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Alkaline textile wastewater biotreatment: A sulfate-reducing granular sludge based lab-scale study

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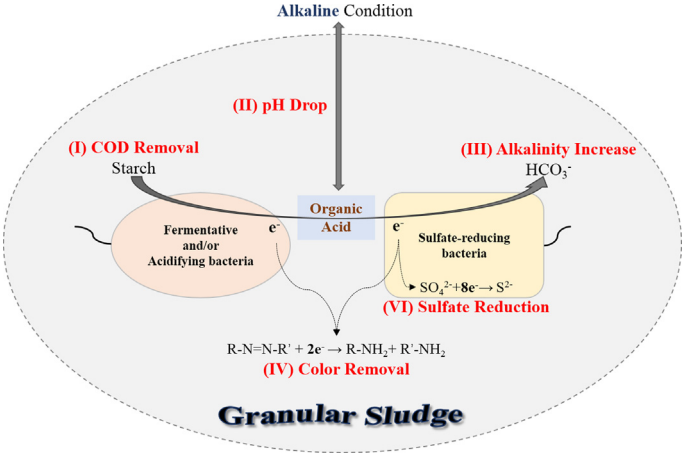
HIGHLIGHTS

- Granular-sulfidogenic bioreactor studied for treatment of textile dye wastewater.
- System achieved 90% azo dye removal without prior acclimation.
- Granular system outperformed floc system for organic and color removal.
- High organic strength induced faster hydrolysis, degradation and decolorization.
- 16S sequencing shows symbioses of sulfate reduction, fermentation and acidogenesis.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study the feasibility of treating dyeing wastewater with sulfate reducing granular sludge was explored, focusing on decolorization/degradation of azo dye (Procion Red HE-7B) and the performance of microbial consortia under alkaline conditions (pH = 11). Efficiency of HE-7B degradation was influenced strongly by the chemical oxygen demand (COD) concentration which was examined in the range of 500–3000 mg/L. COD removal efficiency was reduced at high COD concentration, while specific removal rate was enhanced to 17.5 mg-COD/g VSS h^{-1}. HE-7B removal was also improved at higher organic strength with more than 90% removal efficiency and a first-rate removal constant of 5.57 h^{-1} for dye degradation. Three dye-degradation metabolites were identified by HPLC–MS. The granular structure provided enhanced removal performance for HE-7B

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and COD in comparison to a near-identical floc SRB system and the key functional organisms were identified by high throughput sequencing. This study demonstrates an example of a niche application where SRB granules can be applied for high efficient and cost-effective treatment of a wastewater under adverse environmental conditions.

1. Introduction

Azo dyes, which are characterized by containing one or more azo bonds (—N=N—), are the largest and most versatile class of dyes used in the textile industry [1]. Many of them, including their byproducts, are carcinogenic and/or mutagenic, with potential to cause other severe health impacts such as methemoglobinemia and liver or kidney damage [2]. Textile wastewater typically contains between 2 and 50% of such dyes [3]. The discharge of such wastewaters can also impair light penetration in receiving waters due to the presence of color, while the high organic concentrations and strong alkaline characteristics add a further pollution threat to the water environment [4].

A range of physicochemical methods have been developed to effectively decolor textile effluents, such as coagulation/flocculation, activated carbon adsorption, membrane filtration and advanced oxidation processes. However, high capital expenditure, chemical handling, operational costs and/or energy demand are significant drawbacks to their application, especially when large or concentrated effluents are to be treated [5,6]. Biological treatment methods provide a potentially low operation cost option [7], particularly if made by anaerobic treatment [8]. However, during the scouring and washing processes in textile preparation, alkaline solution is applied to remove the natural waxes, pectins, spinning oils and other non-cellulosic components, raising the effluent's pH to 8–12.5 [9]. When biological treatment is conducted under such alkaline conditions, bioactivity can be significantly reduced due to the inhibition of specific enzymatic reactions and disruption of cell homeostasis [10]. Consequently, long hydraulic retention times in the order of a few days are required to complete the decolorization process [11], increasing reactor footprints and capital costs. Hence, further improvements are required in this aspect.

In a typical textile dyeing process (TDP) sulfate is the anion used as a dyebath additive for ionic strength adjustment [12], resulting in sulfate concentrations of 20–42 g/L in the dyeing effluent [13]. Starch is another major additive used as the sizing agent in the initial step of TDP, responsible for organic matter concentrations in dyeing effluents of 200–3000 mg/L of COD [1]. These two major pollutants induce sulfidogenesis by sulfate-reducing bacteria (SRB), a faster anaerobic treatment than conventionally applied methanogenic anaerobic treatment [14,15]. Sulfide produced during organic oxidation by SRB can provide electron equivalents to reduce the azo bond (—N=N—) through extracellular chemical reduction. The result is that dye decolorization can also occur at a much faster rate than that of conventional anaerobic treatment [16,17]. However, the alkaline conditions propose a potential barrier to the use of SRB [10]. The excellent resilience of sulfate reducing granular sludge to pH allows this challenge to be resolved [18]. This is not only because chemical gradients through the granules create niche zones for collective defense against higher or lower pH and toxic compounds such as azo dyes [19,20], but also the structured communal environment may enhance synergy of microbial consortia for enhanced removal of azo dyes.

