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ORIGINAL RESEARCH PAPER

A novel microbial consortium from sheep compost for decolorization and degradation of Congo red

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ABSTRACT

Congo red is a synthetic azo-dye dye with many industrial applications. The effluents containing azo dyes are causing several environmental hazards and thus should be treated prior to their discharge. The present work investigates the possible use of a novel microbial consortium from sheep compost for the decolorization of Congo red dye. The effect of different parameters including contact time, dye concentration and inoculum concentration on dye decolorization were investigated. The kinetic of dye decolorization was also assessed and the biodegradation of the dye was confirmed by different techniques. The results showed that the microbial consortium decolorized about 98% of Congo red (500 mg/L) after 24h. The efficiency of the decolorization decreased from 95% to 62% when the dye concentration increased from 100 to 500mg/L. Also, it was noticed that 75% of Congo red (25 mg/L) was decolorized at an inoculum rate of 2.5%. The kinetic results suggested that the decolorization of Congo red by the studied consortium follows the first order kinetic model. Also the maximum substrate consumption rate (V_{max}) according to Michaelis- Menten model was found to be 19.30 mg/h/L and the decolorization rate constant (K_m) was 116.93 mg/L. The biodegradation of Congo red was further confirmed by HPLC and GC-Ms analysis which revealed the presence of some spectral differences between the untreated dye sample and the treated one. In conclusion, the results of the present work suggest that microbial consortium from sheep compost could have potential application for bioremediation of industrial effluents containing Congo red dye.

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INTRODUCTION

In developing countries wastewater is a great source of pollution; the discharge of industrial effluents contaminates water resources such as rivers and ground water (Gajera *et al.,* 2015; Imran *et al.,* 2016) only YE stimulated azoreductase activity (increased from 1.32 to 4.19U/mg protein. Wastewater is released from many industries such as textiles, papers, wood, leather, cosmetics, medicine and plastics in which synthetic dyes are used (Sharma *et al.,* 2014). There are more than 100000 different commercial synthetic dyes present in the markets with a production of more than $7x10^5$ ton annually (Saroj *et al.,* 2014; Sharma *et al.,* 2014). These dyes are characterized by their stability and low cost

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compared to natural dyes (Yamjala *et al.,* 2016).About 60-70% of the synthetic dyes belong to azo dyes group which is characterized by possessing the high stable azo group (N=N) (Bankole *et al.,* 2018; Imran *et al.,* 2016; Mittal *et al.,* 2010)Achaetomium strumarium were investigated. Molecular studies of 23S rRNA sequence data confirmed the phylogenetic clade relationship of the isolate with members of the same genus, Achaetomium. Achaetomium strumarium decolorized (99%.These dyes are widely used in almost all coloring industries due to their shining colors (Yamjala *et al.,* 2016). During dyeing processes about 10-15% of the dye go to through the wastewater (Tan *et al.,* 2016). These dyes and their degradation products have toxic, allergic, mutagenic and carcinogenic effects, they also could effect on the plant growth, aquatic organisms and even humans if the industrial effluents could reach to rivers(Costa *et al.,* 2018; Das and Mishra, 2017; Imran *et al.,* 2016; Tan *et al.,* 2016, 2014; Vijayalakshmidevi and Muthukumar, 2015). For those reasons the use of a lot of azo dyes became restricted by many countries especially those used as food colorants. The discharge of effluents containing dyes may affect the aquatic plants photosynthesis due to the reduction of light and oxygen penetration into water resources(Das and Mishra, 2017; Gupta, 2015; Imran *et al.,* 2016). Congo red dye is one of the most widely used azo dyes. It is a sodium salt of benzidinediazo-bis-1-naphtylamine-4-sulfonic acid (Ghorai *et al.,* 2013). The dye has several biological and industrial applications including its use as a biological dye to examine amyloidosis, determination of hydrochloric acid in stomach as well as paper, wood and textile industries (Bentahar *et al.,* 2016; Satheesh Babu *et al.,* 2015). It is thus necessary to treat these industrial effluents before discharging. Wastewater treatment techniques comprise chemical, physical and biological methods. According to Wang *et al.*(2015) the physical and chemical methods are high costly so the biological methods are preferred because they are less costly, highly efficient and environmental friendly (Bankole *et al.,* 2018; Kong *et al.,* 2018; Nguyen *et al.,* 2016; Tan *et al.,* 2016). Microorganisms have a superior effect in wastewater containing dyes treatment than other organisms (Gajera *et al.,* 2015). These microbes have the ability to adapt to toxic and harmful contaminants so they may degrade and convert the toxic contaminants into less toxic compounds (Saranraj *et al.,* 2014; Selva Raj *et al.,* 2012; Senan *et al.,* 2003). Although these microbes can be used singly or mixed, several reports approved that the mixed culture has a superior effect in degrading azo dyes (Das and Mishra, 2017; Vijayalakshmidevi and Muthukumar, 2015). Due to the high ability of bacteria to acclimatize, widely spread and their high efficiency in azo dye degradation, they are used extensively in wastewater treatment (Tan *et al.,* 2014). In this study, a microbial consortium obtained from sheep farm compost was used to examine its ability to decolorize wastewater containing Congo red synthetic dye under anaerobic conditions at 30°C. Also, this consortium was used to examine the effect of different operating parameters on the decolorization efficiency. To the best of our knowledge the present work could be of the first reports which used the sheep compost as a source of microbes to decolorize and degrade wastewater containing azo dyes. This study has been carried out in the Agricultural Research Center, Egypt in 2018.

