

The effect of $\text{Fe}^0/\text{Fe}^{2+}/\text{Fe}^{3+}$ on nitrobenzene degradation in the anaerobic sludge

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Abstract

The influence of $\text{Fe}^0/\text{Fe}^{2+}/\text{Fe}^{3+}$ on the nitrobenzene (NB) degradation in the anaerobic granular sludge was studied and the results demonstrated that: adding iron powder into the anaerobic sludge could exert an accelerative effect on the NB degradation and the degradation rate was faster than that by using iron or the anaerobic sludge alone. The external addition of $\text{Fe}^{2+}/\text{Fe}^{3+}$ exhibited different influences on the NB degradation depending on the concentrations of $\text{Fe}^{2+}/\text{Fe}^{3+}$ and the solution's pH. When $\text{Fe}^{2+}/\text{Fe}^{3+}$ are less than 100 mg/L at pH 6, $\text{Fe}^{2+}/\text{Fe}^{3+}$ inhibited the NB degradation slightly, and when $\text{Fe}^{2+}/\text{Fe}^{3+}$ was 100–200 mg/L, the NB degradation was enhanced. When pH was shifted to 9, Fe^{2+} of lower than 100 mg/L promoted the NB degradation, and 200 mg/L Fe^{2+} inhibit the NB degradation. The synergism of combined use of iron and the anaerobic sludge in treating NB wastewater was proposed, and adjusting the concentrations of $\text{Fe}^{2+}/\text{Fe}^{3+}$ in the anaerobic sludge according to the pH of the wastewater could be an effective method to obtain a high removal rate of NB.

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1. Introduction

Nitrobenzene (NB) is a toxic and suspected carcinogenic compound that has been used by many industries for the production of aniline, aniline dyes, explosives, pesticides and drugs. Nitrobenzene is also used as a solvent in products like paint, shoe, floor and metal polishes [1,2]. As a result of improper handling of industrial wastewaters, nitrobenzene might be released to the surface and groundwater and pose an adverse effect on human health [3]. It should be noted that nitrobenzene does not occur naturally in water and the concentrations in water depend on the degree of contamination that originates from the source [4]. The half-life of nitrobenzene in aquatic environments has been estimated at 0.3 days by the US EPA [5]. For the safety of aquatic animals and the health of human beings, specific studies on removal of nitrobenzene from water and mineralization into harmless end products (CO_2) are necessary. Previous studies indicated that the electron-deficient character

of the nitro-group prevents mineralization of NB by microorganisms [6]. Therefore, conventional biological treatment processes are usually not effective for the treatment of NB-rich wastewaters [7]. But anaerobic biodegradation is generally believed to be a feasible technique for the removal of nitrobenzene. Compared to physical–chemical processes, biodegradation technology is applied widely in wastewater treatment plants due to its low cost and other important advantages [8]. Frieda and Nava [9] combined the simultaneous adsorption and biodegradation process to treat wastewater containing nitrobenzene. The active carbon was used to absorb a great amount of toxic chemicals and microorganisms subsequently degrading the nitrobenzene to a lower concentration. The whole process well combines both advantages of physical absorption and biodegradation. Cai [10] studied the characteristics of micro-electrolysis-biochemical process in treating nitrobenzene wastewater and the removal rate of COD and NB reached 86 and 99%, respectively.

Because nitrobenzene consists of electron withdrawing group- NO_2^- attached to a benzene ring, which makes it less susceptible to attack from oxidants. Nitrobenzene is more open to be decomposed in reductive conditions than in oxidizing conditions. The effect of iron has attracted more attention in water

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treatment since they may interact with the chemicals through catalytic reactions [2,11–14]. The oxidized products of elemental iron were Fe^{2+} and Fe^{3+} which may result in metal pollution by improper discharge from industry plants like Smelters. Therefore, research on the reductive potential of Fe^{2+} and the oxidation/coagulation of Fe^{3+} on NB reduction degradation is promising but by far the relevant reports are still sparse.

The purpose of this paper is to undertake a systematic approach to study the influence of $\text{Fe}^0/\text{Fe}^{2+}/\text{Fe}^{3+}$ on the NB degradation in the anaerobic sludge. The NB degradation by iron or the anaerobic sludge alone was compared with that by the anaerobic sludge added with iron, and the synergism of the combined use of iron and the anaerobic sludge was discussed. The influence of $\text{Fe}^{2+}/\text{Fe}^{3+}$ on the NB degradation was investigated at different $\text{Fe}^{2+}/\text{Fe}^{3+}$ concentrations (0, 50, 100 and 200 mg/L) and pHs (6 and 9).

