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# Biodecolorization of methyl orange by bacteria isolated from textile industrial wastes: optimization of cultural and nutritional parameters

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## Abstract

Bioremediation of textiles wastewater contaminated with dyes has been widely described since their cost/efficiency ratios are lower than other physico-chemical processes. In the present study, effluent samples were collected from textile industries located in Settat city in Morocco, and were used for the isolation of bacterial strains that are able to decolorize the textile dye Methyl Orange. Physico-chemical properties of the effluent samples were analyzed and five bacterial strains S3, S4, S6, S7 and S8, capable of decolorizing Methyl Orange were screened and isolated. They showed more than 90% of decolorization activity at 37°C and at neutral pH. UV-Visible absorption spectra before and after decolorization showed significant change in the positions of peaks, when compared to control dye spectrum, indicating that decolorization was due to biodegradation.

## 1. Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life [1,2]. Reactive dyes, in particular, azo dyes are used by the majority of the textile industries for the coloring process [3]. The synthetic dyes, especially azo dyestuff, have been produced and widely used in paper, textile and coating industries, which have caused serious environmental pollution, especially in developing countries [2-5]. Synthetic azo dyestuff is very difficult to remove or to biodegrade due to its complex aromatic molecular structure. It is also highly coloredand can stain the receiving aquatic ecosystems, toxic as well as carcinogenic [2, 3, 6].

Traditional wastewater treatment technologies have proven to be markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of these pollutants. A wide range of methods has been developed for the removal of dyes from the textile waste effluent by physical and chemical methods, such as flocculation, membrane filtration, electrochemical techniques, ozonation, coagulation and adsorption [7, 8].The Bioremediation of textile effluents has been of considerable significance since it is inexpensive, nontoxic, ecofriendly, economically potential [9] and produces a less amount of sludge [10]. Many bacteria and fungi can efficiently biodegrade azo dyes and decolorized them, including *Aeromonas sp., Pseudomonas*, *Bacillus, Rhodococcus*, *Shigella, Klebsiella*, *Rhizopus oryzae*, *Penicillium oxalicum* and *Phanerochaete chrysosporium* [11, 12]. However, their effectiveness depends on their adaptability and their activities[13, 14].

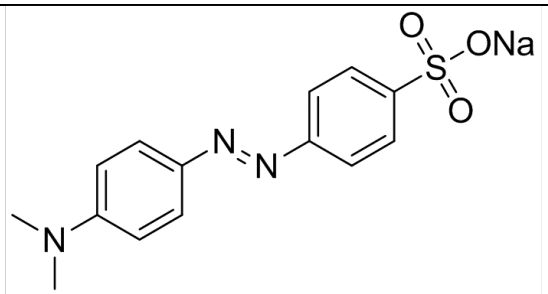
In this context, this study put emphasis on the degradation of Methyl Orange (MO) by the bacteria isolated from local industrial waste, with an aim to optimize various cultural (pH, temperature, dye concentration) and nutritional (carbon source) parameters for maximization of MO dye decolorization in simulated synthetic Minimal Medium (MM), which can be useful in providing an alternate method to accomplish dye degradation of a wide range of dyes in an ecofriendly manner.

## 2. Material and Methods

*2.1. Adsorbate*

The main characteristics of the used Methyl Orange dyeare presented in Table 1.

**Table 1:** Physicochemical characteristics of used dye.

| Name | Molecular structure | Chemical formula | λmax (nm) | Mw (g/mol) |
|---|---|---|---|---|
| Methyl Orange C.I. 13025 |  | $C_{14}H_{14}N_3NaO_3S$ | 463 | 327.34 |

*2.2. Sampling Site, Sample Collection*

Wastewater and sludge samples were taken from the dying effluents of a textile plant in the city of Settat (Morocco) and used for isolation of decolorizing bacteria textile dye.

*2.3. Isolation and screening of microorganisms*

Samples of sludge and wastewater were collected aseptically, in plastic bags and plastic bottles respectively, in the effluent discharges of the textile mills and carefully transported to the laboratory.

