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Halomonas sp. strain A55, a novel dye decolorizing bacterium from dye-uncontaminated Rift Valley Soda lake

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HIGHLIGHTS

- *Halomonas* sp. strain A55 was found the potential candidate for dye removal.
- Organic carbon and nitrogen sources contributed ≥98% dye decolorization efficiency.
- Under oxygen tension conditions, RR 184 dye used as a final electron accepter.
- RR 184 addition to media significantly enhanced *Halomonas* sp. enzyme induction.
- Reactive Red 184 dye decolorization follows pseudo-first-order kinetic model.

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ABSTRACT

Considering the saline-alkaline nature of textile wastewater and treatment requirements, microbial samples were collected from Ethiopian Rift Valley Soda Lakes. A large number of bacteria (121) were isolated from dye-uncontaminated Lakes Chitu (81.0%), Abijata (15.7%) and Arenguadie (3.3%), of which 95 isolates (78.5%) were found dye decolorizer. Many dye decolorizer from Lake Chitu positively correlated with higher pH (10.3 ± 0.1), salinity (64.6 ± 2.0%), conductivity (6.1 ± 0.3 mS cm^{-1}) and Na+ (18.4 ± 0.6 g L^{-1}) values observed than Abijata and Arenguadie Lakes. Through subsequent screening mechanism, strain A55 was selected to investigate the effect of nutrient (carbon and nitrogen), dissolved oxygen and dye concentration using Reactive Red 184 (RR 184). Based on morphological, biochemical and 16S rRNA gene sequence analysis, the strain was identified as *Halomonas* sp. Decolorization efficiencies were significantly enhanced with carbon (≥98%) and organic nitrogen (~100%) than non-carbon/nitrogen (both<55%) supplements. Complete decolorization efficiencies were also observed under anoxic and anaerobic growth conditions. However, growing the isolate with nitrate (<30%) and aerobic (<10%) condition significantly decreased ($p < 0.05$) color removal efficiency. Kinetic analysis showed that pseudo-first-order best describes RR 184 decolorization process. Overall, the ability of *Halomonas* sp. strain A55 decolorized different dyes indicate that alkaline soda lake isolates are the potential candidate for treating color containing effluent.

1. Introduction

Discharge of wastewater from textile, paper, leather tanning, food processing, plastics and cosmetics industries causes water body pollution (Kalyani et al., 2008). The Color which is primarily originated from dyeing process is the most concern environmental contaminant in the textile industry. During the dyeing process, approximately 10–15% of the used dye is released into the wastewater and causes serious environmental and health hazards (Arora et al., 2007). Disposal of dye-containing wastewater into the aquatic ecosystem is not only aesthetically displeasing, but also decreases photosynthetic activities by impeding the light penetration into deeper layers (Asad et al., 2007; Saratale et al., 2011). This condition leads to depletion of dissolved oxygen (DO) and loss of biodiversity (Chen et al., 2003).

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Although physicochemical methods are used to treat textile wastewater, they have drawbacks including high cost, hazardous byproduct formation and intensive energy requirements (Isik and Sponza, 2003; Arora et al., 2007; Kurade et al., 2012). A number of biotechnological approaches have been suggested to overcome these problems. Microorganisms such as bacteria (Chen et al., 2003; Arora et al., 2007; Asad et al., 2007; Kalyani et al., 2008), fungi (Patel et al., 2013; Govindwar et al., 2014; Saroj et al., 2014) and algae (Daneshvar et al., 2007) were reported a potential dye decolorizing organisms.

The rationale of the present study was considering the nature of textile wastewater and hypothesized its fit with alkaline soda lake environment. Since textile industries use different salt and sodium hydroxide in various processing steps, its effluents are characterized by high salinity and alkaline medium (pH = 11.0 – 11.5) (Ali et al., 2014). Bioremediation in such environments inevitably requires the application of halo-alkaliphilic microorganisms, which are able to adapt and physiologically function under such harsh conditions. In this regard, most Rift Valley Lakes are known by their high salinity and alkaline nature (Kebede et al., 1994; Zinabu, 2002; Lanzen et al., 2013; Sorokin et al., 2014). For instance, Lanzen et al. (2013) have studied five Rift Valley Ethiopian Soda Lakes (Abijata, Arenguadi, Chitu, Beseka and Shalla) and reported salinity and pH values varying between 0.21 – 5.8% and 9.6 – 10.4, respectively. However, no research has been yet conducted on these lakes microorganism targeting textile wastewater treatment.

2. Materials and methods

2.1. Experimental setup

The batch experiment of dye biodegradation study was conducted in 1000 mL capacity reactor. The diagrammatic representation of the decolorization reactor set up is provided in supplementary data (Supplementary Fig. 1). The reactor was operated in an airtight condition to ensure the prevalence of anoxic conditions. It has tightened lid with two holes which function gas removing and sampling tube insertion. Metabolic product of gas from the reactor was removed by using potassium hydroxide (KOH) solution. The sampling tube was inserted deep in dye-containing wastewater at one side and attached to a sterile syringe on the other side. When the sample was drawn with a sterile syringe, the opening and closing of the tube were regulated with a control valve located at the center.

2.2. Sample collection for microbial isolation and physicochemical parameters

Mud samples were collected from Ethiopian alkaline soda lakes such as Abijata, Arenguadie and Chitu to use as a source of microbial culture. For these lakes, some of the physicochemical parameters were also measured in the field and laboratory following standard methods. The value of pH and temperature were measured *in situ* using digital pH meter (HQ40d, HACH, USA) and thermometer, respectively. The value of conductivity was also measured using conductivity meter (430, Jenway, UK).

Surface water also collected from the three lakes using sterile 2-L plastic containers for the analysis of chemical features in the laboratory. Samples acidified to a pH of 2 with HNO_3 were used for the analysis of major ions and some micronutrients. To detect the level of calcium, magnesium, sodium and potassium flame atomic absorption spectrophotometer (PE-AA400, Perkin Elmer, USA) were used.

2.3. Media composition for isolation and screening of dye degrading microorganisms

Mineral salt medium (MSM) used by Arora et al. (2007) with some modification (maintaining alkaline pH using Na_2CO_3) were followed. During MSM-agar plate preparation, 2% agar was added to MSM.

Reactive Red 184 (RR 184), Reactive Red 141, Reactive Red 120, Reactive Yellow 84, Reactive Yellow 160, Reactive Blue 198 and Vat Blue 43 dyes which are mostly used in textile industries were collected from Akaki and Adey Abeba textile industries in Ethiopia. Among these dyes, RR 184 ($\lambda_{max} = 540$ nm) was selected as a representative dye used for acclimatization, isolation and decolorization evaluation (Supplementary Table 1). Lake mud samples (10%, w/v) were enriched on azo dye containing (10, 25, 50, 100 and 150 mg L^{-1}) MSM and morphologically different colonies were isolated and identified.

The best dye decolorizer isolate has been characterized based on morphological, physiological, biochemical and molecular techniques. Genomic DNA from a pure isolate was extracted by a freeze-thaw method as modified by Moore and his colleagues (Moore et al., 1999). 16S rDNA was amplified by polymerase chain reaction (PCR) using universal eubacteria specific primers of A8f and H1542r (Giovannoni, 1991). The sequence obtained was deposited to the NCBI under GenBank accession number KU860140.

2.4. Effect of different conditions on dye decolorization

The constant operating parameters and the effect of different conditions were summarized in Table 1. When the effect of carbon sources (treatment 1), nitrogen sources (treatment 2), culturing conditions (treatment 3), dye types (treatment 4) and dye concentration (treatment 5) investigated, the volume of the reactor, inoculum size, pH and temperature values were kept constant. For each treatment, the dye concentration was fixed to 150 mg L^{-1} and the preparations were incubated with 10% inoculum size.