2. Materials and methods

2.1. Experimental set-up

2.1.1. The anaerobic sludge and the operation of bioreactors

The anaerobic sludge used for inoculation was obtained from a full-scale internal circulation (IC) reactor that treated the wastewater containing citric acid in Jiangsu province, China, and the sludge was acclimated with nitrobenzene in a laboratory scale UASB (effective volume 3.5 L). Fig. 1 presents the reactor body, which was controlled at $35 \pm 2^\circ\text{C}$ by a constant temperature chamber. The anaerobic sludge was first fed with 2000 mg/L glucose as the carbon source, which enhanced the biological activity of the anaerobic microorganisms. This period lasted 10 days and the COD removal rate was over 80% before the addition of NB in the feeding water. In the following 60

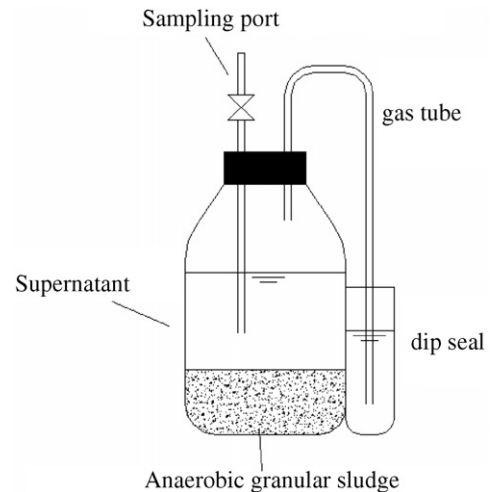


Fig. 2. Schematic diagram of anaerobic serum bottle reactor.

days, the NB concentration was gradually increased in the feeding water which was from 0 to 50 mg/L. The NB concentration of the influent was not increased until the removal rate of NB was over 80%. The pH in the influent was adjusted to 6.8–7.2 using NaHCO_3 .

2.1.2. Batch experiment set-up

The batch experiment was carried out through shake flask study. Fig. 2 shows the schematic diagram of the serum bottle used for the batch experiment. The effective volume of the serum bottle is 200 with 60 mL anaerobic sludge and 140 mL nutrient medium. The composition of nutrient medium in serum bottle included 100 mg/L yeast extract and trace elements containing (mg/L): $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 200; H_3BO_3 , 10; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 25; NiCl_2 , 25; CuSO_4 , 10; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 100; $(\text{NH}_4)_6\text{MoO}_{24} \cdot 4\text{H}_2\text{O}$, 10; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 25; NaCl , 50. NaHCO_3

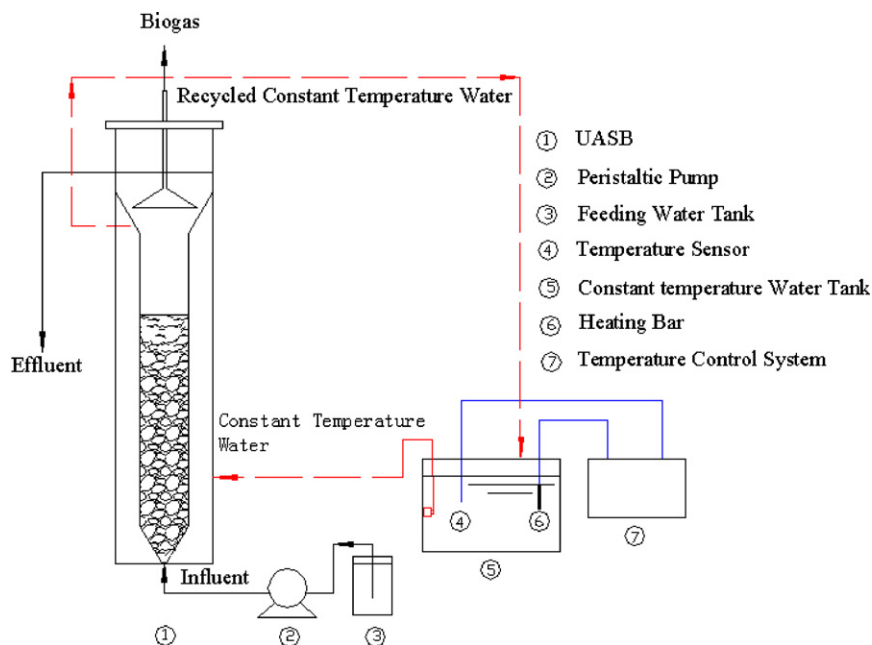


Fig. 1. Schematic diagram of the continuous UASB.