Approximately, 1 g of sludge was suspended in 10 ml of sterile sodium chloride solution 0.95% (w/v) and mixed thoroughly. The samples were serially diluted by following the standard protocol, and the dilution series of $10^{-2}$ to $10^{-8}$ were spread onto Minimal Medium (MM)plates containing: 1.36 g $L^{-1}$ $K_2HPO_4$ ; 0.1 g $L^{-1}$ $MgSO_4$ ; 0.6 g $L^{-1}$ $SO_4(NH_4)_2$ ; 0.02 g $L^{-1}$ $CaCl_2$ ; 0.5 g $L^{-1}$ NaCl ; 1.1 mg $L^{-1}$ $MnSO_4$ ; 0.2 mg $L^{-1}$ $ZnSO_4$ ; 0.2 mg $L^{-1}$ $CuSO_4$ ; 0.14 mg $L^{-1}$ $FeSO_4$ and 15g $L^{-1}$ of Agar. pH was adjusted to 7. Media was then supplemented with 100 mg $L^{-1}$ of dye tested [15].Each dilution was maintained in triplicates. All plates were incubated at 37 °C for 3-5 days. Colonies surrounded by decolorized zones were picked and streaked onto MM plates containing 100 $mgL^{-1}$ of MO. The plates were again incubated at the same conditions to confirm their abilities to decolorize MO.

The pure culture was preserved and maintained at 4°C on nutrient agar (oxoid, LP0011, AGAR N°.1)

*2.4. Bacterial MO Decolorization protocol*

A preculture of bacterial isolates was prepared by growing a single colony in 10 ml of MM containing 1% of glucose at 37°C during 24h in a rotary shaker. An aliquot of 5ml of precultures was inoculated into 200 ml of MM with 1% of glucose and 100 $mgL^{-1}$ of MO. This culture was incubated under the same conditions at 37 °C.

At several time intervals, 10 ml aliquots of each culture were sampled and their cell densities were measured spectrophotometrically at 620 nm using MAPADA V- 1200 UV/Visible spectrophotometer in order to determine the bacterial growth. Cells were then pelleted by centrifugation at 15,000 × g for 15 min and the clear supernatant obtained was 10-fold diluted and the concentration of MO was determined spectrophotometrically at 463 nm, where medium without dye and inoculum was used as blank, while medium with dye but without inoculum was taken as control. The decolorization of dyes was calculated by the given formula [16], as decolorization efficiency (%):

$$Decolorization(\%) = \frac{I - F}{I} \times 100$$

Where I=Absorbance of media prior to incubation, F=Absorbance of decolorized media.

*2.5. Optimization of physicochemical parameters*

The inoculated cultures of strains showing interesting activity, were then incubated at different experimental conditions to determine the effects of physicochemical parameters on the MO decolorization and the bacterial growth.

*2.5.1. Effect of temperature:* The culture medium used to determine the effect of temperature was MM containing 100 $mgL^{-1}$of MOand 1% of glucose at pH 7. The inoculated cultures were incubated at various temperatures (25, 37 and 44 °C) in rotary shakers running at 100 rpm.

*2.5.2. Effect of pH of culture medium:* Procedures to determine the effect of pH were similar to those described above for the temperature, except that the pH of the culture media was adjusted to 4, 6, 7 and 9 with 1 M HCl or NaOH and the inoculated cultures were incubated at 37°C.

*2.5.3. Effect of dye initial concentration:* The culture medium used to determine the effect of the concentration of MO dye was MM containing 1% of glucose and various concentrations of dye (50, 100, 150 and 200 $mgL^{-1}$). The inoculated cultures were incubated at 37 °C at pH7 under shaking condition (100 rpm).

*2.5.4. Effect of glucose concentration:* The culture medium used to determine the effect of the concentration of glucose was MM containing 100 $mgL^{-1}$ of MO and various concentrations of glucose (0.25, 0.5, 1 and 1.5%) at pH7. The inoculated cultures were incubated at 37 °C under shaking condition (100 rpm).