HE-7B, a reactive dye employed in cellulosic fiber dyeing, is widely used as a model compound [27]. This study investigates the role of a granular sludge of SRB for HE-7B removal in textile wastewater in comparison to a near identical flocculent sludge system with consideration of microbial consortia, buffering capacity (pH and alkalinity), stability and tolerance of the system. Analysis of intermediate products of the HE-7B degradation was also performed to understand the degradation pathways.

2. Materials and methods

2.1. Reactor set-up and sulfidogenic granular sludge cultivation

A lab-scale upflow anaerobic sludge blanket (UASB) reactor was used in this study. It had an internal diameter of 45 mm, a height of 500 mm, and both ends covered with plastic plates sealed by silicone rubber. The effective liquid volume was 0.65 L and the headspace was 0.15 L. Anaerobic sludge from a sulfidogenic system was used as seeding sludge. The reactor was operated at room temperature ($22 \pm 1\,^{\circ}C$) with an internal recirculation rate of 4–5 Q (influent flow rate) to maintain a good mixing condition in the reactor. The influent used to cultivate the granules contained a mixture of organics and trace elements as described in Lau et al. [21]. By varying the organic loading, up-flow velocity and HRT [22], granular sludge was formed in the UASB reactor and was operated for more than 150 days with stable biological sulfate reduction. The performance data for the reactor can be referred to in Table S1 (Supporting information).

2.2. Experimental set-up

The synthetic textile wastewater (STW) was prepared as described in O'Neill et al., wherein, HE-7B, starch and sulfate are the main components with a ratio of 3:30:40 by compound mass [1]. Four assays of STW (groups A–D) were conducted containing 8, 28, 56 and 82 mg/L of HE-7B, while maintaining the mass ratio indicated. These four STW assays had corresponding COD concentrations of 500, 1000, 1500 and 3000 mg/L respectively.

Dye-free tests for each STW assay (A–D) were also conducted to analyze the toxicity of HE-7B to SRB granules and the contribution of starch to the COD concentration at beginning and end of each test respectively. An additional autoclaved sludge test was included to exclude the role of physical adsorption onto biomass. An abiotic test was performed to analyze the chemical reaction between sulfide and HE-7B (150 mg/L sulfide was added into STW). The control test (no microorganisms in STW) was used to demonstrate the role of biological removal. Two tests were further conducted to confirm the role of SRB granules in decolorization. The first used molybdate (10 μM) as a chemical inhibitor for SRB to inhibit sulfate reduction [23], the second used riboflavin (20 μM) as a redox mediator for facilitating the bioreaction [24].

To make a comparison to conventional systems a similar set of tests with the standard STW was conducted with SRB flocs sludge. The floc based system was cultivated using the same parameters as the SRB granular sludge (Supporting information, Table S1) with the following exceptions: the seed sludge was taken from the same original source but not pre-cultivated in the granular reactor and the reactor volume was 2.5 L. Like the granular system the floc based system was at steady state after being operated around 100 days on a synthetic brackish municipal wastewater before the batch tests. All SRB floc batch tests were conducted under the same condi-

tions as the granular test with a volatile suspended solids (VSS) concentration of 5000 mg/L.

All previously mentioned batch experiments were carried out on sludge taken from the steady-state SRB parent reactor simultaneously as follows. 15 mL PVC Falcon conical culture tubes (Mexico) were loaded with 7.5 mL of SRB granules (SRB flocs in comparison test and autoclaved SRB granules in autoclaved sludge test) with a VSS concentration of 5000 mg/L, as determined at the beginning of each experiment. An additional 7.5 mL STW (group A–D) was also added to the culture tubes. For the dye-free test and control test, pure water was used to replace the changed substance. For abiotic test, only sulfide was added into STW without bacteria. For confirming SRB involvement in decolorization, additional molybdate and riboflavin were respectively added into group A–D for the two separate tests. The culture tubes were then shaken at 10 rpm for 24 h on an MX-RD-Pro (Dragon Laboratory Instruments Limited, China). For each sample the influent pH was adjusted to 11.0 ± 0.1 with 0.1 M NaOH. Anaerobic conditions were established by flushing the influent with nitrogen gas for 15 min. Each test was conducted in triplicate and the average values were reported. 16S rRNA analysis of SRB granules and SRB flocs from the parent reactors were conducted to further interpret the performance study.

2.3. Chemical and physical analysis

Total suspended solids (TSS), VSS and pH, were measured according to the Standard Methods [30]. Total organic carbon (TOC) was analyzed instead of COD to determine the organic strength of the samples using a high temperature combustion TOC analyzer (TOC-L, Shimadzu, Japan) to avoid chloride and sulfide interferences associated with COD measurements. A correlation between TOC and COD was established with an average ratio of 2.86 g COD–1 g TOC. Sulfate was analyzed by ion chromatography (HIC-20A super, Shimadzu, Japan) and dissolved sulfide was measured with the methylene blue method [25]. Alkalinity and volatile fatty acids (VFAs) were measured by using the 5 pH-point titration method [26].