MATERIALS AND METHODS

Chemicals

Luria Broth (LB) (Condapronadisa) medium (Tryptone 10, sodium chloride 10, yeast extract 5, g/L) was used for enrichment and carrying out the decolorization experiments. Congo red (CR) dye was purchased from Sigma Aldrich ($C_{32}H_{22}N_6Na_2O_6S_2$, M.wt 696.66g/mol). Methanol HPLC grade.

Microorganisms

The microbial culture used in this study was obtained from agricultural compost (from sheep farm in Faculty of Agriculture, Cairo University). The compost was sieved to fine powder then 0.5g were added to 100ml LB medium and incubated at 30°C at static conditions for 2 days. Then, 100ml LB medium supplemented with 25mg/L CR dye were inoculated with 5ml of the previous culture and incubated at the same conditions for enrichment.

Decolorization experiments

Effect of time on removal percentage

The Congo red concentration during all the experiments was determined by spectrophotometer (SpecorD250 plus- Analytik Jena) at 495 nm wavelength (Satheesh Babu *et al.,* 2015). LB medium was inoculated with microbial mixture individually and incubated overnight at 30°C at static conditions. Flasks with LB medium supplemented with different dye concentrations (10, 25, 50, 100, 200 and 500mg/L)

were inoculated with 5% microbial culture and incubated at 30°C at static conditions. Samples were withdrawn at different time periods (0, 2, 5, 10, 24, 36 and 48 hours), centrifuged and their absorbances were measured spectrophotometrically.

Effect of dye concentration

Decolorizing media were supplemented with different dye concentrations (10, 25, 50, 100, 200 and 500ppm) inoculated with 5% microbial culture and incubated at 30°C at static conditions. After 5hours the samples were taken and the remaining dye concentrations were determined.

Effect of inoculum concentration

Flasks containing decolorizing medium with CR dye at a concentration of 25 mg/L were inoculated with different concentrations (2.5, 7.5 and 10% V/V) of microbial culture and were incubated at 30°Cat static conditions. After 5h of incubation a sample from each flask was withdrawn and centrifuged at 10000 rpm for 10min. The remaining dye concentration was then determined.

Calculation

The dye removal percentage was calculated according to Eq. 1.

$$\text{Removal (\%)} = \frac{\text{Initial dye concentration(mg/L)} - \text{Final dye concentration(mg/L)}}{\text{Initial dye concentration (mg/L)}} \times 100 \quad (1)$$

The results are expressed as mean of three replicates.

Kinetic studies

The kinetics of CR removal were investigated using the three kinetic models (first, second and third order) as well as Mechalis-Menten model.

Biodegradation analysis of CR

HPLC analysis was carried out using HPLC (Agilent technologies 1260 Infinity II) equipped with a C18 column (150x 4.6mm) with a flow rate 0.9mL/min and absorbance at 495nm. The mobile phase was acetonitrile: water (60:40) was used as a solvent. Control sampleof25mg/L CR dye in methanol HPLC grade while the treated sample was centrifuged at 3000 rpm for 15 min then 10ml of the supernatant was dissolved in 25ml methanol. Both samples were degassed in an ultrasonic water bath for 50 min at room temperature, filtered through syringe filter 0.45µm and 20µl was injected. The HPLC analysis was carried out according to the procedures of Raj *et al.,* (2012) with some modifications. GC-MS analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass- selective detector (MSD, Agilent 7000) operated with flow rate of 1mL/min. The components were confirmed by coordinating their mass spectra and retention time with the database of National Institute of Standard and Technology (NIST) library.

