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Steady performance of a zero valent iron packed anaerobic reactor for azo dye wastewater treatment under variable influent quality

Yaobin Zhang*, Yiwen Liu, Yanwen Jing, Zhiqiang Zhao, Xie Quan*

Key Laboratory of Industrial Ecology and Environmental Engineering (MOE), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China. E-mail: zhangyb@dlut.edu.cn

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Abstract

Zero valent iron (ZVI) is expected to help create an enhanced anaerobic environment that might improve the performance of anaerobic treatment. Based on this idea, a novel ZVI packed upflow anaerobic sludge blanket (ZVI-UASB) reactor was developed to treat azo dye wastewater with variable influent quality. The results showed that the reactor was less influenced by increases of Reactive Brilliant Red X-3B concentration from 50 to 1000 mg/L and chemical oxygen demand (COD) from 1000 to 7000 mg/L in the feed than a reference UASB reactor without the ZVI. The ZVI decreased oxidation-reduction potential in the reactor by about 80 mV. Iron ion dissolution from the ZVI could buffer acidity in the reactor, the amount of which was related to the COD concentration. Fluorescence *in situ* hybridization test showed the abundance of methanogens in the sludge of the ZVI-UASB reactor was significantly greater than that of the reference one. Denaturing gradient gel electrophoresis showed that the ZVI increased the diversity of microbial strains responsible for high efficiency.

Key words: anaerobic reactor; zero valent iron; azo dye wastewater

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Introduction

Massive discharge of azo dye wastewater from textile factories and chemical industries seriously threats to ecological safety and human health. The azo molecule contains –N=N– system linked with two aromatic rings, which makes it stable and difficult to biologically decompose. Therefore, azo dye wastewater needs to be pretreated by appropriate methods for raising biodegradability prior to the biological treatment. Current methods used to treat wastewater such as Fenton, photocatalysis, electrochemical oxidation, wet catalysis oxidation, have technical or economical limitations including low efficiency, high cost and the creation of secondary pollutants (Kritikos et al., 2007; Manu and Chaudhari, 2003).

Over the last two decades, zero valent iron (ZVI) technology has attracted great interest in wastewater treatment, groundwater purification and soil remediation (Barreto-Rodrigues et al., 2009). As a reductive material, the ZVI is well suited to reduce refractory contaminants such as nitroaromatic compounds, organochlorine pesticides, heavy metals and azo dyestuffs (Chatterjee et al., 2010; Farrell et al., 2000; Keum and Li, 2004; Üzüm et al., 2008). However, a major problem limiting the wider application of ZVI is the rusting (Fan and Ma, 2009). The ZVI is easily oxidized into iron oxides that cover on its surface and attenuate its reactivity. Recently, it was reported that ZVI used in anaerobic conditions could obtain greater contaminants removal efficiencies than that used in aerobic conditions. These results were likely due to the fact that the ZVI is protected from oxygen under anaerobic condition, which allowed it to function stably (Choe et al., 2001; Joo and Zhao, 2008). However, the existing anaerobic-ZVI was focused on remediation of groundwater or soil with low level of contaminants. To our best knowledge, less report has been found to treat wastewater using the ZVI installed in an anaerobic reactor.

Considering its reductive property, the ZVI is expected to be helpful for creating an enhanced anaerobic environment that might benefit to improve the performance of an anaerobic reactor in wastewater treatment. Based on this idea, a ZVI packed upflow anaerobic sludge blanket (ZVI-UASB) reactor is being developed in our laboratory. Our previous work that focused on the observation of the performance of the reactor under a fixed wastewater quality (Zhang et al., 2011b). It is well known that the wastewater quality is quite variable in practice. Generally, fluctuation in wastewater quality often causes obviously negative influences on anaerobic system, even leading to acidification because methanogens are much more sensitive to environmental changes than acidogens and acidogenesis is quicker than methanogenesis (Gutierrez et

* Corresponding author. E-mail: zhangyb@dlut.edu.cn (Yaobin Zhang); quanxie@dlut.edu.cn (Xie Quan)

al., 2009; Yu and Fang, 2003). We believe that the ZVI can help buffer the acidity to maintain a near neutral pH in the reactor, therefore improving methanogenesis and enabling the UASB reactor to better respond to changes of wastewater quality. The objectives of this study were to evaluate the effectiveness of this ZVI-UASB reactor system for azo wastewater treatment under variable influent condition.