Color removal was determined based on absorbance at 544 nm corresponding to HE-7B's absorbance maxima in the UV–vis range. Color removal (%) was calculated as $(A_i - A_e)/A_i{}^*100$; where A_i is the influent absorbance and A_e is the effluent absorbance [27]. The original dye and degradation products were monitored by an Agilent 1260 High Performance Liquid Chromatograph with 6460 Mass Spectrometer and Extend-C18 column (USA). Further details of the acquisition method can be referred to the Supporting information Table S2.

2.4. Kinetic analysis

Batch cultures with various initial dye concentrations (group A–D) were used for determination of dye removal kinetic parameters. Time profiles of HE-7B concentration were measured and assessed against first-order and Monod kinetic models. The former has been reported to be applicable to dye removal [28] and the later more generally for microbial kinetics. The rate constant (k) of the first order model (Eq. (1)) was determined by conducting linear regression of Eq. (2) for all concentration points on the batch degradation curves of HE-7B, where k is given as the slope and t refers to the time.

$$A_e = A_i e^{-kt} \quad (1)$$

$$\ln(A_e) = \ln(A_i) - kt \quad (2)$$

Similarly, the Monod equation is given by Eq. (3) and following integration and rearranging can be put in the linear form of Eq. (4) so that when $\ln(S/S_0)/(S - S_0)$ is plotted against $t/(S - S_0)$ the intercept of the regression line provides the value of k_m, the maximum substrate utilization rate, and the slope gives k_s/k_m, where k_s is the substrate half saturation coefficient [29].

$$dS/dt = -kS/(S + k_s) \quad (3)$$

$$k_s/k_m{*}\ln(S/S_0)/(S - S_0) + 1/k_m = -t/(S - S_0) \quad (4)$$

Where S is the substrate concentration (mg/L), and S_0 is the initial substrate concentration.

It should be noted the above equations are simplifications based on minimal sludge growth during the batch tests. This was deemed applicable as the biomass concentration was greater than the substrate concentration and the true yield of SRB is low, in the order of 0.11 mg-VSS/g-COD. Seven data points were measured for each curve fitting.

2.5. Microbial community analysis

2.5.1. Sampling and PCR amplification

After five months of operation, samples were taken for microbial analysis from both sulfate reducing granular sludge and floc based systems. Total genomic RNA from the sludge pellets for bar-coded pyrosequencing was extracted as described in the Supporting information. Bacterial 16S rRNA genes were amplified by PCR (Bio-Rad S-1000) using amplification primer 515F + 806R targeting the V4 hypervariable regions [30], using two primer barcodes for the granular (G-SRB) and floc (F-SRB) samples:

(G-SRB)(ACGAGACTGATTAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT); and 4 (F-SRB)(AGCGGAGGTTAGAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT).

PCR amplification was carried out under the following thermal cycler: 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s; and a final extension at 72 °C for 10 min. The amplicons and other materials are described in the Supporting information.

2.5.2. Pyrosequencing and data analysis

After filtering of the low quality sequences, FASTA files were generated from the resultant sequences according to the barcodes of individual samples. Reads were assigned to each sample according to their unique barcode. Raw reads were then filtered to obtain the effective reads. A distance matrix was produced by using the software Mothur ver. 1.17.0 [31]. Sequences were assigned to operational taxonomic units (OTU) at 97% and 99% similarities (Mothur v. 1.17.0). Three metrics, Chao1 (estimating the species richness), OTU count (the count of unique OTUs found in the sample), and Shannon diversity index, were determined based on the calculated OTUs using the same software to calculate alpha diversity (Supporting information, Figs. S2–S5).

3. Results and discussions

3.1. Organics removal and sulfate reduction performance

The organics removal and sulfate reduction of SRB granules were evaluated during the tests and the overall efficiency of each batch test is presented in Fig. 1. SRB granules effectively adapted to the new alkaline operational conditions with STW feed. COD removal efficiencies of 75%, 79%, 84% and 65% were achieved at the influent COD concentrations of 500, 1000, 1500 and 3000 mg/L respectively. Sulfide concentration increased and sulfate concentration decreased for assays of groups A, B and C at an almost linear rate while group D behavior was less predictable. However, in all four assays the rate of sulfide production/sulfate reduction measured over the course of the batch increased with higher organic strengths. Total organic removal also showed a generally linear