To understand the effect of pH on the best isolate and its activity, growth and decolonization efficiencies were monitored at different pH values (7.0–12.5). In this study, the best isolate ability to decolorize real effluent was also evaluated. To understand whether the isolate use dye metabolite as a nutrient source, the 24 h decolorized anoxic effluent (AE) feed to the best isolate and incubate under aerobic condition. During this time, other organic nutrient supplement has not provided to the isolate. For comparison purpose, the anoxic and aerobic cultures were also monitored with MSM supplemented with organic matter (glucose and yeast extract) (Table 1). The concentrations of RR 184, chemical oxygen demand (COD) and total organic carbon (TOC) were measured from the anoxic, aerobic and AE-aerobic reactor at different time following standard methods (APHA et al., 1998).

2.5. Biodegradation analysis

For spectroscopic analysis, 10 mL of the liquid sample was taken out aseptically from the reactor every 24 h and centrifuged at 4000 × g for 30 min at 4 °C. Centrifuged cell-free supernatant samples were scanned in the range of 200 – 800 nm using Uv–visible spectrophotometer (Genesys 2 PC, Shimadzu, Japan). The percentage decolorization was calculated using Eq. (1):

$$\text{Decolorization}(\%) = \frac{A_o - A_t}{A_o} \times 100 \quad (1)$$

where, A_o = initial absorbance, A_t = absorbance after time t.

Reactive red 184 biodegradation products were also analyzed

Table 1
The value of different operating parameters at different treatment conditions.

Operating conditions	Treatments				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Reactor volume (mL)	1000	1000	1000	1000	1000
Inoculum size (%)	10	10	10	10	10
pH	10.4±0.6	10.4±0.6	10.4±0.6	10.4±0.6	10.4±0.6
Temperature (°C)	25.0±2.0	25.0±2.0	25.0±2.0	25.0±2.0	25.0±2.0
Decolorization analysis time (h)	24 – 96	24 – 96	24 – 96	24 – 96	24 – 96
Carbon source	C-sources[a]	Glucose	Glucose	Glucose	Glucose
Organic nitrogen source	Not supplied	N-sources[b]	Yeast extract	Yeast extract	Yeast extract
Culturing conditions	Anoxic	Anoxic	Culturing conditions[c]	Anoxic	Anoxic
Dye types	RR 184	RR 184	RR 184	Dye types[d]	RR 184
Concentration (mg L^{-1})	150	150	150	150	50–250[e]

[a] 500 mg L^{-1} each carbon sources (glucose, lactose, sucrose, starch, pyruvate, citrate media without carbon sources).
[b] 1000 mg L^{-1} each nitrogen sources (peptone, yeast extract, $NaNO_3$, $NaNO_2$, $(NH_4)_2SO_4$).
[c] Aerobic, shaker, anoxic, anaerobic.
[d] Reactive Red 184, Reactive Red 141, Reactive Red 120, Reactive Yellow 84, Reactive Yellow 160, Reactive Blue 198 and Vat Blue 43 (150 mg L^{-1} for each dye).
[e] 50, 100, 150, 200, 250 mg.L^{-1}.

using high performance liquid chromatography (HPLC) and fourier transformed infrared spectroscopy (FTIR). Samples were collected at different time and centrifuge 7000 × g for 15 min at 4 °C. The cell-free supernatant was extracted three times using an equal volume of ethyl acetate. The extract was dried in a rotary evaporator and re-dissolved in HPLC grade methanol. For FTIR analysis, the samples were dried and mixed with spectroscopically pure KBr in a ratio of 10:90. The FTIR analysis of biodegraded RR 184 was carried out using Nicolet 5700 FTIR spectrometer (Thermo Scientific, USA). The FTIR analysis was done in the IR region of 400 – 4,000 cm^{-1} with 16 scan speed.

For HPLC analysis, samples were filtered through 0.22 μm membrane filter (ANPEL Laboratory Technology, Shanghai). Chromatography was performed using HPLC system (Shimadzu, Japan) equipped with Shimadzu SPD-M20A binary pump (LC-20AD), online degasser (DGU-20A3), Shimadzu SIL-20A autosampler, Shimadzu CTO-10ASVP column oven and Shimadzu SPD-M20A detector. The chromatographic analysis was performed using a reversed-phase HPLC with Athena C-18 WP column (CNW Technologies, Germany), 5 μm particle size, and 4.6 mm (internal diameter) × 250 mm (length). A mobile phase composed of 65% acetonitrile and 35% ultrapure water has been degassed using sonicator and allowed to operate in isocratic mode. The sample (20 μL) was automatically injected and dye products were allowed to separate for 15 min at the flow rate of 1.0 mL min^{-1}. For the separation column, the temperature was kept at 28 °C. The purification profiles of the degraded metabolite were monitored at 275 nm by running the UV–visible detector in dual wavelength mode.

2.6. Extraction and enzyme assay

Extraction of enzymes after RR 184 decolorization and the control medium was carried out as per the procedure described earlier (Agrawal et al., 2014). The bacterial cells were separated by centrifugation at 7000 × g for 15 min at 4 °C and the cell-free supernatant was used for determination of enzyme activities. The total protein concentration has been determined using bovine serum albumin as standard (Bradford, 1976).

The activities of azoreductase (Zimmermann et al., 1982) and laccase (Agrawal et al., 2014) were assayed spectrophotometrically at 540 and 420 nm, respectively. For azoreductase assay, the reaction mixtures (1 mL) contained: 94 mM potassium phosphate buffer (pH 7.0), 1 mM Nicotinamide adenine dinucleotide (NADH), 50 μM RR 184 dye and 50 μL of protein extract. The reaction was started by the addition of NADH and the decrease in color intensity was monitored at 540 nm. The change in absorbance per min was used to calculate the azoreductase activity. One unit of (U) enzyme activity is defined as the amount of protein required to break down 1 μM dye per min. The laccase activity was also assayed by measuring the oxidation of 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulphonate) (ABTS). The reaction mixture containing 500 M ABTS buffered with 50 mM sodium phosphate buffer (pH 4.5) and 200 μL of enzyme extract. Oxidation of ABTS was followed by an increase in absorbance at 420 nm. An enzyme extracted from the culture medium without adding dye was considered control. All enzyme activity assays were conducted in triplicate at 30 °C and average results were reported.

2.7. Kinetics of decolorization

In order to confirm kinetics of decolorization, experiments were conducted using 10% (v/v) inoculum of the best isolate and 150 mg L^{-1}. Spectrophotometric measurements were made periodically by taking samples at different time interval. To identify the mechanism and the potential rate-controlling steps involved in the process of bioreduction, the data of RR 184 dye was fitted using pseudo-first-order kinetic model Eq. (2) (Mutafov et al., 2006):

$$C_t = C_o e^{[-kt]} \tag{2}$$

where, k stands for the pseudo-first-order rate constant (per hour); C_0 and C*t* (mg L^{-1}) are the concentrations of azo dye (mg L^{-1}) at the zero time and at time t (h), respectively.

For $t > 0$ the decolorization rate (k), was evaluated in the following equation Eq. (3) (Yu et al., 2011).

$$\frac{[C_t]}{[C_0]} = Exp^{[-kt]} \tag{3}$$

The linear relationships are obtained by the plot of RR 184 concentration against time Eq. (4):

$$\ln \frac{[C_t]}{[C_{0]}} = -kt \tag{4}$$

2.8. Statistical analysis

All the data were presented from the mean of three determinations ± standard error. The standard error and significant

level were calculated using SPSS version 20.0 software. The paired-sample *t*-test and one-way analysis of variance (ANOVA) with Tukey's post hoc test were done to calculate statistical significance between mean values. The relationship between a number of isolates and physicochemical values of the lakes were also performed using Pearson correlation coefficient. Differences were considered significant if $p < 0.05$.