Table 1

Design of the experiments for comparing the effect of Fe⁰ and the combined use of iron and the anaerobic sludge on the NB degradation

Group	Anaerobic sludge (mL)	Nitrobenzene (mg/L)	pH	SS (g/L)	Solution volume (mL)	Fe ⁰ (mg/L)			
						Bottle 1	Bottle 2	Bottle 3	Bottle 4
1	60	50 ± 5	6.0	60–80	150	0	20	60	100
2	0	50 ± 5	6.0	60–80	150	0	20	60	100

Table 2

Design of the experiments for investigating the effect of Fe²⁺/Fe³⁺ on the NB degradation

Group	Glucose (mg/L)	Nitrobenzene (mg/L)	pH	SS (g/L)	Solution volume (mL)	Fe ²⁺ /Fe ³⁺ concentration (mg/L)			
						Bottle 1	Bottle 2	Bottle 3	Bottle 4
1,2	2000	50 ± 5	6.0	60–80	150	0	0.5	1.0	2.0
3,4	2000	50 ± 5	6.0	60–80	150	0	50	100	200

was added consistently to maintain a proper pH and buffering capacity. Resazurin was used for monitoring oxygen contamination. The constant temperature shaker was maintained at 35 ± 3 °C and 150 rpm. The flasks were flushed with pure nitrogen (N₂) to expel molecular oxygen (O₂) before the flasks were loaded with sludge and other solutions. The experiments to investigate the effect of Fe⁰/Fe²⁺/Fe³⁺ were conducted as follows: (1) to study the independent effect of iron (no. 200 mesh) and its mixture with anaerobic sludge on the degradation of nitrobenzene, two groups of serum bottles were prepared and their compositions were designed as shown in Table 1; (2) four groups of serum bottles were used to investigate the effect of Fe²⁺/Fe³⁺ at different concentrations as shown in Table 2. The control system is the reaction which contained the same amount of the anaerobic sludge without Fe²⁺/Fe³⁺ added. Ferrous chloride and ferric chloride was used to provide Fe²⁺ and Fe³⁺, respectively. The pH of the mixtures were determined with a digital pH meter and calibrated to pH 6 and 9 using phosphatic buffers. pH 6 and 9 were selected as the initial pH because a too low or too high pH level will inhibit the growth of anaerobic microorganisms.

2.2. Analytical methods

The UV–vis absorption spectrum of NB was determined by a UV spectrophotometer (UV 1700PC, Shimadzu Co.). The

measurement of NB was carried out using an HPLC (Model 1050, Agilent Co.) with a UV detector and a Synchropak RP-Column (250 mm × 4.6 mm). The mobile phase was the mixture of CH₃CN/H₂O in the proportion of 20/80 (v/v) and the flow rate was 1.0 mL/min. The wave length for detection was 275 nm and injection volume per sample was 10 μL. Prior to HPLC analysis, sample solutions were filtered by 0.45 μm membrane [15].

Electron transport system (ETS) activity was employed by an INT method for assessing metal influence on anaerobic sludge [16,17]. Specific methanogenic activity (SMA) was also measured to evaluate the metal influence on methane bacteria.

2.3. Statistical analysis

The results are reported as the mean ± S.E.M. All the experiments were repeated three times and each value of the data was the mean of three samples. Values of *P* were determined using Student's *t*-test. The statistical analysis was processed using SPSS 13.0 and the figures were exported by Origin 7.5.

3. Results and discussion

3.1. UV spectra of NB and aniline

Fig. 3 illustrated the typical UV–vis spectra of the raw NB and aniline. Aniline was the main intermediate product during the

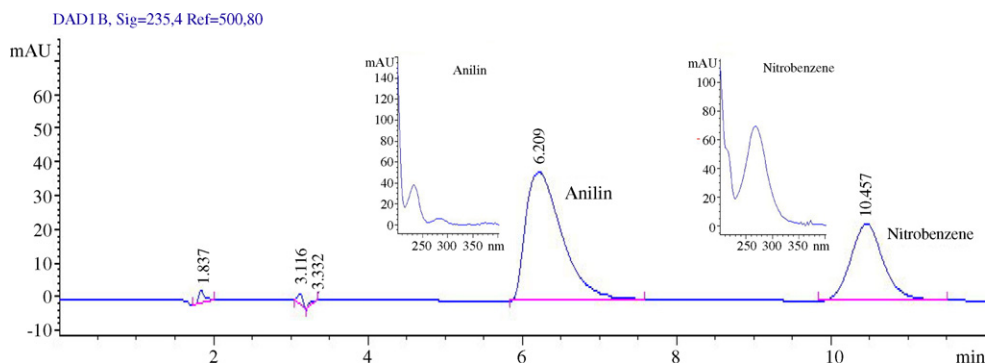


Fig. 3. HPLC spectrum of degradation process of nitrobenzene with UV absorption spectra of aniline and raw NB.