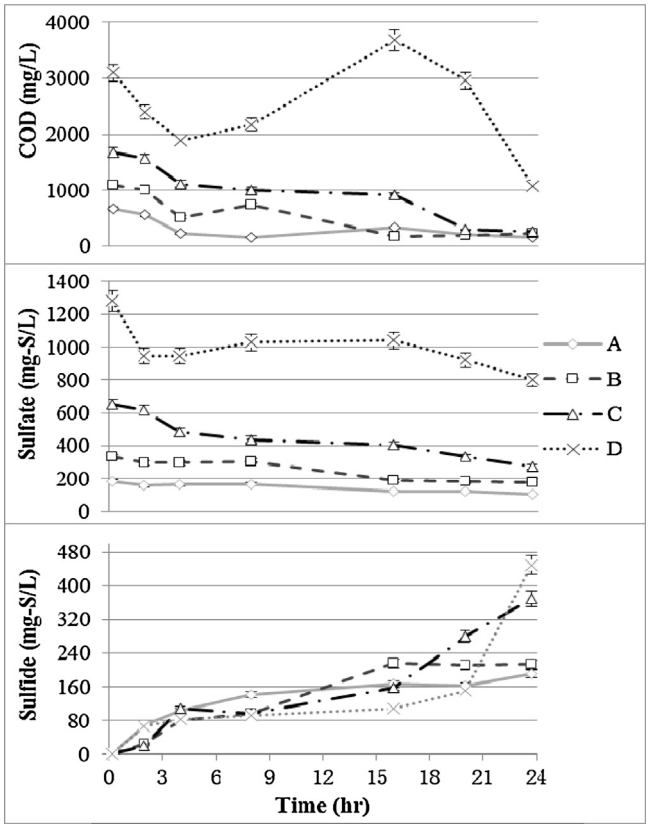

Fig. 1. Performance of SRB granular sludge, A: COD concentration of 500 mg/L with 8 mg/L HE-7B; B: COD concentration of 1000 mg/L with 28 mg/L HE-7B; C: COD concentration of 1500 mg/L with 56 mg/L HE-7B; D: COD concentration of 3000 mg/L with 82 mg/L HE-7B.

behavior except group D, which initially followed the standard behavior but between 4 and 16 h showed a significant increase in soluble COD before decreasing again.

Starch contributes the majority of COD (77.5 ± 3%) to the substrate and requires hydrolyzing before further biodegradation. Generally, starch hydrolysis is the rate limiting step of starch degradation [32]. Nevertheless, under the highest starch and dye concentration of group D (Starch = 3000 mg COD/L, HE-7B = 82 mg/L), bioadsorption of starch onto granules took place first. This is then likely to have temporarily inhibited the organisms (Fig. 1). Hydrolysis of adsorbed starch occurred subsequently at a slow rate and released organics to bulk liquid resulting in COD rising in the system. A similar buildup of starch degradation intermediates has been found in methanogenic anaerobic systems treating high starch laden concentrations of STW [33]. In the study of O'Neill et al. [33], it was found that the highest starch concentration (3.8 g/L) caused a buildup of VFA in the bulk liquid, for which starch rather than the HE-7B concentration was confirmed responsible. The Michaelis-Menten half saturation coefficient, Km, for α-amylases is frequently greater than 2000 mg/L [34]. As there were no significant differences in pH profiles during the different assays of this study to influence the Group D behavior (Supporting information, Figs. S7 and S8) the changes observed in Group D are likely to be associated with the kinetics of the starch amylase.

The role of sulfide produced from sulfate reduction may also be a key factor in the observed intermediates. Sulfide is known to stimulate the activity of β-amylase which is responsible for the cleavage of α-1,4-links from the ends of starch molecules to β-maltose and dextrins [35]. However, most other α-glucosidases responsible for cleavage of internal α-1,4-links are inhibited by sulfide above the range of 150–200 mg/L and sulfate above 1000 mg/L

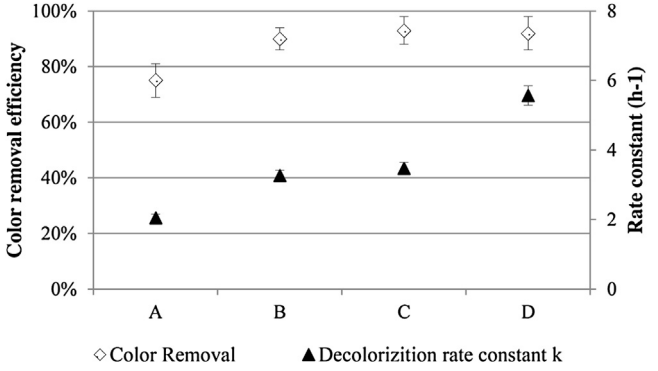

Fig. 2. Color removal and decolorization rate in four groups: A–D.

[36]. The elevated levels of sulfide and sulfate in group D may therefore contribute to increased starch hydrolysis by β-amylase but an overall reduction in SRB utilizable substrate from α-glucosidases activity that could lead to a soluble COD increase.

COD removal efficiency showed a decrease in group D. However, when considering the mass removal rate the higher organic strength was stimulatory, i.e. time was limiting the achievement of higher removal efficiencies for the high concentration STW assays due to the ratio of substrate to available biomass. Over the 24 h batch test it was found 4.2, 7.1, 11.2 and 17.5 mg-COD/g VSS h^{-1} was removed for corresponding influent COD concentrations of 500, 1000, 1500 and 3000 mg/L respectively. To confirm if there was any influence of HE-7B on the COD removal rate, dye-free assays of test A–D were also run. No significant changes in COD removal were observed, indicating that the dye was neither inhibitory nor stimulatory to the overall activity of the microbial biomass.

