

# Biodegradation of Acid Scarlet 3R by a New Salt-tolerant Strain *Alcaligenes faecalis* LJ-3: Character, Enzyme and Kinetics Analysis



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*Alcaligenes faecalis* LJ-3 is highly efficient in degrading various azo dyes (100 mg L<sup>-1</sup>). Almost 100 % degradation was observed within 16 h at the initial Acid Scarlet 3R concentrations of 1000 mg L<sup>-1</sup> under the optimal conditions, which were: 1 g dextrin L<sup>-1</sup>, 3 g yeast extract L<sup>-1</sup>, NaCl ≤ 30 g L<sup>-1</sup>, pH 8.0 and 35–45 °C. Azoreductase, laccase and NADH-DCIP (nicotinamide adenine dinucleotide-dichlorophenol indophenols) reductase were induced during the degradation of Acid Scarlet 3R. Kinetics study of degradation experiments approximated the first-order reaction. The maximum rate ( $V_{max}$ ) and substrate affinity constant ( $K_s$ ) were found to be 115.90 mg L<sup>-1</sup> h<sup>-1</sup> and 1193.23 mg L<sup>-1</sup>, respectively, using Michaelis-Menten kinetics. This work provides new data characterizing Acid Scarlet 3R degradation by bacteria, as well as practical application potential in biological treatment of industrial effluents containing various azo dyes.

**Keywords:**

*Alcaligenes faecalis* LJ-3, degradation, Acid Scarlet 3R, enzyme, kinetics

## Introduction

It is well known that the demographic expansion, economic development, and industrialization have continuously generated increasing water pollution, which not only poses a serious threat to the survival of aquatic organisms, but also causes human health risks<sup>1</sup>.

Azo dyes, aromatic and/or heterocyclic compounds characterized by the presence of one or more azo groups (-N=N-), are the largest chemical class of synthetic dyes and pigments, representing about 70 % of the synthetic dyes used in textile, leather, plastic, cosmetic, and food industries<sup>2</sup>, and 10–15 % azo dyes are lost in aqueous effluents during coloration processes. Most azo dyes are toxic and/or mutagenic to living organisms, and highly stable to light and oxygen due to their complex chemical structures<sup>3</sup>. Therefore, various technologies have been developed to treat azo dyes containing effluents. Compared with physicochemical processes such as adsorption, membrane separation, ion-exchange, coagulation, and oxidation, biological methods are better alternatives due to their lower cost, higher efficiency and less secondary pollution<sup>4,5</sup>.

Hence, increasing research has been performed to screen microbes for azo dyes degradation in the last decades. Some bacterial, yeast, fungal, and algal species such as *Lactobacillus paracase* CL1107<sup>6</sup> and *Scheffersomyces spartinae* TLHS-SF1<sup>7</sup>, etc., have been confirmed to be able to decolorize azo dyes through degradation or adsorption. In particular, bacteria have been proved to be the most promising due to their extensive distribution, strong adaptability, short life cycle, less secondary waste, and high activity<sup>8</sup>. Bacterial consortium AR1 could completely decolorize 100 mg L<sup>-1</sup> of Reactive Red 195 within 14 h<sup>9</sup>. Chen *et al.* developed a thermophilic microflora which biodegraded 97 % of Direct Black G (600 mg L<sup>-1</sup>) within 8 h<sup>10</sup>. Furthermore, much effort has been devoted to isolate bacterial strains capable of efficiently degrading azo dyes. Garg *et al.* isolated *Pseudomonas putida* SKG-1 (MTCC10510) that was capable of decolorizing monoazo dye Orange II efficiently<sup>11</sup>. Guadie *et al.* isolated and characterized Reactive Red 239 decolorization strain (*Bacillus* sp. strain CH12) from alkaline lake<sup>12</sup>. *Bacillus* sp. YZU1 showed approximately 95 % decolorization of Reactive Black 5 (100 mg L<sup>-1</sup>) within 120 h<sup>13</sup>. *Aeromonas* sp. strain DH-6 was also observed to have a remarkable ability to decolorize methyl orange, nearly 100 % decolorization at concentration of 100 mg L<sup>-1</sup> in 12 h<sup>14</sup>.

Azo dye decolorization/degradation varies with varying strains and cultural conditions. Acid Scarlet 3R is an electron-deficient xenobiotic compound

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because of its azo linkage and sulphonic ( $\text{SO}_3^-$ ) groups, which generate an electron deficiency and make the dye less susceptible to degradation by microorganisms. In the present study, a new strain, *Alcaligenes faecalis* LJ-3, capable of decomposing Acid Scarlet 3R, was successfully isolated from textile dye contaminated soil of a printing and dyeing plant. The effects of different parameters on degradation were investigated. In addition, the pertinent enzymes and degradation kinetics were also analyzed to explore the degradation mechanism.

## Materials and methods

### Microorganism, media, dyes, and chemicals

*Alcaligenes faecalis* LJ-3 (CGMCC14108) was screened by the Laboratory of Fermentation, Wenzhou University (Wenzhou, China) and collected in China General Microbiological Culture Collection Center (Beijing, China).

Luria-Bertani medium (LB,  $\text{g L}^{-1}$ ): peptone 10, yeast extract 5 and NaCl 5; Acclimation medium ( $\text{g L}^{-1}$ ): Glucose 1,  $(\text{NH}_4)\text{NO}_3$  2.0,  $\text{Na}_2\text{HPO}_4$  0.5,  $\text{KH}_2\text{PO}_4$  0.5,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2, and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  0.1; Basal medium ( $\text{g L}^{-1}$ ):  $\text{Na}_2\text{HPO}_4$  1.0,  $\text{KH}_2\text{PO}_4$  3.0, NaCl 0.5,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5 and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  0.025.