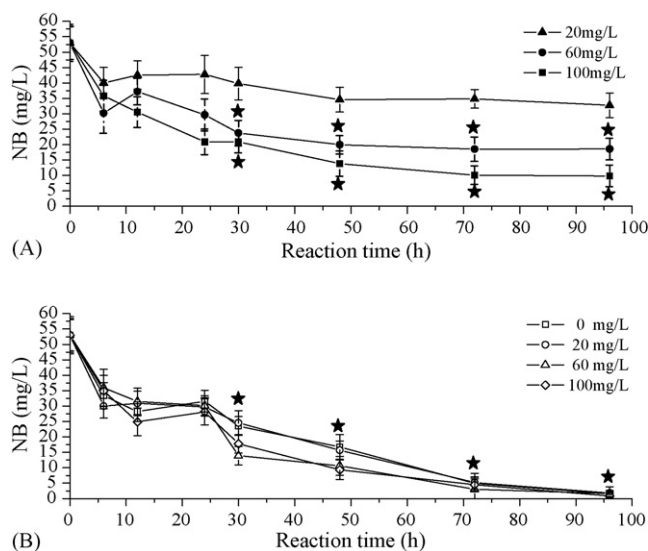
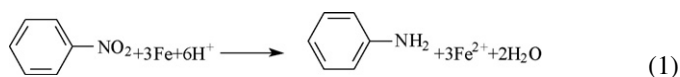


Fig. 4. The reductive degradation of NB by zero-valent iron (A) and by the anaerobic sludge added with Fe⁰ (B) at pH 6. Error bars indicate S.E.M., $n = 3$. * $P < 0.05$ compared with each data of 20 mg/L Fe⁰ for (A) and * for (B) compared with reaction time = 0.

NB degradation in our results which coincided with the previous study [18]. The characteristic absorbance band of NB was at 270 nm and the characteristic absorption bands of aniline were at 229 and 280 nm.

3.2. Comparison of the NB degradation in the reductive reaction by Fe⁰ and the anaerobic sludge added with Fe⁰

Fig. 4A showed the NB degradation curves of the reductive reaction by Fe⁰ at pH 6 and Fig. 4B presented the NB biodegradation curves in the anaerobic sludge with the addition of iron powder. Fig. 4A showed that NB could be degraded by iron at pH 6 and the complete reduction of NB to aniline by iron can be expressed by the following Equation (1) [19]. In our study, three concentrations of iron: 20, 60, and 100 mg/L were added into the sludge and results indicated 100 mg/L iron removed the most NB amount, from 53.0 to 9.8 mg/L and significant differences existed between the data of 100 mg/L Fe⁰ and that of 20 mg/L Fe⁰ according to the Student's *t*-test as indicated by $P < 0.05$ in Fig. 4A.



In the initial 10 h, a sharp decrease of NB concentration was observed in Fig. 4A and soon afterwards the concentration of NB began to increase, and this may be a result of adsorption of NB on the iron powder. Although iron powder is supposed to dissolve slowly in acid solution, in the initial 10 h, most iron powder was not dissolved and still acted as sorbent to organic pollutants. Likewise, the decrease of NB by adsorption of the anaerobic sludge could also be observed in the initial 20 h in Fig. 4B. However, the adsorptive capacity and adsorption time by the anaerobic sludge were much larger than that of iron powder.

Fig. 4B indicated that the iron powder in the sludge did not obviously enhance the NB degradation. Compared to NB degradation curves of single Fe⁰, it is easy to tell that a substantial proportion of NB was actually degraded by the anaerobic sludge in the mixed system of iron and anaerobic sludge. The combined use of iron and anaerobic sludge, however, increased the NB degradation rate compared to that of the single Fe⁰ reaction system or the anaerobic sludge without Fe⁰ added. In less than 100 h, NB was almost degraded completely in the mixed system of iron and anaerobic sludge, but in the single iron reaction, NB concentration remained 9.8 mg/L after 70 h without any further degradation as shown in Fig. 4A. Likewise, NB was degraded to 13.8 with 60 mg/L Fe⁰ in the sludge after 30 h while NB was only removed to 23.5 mg/L in the sludge without Fe⁰. It revealed that the anaerobic biomass and the iron powder could well cooperate and exhibit the synergism in the NB degradation.