3.2. Decolorization performance

The same influents (group A–D) were applied for the investigation of HE-7B degradation (Fig. 2). The color removal efficiencies of different groups were 75%, 90%, 93% and 92% respectively under alkaline conditions. The decolorization process presented first-order kinetics with rate constants of 2.05, 3.26, 3.47 and 5.57 h^{-1} with $R^2 > 0.9$ except group C ($R^2 = 0.82$). Monod kinetics which were also assessed showed poor fitting to the data. The observed first order kinetic rate constants showed an approximately linear increase in the first order removal rate with an increase in dye concentration up to the maximum 82 mg/L used in this study. Therefore the results were also tested against a second order kinetic equation which was a poor fit. As the first order kinetics already account for dye concentration, the increase of the rate constant amongst the four assays indicates a stimulatory impact from other components of the STW on dye decolorization.

The increase of the rate constants could be associated with the increased influent COD concentrations. Degradation of azo dyes typically begins with the anaerobic reduction of the azo bond. This requires an electron donor, which is usually sourced from the other organics present. Increased electron donor concentration (organic substrate) therefore accelerates the decolorization rate [37]. As demonstrated by the kinetic fitting of dye decolorization and results of other studies [28], color removal follows the first order kinetics, the rate increasing linearly with increasing substrate concentration. Similar results by Kapdan and Oztekin [38] and O'Neill et al. [33] support these observations. Kapdan and Oztekin found that 500 mg/L of COD was sufficient to obtain the 90% color removal efficiency, but if the organic concentration was below this threshold color removal was influenced. O'Neill et al. also found that increasing carbohydrate concentrations could improve poor color removal performance. In addition, higher initial HE-7B concentration could

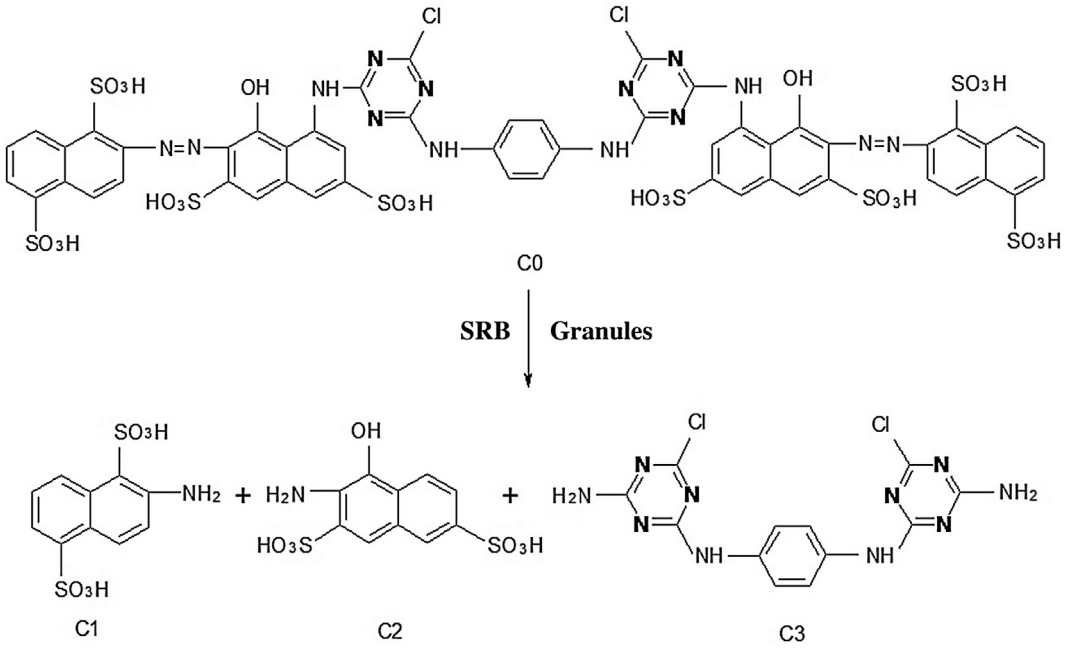

Fig. 3. The proposed reaction products of HE-7B obtained after treatment by SRB granules.

Table 1
HPLC–MS results from the analysis of HE-7B.

Compound	Retention time (min)	Molecular ion (*m*/*z*)	Proposed formula
$\mathbf{C}_0$	0.950	1598	$C_{52}H_{34}O_{26}S_8Cl_2N_{14}$
$\mathbf{C}_1$	18.652	304	$C_{10}H_9NO_6S_2$
$\mathbf{C}_2$	17.721	320	$C_{10}H_9NO_7S_2$
$\mathbf{C}_3$	20.286	366	$C_{12}H_{10}N_{10}Cl_2$

provide a driving force to overcome the mass transfer resistances of the dye between the aqueous and solid phases resulting in an enhancement on decolorization.