1 Materials and methods

1.1 Reactor

A ZVI bed (ϕ80 mm × 300 mm, detailed below) was installed at 2/3 depth in an UASB reactor (ϕ80 mm × 1200 mm) to form a ZVI-UASB reactor (hereafter referred to as R1). A wastewater circulation system was conducted between the top and the bottom of the ZVI bed to intensify the mass transfer using a peristaltic pump (Zhang et al., 2011a). The circulation ratio (of circulation amount to influent amount) was fixed at 18. A similar UASB reactor without the ZVI bed was used as a control reactor (hereafter referred to as R2). The operation temperature was maintained at (35 ± 1)°C by a heating jacket.

The ZVI bed was constructed of stainless steel mesh (10 meshes) packed with waste scrap iron collected from a mechanical factory (20# steel, about 8 mm × 4 mm × 2 mm), which was soaked in 0.1 mol/L of NaOH for 24 hr and then washed with dilute HCl and water.

The sludge collected from an UASB reactor in our laboratory was used as seed sludge. The ratio of volatile suspended sludge to total suspended sludge (VSS/TSS) of the sludge was 0.81. The sludge was added to the two reactors with an initial TSS of 26.0 g/L.

1.2 Wastewater

The azo dye wastewaters used in the present work included an artificial wastewater and an actual wastewater, respectively. The artificial wastewater was composed of X-3B, sucrose and other inorganic elements (N, P, etc.). Sucrose was used as a source of COD.

The actual wastewater was taken from a collecting basin (for regulating wastewater quality) in a textile factory (Haicheng, China). The wastewater was sampled every three days and then stored in a plastic container for experimental use. COD in the wastewater was 1331–1547 mg/L, BOD_5 was 140–170 mg/L, pH was 8–9 and color level was 240–260 dilution times. Tests showed that there was no significant changes of COD and color level (less than 5%) in a batch sample during the three days' store. The main composition in the wastewater included azo dyes (Reactive Brilliant X-3B), polyvinyl alcohol (PVA) and surfactant.

The pH in the wastewater was adjusted to 8 prior to the feeding.

1.3 Operation

After being seeded with the sludge, the two reactors were fed with the artificial wastewater with a COD of 2000 mg/L and were operated under a HRT of 24 hr. After 20 days of operation, the COD removal and decolorization in the two reactors gradually stabilized. Thereafter, the reactors were fed using the following waters in turn: (1) the artificial wastewater with a fixed COD at about 1600 mg/L and increasing X-3B concentration from 50 to 1000 mg/L; (2) the artificial wastewater with a fixed X-3B concentration at 100 mg/L and increasing COD concentration from 1500 to 7000 mg/L; (3) the actual azo dye wastewater.

1.4 Analysis

COD, BOD, VSS and TSS were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Because Fe^{2+} released from the ZVI could exert oxygen demand, the actual COD in the wastewater equaled the total COD subtracted from the COD produced by Fe^{2+}. The oxidant-reductive potential (ORP) was measured using an ORP meter (Sartorius PY-R01, Germany). The concentrations of Fe(II, III) ions were determined by *ortho* phenanthroline spectrophotometer at 510 nm (Techcomp, UV-2301, Shanghai, China). The pH was monitored using a pH analyzer (Sartorius PB-20, Germany). The color level in the wastewaters was measured using method of dilution times (Kong and Wu, 2008). The composition of biogas was determined using a gas chromatogram (Shimadzu, GC-18, Japan) equipped with a thermal conductivity detector (TCD) according to the method described by Jiang et al. (2007). The biogas amount was determined using a wet gas flow meter. Volatile fatty acids (VFAs) were evaluated using a high performance liquid chromatograph (HPLC, Waters 2695, USA) equipped with an ultraviolet (210 nm) detector (Han et al., 2005).

1.5 Fluorescence *in situ* hybridization (FISH)

A cyanine 3 (CY3)-labeled EUB338 probe with the sequence of 5′-ACTCCTACGGGAGGCAG-3′ was used for characterization of Bacteria (red), and a fluorescein isothiocyanate (FITC)-labeled ARC915 probe with the sequence of 5′-GTGCTCCCCCGCCAATTCCT-3′ was used for characterization of Archaea (green) (Amann et al., 1995). The FISH was conducted according to the method described by Hugenholtz and Pace (1996). The sludge hybridized was viewed by a confocal laser scanning microscopy (CLSM, Leica-TCS-SP2, Leica, Germany).

