Scaling-up of a zero valent iron packed anaerobic reactor for textile dye wastewater treatment: a potential technology for on-site upgrading and rebuilding of traditional anaerobic wastewater treatment plant

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ABSTRACT

Anaerobic digestion (AD) is a cost-effective technology for the treatment of textile dye wastewater with clear environmental benefits. However, the need to improve process feasibility of high treatment efficiency as well as to shorten hydraulic retention time has raised interest on several intensification techniques. Zero valent iron (ZVI) packed anaerobic digesters have the potential to become an on-site upgrading wastewater treatment technology through building a ZVI bed in a traditional AD plant. However, the experiences and knowledge of scale-up are limited. In this study, a pilot-scale ZVI packed upflow anaerobic sludge bed (ZVI-UASB) was built up and operated for actual dye wastewater treatment in a textile dye industrial park. Results showed that the treatment performance of this digester is higher than that of a traditional AD plant in terms of chemical oxygen demand (COD) removal and color removal. During 90 days of operation, the average COD removal and color removal in ZVI-UASB was maintained at around 19% and 40%, respectively, while it was only 10% and 20%, respectively, in the traditional AD plant.

Key words | anaerobic treatment, dye, scale-up, zero valent iron (ZVI)

INTRODUCTION

A great amount of dyes and organic agents are produced from textile dye industries, which is liable to cause serious risk to ecological safety and human beings (Weisburger 2002). Every year there has been a large amount of textile dye wastewater, with high chromaticity and complex ingredients resisting decomposition, discharged into the environment, which has increased the processing difficulty (Dos Santos et al. 2007). Application of conventional physical and chemical methods, such as adsorption, flocculation and electrochemical, for treating dye wastewater is limited because of high cost and production of secondary pollutants (Robinson et al. 2001; van der Zee & Villaverde 2005). Alternatively, anaerobic–aerobic processes are economically effective and environment friendly, and have been widely applied for the treatment of dye wastewater (Tomei et al. 2016). During the anaerobic digestion (AD) process, chromophores in the dye molecule, including azo (-N=N), carbonyl (-C=O), methane (-CH=), nitro (-NO₂) and quinoid groups, could be destroyed, resulting in the decolorization of dye wastewater (Bromley-Challenor et al. 2000; Mendez-Paz et al. 2005). However, some organic agents in dye wastewater, such as polyvinyl alcohol (PVA), belong to bio-refractory organics which are hard to be digested. Therefore, the treatment efficiency of dye wastewater is always less than desirable in an independent anaerobic process due to inhibition of microorganisms from the toxicity of dye wastewater (Fernando et al. 2013; Chen et al. 2014). It consequently results in low chemical oxygen demand (COD) removal in the AD unit, and significant COD removal usually occurs in the aerobic process.

Zero valent iron (ZVI), as a strong redox mediator, has been widely used for the decolorization of textile dye wastewaters, dechlorination of organochlorine pesticides and immobilization of heavy metals in underground water purification (Farrell et al. 2000; Jong & Parry 2003). In practice, full-scale iron chipping-filtration processes have also been
applied to pretreat less-biodegradable textile dye wastewater for improving the biodegradability of wastewater and reducing color. However, the application of this technology is limited as ZVI is liable to rust and harden when the surface of ZVI is exposed to air. In our previous works, ZVI was packed into an anaerobic reactor to form a bench-scale ZVI-anaerobic reactor for dye wastewater treatment. Under anaerobic conditions, ZVI was protected from rusting. Both COD removal and color removal are enhanced by the addition of ZVI in anaerobic digesters due to the reducibility of ZVI (Zhang et al. 2011a, 2011b, 2011c, 2011d). The better performance of the ZVI-anaerobic reactor was mainly due to the strong reducibility of Fe0, which could donate electrons for the reduction of dye. Moreover, Fe0 dosing was also helpful in accelerating and improving anaerobic acidogenesis and acetogenesis processes by enhancing the activities of biological enzymes (Liu et al. 2012b; Meng et al. 2013). However, bench-scale tests are usually under ideal conditions while the practical situations are more complex. In China, different kinds of dye wastewaters from various textile dye industries are inclined to be collected and treated together, as centralized processing is much cost-effective. Thus, the composition of actual dye wastewater is quite complex and varied (Tomei et al. 2016). Moreover, the dye wastewater quantity is also changeable according to the different seasons. To further shorten the distance between bench-scale test and large-scale application, ZVI-anaerobic treatment technology needs to undergo a pilot-scale test prior to engineering application.