3. Results and discussion

3.1. Physicochemical characteristics of alkaline soda lakes and bacterial isolation mechanism

Ethiopia has alkaline soda lakes such as Abijata and Chitu which are located South of Addis Ababa in the Rift Valley Region with an altitude of ≥1500 m above sea level (asl). Lake Arenguadie is also one of the Ethiopian soda lake located in the Central Highlands of the country at an altitude of about 1900 m asl (Table 2). In terms of area coverage, Lake Arenguadie is the smallest (0.54 km^2) followed by Lake Chitu (0.80 km^2) and Lake Abijata (176.00 km^2). However, Lake Arenguadie is the deepest (up to a maximum of 32.0 m) than Lakes Chitu and Abijata.

The concentration of major cations in the current study was generally found in the order of $Na^+ > K^+ > Ca^{2+} > Mg^{2+}$ (Table 2). The concentration of mono and divalent cations were found highest in Lake Chitu than Lake Abijata and Lake Arenguadie. In fact, the main Ethiopian Rift region (where these lakes are located) is characterized by rhyolite and trachyte volcanic rocks with excess Na^+ and K^+ ions. As a result of higher concentration of K^+ and Na^+, the Rift Valley Lakes are characterized by higher salinity and alkalinity values. However, divalent cations (Ca^{2+} and Mg^{2+}) showed an inverse relationship with salinity and alkalinity, suggested that they are removed from solution when carbonate precipitates under highly alkaline conditions. Several studies were conducted on Ethiopian Soda Lakes and results were found consistent with the current findings (Wood and Talling, 1988; Kebede et al., 1994; Kebede, 1997; Zinabu, 2002). However, the current results differ from most temperate lakes which have major cations in the order of divalent cations ($Ca^{2+} > Mg^{2+}$) greater than monovalent cations ($Na^+ > K^+$).

There is a significant difference in pH, salinity and conductivity among the three soda lakes. Chitu is the most saline (6.1±0.3%) and alkaline (pH = 10.3±0.1) lake than Abijata and Arenguadie (Table 2). The conductivity value of Lake Chitu was also found twice the conductivity of Lake Abijata. The lakes region higher rate of evaporation under particular conditions of geology, geography and climate have been mentioned a prime factor for the increased alkalinity and salinity nature of the lakes (Wood and Talling, 1988; Zinabu, 2002). The lakes being closed also mentioned to be the main factor for their saline – alkaline nature (Zinabu, 2002).

Alkaliphilic microorganisms were isolated from mud samples obtained from Ethiopian soda lakes in the Rift Valley area. A large number of bacteria (121) have been isolated from dye-uncontaminated Lakes Chitu (81.0%), Abijata (15.7%) and Arenguadie (3.3%) (Table 2). Isolates which showed color removal within 14 days of incubation were considered decolorizer and beyond 14 days non-decolorizer (Supplementary Fig. 2). Among the total bacterial isolates, 95 (78.5%) isolates were found decolorizer (Table 2).

Statistically, the number of decolorizer isolates had a significant strong positive correlation with lakes pH ($R^2 = 0.977$), salinity ($R^2 = 0.926$) and conductivity ($R^2 = 0.969$), suggesting that samples collected from more saline and alkaline environment well adapted the synthetic textile effluent. This finding is consistent with other study conducted on Ethiopian Rift Valley Soda Lakes that showed heterotrophic microbial diversity positively correlated with pH and salinity (Lanzen et al., 2013). It is known that various environmental variables largely shape community structure and community dynamics. Comparing the three soda lakes (i.e, among 95 decolorizers), the highest percentage of dye degrading bacteria were recorded for Lake Chitu (80.0%) than Lake Abijata (16.8%) and Lake Arenguadie (3.2%) most probably related to the highest physicochemical values recorded in Lake Chitu (Table 2). Lanzen et al. (2013) also found surprising results that Lake Chitu with the most extreme conditions yielded the highest operational taxonomic unit (OUT) richness followed by the lake with the second highest pH and salinity (Abijata). Despite the unique and rich ecological characteristics of Lake Chitu and other Ethiopian Rift Valley Lakes, isolating microbes that can be used for biotechnological applications have not yet been fully explored. Of course, there are several studies on Ethiopian Soda Lakes in the recent past, however, most of them are focusing on physicochemical characterization and its effect on algal growth (Wood and Talling, 1988; Kebede et al., 1994; Kebede, 1997; Zinabu, 2002).

Among the decolorizers, 25 isolates that remove 10 mg L^{-1} rapidly were selected for further screening using 25 mg L^{-1}. Eight isolates which decolorize RR 184 ≥ 80% within 24 h and 100% at 96 h were selected and subjected to further screening (data not shown). Among the isolates, strain A55 (isolated from Lake Chitu) was found to be the most efficient decolorizer (86.2 ± 0.7 – 92.1 ± 2.4% at 24 h and ≥98.1 ± 1.5 at 96 h) for the given initial dye concentrations (50 – 150 mg L^{-1}) and was selected for further characterization and parameter evaluation (Supplementary Table 2).

3.2. Identification and phylogenetic position of strain A55

Based on morphological and biochemical characterization, strain A55 was found motile, a rod shape, white color, positive for catalase and oxidase, and negative for spore and Gram staining tests (Table 3). Physiologically, strain A55 was growing in medium without NaCl and managed to tolerate 20% NaCl with an optimum

Table 2
Some physicochemical parameters of Lakes Abijata, Arenguadie and Chitu.

Lakes	Sampling site			Some physicochemical parameters								Decolorizers (num)					Non-Dec (num)
	Altit (m)	Area (km^2)	Depth (m)	Temp (°C)	pH	Cond (mS cm^{-1})	Salinity (%)	Na^+ (g L^{-1})	K^+ (g L^{-1})	Ca^{2+} (mg L^{-1})	Mg^+ (mg L^{-1})	1st day	2nd day	3rd day	4-14 days	Total	>14 days
Abijata	1540	176.00	14.2	24.1 ± 0.8	9.7 ± 0.2	30.4 ± 1.3	3.4 ± 0.2	11.5 ± 0.3	0.4 ± 0.1	<14.2	<8.6	3	8	3	2	16	3
Arenguadie	1900	0.54	32.0	24.3 ± 0.7	9.9 ± 0.3	6.8 ± 0.1	0.4 ± 0.1	1.2 ± 0.1	0.2 ± 0.0	<10.0	<5.2	–	1	1	1	3	1
Chitu	1500	0.80	21.0	22.0 ± 0.3	10.3 ± 0.1	64.6 ± 2.0	6.1 ± 0.3	18.4 ± 0.6	1.2 ± 0.1	<2.5	<0.8	32	22	3	19	76	22
Tot-Is (%)	–	–	–	–	–	–	–	–	–	–	–	**28.9**	**25.6**	**5.8**	**18.2**	**78.5**	**21.5**

Data are shown as mean ± std derived from triplicates. Tot-Is = Total Isolate; Altit = Altitude; Cond = Conductivity; Temp = Temperature; Non-Dec (num) = Non-decolorizer (number).

Table 3
Characterization of strain A55.

Test type	Result	Test type	Result
Morphology:		**Utilization of various nutrients:**	
Shape	rod	Glucose	+
Color	white	Galactose	–
Edge	circular	Glycerol	+
Elevation	convex	Fructose	+
Gram staining	–	Lactose	+
Spore staining	–	Ribose	+
Motility	+	Xylose	–
Physiological:		Sucrose	+
Temperature range/°C	5 – 50	Maltose	+
Temperature optimum/°C	30	Molasses	+
pH range	7.5 – 12	Peptone	+
pH optimum	10	Yeast extract	+
NaCl requirement	0	Beef	+
NaCl optimum/%	10	Starch	+
NaCl tolerance/%	20	Pyruvate	+
Biochemical:		Succinate	+
Anaerobic growth	+	Citrate	+
Aerobic growth	+	Growth with nitrite	+
Catalase	+	Growth with nitrate	+
Oxidase	+		

– = Negative, + = Positive.