ABTS (2,2-Azinobis(3-ethylbenzothiazolin-6-sulfonic acid)), DCIP (2,6-Dichloroindophenol sodium), 3,4-Dimethoxybenzyl alcohol and NADH (reduced form of nicotinamide adenine dinucleotide) were purchased from Sigma-Aldrich Co. (USA). Azo dyes (Acid Scarlet 3R, Methyl Orange, Amino Black 10B, Acid Black 210, Brilliant Scarlet GR, Acid Red 88, Reactive Brilliant Red X-3B, Amaranth, Acid Orange G and Direct Red 80) and other reagents were obtained from Sinopharm Chemical Reagent Company (China). All chemicals used were of the highest purity available and of analytical grade.

### Enrichment and isolation of azo dye-degrading bacteria

The textile dye contaminated soil used for the isolation of bacteria was collected from Lucheng printing and dyeing plant, Wenzhou, China. Ten grams of sludge samples were added into 250-mL Erlenmeyer flasks containing 100 mL of LB medium, and the flasks were incubated at 37 °C for 16 h. Then, 10 mL of each culture was inoculated into 100 mL of acclimation medium containing 50  $\text{mg L}^{-1}$  Acid Scarlet 3R, and incubated under the same conditions described above. Once decolorization was observed, 10 mL of culture was transferred to 100 mL fresh acclimation medium containing 100

$\text{mg L}^{-1}$  Acid Scarlet 3R. With each successive transfer, the concentration of Acid Scarlet 3R was gradually increased to 200  $\text{mg L}^{-1}$ . After the fifth enrichment transfer, each culture was serially diluted with sterile saline and spread on LB plates containing 100  $\text{mg L}^{-1}$  Acid Scarlet 3R for the isolation of single colonies. After being incubated under anoxic conditions at 37 °C for 48 h, the bacterial colonies were screened according to the ability to form a clear zone on the plate. The bacteria showing higher zones of decolorization were isolated and further purified by streaking plate method on LB plates containing 100  $\text{mg L}^{-1}$  Acid Scarlet 3R. Pure culture was maintained on LB agar slants, stored at 4 °C, and sub-cultured monthly.

### Identification of the isolated strain by 16S rDNA sequencing

The bacterial strain with the highest degradation ability of Acid Scarlet 3R was named LJ-3 and selected for further study. The 16S rDNA sequencing of strain LJ-3 was carried out at Sangon Biotech (Shanghai) Co Ltd., China. Briefly, the isolated genomic DNA was used as a template to amplify the 16S rDNA gene by PCR with the universal primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTTGTTACGACTT-3'). The PCR was run for 30 cycles with the following thermal conditions: 45 s of denaturation at 94 °C, 45 s of primer annealing at 55 °C, and 1 min of extension at 72 °C. The final cycle included an extension of 10 min at 72 °C. The resulting sequence (GenBank accession No. MG574872) was compared with known sequences in the GenBank database by BLAST.

### Degradation experiments

Degradation experiments were carried out in 250-mL Erlenmeyer flasks containing 100 mL sterilized medium supplemented with azo dye, and the inoculation size ( $\text{OD}_{600}$  0.8) was 2 % (v/v). To characterize the degradation efficiency of strain LJ-3, the effects of static anoxic/shaking conditions (160 rpm), carbon sources (glucose, maltose, sucrose, lactose, dextrin, fructose, and xylose), nitrogen sources (yeast extract, beef extract, peptone, urea, glycine,  $\text{NH}_4\text{NO}_3$ , and  $\text{NaNO}_3$ ), initial pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0), incubation temperature (15, 20, 25, 30, 35, 40, 45, 50 °C), and salinity (5, 10, 15, 20, 25, 30, 50, 80  $\text{g L}^{-1}$  NaCl) on the degradation of 100  $\text{mg L}^{-1}$  Acid Scarlet 3R were individually monitored. To find out maximum dye degrading ability of strain LJ-3, different concentrations of Acid Scarlet 3R (100, 200, 400, 600, 800, 1000, 1500, 2000  $\text{mg L}^{-1}$ ) were respectively tested. To determine the degradation activity of strain LJ-3

on other azo dyes, the cells were respectively incubated in the media containing 100 mg L<sup>-1</sup> of different azo dyes.

Concentration of azo dyes in supernatant withdrawn at different time intervals was analyzed at the maximum absorption wavelength (Table 1) with a UV-1810 spectrophotometer (Puxi General Instrument Co., Ltd., China) after centrifugation at 4,000 g for 10 min. The percentage degradation was calculated according to the following formula (1).

$$\text{Degradation (\%)} = (A_0 - A_t) / A_0 \cdot 100 \quad (1)$$

where  $A_0$  and  $A_t$  is the absorbance of the dye at initial and different reaction times, respectively.

### Enzyme analysis

The LJ-3 cells were harvested by centrifugation at 12000 g for 10 min. The harvested cells were then suspended in 100 mM sodium phosphate buffer (pH 7.4) and sonicated at 4 °C based on a 100 amplitude output, 99 strokes of 7 s, each at 3 s intervals. The supernatant was collected by centrifugation (4 °C, at 12000 g for 15 min) and used for enzyme analysis.