Inferior color removal efficiency was recorded in group A. This is most likely due to the reduced kinetic rates associated with the low electron donor concentration as detailed previously. However, additional factors could also be influential such as competition between HE-7B and sulfate to accept electrons from the oxidation of starch hydrolysis products. The oxidant-reduction potential (ORP) values of HE-7B are in the range of −316 to −371 mV [39] and the ORP of the sulfate/HSO_3^- couple is −516 mV under standard conditions [40]. Based on these values HE-7B has a higher redox potential compared to sulfate, and should accordingly act as a better electron acceptor with the electrons transferred from COD.

The chemical contribution to the reductive decolorization of azo dyes under anaerobic conditions involves biogenic reductants such as sulfide produced by SRB [41,42]. Sulfide produced by SRB could potentially act as a redox mediator shuttle with sulfide reducing the azo bond then being used as sulfur to oxidize organics. However, dos Santos et al. [43] reported that the contribution of sulfide generated by sulfate reduction seems to be negligible for decolorization. The abiotic tests in these experiments where sulfide was added into STW without bacteria confirmed that abiotic reduction of HE-7B by sulfide was not significant. Therefore, color removal is mainly caused by biological processes, or by sulfide only when mediated by SRB.

The intrinsic property of all biochemical reactions is electron transfer with faster electron transfers resulting in faster bioreactions. Therefore, the crucial role of bacteria in degradation of HE-7B was further verified by the addition of riboflavin, which is a kind of

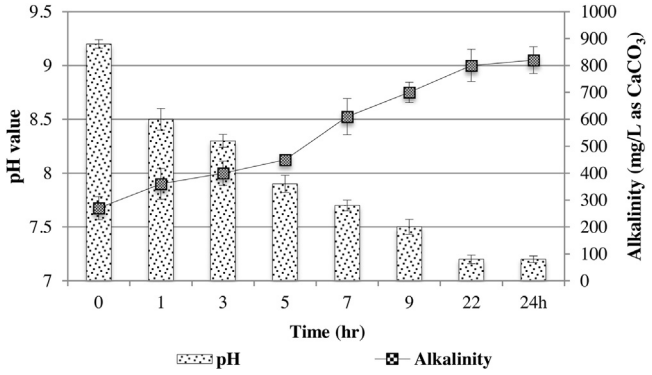

Fig. 4. Alkalinity and pH profile of group A with testing duration of 24 h.

redox stimulator for facilitating extracellular electron transfer [33]. The riboflavin showed a positive effect on decolorization in the biological reaction, further confirming the role of biological activity in the dye reduction. A similar phenomenon was also observed by Yoo et al. [44] for degradation of C. I. Reactive Orange 96. In the final tests to confirm biotic vs abiotic activity and the role of SRB, molybdate was used to inhibit biological sulfate reduction and autoclaved sludge was used to assess removal by sludge adsorption. For molybdate inhibited SRB granules little decolorization (8.7 ± 1.3%) took place over a 24 h period highlighting the crucial role of SRB while little color removal (5.6 ± 2.4%) was observed with the autoclaved sludge tests indicating that removal by biomass adsorption was limited.

3.3. Identification of the degradation products of HE-7B

The intermediates of HE-7B degradation by sulfate reducing granular sludge were analyzed by HPLC–MS (Supporting information, Fig. S1), giving evidence that anaerobic biodegradation of HE-7B produced three types of intermediates ($\mathbf{C}_1$: 2-Amino-1,5-naphthalene disulfonic acid; $\mathbf{C}_2$: 2,7-Naphthalenedisulfonic acid, 3-amino-4-hydroxy-; $\mathbf{C}_3$: 1,3,5-Triazine-2,4-diamine, *N*2, *N*2′-1,4-phenylenebis [6-chloro-). These compounds also suggested