RESULTS AND DISCUSSION

Effect of time on removal percentage

The efficiency of bioremediation processes is usually affected by several physicochemical conditions including time, dye concentration and inoculum concentration. In the current work the effect of contact time on the removal of CR dye at different concentrations is illustrated in (Fig. 1). Each experiment was stopped when complete removal of dye was achieved. It was noticed that more than 80% of the dye was removed within 5h when the initial dye concentration was 10mg/L. By increasing the initial dye concentration (from 10 to 500 mg/L), the time required for the maximum decolorization increased (from 5 to 24h). This may be due to increasing the organic or toxic breakdown products, also the microbial growth rate could be suppressed at higher dye concentration(Tan *et al.,* 2016 and 2013). More than 95% of 50-100 mg/LCR were removed within 5h although the initial dye concentration was increased. Furthermore, the microbial mixture was capable of decolorizing more than 98% of 500 mg/L of Congo red after 24 h.

Effect of dye concentration

As shown in Fig. 2, by increasing the initial dye concentration from 100 to 500mg/L the decolorization efficacy was decreased from 95% to 62% after 5h incubation. Each experiment was terminated once the color was completely removed. The initial concentration of the dye may affect the decolorization efficiency as the toxicity of dye could be more pronounced at higher dye concentrations which may suppress the microbial growth(Guadie *et al.,* 2018; Hameed and Ismail, 2018; Lalnunhlimi and Veenagayathri, 2016; Pearce *et al.,* 2003) this study presents isolation of a bacterial

consortium from soil samples of saline environment and its use for the decolorization of azo dyes, Direct Blue 151 (DB151. It is noted that at an initial dye concentration (200 mg/L) the removal efficiency was greater than 85% which may suggest that the culture used in the present study may have a great potential to be used in the industrial wastewater treatment containing high concentration of dye.

Effect of inoculum concentration

Fig. 3 shows the effect of inoculum concentration on dye decolorization after 5h at initial dye concentration of 25 mg/L. The results revealed that at 2.5%inoculum concentration more than 75% of the dye was removed with no further increase in dye removal at higher inoculum concentration up to 10%.