1.6 Denaturing gradient gel electrophoresis (DGGE)

Genomic DNA extraction was performed according to the methods described previously (Lakay et al., 2007). A primer combination of 341f /907r was used to selectively amplify the 16S ribosomal RNA sequences of eubacteria. A 40-nucleotide GC clamp was added to the forward primer at the 5'-end to improve the detection of sequence variation in amplified DNA fragments by subsequent denaturing gradient gel electrophoresis (DGGE). The PCR products of the proper size were confirmed by electrophoresis through a 1% agarose gel in 0.5 × TAE buffer, stained with Genfinder (Dalian TaKaRa, China). DGGE was performed at 60°C with a D-CODE System Universal Mutation (Bio-Rad Laboratories, USA) accord-

ing to the manufacture's instruction. The PCR products were applied on a DGGE gel of 6% polyacrylamide with a linear denaturing gradient ranging from 30% to 60% (100% denaturing gradient contains 7 mol/L urea and 40% formamide). Electrophoresis was run at a constant voltage of 180 V for 6 hr in 1 × TAE buffer. Subsequently, the gels were stained with SYBR Gold (Dalian TaKaRa, China) in 1 × TAE buffer for 40 min and gel digital images were obtained using the Gel Doc 2000 System (Bio-Rad Laboratories, USA).

Each gel slice that contained an obvious electrophoretic band was excised and placed in a 1.5-mL Eppendorf tube, incubated with TE buffer at 4°C for 12 hr. Then the second PCR was carried out. The PCR products using the corresponding 16S rRNA primers with no "GC" clamp were purified with a PCR purification kit (Dalian TaKaRa, China), and used as template in a cycle sequencing reaction with a BigDye Terminator V3.1 in TaKaRa Company (Dalian, China). Sequencing of 16S rRNA fragments was carried out with a Sequencing System ABI PRISM 3730 (Applied Biosystems, USA). The obtained gene sequences were compared with the reference microorganisms available in the GenBank by BLAST search.

2 Results and discussion

2.1 Effect of the dye concentrations

Dye loading is an important operational factor influencing removal of color and COD in the anaerobic process. It was reported that azo dyes and their degradation products could be used as carbon sources for methanogenesis (Manu and Chaudhari, 2002), but they usually showed apparent inhibitions on anaerobes in most cases due to the toxicity of azo dyes and their intermediate products such as aromatic amines. With increasing dye concentration, the inhibition would generally be augmented, even causing deterioration in the anaerobic process (Kapdan et al., 2003). To make clear the role of ZVI in the anaerobic reactor in response to the increase of azo dye concentration, the two reactors were fed with the artificial wastewater, in which X-3B concentration rose from 50 to 1000 mg/L in seven levels (COD fixed at about 1600 mg/L), and the results are displayed in Fig. 1.

After an adaptation of the influent X-3B of 50 mg/L, the effluent COD decreased to 776.5 mg/L from R1, while it decreased to 850.1 mg/L from R2 (Fig. 1a). Afterwards, with increasing the dye from 50 to 1000 mg/L, the effluent COD from R1 was maintained a relatively steady level, averaging 853 mg/L, while it increased gradually from 850.1 to 1288.4 mg/L from R2. From Fig. 1b, with increasing the dye from 50 to 1000 mg/L the color removal in R2 decreased from 69.6% to 54.3% (except that in the initial days), while the color removal in R1 was maintained well at 89.2%–94.2%. The results indicated that increase of dye concentration had less effect on R1.

The pH changes in Fig. 1c also clearly revealed the difference between the two reactors. When raising the dye concentration to 1000 mg/L, the pH in R2 decreased to