10% NaCl, suggesting that this isolate has evolutionarily developed an osmoregulatory mechanism to a wide range of salinity (hypotonic-hypertonic environments) from where it grows (i.e, Lake Chitu) by adjusting its cell sap. Ethiopian Soda Lake water physicochemical variations (including salinity) has been studied during wet and dry seasons and results showed chemical constituents vary with season (Zinabu, 2002) which is a stimulus for those microorganisms that live in the area to develop a special adaptive mechanism.

Strain A55 was found to grow an optimum temperature and pH value of 30 °C and 10, respectively (Table 3). In order to survive in this extreme alkaline environment, strain A55 has developed various bioenergetic and structural adaptations to maintain pH homeostasis (will be discussed later).

Strain A55 utilized various nutrients including glucose, glycerol, fructose, lactose, ribose, sucrose, maltose, molasses, starch, pyruvate, succinate, citrate, peptone, yeast extract, beef, nitrite and nitrate (Table 3). The ability of this strain metabolizing various sugar and nitrogen compounds indicated that the strain A55 is actively involved in the biogeochemical transformation of carbon and nitrogen in Chitu Lake. According to Lanzen et al. (2013), all soda lakes studied (including Lakes Arenguadie, Abijata and Chitu) harbored remarkably diverse microbial communities that involved in the biogeochemical cycles. Sorokin et al. (2014) also mentioned that low and moderately saline soda lakes are highly productive and harbor fully functional and diverse haloalkaliphilic microbial communities responsible for carbon, nitrogen, and sulfur biogeochemical cycles.

Generally, characterization of A55 through morphological, physiological, biochemical and ability to utilize various substrates has led strain A55 identified to be in the genus *Halomonas*. Using 16S rRNA gene sequencing, the phylogenetic position of strain A55 was also determined. According to the phylogenetic tree (Supplementary Fig. 3), strain A55 closely related to members of *Halomonas* within the phylum Proteobacteria. Strain A55 showed more than 99% 16S rRNA gene sequence similarity to members of *Halomonas* isolated from soda lakes and other hypersaline environments. For instance, the strain showed more than 99% 16S rRNA gene sequence similarity to *Halomonas* sp. MC1-1 and *Halomonas* sp. NBSL10 isolated from Rift Valley Alkaline Soda Lake in Kenya and Sambhar Salt Lake in India, respectively.

Several researchers reported that effluent adapted microbes are involved in the active decolorization of different textile dyes (Chen et al., 2003; Bhatt et al., 2005). Bhatt et al. (2005) were able to isolate 17 microbial cultures using soil samples collected from the vicinities of dyestuff industries by employing Reactive Blue 172 as a substrate. On the contrary, in the present study; bacteria isolated from Rift Valley Alkaline Soda Lakes (which have no contact with textile effluents) were found abundant and efficient dye decolorizer. As results stated above in physicochemical measurement (Table 1) and physiological characterization (Table 3), *Halomonas* sp. strain A55 has been found high pH and salt tolerant organism. Under application condition, the strain will be economically and environmentally sustainable by avoiding pH adjustment done in most biological treatments. So far, most textile wastewater treatment uses neutralophilic microorganisms which require a lot of chemicals for pH re-adjustment.

3.3. Decolorization activity of strain A55 under different conditions

3.3.1. Effects of pH on A55 growth and dye decolorization

In order to confirm the ability of *Halomonas* sp. strain A55 grew and perform decolorization activity at different pH environments, the initial pH of dye containing MSM was adjusted (7.0–12.5) and used for growth of the strain. After treatment, the pH of the media was measured and found to be significantly changed at higher initial pH (Fig. 1). At lower pH, the initial and the final pH values did not show a significant difference. Optimum pH difference was observed between pH 8.5 and 11.0 (0.5–1.8 pH unit change, $p < 0.05$), indicating that metabolic activities played by *Halomonas* sp. have been significantly modified the growth media. This could be most related to the biodegradation of RR 184 dye (and further mineralization) and organic matter (glucose and yeast extract) added as a feed converted into simple organic acids and decreased the pH value. This media acidification might help *Halomonas* sp. strain A55 for pH homeostasis.

As shown in Fig. 1, a strong positive relationship has been also observed between dye decolorization activity and mixed liquor suspended (MLSS) concentration at 24 h ($R^2 = 0.9865$) and 96 h

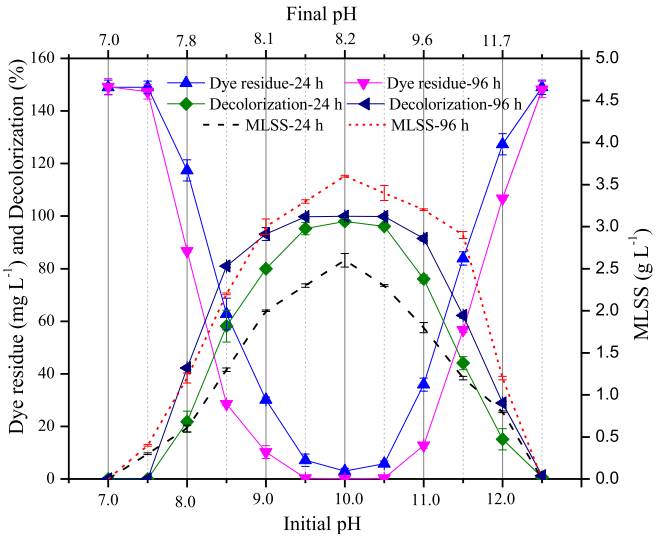

Fig. 1. Effects of different pH on *Halomonas* sp. growth and its decolorization activity on RR 184.

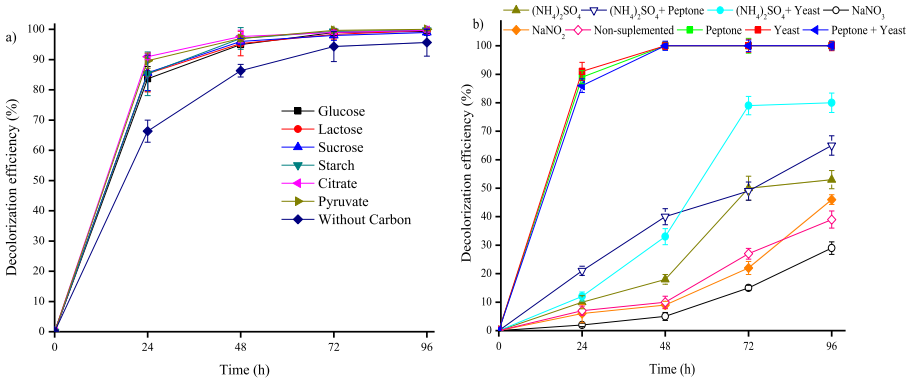

Fig. 2. Effect of different organic and inorganic supplements on decolorization efficiency (a) carbon sources and (b) nitrogen sources. Error bars represent the standard deviation calculated from at least three independent experiments.

($R^2 = 0.9716$). Compared to acidic and alkaline environments, strain A55 tolerated higher pH value than the acidic environment. No growth and decolorization activity occurred at pH 7.0 and 12.5. At pH 7.5, growth has been identified, however, dye decolorization has not been observed. *Halomonas* sp. strain A55 showed optimum growth and dye decolorization at the pH range of 8.5 – 11.5. Especially at pH 10.0, the MLSS concentration has been found highest at 24 h (2.6 ± 0.08 g L^{-1}) and 96 h (3.6 ± 0.01 g L^{-1}) that reduced the dye residue below 3.0 mg L^{-1} and decolorization efficiency ≥98.0 (Fig. 1).