Although iron powder in the acid condition could well react with NB by providing electrons during the transformation of NB to aniline, the surfaces of iron particles were limited and some reaction products like Fe(OH)₂, Fe(OH)₃ might adhere to surfaces of iron particles and blocked the reactions of iron [20,21]. Furthermore, the reductive reaction like Eq. (1) could cause redox and pH changes that might hamper reductive reaction of NB. Therefore, the reductive reaction could not degrade NB completely. On the contrary, in the mixed system of iron and sludge, iron powder could be well distributed in the apertures of anaerobic granular sludge. Fe²⁺/Fe³⁺ from iron oxidation could be adsorbed as the growth element of anaerobic microorganisms. A certain buffer ability of anaerobic sludge also avoided sharp changes of ORP and pH in the solution which is helpful for the reductive reaction. In addition, some intermediate products of microbial metabolism like the reducing agents (e.g. NADPH, sulphide) can provide a huge reductive power for the NB degradation [6].

The synergism of combined use of zero-valent iron and anaerobic sludge for the accelerative effect on NB degradation probably lied in the formation of a stable Fe⁰-bacterial body structure which exhibited the following features of synergism: (1) iron in the sludge could react with the oxidizer by providing electrons as micro-batteries and the reactions lowered the solution ORP which was beneficial for the anaerobic metabolism; (2) the rusting process of Fe⁰ produced Fe²⁺/Fe³⁺ for the anaerobic microorganisms as mineral elements; (3) the electronegative anaerobic bacteria were easily attached on the surfaces of iron particles due to the static function and the iron particles were then covered and embedded in the zoogloea where a stable Fe⁰-bacterial body system gradually formed; (4) the surfaces of iron particles could be a good reaction sites for electron transfer, mass transfer, and transformation of the organic matter [22]. According to the microscopic observation, Fig. 5 was drawn to illustrate the structure of Fe⁰-bacterial body system formed in the mixture of iron powder and anaerobic sludge. The above analysis revealed that adding iron powder in the anaerobic sludge is a feasible method to enhance the degradation of NB and still other governing factors like pH control need further studying.

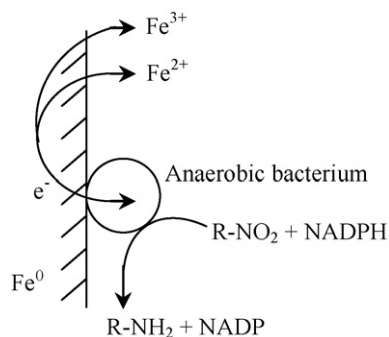


Fig. 5. The Fe^0 -bacterial body structure in the mixture of iron powder and the anaerobic sludge.

3.3. The effect of $\text{Fe}^{2+}/\text{Fe}^{3+}$ on NB degradation under the anaerobic condition

The results showed that the NB degradation in the anaerobic sludge with $\text{Fe}^{2+}/\text{Fe}^{3+}$ (<2 mg/L) was not remarkably influenced mainly because $\text{Fe}^{2+}/\text{Fe}^{3+}$ at low concentrations would be absorbed by the microorganisms as mineral elements [13,23]. Therefore, the NB degradation with $\text{Fe}^{2+}/\text{Fe}^{3+}$ concentration from 0 to 200 mg/L in the anaerobic sludge at pH 6 was investigated systematically and the results were shown in Fig. 6.

Fig. 6A showed that the NB degradation with 0, 50, 100, and 200 mg/L Fe^{3+} in the sludge which is very similar with that of Fe^{2+} . As can be seen, when Fe^{3+} was lower than 100 mg/L, the NB degradation was not obviously enhanced but slightly inhibited by adding Fe^{3+} compared to that of 0 mg/L Fe^{3+} because in 47 h NB was removed from 53.0 to 26.7 with 0 mg/L Fe^{3+} while NB was degraded to 30.9 with 50 mg/L Fe^{3+} . However, Fe^{3+} of 200 mg/L seemed to strengthen NB degradation in the anaerobic sludge by degrading NB from 53.0 to 2.0 mg/L in 47 h which clearly showed the accelerative effect of high concentrations of Fe^{3+} on the NB degradation. The influence of Fe^{3+} on