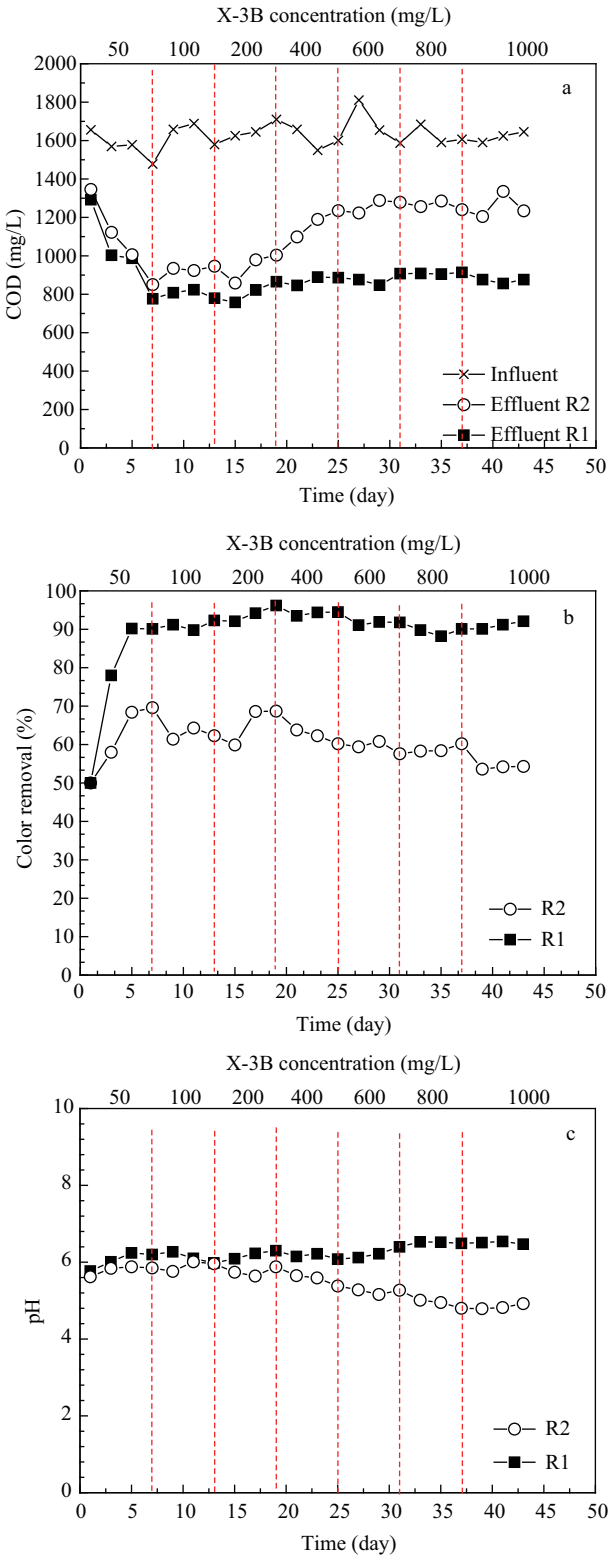

Fig. 1 Effect of the dye concentration on COD (a), color removal (b), pH (c) changes in the reactors. The two reactors were fed with the artificial wastewater with X-3B concentration rising from 50 to 1000 mg/L in seven levels. R1 indicates ZVI-UASB reactor and R2 indicates the reference UASB reactor without the ZVI.

4.8, while the pH in R1 was well maintained between 6.3 and 6.5. It is well-known that the pH is crucial to maintain normal operation of the anaerobic reactor. However, since methanogens are more sensitive, changes of operational conditions such as inhibitors (azo dye) primarily hurt

methanogenesis, which would lead to acidity accumulation to further destroy the pH balance. Unlike that in R2, the pH in this ZVI-UASB reactor was always maintained well. The partial reason was ascribed that the ZVI counteracted acidity due to the fact that Fe^{2+}/Fe^0 (E_0 = –0.44 V) has lower ORP than H^+/H_2 (E_0 = 0).

2.2 Effect of the influent COD

The anaerobic reduction of azo dye is a co-metabolic process, in which the COD may act as a primary electron donor to participate the azo dye reduction (dos Santos et al., 2006). Therefore, low COD is not conductive to azo decolorization. On the other hand, the COD also serves as a source for acidogenesis and methanogenesis. Increasing COD beyond an extent may lead to acidification in the system. We believed the ZVI could reduce the dependence of azo reduction on the COD and make the treatment system more stabilized. To clarify it, the experiment was carried out under different influent COD and the results are shown in Fig. 2.

From Fig. 2a, with increasing influent COD from 1500 to 7000 mg/L (X-3B concentration fixed at 100 mg/L), the effluent COD in R1 ranged from 644.0 to 404.8 mg/L, showing a declining trend. Comparatively, the effluent COD in R2 ranged from 884.7 to 2086.5 mg/L, greater than that in R1. Specially, the COD removal in R1 under the COD of 7000 mg/L even reached its highest efficiency, while an obvious increase of effluent COD was found in R2.

From Fig. 2b, the color removal in R2 increased from 50.4% to 75.6% with increasing COD from 1500 to 7000 mg/L, indicating that COD acted as a co-metabolic substrate to enhance the azo decolorization. As compared, the color removal in R1 maintained well from 91.4% to 96.7% as increasing COD in the same extent. The results suggested that the ZVI could efficiently complement the lack of reducing equivalents, which is meaningful to obtain effective decolorization under low COD concentration.