As stated above in section 3.2, haloalkaliphilic bacteria like *Halomonas* sp. have developed essential strategies for bioenergetic and structural adaptation to maintain pH homeostasis (Horikoshi, 1999; Sorokin et al., 2014). Structurally, the alkaliphilic bacterial cell surface (cell wall and cell membrane) have a significant role in maintaining internal pH homeostasis (Konings et al., 2002; Sorokin et al., 2014). The cell wall of alkaliphilic bacteria has composed of peptidoglycan and certain acidic polymers in which the later components gave a negative charge matrix that might reduce the pH value at the cell surface by adsorbing hydronium and sodium ions (Krulwich et al., 1998; Horikoshi, 1999; Padan et al., 2005). This might offer an advantage for alkaliphilic bacteria like *Halomonas* sp. to grow in alkaline environments.

3.3.2. *Effect of carbon and nitrogen sources on decolorization*

Strain A55 dye decolorization efficiency was significantly influenced by carbon and nitrogen medium composition (Fig. 2). Time was a significant factor ($p = 0.02$) on different carbon source to bring efficient decolorization. Within 24 h, *Halomonas* sp. has shown better decolorization efficiency with different carbon sources (83 – 92%) than in the absence of any carbon source (66%) may indicate that the requirement for the presence of sufficient electron donors for growth and maintenance (Fig. 2a). When organic carbon sources are metabolized, they produce NADH which acts as the electron donor for an azo dye reduction (Nigam et al., 1996). The other probability of higher decolorization using organic carbon source could be the nutritional contribution of the source which results in the fast growth of the organism.

Compared to 48 h, at 72 ($p = 0.551$) and 96 h ($p = 0.376$) incubation period, *Halomonas* sp. did not show significant dye decolorization efficiency for carbon and non-carbon supplemented growth media. For instance, without carbon source addition in the growth media, dye decolorization efficiency was 94% at 72 h (Fig. 2a), suggesting that the yeast extract added as nitrogen source most likely deaminated and used as carbon source. The other possible explanation for this could be related to RR 184 biologically degraded and the end products may have been used as a carbon source.

Observing the strain ability to use starch efficiently (Fig. 2a) also has practical advantages. In most cases, textile wastewater contains a large proportion of starch released during the desizing operation. Thus, under application conditions, the organism may not need any other additional carbon source input to bring about dye decolorization.

Fig. 2b shows the significant effect of ($p = 0.001$) various nitrogen sources on dye decolorization efficiency by strain A55. Compared to inorganic nitrogen sources (≤20%), the highest decolorization efficiency was observed in cultures supplemented with organic nitrogen sources (≥89%) within 24 h of cultivation. Within 96 h incubation period, organic nitrogen sources were contributed almost complete dye decolorization efficiency (Fig. 2b). It was also reported that the metabolism of an organic nitrogen source such as yeast extract is considered essential to the regeneration of NADH that acts as the electron donor for the reduction of the dye functional group. During the entire cultivation period, a lower percentage of RR 184 decolorization (2.0±0.6 – 29.0±2.2%) was observed for $NaNO_3$ supplemented culture (Fig. 2b). The presence of $NaNO_2$ in the strain growth media also significantly decreased ($p < 0.05$) the efficiency RR 184 decolorization. A possible explanation for the low decolorization result by strain A55 in the

Table 4
Effect of various culturing conditions as a function of time.

Culturing Condition	Dissolved oxygen (mg L^{-1})				MLSS (g L^{-1})				Dye residue (mg L^{-1})			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Anaerobic	0.20 ± 0.1	0.10 ± 0.1	0.06 ± 0.0	0.01 ± 0.0	0.20 ± 0.1	2.15 ± 0.3	2.64 ± 0.1	2.57 ± 0.5	62.0 ± 2.1	6.5 ± 1.3	2.6 ± 0.2	0.7 ± 0.1
Anoxic	0.76 ± 0.3	0.46 ± 0.2	0.31 ± 0.1	0.25 ± 0.0	0.88 ± 0.2	2.94 ± 0.1	3.25 ± 0.4	3.29 ± 0.3	55.2 ± 1.7	1.8 ± 0.8	0.6 ± 0.1	0.02 ± 0.0
Shaker	1.33 ± 0.3	1.36 ± 0.1	1.32 + 0.2	1.35 ± 0.1	3.16 ± 0.1	3.33 ± 0.4	3.41 ± 0.2	3.52 ± 0.1	144.0 ± 1.0	138.5 ± 2.2	130.4 ± 1.8	112.8 ± 2.4
Aerobic	2.54 ± 0.3	2.35 ± 0.5	2.50 ± 0.4	2.42 ± 0.2	3.49 ± 0.3	3.63 ± 0.2	3.66 ± 0.3	3.58 ± 0.5	148.1 ± 3.1	142.7 ± 1.6	138.2 ± 0.4	136.0 ± 0.3

Data are shown as mean ± std derived from triplicates. Anaerobic culture condition was maintained with nitrogen. Anoxic and aerobic cultures were achieved by using full volume of the reactor and continuous air flow (6 L h^{-1}), respectively. Shaking/agitation was operated on a rotary shaker running at 120 g.

presence of these inorganic nitrogen sources may be related to the nitrate/nitrite preferentially consumed by the organism and serve as a final electron acceptor than the dye. Others have also reported $NaNO_3$ contribute lower dye decolorization efficiency (Chen et al., 2003; Guadie et al., 2017).

3.3.3. *Effect of culturing condition on decolorization*

Table 4 shows DO, MLSS and dye residue results of anaerobic, anoxic, shaker and aerobic culture conditions. During the entire experimental periods, the DO concentrations were found highest for aerobic ($2.35 \pm 0.3 - 2.54 \pm 0.5$ mg L^{-1}) followed by shaker ($1.32 \pm 0.2 - 1.36 \pm 0.1$ mg L^{-1}) culture conditions, both growth conditions had a significant difference ($p < 0.05$) than anoxic and anaerobic culture growth. This higher DO concentration has been contributed an increased MLSS concentration in aerobic and shaker growth which significantly differs from anaerobic culture. However, anoxic culture showed almost the same MLSS concentration with a shaker ($p = 0.210$) and aerobic culture ($p = 0.109$) which offers higher biomass in anoxic condition to decrease RR 184 from 150 mg L^{-1} initially added to 55.2 ± 1.7 and 1.8 ± 0.8 mg L^{-1} within 24 and 48 h, respectively (Table 4).

There was no significant difference ($p = 0.628$) on decolorization efficiency observed between anoxic and anaerobic culture, however; the difference was significant ($p = 0.010$) on the concentration of DO (Table 4). Compared to shaker (4 – 25%) and aerobic (4 – 25%) growth decolorization, the lower DO value for anaerobic and anoxic conditions has contributed strain A55 to show highest color removal efficiency (58 – 99 and 63 – 100%, respectively). When the level of DO depleted, the organism was forced to switch aerobic metabolism and use dye as a final electron acceptor. In fact, others also mentioned that microbial degradation of azo dyes is often linked to anaerobiosis metabolic process (Patel et al., 2013). The ability of *Halomonas* sp. strain A55 that induce significant amount of azoreductase (Table 5) in the presence of RR 184 as substrate has confirmed the biotechnological advantage of alkaliphilic inoculum that produce enzymes actively function under alkaline conditions.

