharmaceuticals, etc. [1–3]. The release of pyridine and its derivatives into the environment brings about severe health hazards, as they can cause increase in heart rate, heart disease and stroke, blood pressure and even lead to teratogenic and carcinogenic effects [1,4]. Therefore, researchers have devoted themselves to develop effective and economically feasible methods for the remediation of the sites contaminated by pyridine. Various physico-chemical methods have been investigated for the treatment of pyridine-containing wastewater, such as adsorption [5], oxidation [6], microwave radiation [7], liquid–liquid extraction [8], and so on. However, because of their huge economic and energy consumption, the application of these physico-chemical methods is limited. Biological treatment, which is both environmental friendly and cost effective, has become a preferred approach for pyridine removal from wastewater [9].

Previous studies on pyridine biodegradation under aerobic condition have been reported extensively. Most of these studies were concentrated on the isolation and characterization of pyridine-degrading microorganisms [10,11], bioaugmentation of the bioreactors by adding pyridine-degrading microorganisms [11–13], immobilization of the microorganisms capable of degrading pyridine [14,15], and so on. However, considering the nauseating odour at aerobic condition, pyridine degradation under anaerobic or anoxic condition has attracted increasing interest in recent years [16]. Unfortunately, under anaerobic conditions, pyridine biodegradation is relatively slow and thus less attractive for full-scale practical application [17]. However, many organic compounds, which are nondegradable under aerobic conditions or less degradable under anaerobic condition, can be effectively utilized by denitrifying bacteria as carbon sources under anoxic condition [18]. Li et al. [17] investigated the anoxic biodegradation of pyridine, indole, quinoline, isoquinoline and 2-methyl quinoline, demonstrating that anoxic degradation of these refractory NHCs was feasible. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in marine sediment under anoxic conditions was evaluated by Lu et al. [19]. It was also found that biodegradation of these PAHs under anoxic condition was improved to some extent. Nevertheless, a predominance of these present papers focused mainly on the verification of the enhanced degradation performance of the refractory compounds under anoxic condition, whereas papers with technological orientation are in the minority [17].

As is well known, high strength ammonia are often found in most pyridine containing wastewater, such as coking wastewater [20]. Moreover, ammonia can be released during the mineralization of pyridine in the biodegradation process. Predenitrification based on anaerobic and aerobic process (A–O process) was often used for the treatment of coking wastewater [21,22]. In the anoxic reactors, the nitrate produced from the aerobic process could be removed through denitrification, with recalcitrant compounds partially removed simultaneously [23,24]. However, it is still not clear that whether the pyridine biodegradation in the anoxic reactors is closely related to the presence of the nitrate. Systematic investigations are still absent and the mechanisms involved are not fully understood.

Therefore, this study aimed to verify the enhanced biodegradation of pyridine under anoxic condition through both batch and continuous experiments. The key role of the nitrate as electron acceptor during pyridine biodegradation was investigated. An integrated predenitrification process consisted of an anaerobic baffled reactor (ABR) and an aerobic moving-bed biofilm reactor (MBBR) was constructed to study the feasibility and performance of this combined process treating pyridine wastewater. In addition, dominant species in the anoxic degradation system was investigated through high-throughput sequencing analysis.

2. Materials and methods

2.1. Synthetic wastewater and seed sludge

Both batch and continuous experiments were performed in liquid mineral salt medium (MSM) containing phosphate buffer (7 mM for continuous experiment and 70 mM for batch experiment at desired pHs), $MgSO_4{\cdot}7H_2O$ (0.2 g L^{-1}), $CaCl_2$ (0.05 g L^{-1}) and SL-4 (1 mL L^{-1}). In MSM, KH_2PO_4 and $Na_2HPO_4{\cdot}12H_2O$ served as the phosphate buffer. Pyridine was added into MSM as the sole carbon sources at desired concentrations, while $NaNO_3$ was added at desired concentrations as the supplementary electron acceptor. The composition of SL-4 was described previously by Shen et al. [25].

The seed sludge for the anoxic process was collected from a sludge storage tank of a lab-scale bioreactor treating pyridine containing wastewater. In order to increase the pyridine removal efficiency, a pure pyridine-degrading bacterium, *Rhizobium* sp. NJUST18 [26,27], was added into the seed sludge as supplement. The ratio of NJUST18 to the seed sludge is 1:10 (measured in dry weight), and the mixture was used as the inocula for both batch reactors and ABR. The aerobic process was initially inoculated with the sludge taken from a secondary sedimentation tank of a municipal wastewater treatment plant in Nanjing, China. Before use, the seed sludge was washed five times using liquid MSM so as to remove any substrate residues in the sludge.

2.2. Batch experimental procedure

To evaluate the pyridine removal performance under different operating conditions, batch experiment was conducted in a series of 100-mL serum bottles as batch reactors. 100 mL MSM was placed in each batch reactor for batch experiment. These batch reactors was inoculated with the seed sludge mixture as described above at initial mixed liquor suspended solid (MLSS) concentration of 10 g L^{-1} and was incubated at 30 °C in a biochemical incubator. To maintain anaerobic condition, the solution was purged with nitrogen gas for at least 10 min to remove any residual dissolved oxygen, and the reactors were sealed by butyl rubber stoppers.

Three groups of experiment were performed at different initial pHs (5.0, 6.0, 7.0, 7.5, 8.0 and 9.0), different NO_3^--N dosage (0, 82, 165, 247, 329, 389 and 779 mg L^{-1}) and different initial pyridine concentration (250, 500, 1000, 2000 and 3000 mg L^{-1}). To investigate the pH effect, the initial pyridine concentration was controlled at 500 mg L^{-1}, while nitrate was added at the theoretical dosage (theoretically, 389 mg L^{-1} NO_3^--N was required for complete biodegradation of 500 mg L^{-1} pyridine through stoichiometric denitrification). To maintain pHs constant, the phosphate buffer (70 mM) was used. The pHs were changed through changing the proportion of KH_2PO_4 and $Na_2HPO_4{\cdot}12H_2O$ in the buffer system. To study the effect of NO_3^--N dosage, the initial pH and pyridine concentration was controlled at 7.5 and 500 mg L^{-1}, respectively. To investigate the effect of initial pyridine concentration, the initial pH was controlled at 7.5 and NO_3^- was added at the theoretical dosage.

During the experiments, pyridine, NO_3^--N, NO_2^--N and NH_4^+-N concentrations were monitored at regular time intervals. To maintain anaerobic condition, sampling was performed in an anaerobic box.

2.3. Continuous experiment procedure

Continuous experiment was carried out in an integrated ABR/MBBR process, as was illustrated in Fig. 1. The ABR was a rectangular box with effective volume of 6 L. It was divided into four