From Fig. 2c, the ORP in R1 averaged –157.3 mV, about 80 mV lower than that in R2. Low ORP benefited to transfer electron for azo reduction and to improve the survival environment for methanogens (Bromley-Challenor et al., 2000; Georgiou et al., 2004). CH_4 production in the two reactors is shown in Fig. 2d. Greater CH_4 production was observed in the biogas of R1, suggesting that methanogenesis were more active in R1. Consistently, Zandvoort et al. (2003) and Karri et al. (2005) reported that addition of iron improved the methanogenic activity of the sludge.

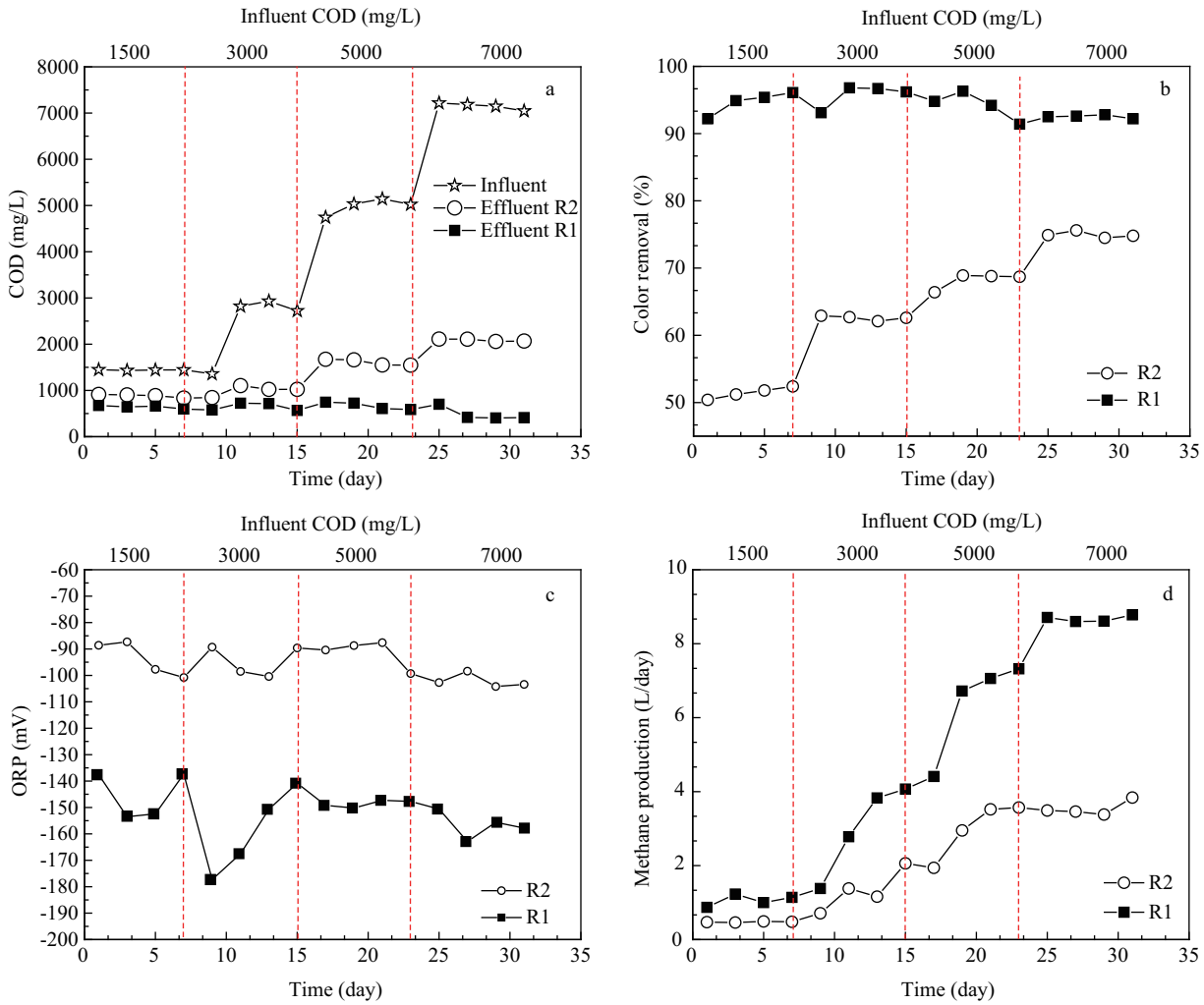

Fig. 2 Effect of the influent COD on the performance of the two reactors: (a) COD, (b) color removal, (c) ORP, (d) methane production. The influent COD increased from 1500 to 7000 mg/L in four levels, X-3B concentration was fixed at 100 mg/L.