In order to figure out more about the effect of culture incubation using *Halomonas* sp. strain A55 and decolorized media as a sole nutrient source, the end products of RR 184 dye operated for 24 h under anoxic condition has been fed to aerobic reactor (AE-aerobic) and compared with MSM supplemented anoxic and aerobic incubations (Fig. 3). The dye decolorization efficiency for anoxic and aerobic incubations has been found 87.4 ± 2.5 and 5.7 ± 1.4 at 24 h, respectively. The dye residue left during 24 h anoxic incubation didn't significantly decrease with *Halomonas* sp. strain A55 incubation under AE-aerobic condition (8.1 ± 0.2). Further incubation of the strain (240 h) under aerobic (18.5 ± 6.3) and AE-aerobic (24.2 ± 3.4) also found to be lower for dye removal (than oxic which is complete decolorization), suggesting that the strain shift to using oxygen as a preferential electron acceptor than RR 184. However, in the first 24 h, the COD and TOC removal efficiencies were identified highest for AE-aerobic followed by aerobic

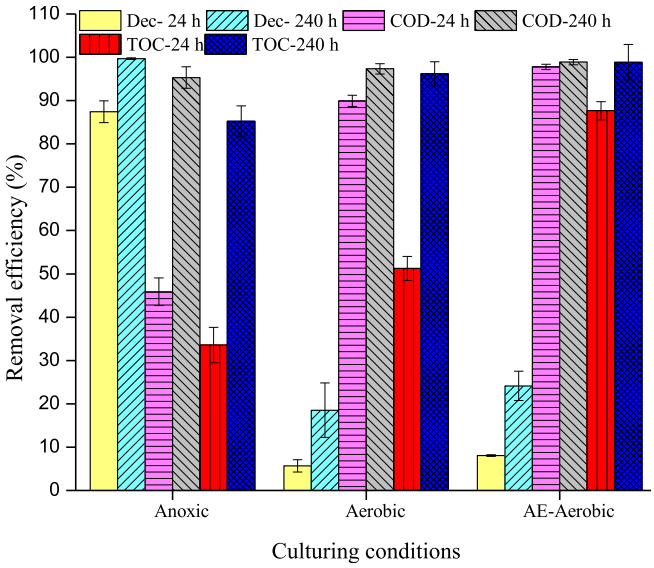

Fig. 3. Effect of incubation on COD, TOC and dye mineralization by *Halomonas* sp. strain A55.

culturing conditions confirming that the ability of *Halomonas* sp. strain A55 grow very well in both conditions (data not shown) and efficiently mineralized the organic matter. Interestingly, after a week incubation (240) under anoxic condition, the COD and TOC reductions were significantly enhanced and has no difference with aerobic and AE-aerobic culture (Fig. 3), indicating that organic matter mineralization has occurred through time under anoxic condition. This could be due to the efficient utilization of dye end product and organic matter added in the MSM (glucose and yeast extract) for growth and metabolic activity. The pool of reducing agents like NADH and sulphide resulted from organic matter and dye degradation metabolism has been stated as an excellent electron donor for azo dye degradation (Nigam et al., 1996; Van der Zee et al., 2001; Isik and Sponza, 2003; Yemashova and Kalyuzhnyi, 2006; Dos Santos et al., 2007). Moreover, complete decolorization and enhanced mineralization of COD and TOC achieved by using *Halomonas* sp. in the current study might be due to the advantage of using alkaliphilic inoculum that have developed a mechanism to adapt the changing environment (i.e, accumulation of degradation product) and performed further mineralization which may not true for non-alkaliphilic microorganisms (Dos Santos et al., 2007; Almeida and Corso, 2014). The ability of alkaliphilic bacteria to respond rapidly the changing environment has been reported as a prerequisite for survival in their habitat (Pinner et al., 1993; Krulwich et al., 1998; Horikoshi, 1999; Sorokin et al., 2014; Preiss et al., 2015). Indeed, Yemashova and Kalyuzhnyi (2006) also observed better aromatic amine degradation using anaerobic sludge inoculum than activated sludge, indicating that the source of

Table 5
Enzyme activity during decolorization of dye RR 184 by *Halomonas* sp.strain A55.

Time	Azoreductase (U per mg protein)			Laccase (U per mg protein)		
	Control without dye	Treated with dye	*p*-value	Control without dye	Treated with dye	*p*-value[b]
Initial	0.161±0.002	0.160±0.015	0.9897	0.212±0.079	0.218±0.016	0.999
24 h	0.165±0.012	0.289±0.006	4.9394E-13	0.215±0.010	0.248±0.017	0.7264
48 h	0.156±0.027	0.336±0.054	2.4401E-05	0.228±0.062	0.345±0.020	0.0045
72 h	0.162±0.006	0.427±0.008	2.1619E-14	0.232±0.015	0.396±0.042	0.0004
p-value[a]	0.3632	8.1054E-09	–	0.4217	0.0017	–

[a] *p*-value computed for the control and treated sample before (initial) and after 72 h incubation with strain A55.
[b] *p*-value computed for the control and treated samples at the same time.

seed microorganism matters for effective treatment. Moreover, the anoxic culturing condition (DO = 0.25 – 0.75 mg L^{-1}) in the current investigation has not only favored the rapid azo dye decolorization but also enhanced COD mineralization. In agreement with this study, Isik and Sponza (2003) also investigated the effect of oxygen on decolorization of azo dyes and further mineralization using *Pseudomonas* sp. by incubating the bacteria for five to nine days under aerobic and microaerophilic conditions. The organic matter mineralization results have been found significant under both conditions.

Based on the anoxic, aerobic and AE-aerobic incubation removal efficiency of dye, COD and TOC results observed at different time, it is possible to conclude that the dye metabolites are further transformed and used as nutrient under anoxic and aerobic conditions.

3.3.4. *Effect of dye concentration and dye types on decolorization*

The dye concentration of textile industries wastewater is believed to be in the range of 16 – 20 mg L^{-1} (Banat et al., 1996). The effect of initial dye concentration (50 – 250 mg L^{-1}) on strain A55 decolorization potential was studied. In the first day of culture incubation, the decolorization efficiency of the strain was found ≥85% for initial lower dye concentration (50 – 150 mg L^{-1}) and 25 – 55% for higher dye concentrations (200 – 250 mg L^{-1}) (Fig. 4a, Supplementary Fig. 4). Within 96 h of the incubation period, strain A55 showed almost equal percentage of decolorization for all dye concentrations ($p = 0.105$). This means that an acceptable high color removal can be achieved by strain A55 in an extensive range of dye concentrations. Previous studies showed that dye concentration can influence the efficiency of microbial dye decolorization through a combination of factors including toxicity imposed by the dye at higher concentrations (Bhatt et al., 2005). However, results of the present study showed that higher concentration of dye (250 mg L^{-1}) was not toxic to strain A55 (Fig. 4a). Thus, this culture which could decolorize dyes above the reported dye concentration in wastewater can be successfully employed for treatment of dye bearing industrial wastewater. Textile industries are known to use a battery of dyes. Thus, wastewaters from these industries are reported to contain different dyestuffs (Hao et al., 2000; Saratale et al., 2011). To check if strain A55 degrades other commonly used textile dyes, the culture medium was supplemented with 150 mg L^{-1} of six different additional azo dyes.