The activities of azoreductase, laccase, veratryl alcohol oxidase, and NADH-DCIP reductase were assayed spectrophotometrically by reference to the methods of Shah *et al.*<sup>15</sup>. Manganese peroxidase activity was measured by reference to the method of Bilal and Asgher<sup>16</sup>. Protein concentration was determined by the Lowry method using bovine serum albumin as the standard<sup>17</sup>. One unit of enzyme activity was defined as the amount of enzyme required to reduce or oxidize 1 µg (or µM) of substrate per minute per mg of protein.

### Kinetics study

To determine the order of the degradation reaction of *A. faecalis* LJ-3, the rate constants of degradation reaction and coefficients of least square method analysis were calculated. The zero-, first- and second-order reaction kinetics were respectively expressed as equations (2)–(4) below:

$$[D]_t = [D]_0 - k_0 t \quad (2)$$

$$\ln[D]_t = \ln[D]_0 - k_1 t \quad (3)$$

$$1/[D]_t = 1/[D]_0 + k_2 t \quad (4)$$

where  $[D]_0$  and  $[D]_t$  are the concentrations of dye at reaction time 0 and  $t$ , respectively.  $k_0$ ,  $k_1$  and  $k_2$  are the kinetic rate constants of zero-, first-, second-order reaction kinetics, respectively.  $t$  is the reaction time.

Michaelis-Menten model (equation 5) was used to fit the relationship between the degradation rate and initial dye concentration.

$$V = \frac{V_{\max} [D]}{K_s + [D]} \quad (5)$$

where  $[D]$  (mg L<sup>-1</sup>),  $V_{\max}$  (mg L<sup>-1</sup> h<sup>-1</sup>) and  $K_s$  (mg L<sup>-1</sup>) are dye concentration, maximum dye degradation rate and substrate affinity constant, respectively.

## Results and discussion

### Identification and azo dye biodegradation of strain LJ-3

In the present study, a bacterial strain (named LJ-3), capable of decolorizing Acid Scarlet 3R (100 mg L<sup>-1</sup>) on solid agar plate, was successfully isolated from textile dye contaminated soil of a printing and dyeing plant in Wenzhou, China. It was Gram-negative, and the colony on LB plate was smooth, lustrous, light brown and regular on the edge. The 16S rDNA gene sequence (1337 bp) of strain LJ-3 was determined and deposited in GenBank database under the accession number MG574872. Strain LJ-3 was identified as *A. faecalis* based on the sequence similarity analysis, which exhibited 100 % homology to *A. faecalis* Fa1.3 (accession number KF383272).

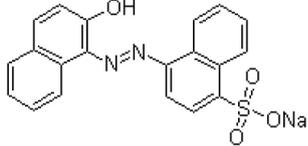
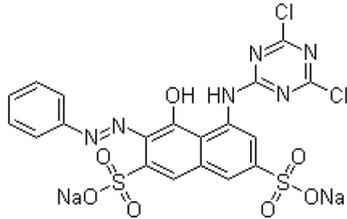
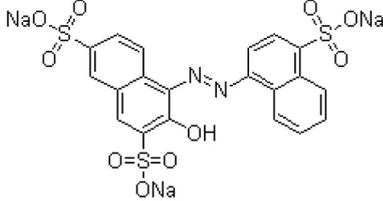
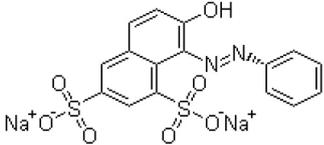
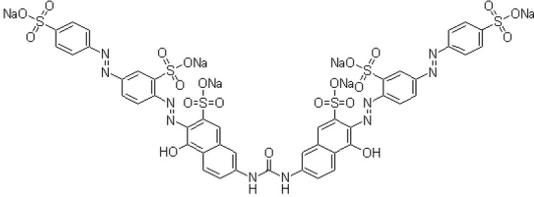
It had been reported that some isolates belonging to *A. faecalis* could biodegrade and detoxify a series of xenobiotic/recalcitrant compounds such as endosulfan<sup>18</sup>, nicosulfuron<sup>19</sup> and ochratoxin A<sup>20</sup>. Strain *A. faecalis* PMS-1 could also decolorize Reactive Orange 13<sup>15</sup>. As shown in Table 1, strain *A. faecalis* LJ-3 could efficiently biodegrade a wide variety of azo dyes, including Acid Scarlet 3R, Methyl Orange, Amino Black 10B, Acid Black 210, Brilliant Scarlet GR, Acid Red 88, Reactive Brilliant Red X-3B, Amaranth, Acid Orange G, and Direct Red 80. Except for Acid Black 210, the highest degradations of the other nine dyes by strain *A. faecalis* LJ-3 were more than 92 % within 4–24 h. Obviously, strain *A. faecalis* LJ-3 has great potential in treating industrial effluents containing azo dyes due to the high dye adaptability and short removal time.