2.3 Treatment of the actual wastewater

The performances of the two reactors for the actual wastewater treatment are displayed in Fig. 3. Due to more complex composition in this wastewater, the reactors needed time to adapt. After 19–25 days, the COD and color removal efficiencies in the two reactors gradually stabilized. Thereafter, the effluent COD in R1 averaged 509 mg/L (day 19), while it was 795.9 mg/L in R2 (from day 25). The color removal in R1 averaged 80.9%, while it was 61.2% in R2. These indicated that R1 could adapt well to the actual wastewater.

2.4 Iron dissolution under different influent COD

Iron leaching under anaerobic conditions resulted in ferrous iron. Changes of iron concentrations in the reactor under the different influent COD are shown in Fig. 4. When increasing COD from 5000 to 7000 mg/L, the mean concentration of iron ion increased from 57.1 to 65.4 mg/L. Afterwards, the iron concentration decreased to 33.8 mg/L when the reactor was fed with the actual wastewater with COD concentration of 1400 mg/L. The results indicated that the COD concentration had a significant effect on the iron dissolution. It is well known that the COD is a source for producing VFAs during acidogenesis. High COD loading increased VFAs production and thus decreased pH level. Consistent with the analysis, VFAs amount in R2 increased from 879 to 1214 mg/L then decreased to 395.6 mg/L with changing the influent COD from 5000 to 7000 mg/L then to about 1400 mg/L. As a result, the iron dissolution changed with the variation of influent COD.

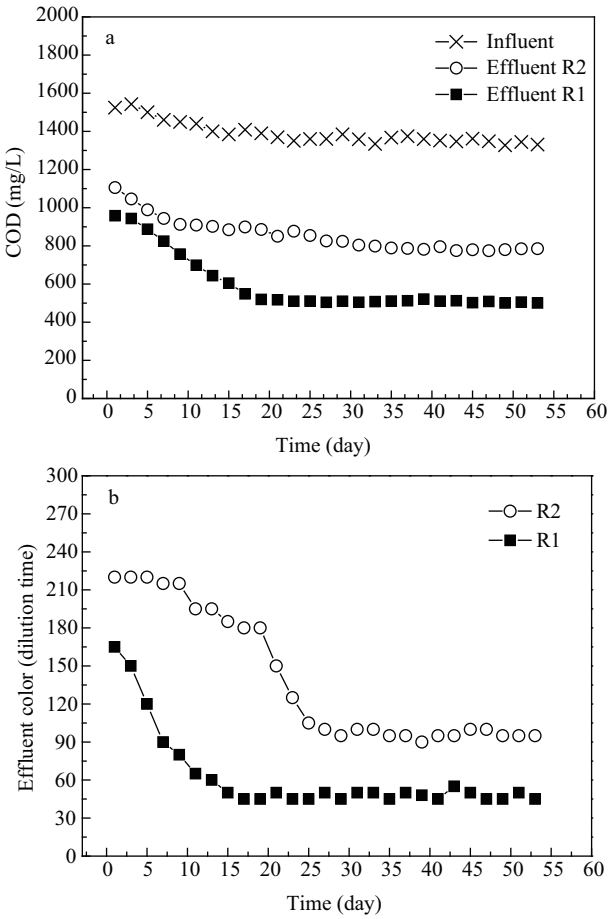

Fig. 3 COD and color removal in the actual wastewater treatment. The two reactors were fed with the actual dye wastewater with COD ranging from 1331 to 1547 mg/L and color level was about 250 dilution times.

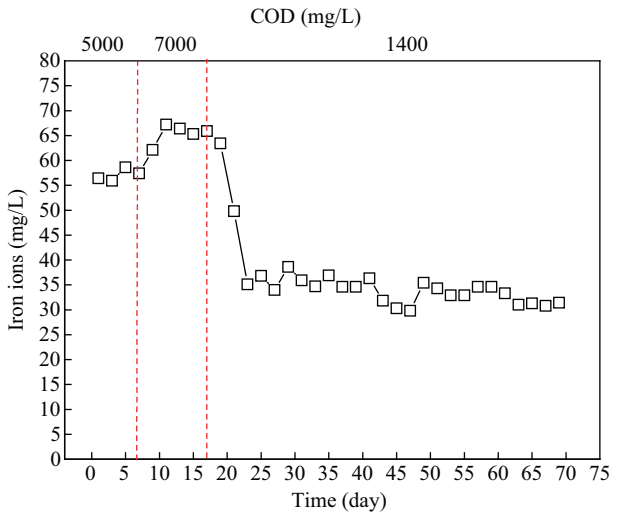

Fig. 4 Iron dissolution with changing influent COD from 5000 to 7000 to 1400 mg/L.