Time was the significant factor ($p = 0.002$) for decolorization of the different dye types. Compared to 24 h, the strain showed significant decolorization efficiency with 72 h ($p = 0.037$) and 96 h ($p = 0.011$) for the different dye types (Fig. 4b). However, there was no significant difference ($p = 0.055$) in decolorization efficiency after 96 h for the different dyes, suggested that under application condition strain A55 could be used to decolorize complex dyestuff effluent without difficulty. Similar results were also obtained by Chen et al. (2003) but with an extended period. They were successful to get decolorization results ranging from 20 – 100% within 168 h of incubation using *Aeromonas hydrophila*. The overall decolorization variation (51 – 100%, $p = 0.006$) among different dye types observed using strain A55 might be attributable to the structural diversity of the dyes (Fig. 4b). In fact, it was reported that decolorization variation depends on the structure and complexity of the dyes, particularly on the nature and position of the substituent in the aromatic rings (Saratale et al., 2011; Rauf and Ashraf, 2012; Patel et al., 2013). For instance, the half-life of hydrolyzed Reactive Blue 19 is about 46 years at pH 7 and at a temperature of 25 °C (Hao et al., 2000). In the present study, strain A55 also showed least color removal efficiency when Reactive Blue 198 was used (Fig. 4b), suggesting that Reactive Blue dyes are less attacked by microorganisms.

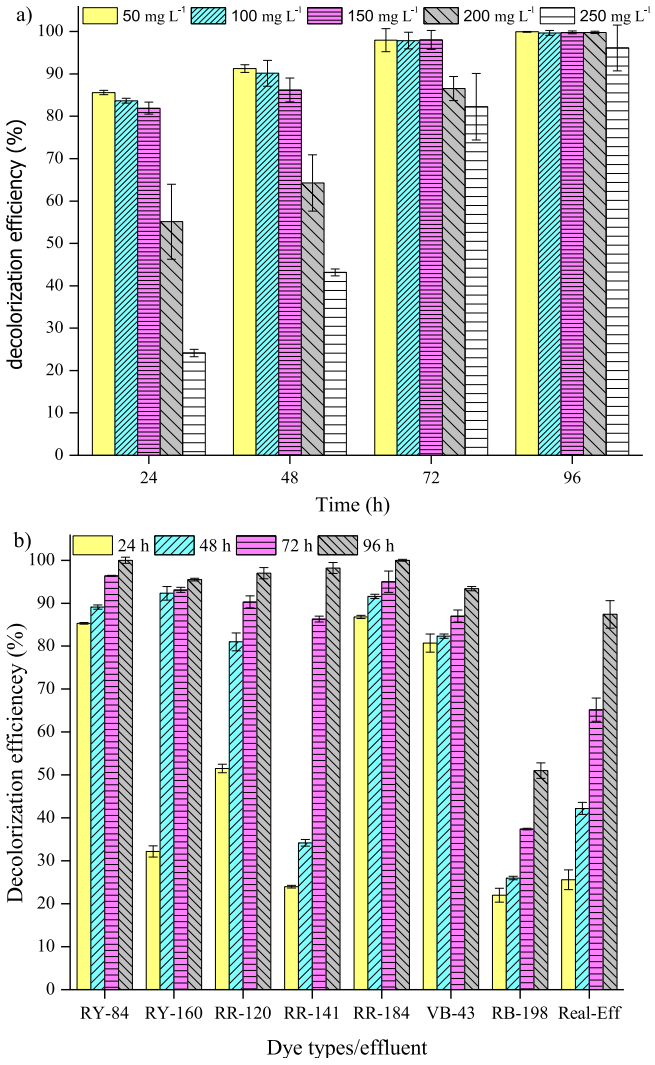

Fig. 4. The effect of dye on strain A55 decolorization efficiency (a) initial dye concentration (150 mg L^{-1}) and (b) different textile dye types (150 mg L^{-1}). RY, RR, VB, RB and Real-Eff represents, Reactive Yellow, Reactive Red, Vat Blue, Reactive Blue dyes and real effluent, respectively. Error bars represent the standard deviation calculated from at least three independent experiments. For real effluent, the percentage results are based on ADMI. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The ability of Halomonas sp. strain A55 decolorizing real effluent was also found interesting for this study (Fig. 4b). Initially, the wastewater used for treatment was characterized using standard methods (APHA et al., 1998). The strain showed 86.4±3.2% of real effluent American Dye Manufactures Institute (ADMI) removal efficiency at 96 h, suggesting that this bacterium could be used effectively for the treatment of textile effluent which comprises a mixture of many dyes.

3.3.5. *Kinetics of decolorization and mechanism of RR 184 degradation by strain A55*

Reduction kinetics of azo dyes has been expressed in several ways (Yu et al., 2011). In order to approximate the reaction by first-order kinetics with respect to the dye concentration, the values of $\ln\left[\frac{C_t}{C_o}\right]$ were plotted against time, using experimental data of decolorization (Fig. 5a). The data best fit on the straight line in which the slope equals the value of decolorization rate. The highest coefficient of correlation ($R^2 = 0.9940$) was obtained, implying that

the pseudo-first-order kinetic model better describes the decolorization kinetics of RR 184 by strain A55. Pseudo-first-order kinetic model as dye decolorization process was also reported in other studies (Mutafov et al., 2006; Yu et al., 2011).

Fig. 5b shows a typical time-dependent UV–visible spectrum of RR 184 dye solution during biodegradation. Peaks were observed in the spectral ranges of 470–600 nm. Peak intensity was observed in UV region near 540 nm, which corresponds to the azo group of RR 184. The absorbance peaks corresponding to RR 184 dye were diminished with time indicates that the dye had been removed. Moreover, the control did not show any color change confirmed rather than abiotic factor the color removal is due to microbial biodegradation (data not shown). The UV–visible the results of RR 184 dye reduction experiments as well as the controls demonstrate that RR 184 dye reduction by A55 is a bioreduction process, which also agrees with other studies (Yu et al., 2011; Saroj et al., 2014).

Fig. 6 shows the FTIR spectrum of the control dye (untreated RR 184) and extracted products. The FTIR spectrum of the control dye exhibits specific peaks at 1624.9 and 1535.0 cm^{-1} due to the presence of azo groups –N=N–stretching (Fig. 6a). The peak at 1126.8 cm^{-1} corresponds to S=O stretching while the presence of the peak at 1042.7 cm^{-1} and 804.6 cm^{-1} showed C–F and C–Cl stretching, respectively.

Compared to the control dye spectrum, the FTIR spectrum of extracted products after decolorization of RR 184 by *Halomonas* sp. strain A55 showed variation in the positions of peaks (Fig. 6b). The disappearance of peaks at 1624.9 and 1535.0 cm^{-1} indicates the reductive cleavage of RR 184 at the azo bond position. The peak observed at 3578.6 cm^{-1} is also due to the stretching of O–H group of the naphthalene. The peak at 2942.3 cm^{-1} shows the C–H stretching of alkanes while the peak at 2830.5 cm^{-1} shows C–H stretching of aromatics. The peak observed at 1580.3 also indicates the existence of C=C stretching in the benzene ring. In addition, peak at 1030.0 cm^{-1} shows the C–F stretching from triazine ring. These changes in the FTIR spectrum are clear evidence for the biodegradation of RR 184 into simpler molecules. Fig. 7 also shows the result of HPLC analysis on the metabolites of RR 184 decolorization by strain A55. The parent compound analyzed from the control has been detected at a retention time of 1.651 min with high intensity and lower intensity at a retention time of 2.159 min (Fig. 7a). After anoxic treatment, the peaks observed in the control disappeared and seven new peaks were formed from the anoxic decolorized dye metabolite. The three major peaks were observed at the retention times of 2.462, 2.585 and 10.308 min. Other four peaks were also observed at a retention time of 3.425, 5.138, 5.798 and 13.013 min (Fig. 7b). The appearance of new peaks (major and minor) in the treated dye end product and disappearance of the major peak in the control