### Effects of parameters on biodegradation of Acid Scarlet 3R by strain LJ-3

As shown in Fig. 1, 96.94 % degradation of Acid Scarlet 3R by strain *A. faecalis* LJ-3 was determined under static anoxic conditions, whereas only 3.77 % degradation was observed under shaking conditions at 160 rpm after 8 h of incubation. Similar results were also reported previously on

Table 1 – Degradation of various azo dyes (100 mg L<sup>-1</sup>) by *A. faecalis* LJ-3

Azo dyes		Before degradation	After degradation	Maximum wavelength (nm)	Degradation (%)	Time (h)
Name	Molecular structure					
Acid Scarlet 3R				508	98.95	4
Methyl Orange				462	95.51	6
Amino Black 10B				618	92.62	12
Acid Black 210				606	85.3	24
Brilliant Scarlet GR				506	96.7	10

Azo dyes		Before degradation	After degradation	Maximum wavelength (nm)	Degradation (%)	Time (h)
Name	Molecular structure					
Acid Red 88				502	94.65	6
Reactive Brilliant Red X-3B				538	93.89	12
Amaranth				520	98.72	6
Acid Orange G				478	91.84	12
Direct Red 80				528	93.39	24

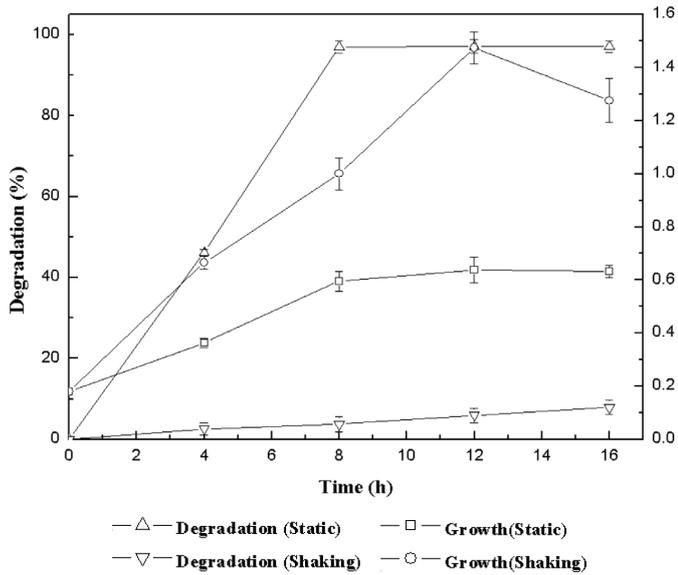


Fig. 1 – Effects of shaking (160 rpm) and static anoxic conditions on the degradation of Acid Scarlet 3R by *A. faecalis* LJ-3

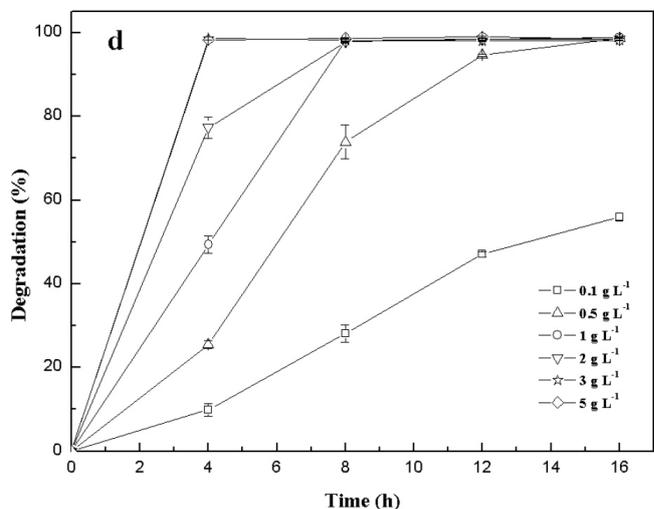
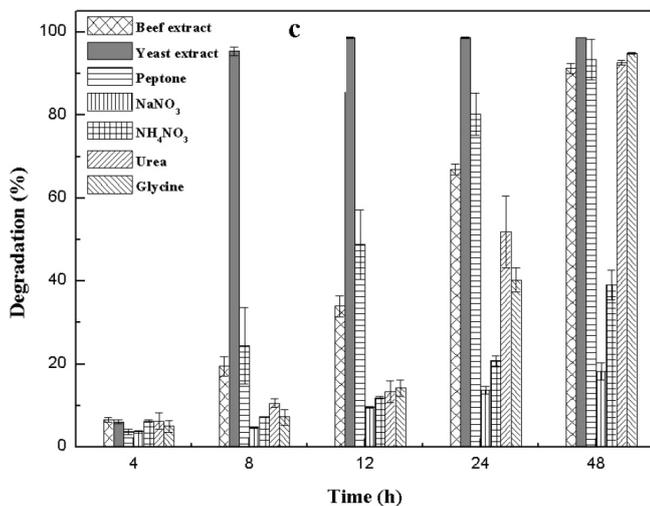
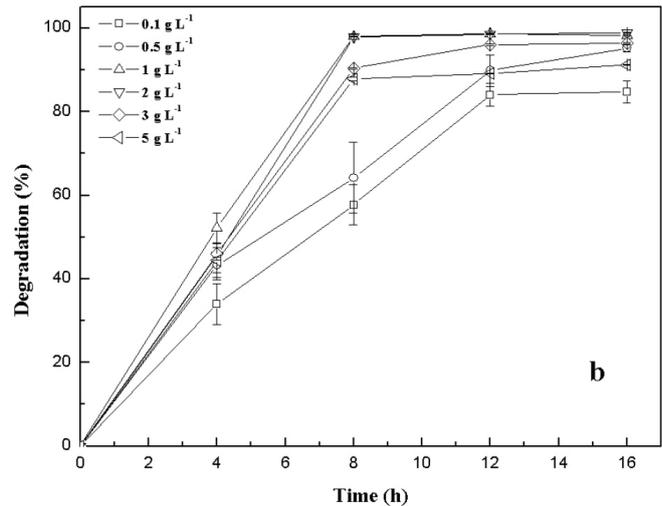
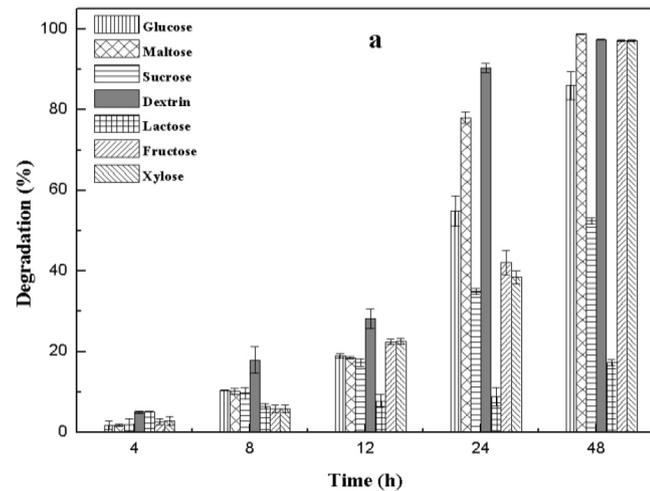