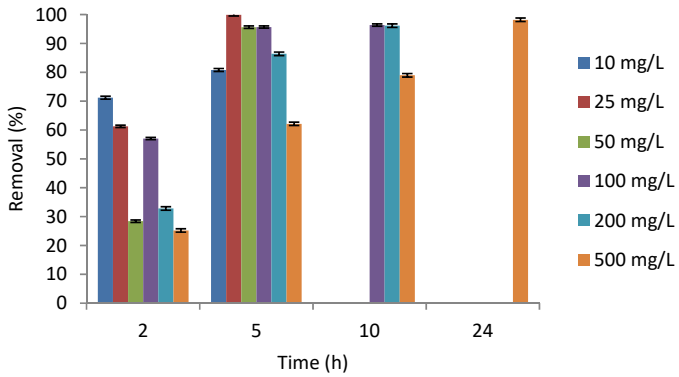


Fig. 1: Effect of time on dye removal percentage

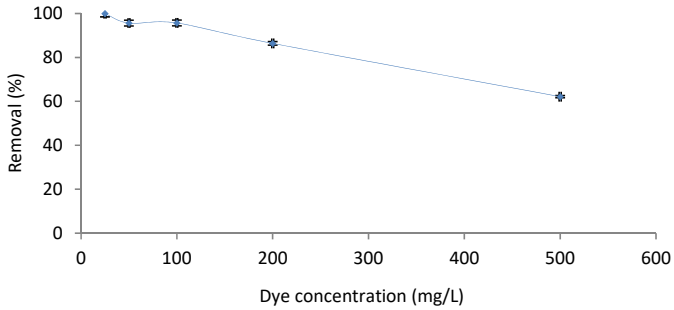


Fig. 2: Effect of dye concentration on the removal percentage

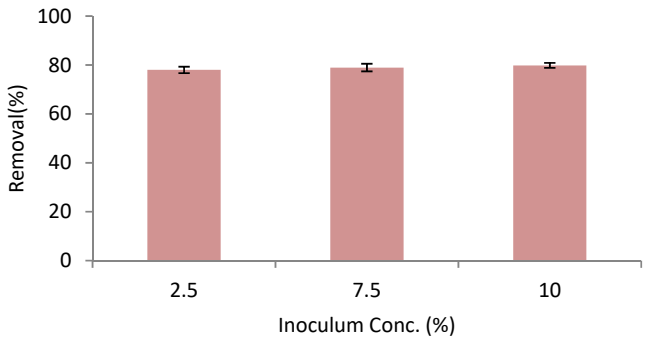


Fig. 3: Effect of inoculum concentration on the removal percentage

Kinetic studies

Determination of reaction order

Three kinetic models were applied to fit the experimental data for the decolorization of Congo red by the studied consortium in order to determine the reaction order. The three models are zero, first and second order kinetic presented in their linear forms by Eqs. 2, 3 and 4, respectively.

$$C_t = C_i - K_0 t \quad (2)$$

$$\ln C_t = \ln C_i - k_1 t \quad (3)$$

$$1/C_t = 1/C_0 + k_2 t \quad (4)$$

In these equations C_i represents the initial dye concentration (mg/L), C_t (mg/L) is the remaining dye concentration at time (t), t is the time (h), K_0, k_1 and k_2 are the rate constants for zero, first and second order kinetic, respectively. Plots of the three kinetic models are shown in Figs. 4, 5 and 6.The rate constants K_0,

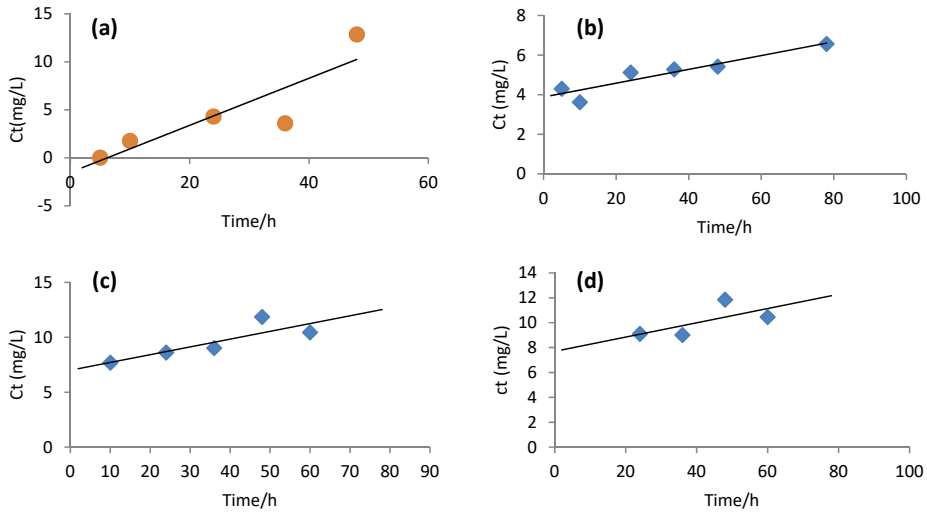


Fig. 4: Zero order kinetic plot at different dye concentrations: (a) 25 mg/L, (b) 100 mg/L, (c) 200 mg/L and (d) 500 mg/L