the NB degradation is complicated since high concentration of Fe^{3+} at pH 6 would transform into amorphous colloid in water phase and the coagulation process generally occurred before the biological degradation which meant that $\text{Fe}(\text{OH})_3$ precipitates could have NB directly removed by adhering on surface of colloid as a phase change. Besides this, NB could also be removed from the solution by forming metal–organic complexes through electrical neutralization or adsorbing onto metal hydroxide colloid surfaces [24]. In most cases, the mechanism of electrical neutralization occurs at low-coagulant doses and acidic conditions, while the latter mechanism occurs at high-coagulant doses and alkalic conditions [25,26]. Therefore, for lower than 100 mg/L Fe^{3+} , NB was supposed to be removed by forming metal–organic complexes through electrical neutralization and the low NB removal rate might be due to the drop of pH caused by the hydrolysis reaction of Fe^{3+} that decreased the microbial activity and lowered the biodegradation amount of NB. Likewise, main mechanism of removing NB when Fe^{3+} increased to more than 100 mg/L was the colloid adsorption and precipitation. Another effect of Fe^{3+} on the degradation of NB is that under low pH, a complex reaction may occur resulting in a soluble complex compound that cannot be removed by settling or filtering [27]. As shown in Eq. (2), the functional groups $-\text{OH}$ of phenyl hydroxylamine (an intermediate product of NB) could coordinate with Fe^{3+} to form a soluble complex compound that lessens the hydrolysis reaction of phenyl hydroxylamine which would lead to incomplete degradation of NB.



Analogous results were obtained for the effect of Fe^{2+} on the degradation of NB as shown in Fig. 6B. After about 45 h, the NB removal rates with Fe^{2+} concentrations of 0, 50, 100 and 200 mg/L were 86, 76, 71 and 93%, respectively, indicating that 100 mg/L was believed to be a critical point at which the effect of Fe^{2+} began to change by inhibiting or promoting NB degradation. Lower than 100 mg/L, Fe^{2+} exerted slight inhibition to the NB degradation by removing 80.4% NB in 48 h comparing to the removal rate of 86.6% in the sludge without Fe^{2+} . However, 200 mg/L Fe^{2+} increased the NB degradation speed by removing 95.2% NB in 48 h. The abatement of the NB removal rate as Fe^{2+} approached to 100 mg/L might be a result of the inhibition of Fe^{2+} on the microbial metabolism if the anaerobic biodegradation was believed to play the key role of degrading NB. When the concentration of Fe^{2+} continued to increase, Fe^{2+} as the electron donor could promote the reductive removal of NB. Meanwhile, other types of the removal of NB like the anaerobic degradation and even the precipitate of $\text{Fe}(\text{OH})_2$ should co-exist and finally resulted in the higher removal efficiency of NB than that of control system.

In order to further investigate the effect of $\text{Fe}^{2+}/\text{Fe}^{3+}$ on the NB biodegradation under alkalic conditions, pH was changed from 6.0 to 9.0 and interesting results were observed that Fe^{2+} under alkalic condition displayed a different effect on the NB degradation from the acid condition as Fig. 7 showed that the degradation speed of NB with 100 mg/L Fe^{2+} in the sludge was faster than the other three conditions which removed NB from

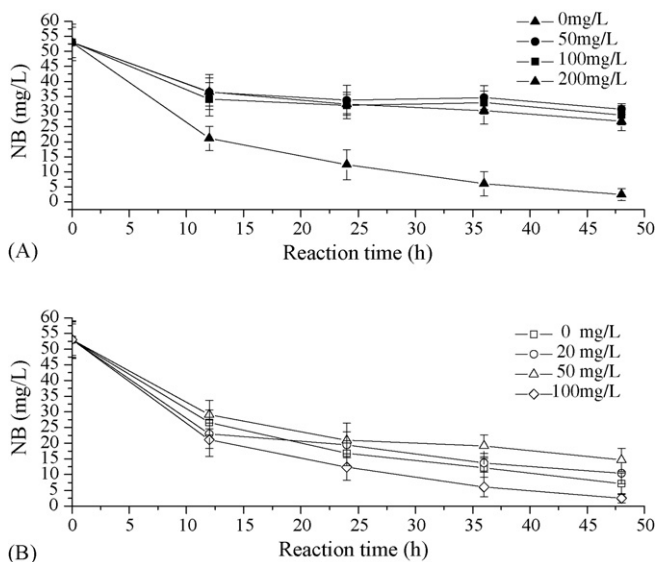


Fig. 6. The NB degradation in the anaerobic sludge with additions of Fe^{3+} (A) and Fe^{2+} (B) at pH 6. Error bars indicate S.E.M., $n = 3$.

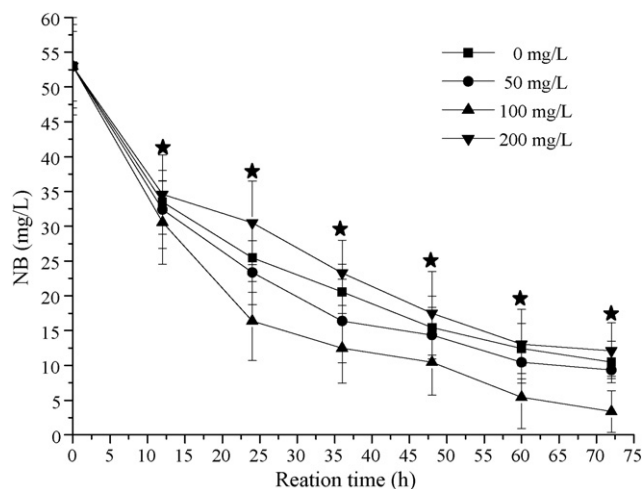


Fig. 7. The NB degradation in the anaerobic sludge added with Fe^{2+} at pH 9. Error bars indicate S.E.M., $n=3$. * $P < 0.05$ compared with reaction time = 0.