Fig. 2 – Effects of carbon sources (a), dextrin concentration (b), nitrogen sources (c), and yeast extract (d) on the degradation of Acid Scarlet 3R by *A. faecalis* LJ-3

pure bacterial strains such as *A. faecalis* PMS-1<sup>15</sup>, *Pseudomonas* sp. SUK1<sup>21</sup> and *Micrococcus glutamicus* NCIM-2168<sup>22</sup>. Because reduction is the basic mechanism of bacterial biodegradation, anaerobic or anoxic conditions are beneficial to the degradation of azo dyes by bacterial strain<sup>23</sup>.

Azo dye degradation by microbes generally requires supplement of carbon or nitrogen sources. Jain *et al.* suggested that glucose is the optimal carbon source for Reactive Violet 5R degradation by bacterial strains such as *Bacillus* sp. V1DMK and *Lysinibacillus* sp. V3DMK<sup>24</sup>. However, our results showed that, when dextrin was supplemented as a carbon source, the strain *A. faecalis* LJ-3 showed a faster removal of Acid Scarlet 3R (Fig. 2a). The results were supported by Mathew and Madamwar, who confirmed that soluble starch was the optimal carbon source for azo dye degradation by bacterial consortium SV5<sup>25</sup>. The biodegradation increased with the increase in dextrin concentration from 0.1 to 1.0 g L<sup>-1</sup>, and reached 98.05 % within 8 h (Fig. 2b).

Of all tested nitrogen sources in this work, glycine,  $\text{NH}_4\text{NO}_3$  and  $\text{NaNO}_3$  exhibited less than 10 % degradation of the dye within 8 h, followed by urea, beef extract, and peptone with degradation of 10.61 %, 19.52 % and 24.41 %, respectively (Fig. 2c). In accordance with the previous studies<sup>24,25</sup>, yeast extract showed best degradation efficiency (95.42 %) because yeast extract is helpful for NADH regeneration<sup>23</sup> and stimulating for azoreductase activity<sup>26</sup>. The degradation reached 98.69 % within 4 h when 3 g  $\text{L}^{-1}$  yeast extract was added, while the further increase in yeast extract concentration could not significantly enhance degradation efficiency (Fig. 2d).

Temperature and pH-value are important parameters for azo dye biodegradation by bacteria. Strain *A. faecalis* LJ-3 could degrade Acid Scarlet 3R in the pH range of 5.0–10.0 with degradation above 80 % within 16 h, but the optimal degrading efficiency was found at pH 8.0 with 97.49 % degradation within 8 h (Fig. 3a). With an increase in tem-

perature from 15 to 35 °C, the degradation increased from 7.12 % to 97.38 % within 8 h, and similar degradation was observed at 40 °C and 45 °C, while the dye almost could not be degraded at 50 °C (Fig. 3b). The results suggest that the optimum temperature range for degradation was between 35 °C and 45 °C, which was in accordance with the conclusion of Pearce *et al.*<sup>27</sup>

Generally, the high concentrations of salts existing in textile industrial effluents will affect the microbial growth and dye biodegradation efficiency by causing plasmolysis of cells<sup>7,13</sup>. As shown in Fig. 3c, the degradation by *A. faecalis* LJ-3 reached 93.64 % within 16 h in the presence of 30 g  $\text{L}^{-1}$  NaCl. However, there are negative effects on the degradation with further increased salinity, and lower than 57 % and 14 % degradation efficiency were observed within 16 h in NaCl concentrations of 50 g  $\text{L}^{-1}$  and 80 g  $\text{L}^{-1}$ , respectively. Different strains show a wide range of salt tolerance. The maximum salt tolerance of *Pseudomonas* sp. ACT 1