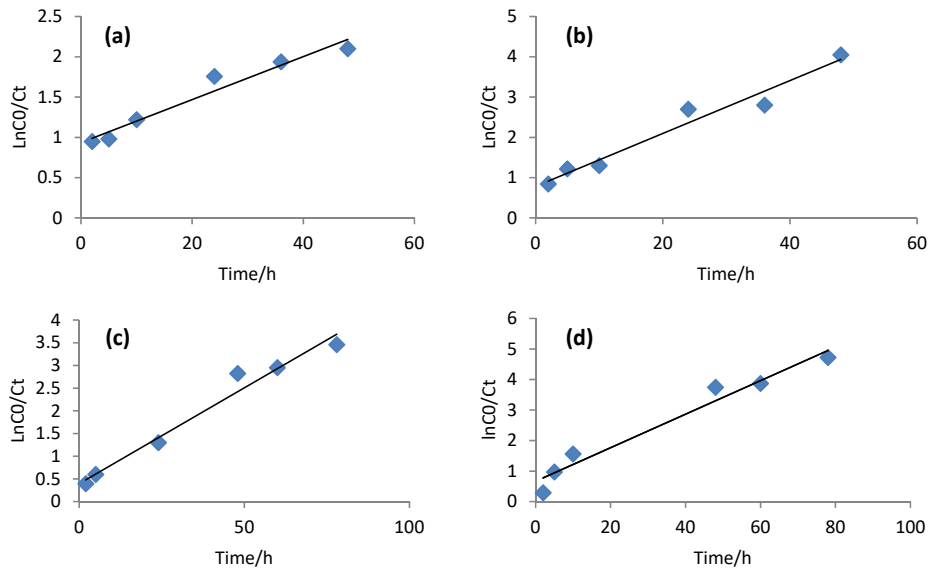


Fig.5: 1st order kinetic plot at different dye concentrations: (a) 25 mg/L, (b) 100 mg/L, (c) 200 mg/L and (d) 500mg/L mg/L

k_1 and k_2 were obtained from the slopes of the plots of (C_t) versus (t), ln C_t versus (t) and ($1/C_t$) versus (t) for Eqs. 2, 3 and 4, respectively. Also the regression coefficients (R^2) for each equation were obtained from these plots.

Table 1 gives the rate constants of decolorization experiments and regression coefficients of the three studied kinetic models. The correlation coefficients (R^2) for the first order reaction kinetics were in the range of 0.90-0.96 implying the suitability of this model to fit the experimental data. On the other hand the data did not fit the zero or the second order model as seen from their very low R^2 values. In agreement with previous studies, the decolorization of CR followed first order kinetics which reveals that the rate of decolorization increases with the increase of initial dye concentration (Das and Mishra, 2017). Kinetic behavior is a critical subject in the completion of any biological process, as it can facilitate the control of the process, which is very important while working at industrial scale (Deive *et al.*, 2010).

The data were further investigated by applying Michaelis-Menten equation in its reciprocal form given by Eq. 5 known as Line weaver- Burk equation.

$$1/V = K_m/(V_{max}.S) + 1/V_{max} \quad (5)$$

The applicability of the model is checked by plotting (1/V) against (1/S) as shown in Fig. 7.As seen from the figure the experimental data showed a good fitting with the model equation with R^2= 0.907. The maximum substrate consumption rate (V_{max}) and decolorization rate constant (K_m) as calculated from the plot were 19.30 mg/h/L and 116.93 mg/L, respectively.

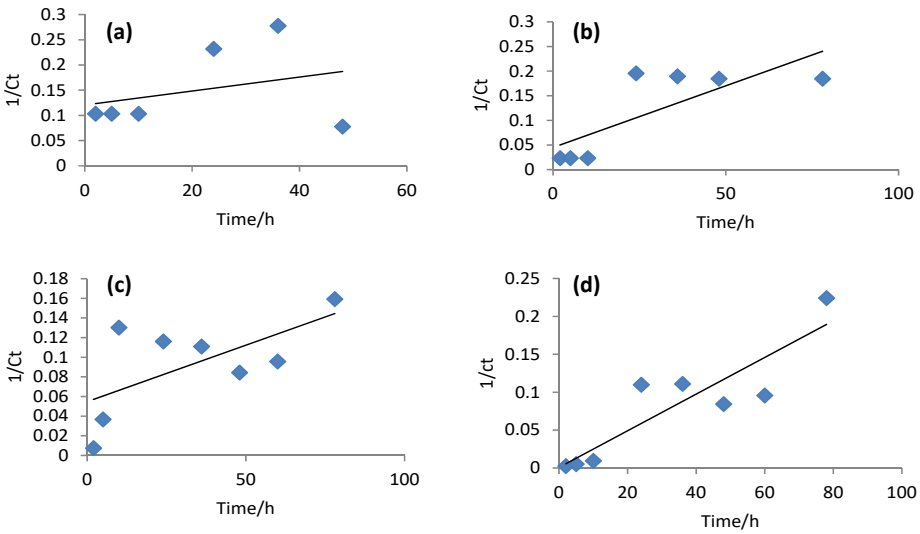


Fig. 6: 2nd order kinetic plot at different dye concentrations: (a) 25 mg/L, (b) 100 mg/L, (c) 200 mg/L and (d) 500 mg/L