When the ZVI bed was taken after the operation for nine months, the scrap iron posed a black surface (Fig. 5). After water-rinsed, it appeared a fresh surface with metallic sheen, suggesting the ZVI in the anaerobic reactor was well protected from rusting. During the nine months, the weight of ZVI was found to drop from 200 to 99 g, amounting that the consumption of ZVI was about 10 mg/g COD.

2.5 FISH analysis

It has been suggested that H_2 production accompanies the ZVI reaction (acid buffering). Thus, H_2 content in the biogas of R1 should be greater than that of R2. Interestingly, almost no H_2 could be detected from R1, while 2.2%–5.4% of H_2 (data not shown) was found in the biogas of R2. The reason might be related to the different activity of methanogens in the two reactors. It is well known that acidogens break down the substrates into mainly H_2, acetic acid and CO_2, and methanogens convert acetic acid, H_2 and CO_2 to CH_4. Generally, H_2 in the biogas indicates low activity of methanogenesis or imbalance between methanogenesis and acidogenesis (Cooney et al., 2007). We assumed that methanogenesis in R1 were more active so that it consumed all of H_2 produced by acidogenesis and the ZVI reaction (Daniels et al., 1987).

To further clarify this speculation, ARC915 and EUB338 as domain-specific probes, were used to *in situ* hybridization of Archaea and Bacteria in the sludge from the two reactors, respectively. It is well known that methanogens are the members of Archaea and acidogens are the members of Bacteria. In most anaerobic reactors, methanogens represented Archaea (Sekiguchi et al., 1999). As shown in Fig. 6, the abundance of Archaea in the sludge of R1 was significantly greater than that of R2. The FISH experiment confirmed that the presence of ZVI was beneficial to the growth of methanogens in a UASB

Fig. 5 Pictures of the ZVI after 9 months of operation. (a) ZVI just taken from the reactor; (b) ZVI after water rinsed.

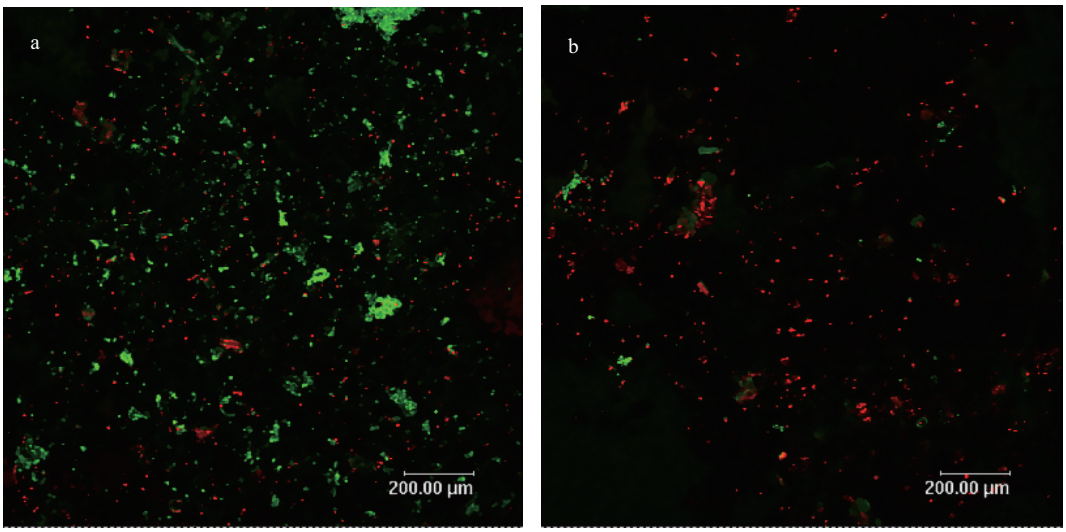

Fig. 6 Fluorescence *in situ* hybridization of sludge viewed by CLSM: (a) sludge from R1, (b) sludge from R2. Simultaneously hybridized with a CY3-labeled Bacterial-domain probe (EUB338) (red) and a FITC-labeled Archaeal-domain probe (ARC915) (green). It can be observed that the abundance of Archaea in the sludge of R1 was significantly greater than that of R2.

reactor, which enabled the reactor to more effectively degrade organic matters in wastewater.