On the basis of the above, a pilot-scale ZVI-upflow anaerobic sludge bed (ZVI-UASB) reactor was developed in a textile industrial park for actual dye wastewater treatment. The primary objective of this study is expected to provide more experience of this ZVI-UASB reactor to treat real dye wastewater. Its performance was compared with the local anaerobic treatment plant for effectively treating dye wastewater. The study is expected to provide more experience and knowledge for the scale-up of a ZVI-UASB reactor, which is meaningful for the upgrade and reform of traditional anaerobic treatment plant for high efficiency dye wastewater treatment.

MATERIALS AND METHODS

Reactor specification and operation

A ZVI bed (Φ 1,200 mm × 300 mm) was inserted at two-thirds depth in a UASB (Φ1,200 mm ×4,500 mm) to form a pilot-scale ZVI-UASB reactor (R1). The ZVI-UASB reactor replaced the traditional UASB reactor (anaerobic process) to create an upgraded anaerobic–aerobic process on April 20, 2016. The picture of reactor R1 is shown in Figure 1. The ZVI bed was constructed of cylindrical stainless steel mesh packed with 200 kg of scrap iron. The scrap iron was obtained from a mechanical factory (Hong Tai Steel Co., Ltd, Dalian, China). The pilot-scale reactor was built up in a dye sewage treatment plant that is used to treat dye wastewater from a textile dye industrial park. The working volume of this reactor was 5 m³. The control experiment was conducted with a practical anaerobic tank in a dye sewage treatment plant (R2). After feeding seed sludge, both R1 and R2 were operated with the hydraulic retention time (HRT) of 18 h at room temperature. Eighteen hours of HRT was selected to reduce the handling expense for longer HRT.

Sludge and wastewater

Seed sludge was obtained from an anaerobic treatment tank in the dye sewage treatment. The ratio of volatile suspended solids to total suspended solids (VSS/TSS) of the sludge was 0.7 with an initial TSS of 29.6 g/L. The sludge age of the UASB reactor was longer than 80 days.

The dye wastewater was taken from an adjustment tank in the sewage treatment plant. The influent COD ranged from 400 to 1,460 mg/L and color level ranged from 300 to 500 dilution-times. The influent COD load was 0.02 to 0.07 kg COD/kg TSS-d. The flow rate was 6.67 L/day. The wastewater contained many types of azo dyes, including Helianthin B, Azogeramine, and Magdala red, and additives such as PVA, dimethylformamide, carboxymethyl cellulose and surfactants. Fifty millilitres of sample was collected from the sample connection of reactors to analyze the relevant parameters every day.

Analytical methods

COD, VSS and TSS were determined in accordance with Standard Methods (APHA 2005). The concentration of Fe(II) was determined using ortho-phenanthroline spectrophotometry at 510 nm (Techcomp UV-2301, Shanghai, China). The pH and oxidation reduction potential (ORP) were monitored by an on-line pH analyzer (Sartorius PB-20, Germany) and ORP combination class-body redox electrode (Sartorius PY-R01, Germany). The color level was measured using the method of dilution-times (Kong & Wu 2008). The size of the granular sludge was measured by a
Mastersizer (Malvern 2000, UK). The morphology of the sludge was observed by a scanning electron microscope (SEM) (Quanta 200 FEG, The Netherlands). Briefly, the sludge samples were fixed with 2.5% glutaraldehyde solution for 2 h and then dehydrated in a graded ethanol/water series (30-50-70-90-100%), each concentration for 10 min. The samples were dried and sputter-coated with gold. Afterwards, the structure of the sludge was obtained by SEM.

**Fluorescence in situ hybridization**

A cyanine-3-labeled EUB338 probe with a sequence of 5'-ACTCCTACGGGAGGCAG-3' was used for characterization of bacteria (red), while a fluorescein-isothiocyanate-labeled ARC915 probe with a sequence of 5'-GTGCTCCCCCGCCAATTCCT-3' was used for characterization of archaea (green) (Amann et al. 1995). Fluorescence in situ hybridization (FISH) was conducted according to the method described by Hugenholtz & Pace (1996). The sludge hybridized was viewed by confocal laser scanning microscopy (Leica SP2 Heidelberg, Germany) under the X200 objective lens, and the microscopic fields were acquired at random. The abundance of the microorganisms was analyzed using the Image-Pro plus 6.0 software based on the FISH images.