Table 1: Rate constants and regression coefficients of kinetic models

Constants	Dye Concentration (mg/L)			
	25	100	200	500
		Zero order		
K_0 (mg/h)	0.2456	0.0349	0.0708	0.0574
R^2	0.7826	0.8753	0.7174	0.4418
		First order		
K_1/h)	0.03	0.066	0.0414	0.0549
R^2	0.97	0.90	0.96	0.96
		Second order		
K_2 (Lmg/h)	0.0004	0.0036	0.0011	0.0024
R^2	0.0079	0.7035	0.4105	0.7913

HPLC and GC–MS analysis

As shown in Fig. 8, the HPLC profile for the control sample showed one main peak at retention time 1.034 min. with area 751.47. While the treated sample's profile showed noise peaks which indicates that complete degradation of CR may have occurred. Degradation was further confirmed by GC-MS analysis as shown in Fig. 9. The presence of CR before treatment was confirmed by GC-MS spectrum at retention time 16.38 min and area 44.67% while the spectrum for the treated sample showed a peak at the same retention time but with much smaller area of 9.25% indicating the degradation of the dye. According to Raj *et al.* (2012), the spectral differences between the control and treated samples could be attributed to the dye biodegradation in the decolorization medium or may be dye biotransformation to other components. Similar results were achieved by other authors for different types of synthetic dyes (Das and Mishra, 2017; Gajera *et al.*, 2015) removal of a textile dye Reactive Green-19 from the aqueous medium was investigated using a developed bacterial consortium. Optimal combinations of three significant process parameters pH (5-10.

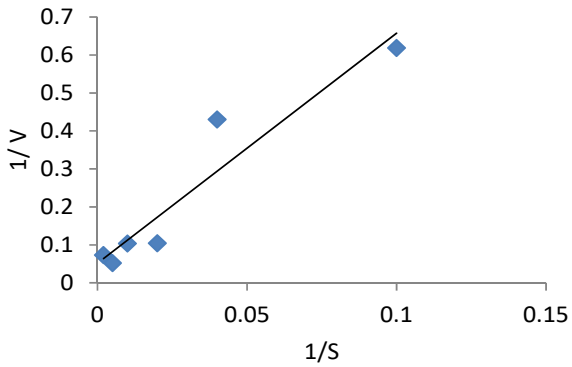


Fig. 7: Line weaver- Burk plot of (1/V) against (1/S)

Comparing the results obtained during the present work with previously published sudies, it can be concluded that sheep compost consortium was capable ofdecolorizing 98% of CR after 24hours. While Dietzia sp. decolorized 93% of CR after 30h (Satheesh Babu *et al.*, 2015). Also the decolorization efficiency of Penicilliumoxalicum SAR-3 d for three different azo dyes (Acid Red 183, Direct Blue 15 and

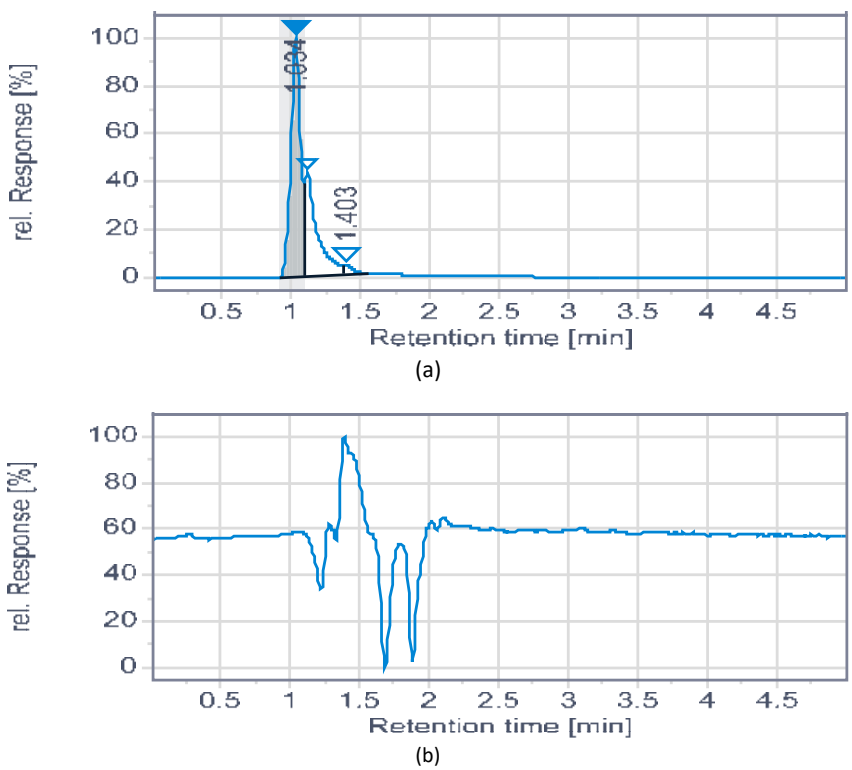


Fig. 8: a) HPLC chromatogram of Congo red (Control) and b) HPLC chromatogram of treated sample