53.0 to 3.3 mg/L in 72 h. The NB degradation was enhanced under low Fe^{2+} concentrations (≤ 100 mg/L) and conversely 200 mg/L Fe^{2+} in the sludge inhibited the NB degradation by removing NB from 53.0 to 12.1 mg/L in 72 h. But adding Fe^{2+} in the anaerobic sludge at pH 9 produced a lower NB degradation rate when compared with the results at pH 6. Anaerobic microorganism would be inhibited at pH 9, and the biodegradation of NB should not play the key role of removing NB. Therefore, chemical reduction of NB was the only reasonable explanation which included that the reductive degradation and the removal by precipitation were both involved as $\text{Fe}(\text{OH})_2$ appeared in the solution. In the test, it was found the yellow precipitates attached on the serum wall and accumulated more with higher Fe^{2+} concentrations which was obviously the Fe^{3+} salts and testified that NB was degraded in the reductive reaction with Fe^{2+} . In the other way, NB was adsorbed and removed from water phase by the adsorption of the negative colloid. The influence of Fe^{3+} at pH 9 was not tested due to the fact of extremely rapid and uncontrolled hydrolysis to form $\text{Fe}(\text{OH})_3$ precipitates.

3.4. The influence of $\text{Fe}^0/\text{Fe}^{2+}$ on activity of anaerobic sludge

Electron transport system (ETS) activity was employed by the INT-method to describe the effect of the iron and iron ions on dehydrogenase activity of anaerobic sludge [16]. The iron powder did not exert much influence on the activity of the anaerobic bacteria in term of ETS because iron was dissolved slowly after being mixed in the sludge. But SMA results showed that gas production rate of the sludge with iron added was higher than that of control system which was possibly caused by the extra production of hydrogen gas by the reactions of iron powder in the acid solution.

Fe^{2+} is an influencing factor for the activity of the anaerobic bacteria and Fig. 8 illustrated the influence of different Fe^{2+} concentrations on ETS by means of INT method. The changes of ETS of the original sludge and the sludge that was acclimated for more than 40 days by feeding NB were both displayed in

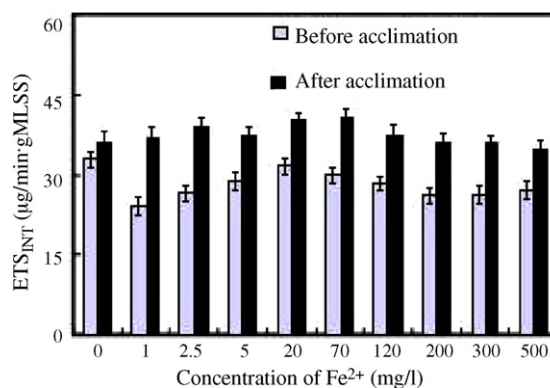


Fig. 8. The changes of ETS_{INT} of the anaerobic sludge under different concentrations of Fe^{2+} before and after acclimation.

Fig. 8. The activity of the original sludge was lower at each level of Fe^{2+} concentrations than that of the acclimated sludge. The activity of the acclimated sludge was not significantly inhibited by Fe^{2+} and even ETS was increased when Fe^{2+} was from 1 to 70 mg/L and as Fe^{2+} concentration further increased, the ETS began to shift down slightly. However, it can be observed that long-term acclimation could enhance the activity of sludge and reduce the influence of Fe^{2+} . As Takashima and Speece [28] reported, ferrous chloride in anaerobic system could dramatically increase the utilization rate of acetate ($4\text{--}8$ kg/ m^3) and cause the changes of predominant methanogenic bacteria (predominant bacteria transformed from *Methanosarcina barkeri* to *Methanotrix soehngenii*). Our results showed that when Fe^{2+} is less than 50 mg/L, gas production rate obviously increased; when Fe^{2+} was shifted to a higher concentration, the SMA of the anaerobic sludge changed slightly.