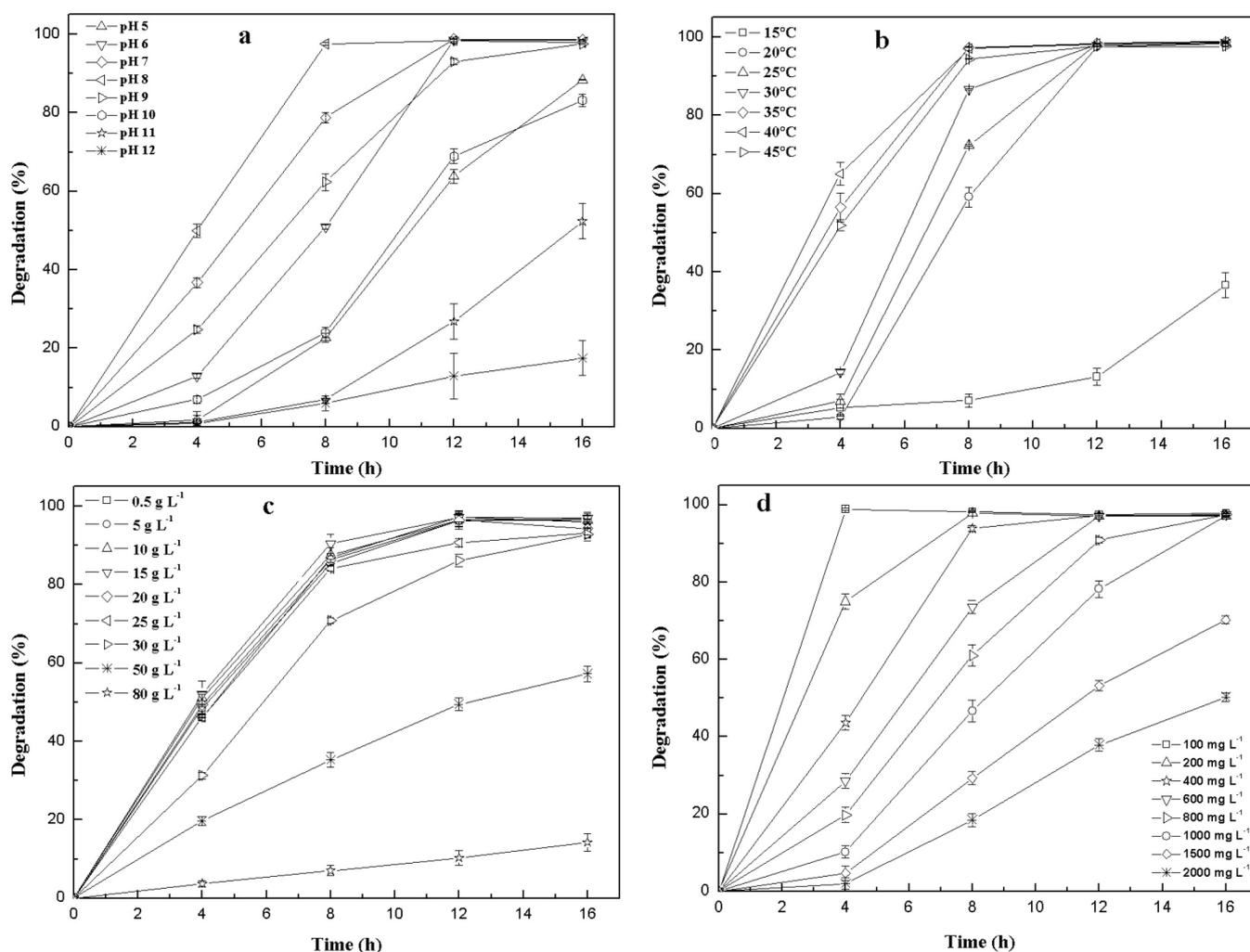


Fig. 3 – Effects of pH (a), temperature (b), salt concentration (c), and initial dye concentration (d) on the degradation of Acid Scarlet 3R by *A. faecalis* LJ-3

Table 2 – Comparison of azo dye removal efficiency of this study and other studies

Microbial isolate	Azo dye	Concentration (mg L <sup>-1</sup> )	Time (h)	Degradation (%)	Reference
<i>Scheffersomyces spartinae</i> TLHS-SF1	Acid Scarlet 3R	100	16	80	7
<i>Pseudomonas putida</i> SKG-1	Orange II	100	96	85.2	11
<i>Bacillus</i> sp. strain CH12	Reactive Red 239	100	24	95	12
<i>Bacillus</i> sp. YZU1	Reactive Black 5	100	120	95	13
<i>Aeromonas</i> sp. DH-6	Methyl Orange	100	12	Almost 100	14
<i>Alcaligenes faecalis</i> PMS-1	Reactive Orange 13	400	16	Almost 100	15
<i>Brevibacillus laterosporus</i> MTCC 2298	Disperse Red 54	200	72	94	30
<i>Enterobacter</i> sp. EC3	Reactive Black 5	500	120	91.2	34
<i>Enterobacter aerogenes</i> PP002	Direct Green 28	100	168	99.23	35
<i>E. aerogenes</i> PP002	Direct Blue 71	100	168	99.0	35
<i>A. faecalis</i> LJ-3	Acid Scarlet 3R	100	4	98.95	This study
<i>A. faecalis</i> LJ-3	Acid Scarlet 3R	1000	16	99.17	This study

in Congo Red biodegradation was 8 g NaCl L<sup>-1</sup> after acclimatization<sup>28</sup>. *Psychrobacter alimentarius* KS23 and *Staphylococcus equorum* KS26, isolated from Arabian seawater sediment, could efficiently degrade Reactive Black 5 in the presence of 40 g NaCl L<sup>-1</sup>.<sup>29</sup> The result of this study showed that *A. faecalis* LJ-3 was salt-tolerant because of its good degradation efficiency at high salinity.