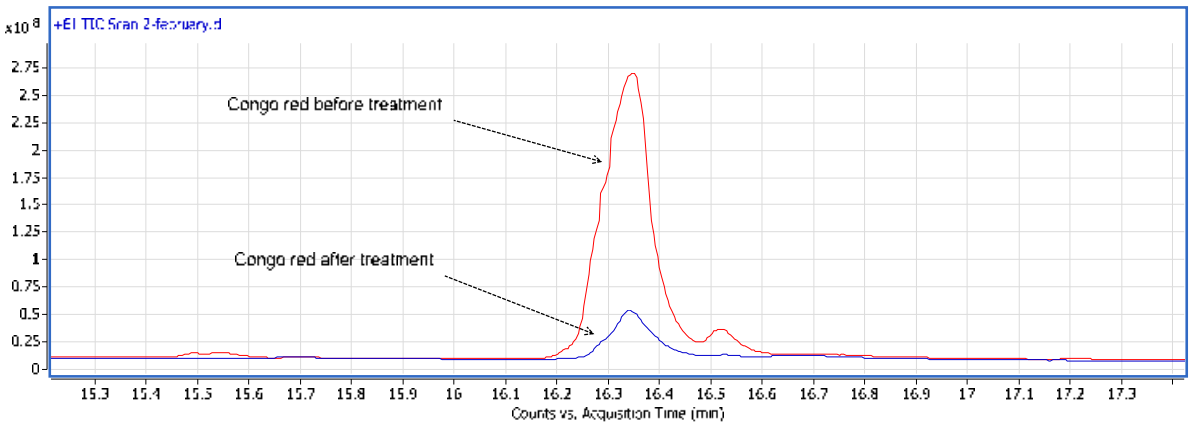


Fig. 9: GC-MS Chromatogram of control sample vs treated sample

Direct Red 75) was about 95% (Saroj *et al.*, 2014), and Scheffersomycesspartinae TLHS-SF1 degraded 90% ofAcid Scarlet 3R dye after 16h (Tan *et al.*, 2016). Thus the studied sheep compost consortium showed high decolorization percentage within reasonable time comparedto other microorganisms.

CONCLUSION

In the present study, sheep farm compost was successfully employed a source of microbial consortium. The microbial consortium was effective in the decolorization of about 98% Congo red dye from wastewater after 24h of incubation. An inoculum concentration 2.5% was capable of removing more than 75% of the dye from wastewater. The biodegradation of the dye was confirmed by gas-chromatography and high performance liquid chromatography analysis. In conclusion, this consortium from sheep farm compost could have potential applicability for the remediation of effluents containing azo dyes in an economic and efficient way. Future work will be addressed to the purification and identification of the microorganisms present in sheep compost consortium.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS

C_i	initial dye concentration
CR	Congo red
C_t	dye concentration at time (t)
$C_{32}H_{22}N_6N$-$Na2O_6S_2$	Chemical formula of Congo red
°C	Degree Celsius
Eq.	equation
Eqs.	Equations
Fig.	figure
g	gram
g/l	Gram per liter
g/mol	Gram per mole
GC	Gas chromatography
GC-Ms	Gas chromatography- mass spectroscopy
h	hour
HPLC	High performance liquid chromatography
K_0	rate constant for zero order kinetic
k_1	rate constant for first order kinetic
k_2	rate constant for second order kinetic

K_m	decolorization rate constant
LB	Luria Broth
L	liter
Min.	minutes
M.wt	Molecular weight
ml	Milliliters
mg/L	Milligram per liter
mg/L/h	Milligram per liter per hour
NIST	National institute of standard and technology
nm	Nanometer
R^2	Regression coefficient
Rel. response	Relative response
rpm	Round per minute
µl	Micro liter
µm	Micro meter
V_{max}	maximum substrate consumption rate
V/V	Volume per volume
vs	versus

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