**PCR amplification, DGGE and sequences**

Genomic DNA was extracted using an extraction kit (Biotek Corporation, Beijing, China) according to the manufacturer's instructions. A primer combination of 341f/907r was used to selectively amplify the 16S ribosomal RNA sequences (Teske et al. 1996). A 40-base GC clamp was added to the forward primer at the 5'-end to improve the detection of sequence variation in DNA fragments by subsequent denaturing gradient gel electrophoresis (DGGE). 16S rDNA fragments were amplified using a polymerase chain reaction (PCR) thermal cycler Dice (BioRad Co., Ltd, USA) with a ‘touchdown’ PCR method. The PCR products obtained were applied in DGGE analysis using a BioRad Dcode system (BioRad Co., Ltd, USA). A DGGE gel of 6% polyacrylamide with a linear denaturing gradient ranging from 30% to 60% (100% denaturing gradient contains 7 M urea and 40% formamide) was applied. Electrophoresis was run at a constant voltage of 180 V in 1×TAE buffer and 60 °C for 6 h. Afterwards, the gels were stained with SYBR Gold (Dalian TaKaRa, China) in 1×TAE buffer for 40 min and the gel's UV transillumination image was photographed using the Gel Doc 2000 system (BioRad Co., Ltd, USA). DGGE profiles were analyzed by the software ‘Quantity One’.
RESULTS AND DISCUSSION

Pilot-scale test

On the basis of laboratory-scale tests, a pilot-scale ZVI-UASB reactor (R1) was developed for actual dye wastewater treatment. The treatment performance of R1 in terms of COD removal and color removal was compared with a traditional anaerobic treatment tank (R2) in a dye sewage treatment plant. As shown in Table 1, the COD of dye wastewater in the influent ranged from 400 to 1,460 mg/L with the colority from 300 to 500 times. During 90 days’ operation, the average COD removal was maintained at 19% in R1 (Figure 2(a)) while it was only 10% in R2 (Figure 2(b)). Our previous studies demonstrated that, under the different conditions employed (temperature, pH, substrate, salinity and so on), the COD removal efficiency in the ZVI-UASB reactor was improved from 58%–32% to 87%–53%, compared with that in the control reactor without ZVI supplementation (Zhang et al. 2011a, 2012b, 2012; Liu et al. 2012a, 2012b; Meng et al. 2013). The better performance of the ZVI-UASB reactor was mainly attributable to the favorable condition created by addition of ZVI. ZVI could react with H\(^+\) to neutralize the acidic pH resulting from the accumulation of fermentative intermediates (volatile fatty acids) and sustain a lower ORP for anaerobic microorganisms to metabolize organics in the AD process. In this study, when treating practical wastewater, adding ZVI in the anaerobic reactor also showed a difference in COD removal (19% vs 10%, R1 vs R2). The colority removal efficiency showed a similar trend to the COD removal in R1 and R2. From Table 1, the colority of influent fluctuated between 100 times and 500 times. The average removal rate of colority in R1 was still higher than 40% (Figure 2(c)), while that in R2 was only 20% (Table 2). In our previous study, colority removal rate was also enhanced by adding ZVI, from 32%–54% to 60%–89% when treating synthetic wastewater (Zhang et al. 2011a, 2012, Liu et al. 2012a, 2012b). The high decolorization in synthetic wastewater can be explained by ZVI, as an electron donor, being able to provide electrons for the destruction of dye molecule, effectively enhancing the decolorization of dyes. The better performance of the ZVI-UASB reactor in laboratory scale was because the relative acidic pH resulting from the organic acids accumulation in the AD process was favorable for the reduction of ZVI. However, in practical dye wastewater, the influent organic load and pH are variable. The result of colority removal here in the pilot-scale ZVI-UASB reactor was still higher than that in the traditional anaerobic treatment tank (40% vs 20%), although the difference between the two reactors was not as significant as that in laboratory scale. It meant that even under a changeable pH condition, addition of ZVI into the anaerobic reactor could also enhance the decolorization of dye wastewater.