Because the INT agent can be oxidized by Fe^{3+} and therefore the ETS indicator can not precisely reflect the activity of the anaerobic sludge. The SMA of the anaerobic sludge was found to be lower than the control system when FeCl_3 was added into the sludge without adjusting pH because the low pH caused by the hydrolysis of Fe^{3+} in the solution decreased the activity of the anaerobic sludge and also the granular sludge might be surrounded and buried by the colloid of $\text{Fe}(\text{OH})_3$ which hampered the metabolism of anaerobe and blocked the bio-gas production.

3.5. Kinetic analysis of reductive reaction rate in the mixture of zero-valent iron and sludge

In the previous studies [18,29], the reductive degradation rate of reactants was described by an empirical equation analogous to the Michaelis–Menten equation as follows:

$$\frac{dC_t}{dt} = \frac{V_m C_t}{K_{1/2} + C_t} \quad (3)$$

where C_t is the NB concentration at reaction time, V_m the maximum reaction rate (mg/L h), and $K_{1/2}$ defined as C_t at $V_m/2$ (mg/L) [29]. It can be presumed that $K_{1/2}$ and V_m are constants for a certain substrate. Eq. (3) was used for simulating the process of the reductive reaction of NB with the anaerobic sludge

and zero-valent iron mixed together in Fig. 4B and determined that $K_{1/2}$ is the 19.6–35.0 mg/L and V_m is the 1.3–1.7 mg/L h.

3.6. HPLC analysis of the reduction product of NB

The effluent sample from bioreactors was injected to HPLC equipment to further test the reductive pathways of NB. Fig. 3 shows the chromatogram of the NB and its intermediate product aniline. The peaks with retention time of 10.457 and 6.209 min were identified as nitrobenzene and aniline, respectively, based on the UV absorption spectra. It is known that the biodegradability of aniline is much better than that of NB in the aerated active sludge process [7]. Therefore, after the initial treatment by Fe^0 or anaerobic sludge, the NB-laden wastewater would be readily treated in a subsequent biological process.

Research on elucidation of the reaction mechanism of the reductive carbonylation of nitrobenzene to carbamate has been in progress for many years. However, this mechanism has not as yet been conclusively learnt. One of the suggested mechanisms is a direct carbonylation of nitrobenzene to carbamate by an intermediate nitrene stage [30]. Some authors agree that this reaction proceeds via an intermediate stage of aniline formation, which is subsequently directly carbonylated to carbamate [30,31]. An alternative mechanism postulated involves aniline carbonylation to isocyanate, which immediately reacts with the excess aniline to afford diphenylurea [32,33].

4. Conclusions

Bench-scale UASB reactors were operated to cultivate and acclimate the anaerobic sludge by feeding NB in the influent. The influence of $\text{Fe}^0/\text{Fe}^{2+}/\text{Fe}^{3+}$ in the anaerobic sludge on the NB degradation were investigated through shake flask study. The results illustrated that there is a good correlation between the NB degradation rate and the concentration of iron powder under acid condition and significant differences existed between the data of 100 mg/L Fe^0 and that of 20 mg/L Fe^0 proving that the more iron was used, the higher the NB removal rate was obtained. The accelerative effect of the combined use of zero-valent iron and the anaerobic sludge on the NB degradation was observed. The degradation rate of NB by the anaerobic sludge added with iron is faster than that of the single Fe^0 reaction system or the anaerobic sludge without Fe^0 added. The synergism of the anaerobic sludge and iron on the NB degradation primarily lied in the formation of a stable Fe^0 -bacterial body structure based on the microscopic observation.

The external addition of $\text{Fe}^{2+}/\text{Fe}^{3+}$ could either inhibit or promote the NB degradation depending on their concentrations and pH. When $\text{Fe}^{2+}/\text{Fe}^{3+}$ are less than 100 mg/L at pH 6, $\text{Fe}^{2+}/\text{Fe}^{3+}$ inhibited the NB degradation slightly compared to the control system, while 100–200 mg/L, $\text{Fe}^{2+}/\text{Fe}^{3+}$ displayed promotion to the NB degradation. However, when the solution's pH was shifted from 6 to 9, 0–100 mg/L Fe^{2+} promoted the NB degradation; on the contrary, 200 mg/L Fe^{2+} lowered the NB degradation rate. Significance analysis also indicated that 100 mg/L Fe^{2+} at pH 9 and 200 mg/L Fe^{2+} at pH 6 could promote the NB degradation with the reliability of 99.5%.

In conclusion, adding iron powder in the anaerobic sludge is a promising and effective method to treat the NB-laden wastewater. Furthermore, this paper also demonstrated that $\text{Fe}^{2+}/\text{Fe}^{3+}$ in the anaerobic sludge could exert an accelerative effect on the NB degradation if appropriately adjusting the concentrations of $\text{Fe}^{2+}/\text{Fe}^{3+}$ according to the solution's pH.

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