Dye concentration significantly influences degradation efficiency of microbes and faster degradation is achieved in lower dye concentrations. Almost 100 % degradation was observed within 4 h at the initial Acid Scarlet 3R concentration of 100 mg L<sup>-1</sup>, 8 h at the initial concentration of 200 mg L<sup>-1</sup>, 12 h at the initial concentrations of 400–600 mg L<sup>-1</sup>, and 16 h at the initial concentrations of 800–1000 mg L<sup>-1</sup>. Further increasing dye concentrations to 1500 and 2000 mg L<sup>-1</sup>, only 71.26 % and 51.28 % degradation, respectively, was determined after incubation for 16 h (Fig. 3d). Increasing the initial dye concentration decreased the degradation over the same time interval and inhibition was observed at high concentration. Similar results were also reported by Garg *et al.*<sup>11</sup>, Shah *et al.*<sup>15</sup> and Kurade *et al.*<sup>30</sup> due to the toxic effect of azo dyes on the degrading microorganisms and blockage of azoreductase active sites by complex dye molecules.

Table 2 summarizes some previous studies on azo dyes degradation. Compared with the reported strains, *A. faecalis* LJ-3 shows faster degradation rate and stronger tolerance to high concentrations of Acid Scarlet 3R.

### Enzyme analysis

Enzyme catalysis is considered as the major mechanism in microbial degradation of azo dyes,

and various oxidative and reductive enzymes like veratryl alcohol oxidase, laccase, manganese peroxidase, azoreductase and NADH-DCIP reductase were recorded in azo dye degrading microbes for decolorization/degradation of azo dyes<sup>15,30,31</sup>.

As shown in Table 3, azoreductase, laccase and NADH-DCIP reductase were determined in cells of *A. faecalis* LJ-3. As compared with that of control, activities of azoreductase, laccase and NADH-DCIP reductase in the dye degrading sample had increased 3.77-fold, 1.24-fold, and 3.13-fold, respectively. A similar inductive pattern of pertinent enzymes was also observed during degradation of azo dyes by *Aeromonas* sp. strain DH-6<sup>14</sup>, *M. glutamicus* NCIM-2168<sup>22</sup>, *Brevibacillus laterosporus*<sup>30</sup>, *Bacillus* sp. strain UN2<sup>32</sup> and *Morganella* sp. HK-1<sup>33</sup>. Furthermore, no dye was adsorbed on the cell pellets of *A. faecalis* LJ-3 (Fig. 4a), and UV-vis spectral scanning (200–700 nm) of supernatants displayed that

Table 3 – Enzyme activity in control *A. faecalis* LJ-3 cells (0 h) and the cell obtained after degradation of Acid Scarlet 3R (4 h)

Enzyme	Control cells (0 h)	Cells obtained after degradation (4 h)
Azo reductase <sup>a</sup>	4.33 ± 0.220	16.33 ± 0.530***
Laccase <sup>b</sup>	0.033 ± 0.001	0.041 ± 0.001*
NADH-DCIP reductase <sup>c</sup>	13.42 ± 0.300	42.02 ± 0.531**
Veratryl alcohol oxidase	–	–
Manganese peroxidase	–	–

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

<sup>a</sup>μg of Acid Scarlet 3R reduced min<sup>-1</sup> mg protein<sup>-1</sup>.

<sup>b</sup>μM ABTS oxidized min<sup>-1</sup> mg protein<sup>-1</sup>.

<sup>c</sup>μg DCIP reduced min<sup>-1</sup> mg protein<sup>-1</sup>.

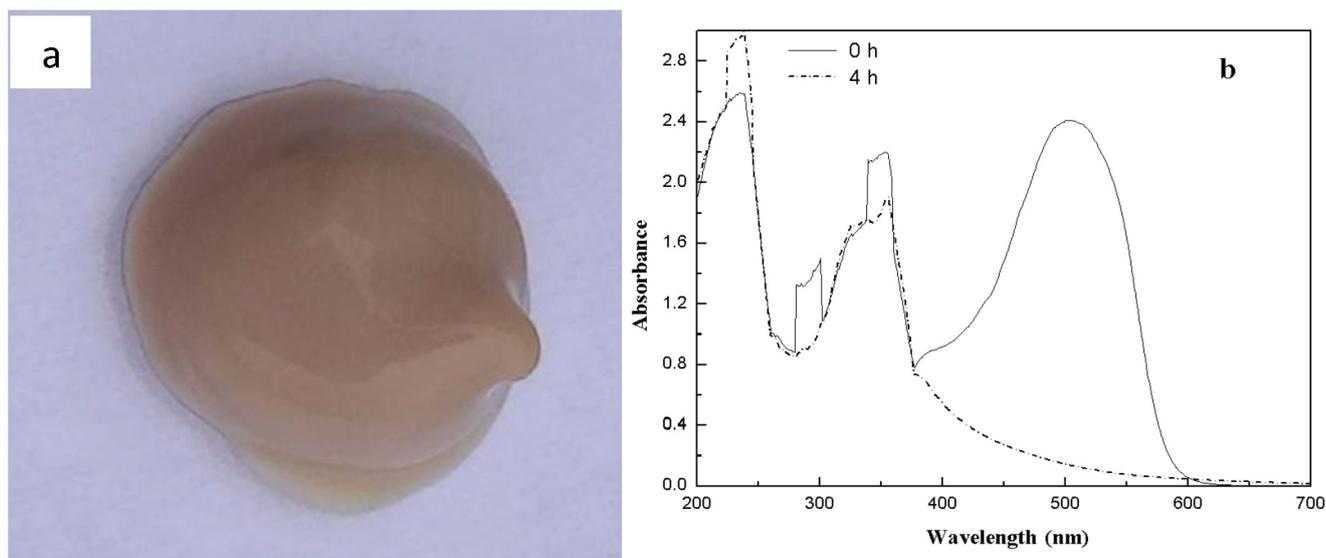


Fig. 4 – Cell pellets of *A. faecalis* LJ-3 after decolorization (a) and UV-vis spectra of Acid Scarlet 3R before (0 h), and after (4 h) degradation (b)

the intensity at the maximum absorbance wavelength (508 nm) of Acid Scarlet 3R drastically decreased after degradation in 4 h (Fig. 4b), which indicated that the azo groups were destroyed<sup>7</sup>. From these results, it could be primarily speculated that *A. faecalis* LJ-3 degrades the azo dye via enzymatic mechanism, which supports the previous conclusion that azo dye degradation by bacteria is due to biodegradation, rather than surface adsorption<sup>34</sup>.