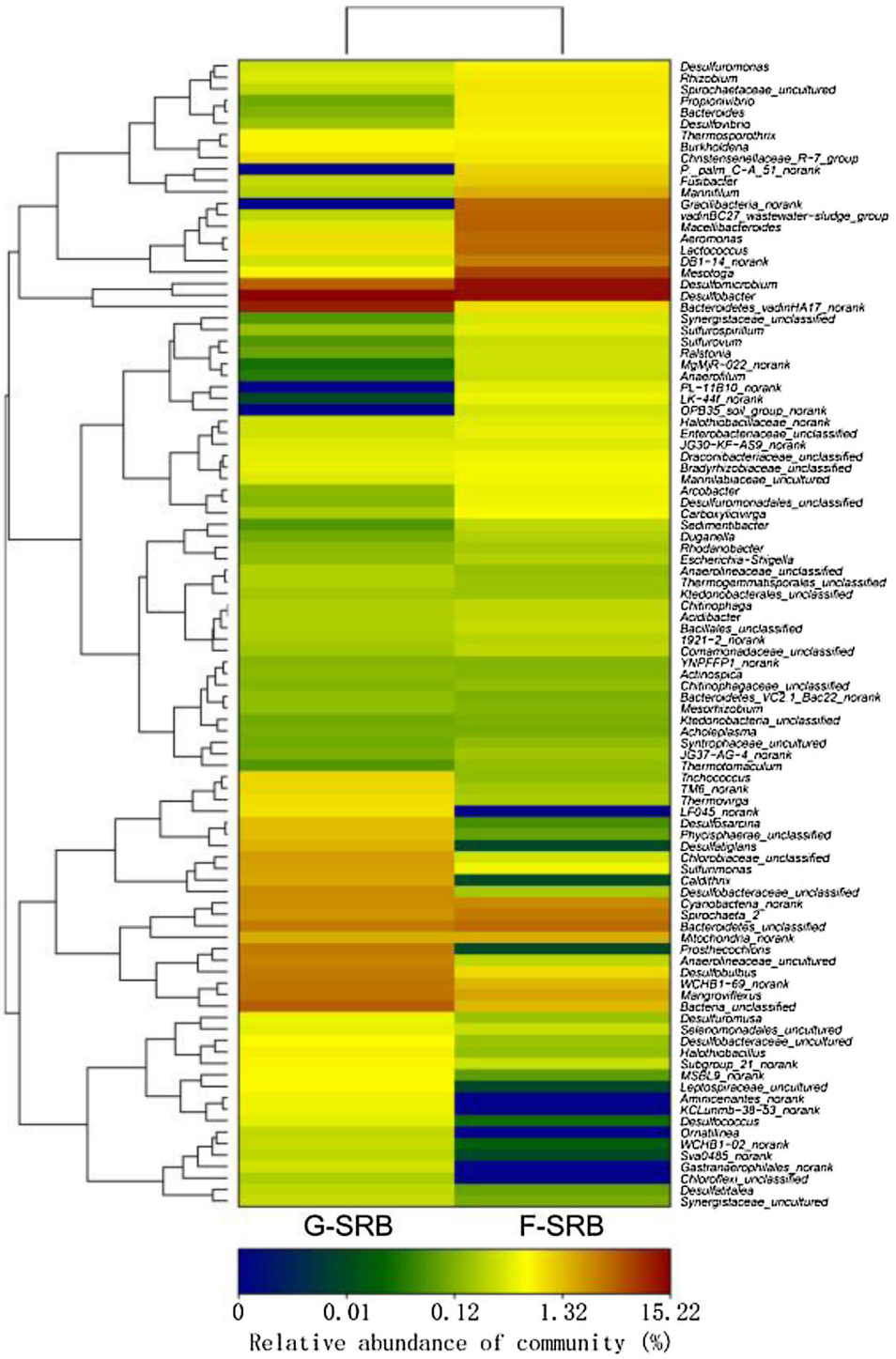

Fig. 5. Taxonomic classification of bacterial 16S rRNA gene reads retrieved from SRB granules (G-SRB) and SRB flocs (F-SRB) at genera level using RDP classifier with a confidence threshold of 80%.

decolorization occurs by cleavage of the azo bond involved with the presence of SRB. Retention times and the most intense ions in the MS spectrum of the dye degradation products are shown in Table 1 ($\mathbf{C_0}$ represents the HE-7B; $\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_3}$ represents the three types of intermediates).

Supported with prior knowledge of the molecular pattern and the potential biochemical pathways [8,45], the proposal of the best chemical structures are shown in Fig. 3. While these are still quite complex intermediates that may require further aerobic oxidation for complete removal [33], the toxicity of HE-7B intermediates from anaerobic degradation is significantly lower than the parent dyes, both for humans [46] and agriculture [47]. On the other hand, it was observed that different chemical structures affect the rates of biodegradation for different dyes. Mahmood et al. [48] demonstrated that azo dyes with electron-withdrawing groups (e.g. sulfo group in Reactive Red 198, Reactive Black 5 and HE-7B) decolorize faster than the azo dyes with the electron-releasing groups (–NH-triazine in RB171 and RG19), indicating the important role of electron withdrawing groups in biodegradation of azo dyes.

3.4. Alkalinity, VFA and pH of effluents

Time profiles of pH and buffer capacity are shown in Fig. 4 for the treatment of STW by SRB granules in group A. Similar behaviors were also observed for groups B, C and D (Supporting information, Figs. S7–S10). The pH decreased immediately from 11 in the STW to 9.2 when the STW was added into the SRB granular treatment system, possibly due to charge neutralization by reaction of the dye on the cell surface. Thereafter the pH gradually dropped to 7.4 during the treatment. Conversely, alkalinity increased from 273 ± 28 mg/L to 824 ± 35 mg $CaCO_3$/L. During the experiments VFA was not detected.

In order to explore whether the pH and alkalinity profiles observed were associated with SRB granule activity, two experiments, a control test (no microorganism in STW) and a comparison test (SRB flocs in STW), were conducted. In the control test the pH and alkalinity remained unchanged. In the presence of SRB flocs during the comparison test, the pH also dropped significantly from 11 to 7.8 and alkalinity increased to 668 ± 42 mg $CaCO_3$/L within 24 h. For comparison tests of group B,C and D with SRB flocs, the alkalinity increased with a higher influent COD concentration as well (Supporting information, Figs. S9 and S10). However, approximately 40% more COD and 10% more color removal from the STW were achieved by SRB granules compared to SRB flocs.