Compared with the traditional anaerobic treatment tank in practical wastewater treatment, the pilot-scale ZVI-UASB reactor had a better performance. It was attributed to ZVI being able to sustain a stable condition (pH and ORP) for functional microorganisms, while the microorganisms in R2 were influenced powerfully by the impact of the practical wastewater. The organic load of the practical wastewater was variable, which would lead to the accumulation of intermediate products (volatile fatty acids), and resulted in an acidic pH condition. The functional microorganisms could not survive in the acidic circumstance and low treatment efficiency was inevitable. The ORP value and Fe\(^{2+}\) leaching in R1 are −330 mV to −290 mV and 2 mg/L to 10 mg/L, respectively, forming a favorable anaerobic environment for anaerobic bacteria growth (Table 2). Even though ORP in R1 is significantly higher than that in laboratory scale, it is still much lower than that of −210 mV to −150 mV in R2 (Table 2). Our previous studies proved sufficiently that ZVI could decrease ORP to

![Table 1](image)

<table>
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<tr>
<th>Parameters</th>
<th>Influent COD (mg/L)</th>
<th>Influent colority (times)</th>
<th>Influent pH</th>
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<tr>
<td>R1</td>
<td>400–1,462</td>
<td>100–500</td>
<td>5.3–8.1</td>
</tr>
<tr>
<td>R2</td>
<td>400–1,462</td>
<td>100–500</td>
<td>5.3–8.1</td>
</tr>
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![Table 2](image)

<table>
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<tr>
<th>Parameters</th>
<th>Average decolorization rate (%)</th>
<th>Average COD removal (%)</th>
<th>pH</th>
<th>ORP</th>
<th>Fe(^{2+}) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>40 ± 5.6</td>
<td>20 ± 3.5</td>
<td>6.5–8.0</td>
<td>−330 to −290</td>
<td>2–10</td>
</tr>
<tr>
<td>R2</td>
<td>20 ± 2.3</td>
<td>10 ± 1.8</td>
<td>5.5–8.0</td>
<td>−210 to −150</td>
<td>−</td>
</tr>
<tr>
<td>Final effluent</td>
<td>98 ± 6.4</td>
<td>91 ± 3.2</td>
<td>6.5–8.1</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Data of ORP, pH and Fe\(^{2+}\) are the range throughout the whole experiment (90 days). Data of decolorization and COD are the averages of the values obtained during the whole experiment (90 days). Errors represent standard deviations of statistical analysis (n = 90).
create a more favorable environment for AD with synthetic dye wastewater (Zhang et al. 2014a, 2014b). This study further confirmed that, besides for synthetic wastewater, when facing complex practical dye wastewater, a pilot-scale ZVI-UASB reactor could still sustain a more favorable ORP for bacteria and achieve a higher COD and colority removal efficiency than a traditional anaerobic treatment tank.

Characteristics of granular sludge and bioinformatics analysis

After 90 days of operation, sludge fragments were washed out from R1. Most of the brown non-granular sludge became ellipsoidal granular sludge of black color. The average particle size of the sludge was 486 μm. In our previous studies, granular sludge with an average size of 695 μm was also formed by adding ZVI in an anaerobic digester for synthetic wastewater treatment (Liu et al. 2011). The size of granular sludge (486 μm) formed in the practical wastewater was much smaller than the size of 695 μm in synthetic wastewater, which might be because of the negative impact of the variable water quality in the practical wastewater. However, the granular sludge with a mean size of 486 μm was still bigger than that of 269 μm in R2, maintaining good performance for textile dye wastewater treatment. The formation of granular sludge was mainly due to the release of Fe^{2+} (Liu et al. 2011; Zhang et al. 2011a, 2011b, 2011c). The divalent metal ions could decrease electric repulsion and facilitate cell-to-cell interaction between bacteria, consequently enhancing cell aggregation (Liu et al. 2011). Also, Fe^{2+} leaching increased the iron content of the sludge, which benefited the formation of the granule matrix with extracellular polymeric substances (EPS). EPS secreted by bacteria can mediate cohesion as well as adhesion of cells, which is crucial to maintenance of the structural integrity of anaerobic granules (Liu et al. 2011). Therefore, adding ZVI in the anaerobic reactor enhanced the granulation of sludge efficiently.

SEM pictures show that the surface of the granular sludge is dense and sharply demarcated with a layer of furry white matter (Figure 3). Even so, SEM can only give the shape of microbes while it cannot identify the microbial communities of bacteria and methanogenic archaea. To clarify this, FISH analysis was conducted to determine the relative abundance of archaea (green) and bacteria (red) (Figure 4). The results showed that the relative abundance of archaea increased to 63% in R1 after 90 days of operation, 32% higher than that in R2. The result was quite consistent with that in synthetic wastewater, which indicated that ZVI could also help enrich methanogenic archaea (in most anaerobic reactors, methanogens represent the majority of archaea) even in a complex environment. The increased relative abundance of archaea might be attributed to the more comfortable circumstance for archaea and bacteria.
to multiply by ZVI, which was because ZVI lowered the ORP value (−290 mV to −330 mV) and increased the Fe²⁺ supplement in the ZVI-UASB reactor. And it consequently accelerated the COD removal and decolorization.