### Kinetics study

The biodegradation kinetics is essential for reactor design to treat dye-containing effluents efficiently. Sudha *et al.* reported that the degradation of Direct Blue 71 and Direct Green 28 by *Enterobacter aerogenes* PP002 follows the zero-order reaction<sup>35</sup>. The results from Wang *et al.* showed that the degradation of Reactive Black 5 by *Bacillus* sp. YZU1 approximates the first-order reaction<sup>13</sup>. Reactive Orange 13 degraded by *A. faecalis* PMS-1 was also well described by the first-order model<sup>15</sup>.

In this work, the degradation decreases with the increase in initial Acid Scarlet 3R concentration. As shown in Table 4, the correlation coefficients ( $R^2$ ) were in the range of 0.855–0.952, and the first-order reaction model approximates the degradation process of Acid Scarlet 3R.

Furthermore, data also revealed that the initial dye concentration significantly affected the degradation rate, and the highest degradation rate was observed at the initial stage (0–4 h) in dye concentrations from 100 to 200 mg L<sup>-1</sup>, while that was determined at 8 h when more dye (> 400 mg L<sup>-1</sup>) was added.

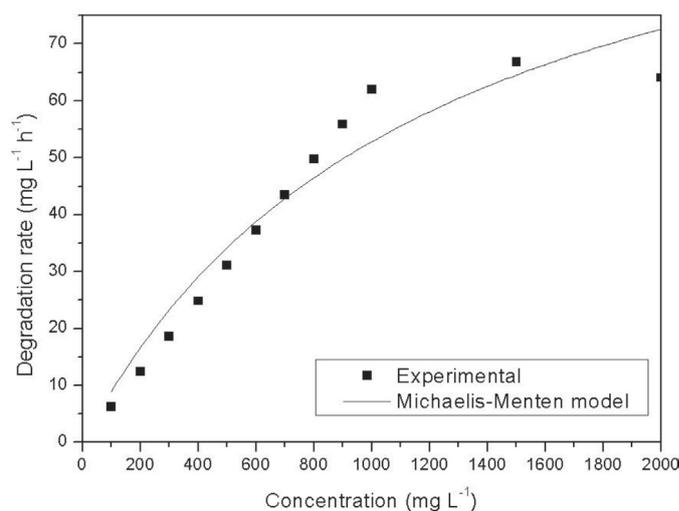


Fig. 5 – Model fitting for *A. faecalis* LJ-3 in degradation of Acid Scarlet 3R

Table 4 – Rate constants of degradation experiments with respect to dye concentration

	Concentration (mg L <sup>-1</sup> )					
	100	200	500	1000	1500	2000
$k_0$ (mg L <sup>-1</sup> h <sup>-1</sup> )	5.667	12.42	34.49	68.83	71.62	69.19
$R^2$	0.704	0.796	0.877	0.938	0.968	0.955
$k_1$ (h <sup>-1</sup> )	0.297	0.343	0.355	0.288	0.080	0.047
$R^2$	0.855	0.911	0.952	0.858	0.948	0.937
$k_2$ (L mg <sup>-1</sup> h <sup>-1</sup> )	0.057	0.045	0.024	0.006	0.0001	0.00003
$R^2$	0.921	0.883	0.628	0.524	0.853	0.904

With the use of Origin 8.0 software, the degradation kinetics parameters and correlation coefficient were calculated. Previous works showed that Haldane equation displayed well the azo dye degradation of *Bacillus* sp. YZU1, while Michaelis–Menten equation fits well the degradation process of *A. faecalis* PMS-1<sup>13,15</sup>. In this work, the experimental data fitted well with Michaelis–Menten model (adj.  $R^2 = 0.934$ , Fig. 5), and the  $K_s$  and  $V_{max}$  values were  $1193.23 \text{ mg L}^{-1}$  and  $115.90 \text{ mg L}^{-1} \text{ h}^{-1}$ , respectively.

## Conclusions

In summary, *A. faecalis* LJ-3 has excellent ability to degrade Acid Scarlet 3R under a broad range of pH, temperature, and initial dye concentrations, and can tolerate high salinity. The enzyme and UV-vis spectroscopy analysis indicated that the strain degrades Acid Scarlet 3R by enzyme reaction, not simply by physical surface adsorption. The kinetics study showed that the degradation of Acid Scarlet 3R by *A. faecalis* LJ-3 approximates first-order reaction. Michaelis–Menten kinetics is suitable to fit the relationship between degradation rate and initial dye concentration, and  $V_{max}$  and  $K_s$  were found to be  $115.90 \text{ mg L}^{-1} \text{ h}^{-1}$  and  $1193.23 \text{ mg L}^{-1}$ , respectively. Furthermore, *A. faecalis* LJ-3 displays high degradation efficiency of various azo dyes used in the textile industries. It can be concluded that *A. faecalis* LJ-3 possesses practical application potential in biological treatment of industrial effluents containing various azo dyes.

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