Further, bar-coded pyrosequencing was used to investigate the bacterial communities of SRB granules and flocs (Fig. 5). Altogether, 25 and 22 bacterial phyla were recovered from the granules and flocs systems respectively. The majority of 16S rRNA gene sequences in granules belonged to the phyla Proteobacteria (41%), Bacteroidetes (22%), Chloroflexi (6%) and Chlorobi (5%), which was similar for SRB flocs. At the genus level, the most dominant functional genera of the microbial community in the SRB granules and flocs were the complete oxidizers (*Desulfobacter and Desulfomicrobium*) as well as the acidifying bacteria (e.g. *Lactococcus*) and *Macellibacteroides*. Additionally, *Bacteroidetes_vadinHA17_norank* and *Mangroviflexus* were also dominant in granules while *Mesotoga, Gracilibacteria_norank, Aeromonas* and *Spirochaeta_2* were major genera in flocs.

Starch is the major component of COD in the STW because degumming is the first stage of industrial textile processing. This macro-organic is hydrolysed then degraded through several steps to VFA prior to utilization by SRB. This could cause a decrease in pH and adverse impacts on reactor performance. In this case, VFA was not detected during entire experiment, which explains the presence of the complete oxidizers (*Desulfobacter* and *Desulfomicrobium*) detected in the microbial community, which completely oxidize VFA to CO_2. As starch hydrolysis is typically the rate limiting step, the ability to completely oxidize VFA allows SRB organisms to maximize their energy yield in an environment where strong competition exists for limited soluble substrate.

Pearce et al. [49] pointed out that the biological reduction of the azo bond can result in an increase in the pH due to the formation of aromatic amine metabolites, which are more basic than the original azo compound. But Chen et al. [50] inferred that the degradation of glucose to organic acids in the medium would result in a pH drop with inferior decolorization performance. The variability may be due to the different microbial degradation pathways and dye structures. In the sulfidogenic system, the pH could decrease (e.g. with lactate or ethanol) or increase (e.g. with H_2 or formate) depending on the electron donor type (Supporting information, Table S3), while the carbonate alkalinity is independent from electron donor type and will increase [51]. In this study, starch is the main carbon source of STW. For starch degradation under SRB metabolism pH decrease and alkalinity increase can be expected [52].

On the other hand, the pH and alkalinity profiles in SRB granules and SRB flocs could be explained by the combination of acidogenesis and sulfate reduction processes in the microbial consortia. Acidogenesis is conducted by biomass including *Lactococcus* (0.9% and 3.9% in SRB granules and flocs respectively) and additionally, *Macellibacteroide*s (0.5% and 4.0% respectively in SRB granules and flocs) which has been reported as a fermentative microorganism associated with starch digestion [53]. These genera, under the protection of granules, exist synergistically with the SRB community by producing acids that lower the high pH environment. However, SRB flocs lack the compact structure of granules and are therefore at greater risk from the adverse environmental conditions. This might be one of the reasons for inferior COD and color removal at high pH, even though the dominant genera such as *Mesotoga* and *Aeromonas* in SRB flocs are complimentary in their functions.

Further, the acidogenic process is followed by sulfate reduction where VFAs are quickly consumed due to the activity of complete oxidizers such as *Desulfobacter* and *Desulfomicrobium* (total 19.0% and 24.4% respectively in SRB granules and SRB flocs). Additionally, *Bacteroidetes_vadinHA17_norank* (9.7%) and *Mangroviflexus* (3.4%) in the granular biocommunity may cooperatively degrade the macro-organic substrates to micro-organic substrates which are preferable for SRB [54,55]. SRB then produce dissolved inorganic carbon (as bicarbonate ion) which contributes to the alkalinity and buffering capacity of the biological system. From this phylogenetic analysis, it is apparent that the SRB granules showed a broad diversity of species while possessing a few dominant genera associated with the key functions of the process. This information will be useful in allowing the specific manipulation and optimization of the system while retaining the resilience of the diverse community.

4. Conclusions

Application of the SRB granules for alkaline textile wastewater treatment was explored in this study. SRB were demonstrated to be highly involved in both the COD removal and decolorization through a variety of batch tests. Higher wastewater strengths enhanced kinetic rates for both COD removal and decolorization demonstrating the resilience and suitability of the granular system to this industrial waste stream. In all assays pH was reduced to neutral conditions through combined acid and alkalinity production from the two main bioprocesses. Bar-coded pyrosequencing of the 16S rRNA gene revealed diverse microbial populations in SRB granules and SRB flocs, both including acidifying bacteria (*Lactococcus*) and complete oxidizers (*Desulfobacter and Desulfomicrobium*). Future work should focus on enhancing the efficiency and functionality of SRB and investigating the specific metabolism involvement of SRB in HE-7B degradation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2017.03.005.

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