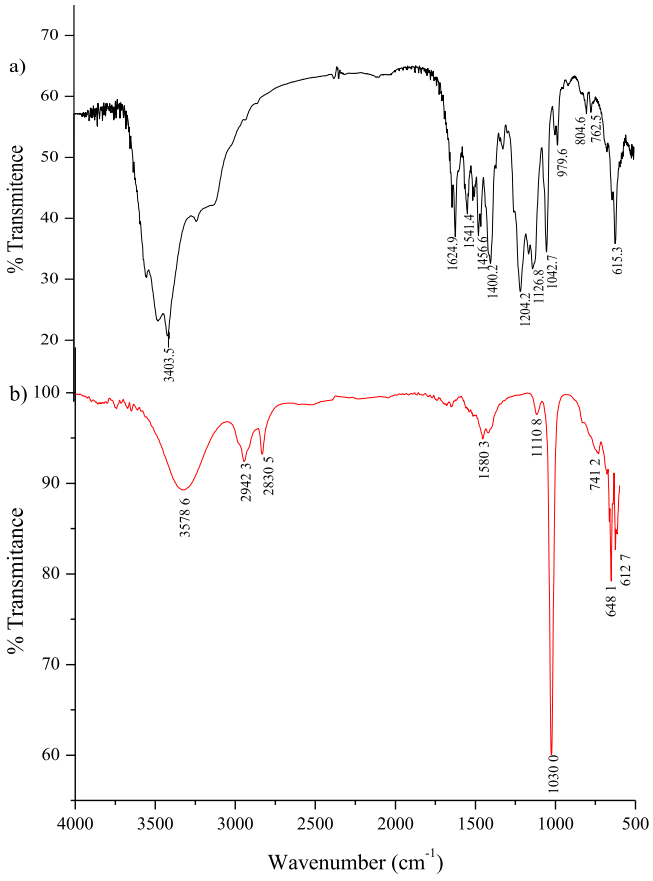

Fig. 6. The FTIR spectrum of RR 184 (a) without (control) bacteria, (b) with strain A55 treatment.

dye suggested that biodegradation of RR 184 has been carried out by *Halomonas* sp. strain A55.

To confirm the presence of RR 184 dye degrading enzymes, the decolorized samples were collected at different time and compared with the control (Table 5). Results showed that *Halomonas* sp. strain A55 possesses azoreductase and laccase enzymes that play a significant role in the break down of the azo bond of the dye. Compared with the control, the presence of RR 184 as substrate has significantly stimulated the strain to induce azoreductase and laccase enzymes. At 24 h incubation period, the induction of azoreductase and laccase were found to be 80.2% ($p = 4.9394E\text{-}13$) and 15.3% ($p = 0.7264$), respectively. At 72 h sample enzyme assay, the

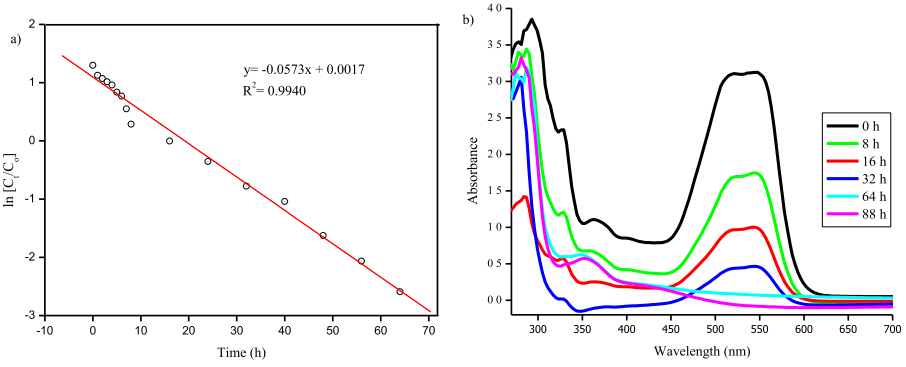

Fig. 5. (a) First order kinetic plot for RR 184 and (b) variation in Uv–visible spectra of RR 184 (150 mg L^{-1}) biodegraded by strain A55 with respect to time intervals.

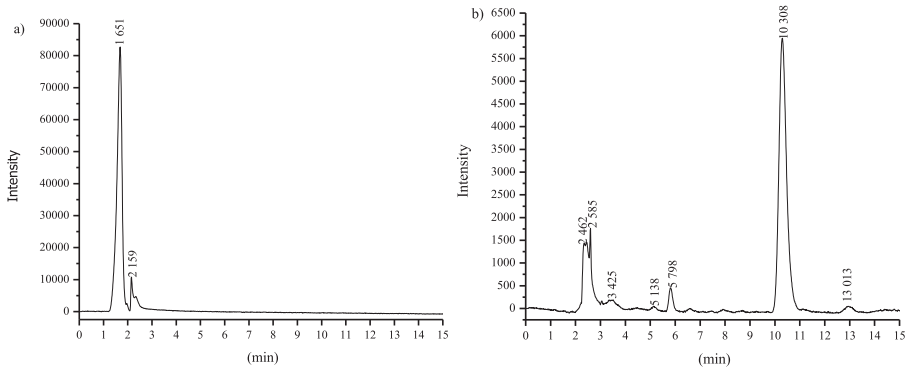

Fig. 7. (a) HPLC profile of RR 184 (control), (b) RR 184 after treatment with *Halomonas* sp. strain A55.

azoreductase induction has been also found 2.63 fold (163.14%) than the control ($p = 2.1619E-14$), suggesting that azoreductase could be the major enzyme involved in RR 184 reduction. Comparison of the same treatment conditions (treatment with dye = before/initial and final/after 72 h) also showed that significant amount of azoreductase ($p = 8.1554E-09$) and laccase ($p = 0.0017$) induction through time, while the control remains the same (Table 5). Azoreductase which showed specific activity on azo dye decolorization has been extensively investigated to elucidate azo dye reduction mechanism (Zimmermann et al., 1982; Ayed et al., 2010; Agrawal et al., 2014; Almeida and Corso, 2014). Microbial azoreductase has great importance for designing the biotreatment process to treat azo dye-containing wastewater (Banat et al., 1996; Dos Santos et al., 2007; Saratale et al., 2011; Rauf and Ashraf, 2012). Laccase enzyme also investigated by many scholars (Ayed et al., 2010; Agrawal et al., 2014). It is low molecular weight multicopper oxidase family enzyme with less substrate specificity and is potent to degrade a wide range of recalcitrant compounds (Banat et al., 1996; Hao et al., 2000; Saratale et al., 2011; Rauf and Ashraf, 2012). It has great importance in biotechnological approaches as it has bioremediation capacity and does not use readily available oxygen as an electron acceptor (Dos Santos et al., 2007; Agrawal et al., 2014).

4. Conclusions

Batch treatment of synthetic wastewater containing dye was evaluated using Rift Valley Alkaline Soda Lake microbial inoculum. Although the microbial isolates have no previous exposure to textile effluent, they have been found an abundant and potential candidate for dye removal. Among the isolates, strain A55 which belongs to the genus *Halomonas* was found the best candidate that decolorize seven dye types efficiently without significant effect at higher dye concentration. The strain grows at a range of pH (7.5 – 12.0) and NaCl concentration (0 – 20%) also indicates the potential candidacy of the strain to treat alkaline textile effluent. The presence of organic carbon and nitrogen sources were found enhancing decolorization of RR 184 than inorganic nitrogen sources. *Halomonas* sp. strain A55 was also completely decolorized RR 184 under anaerobic and anoxic condition which indicates the involvement of oxygen sensitive metabolic process. Indeed, a significant increase in the activities of azoreductase in cells obtained after RR 184 decolorization indicates the involvement of this enzyme in the decolorization process. Color removal was significantly decreased when $NaNO_3$ and $NaNo_2$ used as MSM. The effect of shaker and aerobic growth condition also significantly decreased the decolorization ability of *Halomonas* sp. strain A55. Reactive Red 184 dye decolorization by strain A55 was found following pseudo-first-order kinetics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.04.134.

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