2.6 PCR-DGGE analysis

PCR-DGGE analysis of the sludge taken from the two reactors at the end of operation was performed. The DGGE profiles (Fig. 7) suggested that the bacterial community in R1 was more diverse than in R2. Especially in R1, the bands 1, 2, 5, 7, 8 and 9 in lane R1 was stronger compared with those of lane R2. Cluster analysis of the fingerprints from the two reactors confirmed that their communities were quite different (data not shown), with a low similarity of 63%.

In order to provide further insight into the microbial diversity, the predominant species extracted from DGGE bands were sequenced and compared with the published species in National Central for Biotechnology Information (NCBI, Table 1). The clone from bands 1, 2, 5 and 9 showed 99%, 100%, 100% and 98% respectively sequence similarity to uncultured bacterium gene for 16S rRNA,

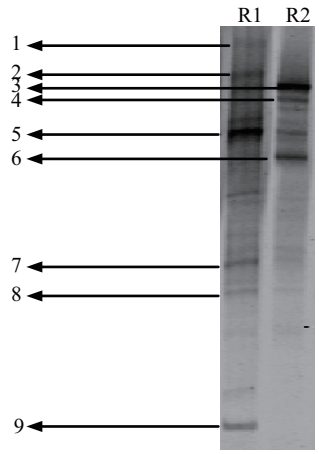

Fig. 7 DGGE analysis of PCR-amplified 16S rRNA gene fragments of sludge samples from each reactor.

partial sequence, clone, which was observed in mesophilic UASB sludge granules. It indicated that less negative effects of increasing dye concentration on anaerobic sludge

Table 1 Characteristics of 16S rRNA fragments obtained from DGGE gel

DGGE band	Phylogenetic affiliation	GenBank accession number	Sequence similarity	Isolation source
1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: KsuB79fl	AB267033.1	99%	Mesophilic UASB sludge granules
2	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: KsuB36	AB291457.1	100%	Mesophilic UASB sludge granules
3	Uncultured bacterium clone C9.26 16S ribosomal RNA gene, partial sequence	GU559765.1	99%	12-day-old anaerobic fermentation course of Microcystis blooms
4	Uncultured bacterium clone BXHA16 16S ribosomal RNA gene, partial sequence	GQ479974.1	98%	Sewage from wastewater treatment plant
5	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: KsuB123fl	AB267037.1	100%	Mesophilic UASB sludge granules
6	Uncultured compost bacterium partial 16S rRNA gene, clone PS3297	FN667451.1	100%	Pilot scale municipal drum compost
7	Propionibacteriaceae bacterium WR032 gene for 16S ribosomal RNA, partial sequence	AB377179.1	100%	Rice straw residue in a methanogenic reactor of cattle farm waste
8	Propionibacteriaceae bacterium SH081 gene for 16S rRNA, partial sequence	AB298752.2	100%	Suspended plant residue in a methanogenic reactor of cattle farm waste
9	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: N2B39	AB291353.1	98%	Mesophilic UASB sludge granules

granule of R1, which was possibly related to Fe^{2+} roles in enhanced granulation. This was also coincident with our observation that there were more clear-cut and intact granules in R1 (data not shown). Therefore, intact structure of the sludge of R1 could protect susceptible methanogens inside the sludge from unfavorable conditions, resulting in high removal efficiency. The clone from bands 7 and 8 presented 100% sequence similarity to Propionibacteriaceae bacterium, which might play an important role in dye reduction. R1 presented the higher level in these bands above. On the other hand, the bands 3 and 6 were stronger in R2, but they might be not responsible for treatment of azo dye wastewater.

3 Conclusions

The ZVI-UASB reactor displayed a steady performance in the azo dye wastewater treatment under variable influent quality. The pH in the reactor was maintained at near neutral levels, whereas it dropped to 4.8 in the reference reactor without the ZVI. Greater removal of COD and color was achieved in this ZVI-UASB reactor. The ZVI decreased the ORP and reduced the acidity in the reactor, which was helpful for the growth of methanogens. Iron dissolution could decrease acidity in the ZVI-UASB reactor, the amount of which was depended on the COD concentration. It implied that the more VFAs were produced, the more Fe^{2+} leached from the ZVI to neutralize. FISH test indicated that the abundance of methanogens in the sludge of ZVI-UASB reactor was significantly greater than that of the reference one. The synergetic effects between ZVI and UASB not only could improve the performance in the azo dye wastewater treatment with changing loadings, but also is promising to treat other refractory wastewaters.

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