As shown in Figure 5, bacterial community structure was investigated by PCR-DGGE analysis after 90 days of operation. The results showed that a total of nine bands were detected as dominant bands in the two lanes of R1 and R2. The bands in DGGE profiles of R1 are much stronger than that of R2, especially for the band 7, 8 and 9. This result indicated that some of the dominant microorganisms are enriched in R1, which might be attributed to the coupling effect of the ZVI function and favorable anaerobic environment with low ORP. Different bands represent different bacterial species, which usually have different functions for dye wastewater treatment. The effective enrichment of functional microorganisms by adding ZVI may be the biological reason for better COD and colority removal in the pilot-scale ZVI-UASB reactor.

**On-site upgrading of traditional textile dye sewage treatment plant by the addition of ZVI bed in the anaerobic digester**

Figure 6(a) shows the main treatment units of a traditional textile dye sewage treatment plant including primary settling basins, anaerobic digester, aerobic treatment tank and secondary clarifiers. The treatment capacity of this plant is 40,000 t/day. After adjusting wastewater quality in primary settling basins, dye wastewater is pumped into the anaerobic digester for COD removal and decolorization. However, to realize the national emissions standards the effluent will have to be processed further by the subsequent aerobic treatment and coagulation sedimentation. There is no doubt that too low a
COD and colority removal in R2 will increase the processing difficulty for the follow-up treatment units. Moreover, the usage of a large amount of flocculants may increase the cost. As an important product of the ZVI reaction (Fe→2e− = Fe2+), Fe2+ concentration in the effluent of R1 ranged from 2 to 10 mg Fe2+/L. It can be transformed to Fe3+ after the aerobic treatment process, forming a useful flocculant agent in R1. If the ZVI bed can be used in the upgrade of R2, Fe2+ produced in R1 was calculated to range from 80 to 320 kg Fe2+ (40,000 × 1,000 kg × 2 mg to 40,000 × 1,000 kg × 10 mg Fe2+ /kg wastewater). If ignoring process loss, about 1.43 to 5.71 kmol Fe3+ can be produced during the aerobic treatment process. FeCl3·6H2O is a commonly used flocculant agent in R1. Using the ZVI bed in R2, the produced Fe2+ is expected to save about 1.43 to 5.71 kmol FeCl3·6H2O dosage everyday, i.e. 386 to 1,540 kg FeCl3·6H2O. According to the commercial price of FeCl3·6H2O, the addition of ZVI in R1 will save about $129–526 every day. Furthermore, waste iron scraps are cheap and easily available. One-time dosage in an anaerobic reactor will have a role to play for high COD and colority removal for a long time to alleviate the pressure of subsequent processing units.

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**Figure 5** | DGGE fingerprints of microbial communities in R1 and R2 after 90 days of operation.

**Figure 6** | (a) Process flow chart of traditional textile dye sewage treatment plant. (b) Schematic diagram of upgrading textile dye sewage treatment plant by the addition of ZVI bed in the anaerobic digester.
Therefore, constructing the pilot-scale ZVI-UASB reactor to realize in situ upgrading and rebuilding of sewage treatment plant provided a cost-effective method with high treating performance. The schematic diagram of upgrading the textile dye sewage treatment plant by the addition of a ZVI bed in the anaerobic digester is shown in Figure 6(b). By adding a certain amount of low-cost ZVI only once, the treatment performance will be increased in terms of COD removal and colority removal without increasing the original floor space, construction cost and operation cost. The increase of COD and colority removal in R2 by the addition of the ZVI bed is also expected to reduce the cost of aeration in the aerobic treatment tank, as the effluent of R2 contains lower COD and colority to be treated by an anaerobic process. Also, the application of ZVI into the anaerobic process overcame the corrosion of ZVI and realized the function of ZVI (hyperslow dissolving out). The result would be promising and attractive for the anaerobic treatment of dye wastewater, which may also be acceptable for other industrial wastewaters.

CONCLUSION

A pilot-scale anaerobic reactor packed with a ZVI bed has been fabricated and operated successfully for actual dye wastewater treatment. The application of ZVI in AD effectively improved the performance of the reactor by way of both COD and color removal. The attempt of enlargement from laboratory-scale test to pilot-scale test was completed smoothly, and provided a useful and reliable approach for actual dye wastewater treatment. This novel anaerobic-ZVI technology can be employed to upgrade existing sewage and other wastewater treatment systems.

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