## 3. Results and discussion

*3.1. Isolation and Screening of Methyl Orange decolorizing bacteria*

We have obtained 24 isolates and were subjected to decolorization of MO ($100mgL^{-1}$) in minimal medium agar. All of them exhibited various levels of efficiency at 37°C and pH 7. Five strains designated as S3 and S7 isolated from sludge, S4, S6 and S8 from water, were found to be effective in decolorizing MO.

*3.2. Effects of physicochemical parameters on the decolorization of Methyl orange and bacterial growth of selected bacterium*

In general, pH and temperature play a crucial role for the optimal physiological performance of microbial cultures, and pH is particularly critical for transport of nutrients across the cell membrane. Thus, these factors are important in the dye decolorization process as they have marked effect on bacterial cell growth and production/activity of various enzymes[9]. Dye decolorization properties by five strains were investigated as a function of changes in the initial pH values, initial dye concentrations, temperature and glucose concentration.

*3.2.1. Effect of temperature*

The ability of the selected microorganisms to grow and biodegrade MO was tested by culturing them on MM supplemented with 100 mg $L^{-1}$ of MO under agitation and controlled temperature, 37°C and 44°C. Figure 1 shows the growth (as $OD_{620\ nm}$) and MO degradation (residual MO) by the selected bacterial strains during the incubation at different temperature conditions. Incubation of S3, S4, S6, S7 and S8 strains at 25, 37and 44°C, revealed that these bacterial strains are unable to discolor the MO present in the culture medium at 25 °C while they are capable of degrading at 37 and 44 °C with variable discoloration kinetics.The lowest dyes degradation of 39.4 and 39.09% were observed at temperature of 44°C (after 4 days of incubation), while maximum dye degradation, after 9 days, was about 95.2 and 94.05% at 37°C for strains S3 and S6, respectively. The S4 and S7 strains represented respectively an average dye degradation of 67.97 and 73.4 % (4 days) at 44°C, while maximum degradation of 95.3 and 94.8% for an incubation time of 9 days were observed at temperature of 37°C. The S8 strain revealed a similar degradation pattern of the order of 65% for 3 days for the two temperatures tested (37 and 44°C). Strains reached their maximum growth at 37°C, showing their highest growth (OD 0.387- 0.555)for S3, S4, S6, S7 and S8 at6 to 8days of incubation, respectively. However, the growth of different strains tested is low at 44°C.

It is important to highlight that the greatest degradation and bacterial growth were observed by bacterial strains at 37°C. This performance in the decolorization of MO was explained by the importance of bacterial growth which is directly influenced by the temperature of incubation. These results are consistent with those reported by Pearce et al. [17] while the decline in color removal activity at higher than optimum temperature, can be attributed to decreased cell viability and/or slight denaturation of azoreductase [12, 17].

*3.2.2. Effect of pH*

The effect of pH on decolorization of MO by the selected strains was determined over a range of pH 4.0 to 9.0. S3, S4, S6, S7 and S8 isolates showed the decolorization maximum which exceeds of 90% at pH 7.0 at 37°C during 5-7days of incubation (Figure 2).Following increases from either side of neutral pH, the percentage of decolorization decreased from 95% to 86% for S3, from 95% to 87% for S4, from 94% to 87% for S6, from 94% to 87% for S7 and from 93% to 78% on the alkaline side (pH 9), while steep decline in percent decolorization from 90% to less than 20% on acidic side (pH 4) was found (Figure 2). These results are consistent with those of Pearce et al. [17]; Chan and Kuo 2000 [18] and Shah 2014 [19], who reported that the neutral pH would be more favorable for decolorization of the azo dyes and is suitable for industrial applications.Incubation of the strains in the presence of MO at pH 4, 6, 7 and 9 showed that their growth is influenced by the pH of culture medium. Indeed, the growth was more important at pH 9 and pH 7 whereas at pH 4 and pH 6 growth was very low.

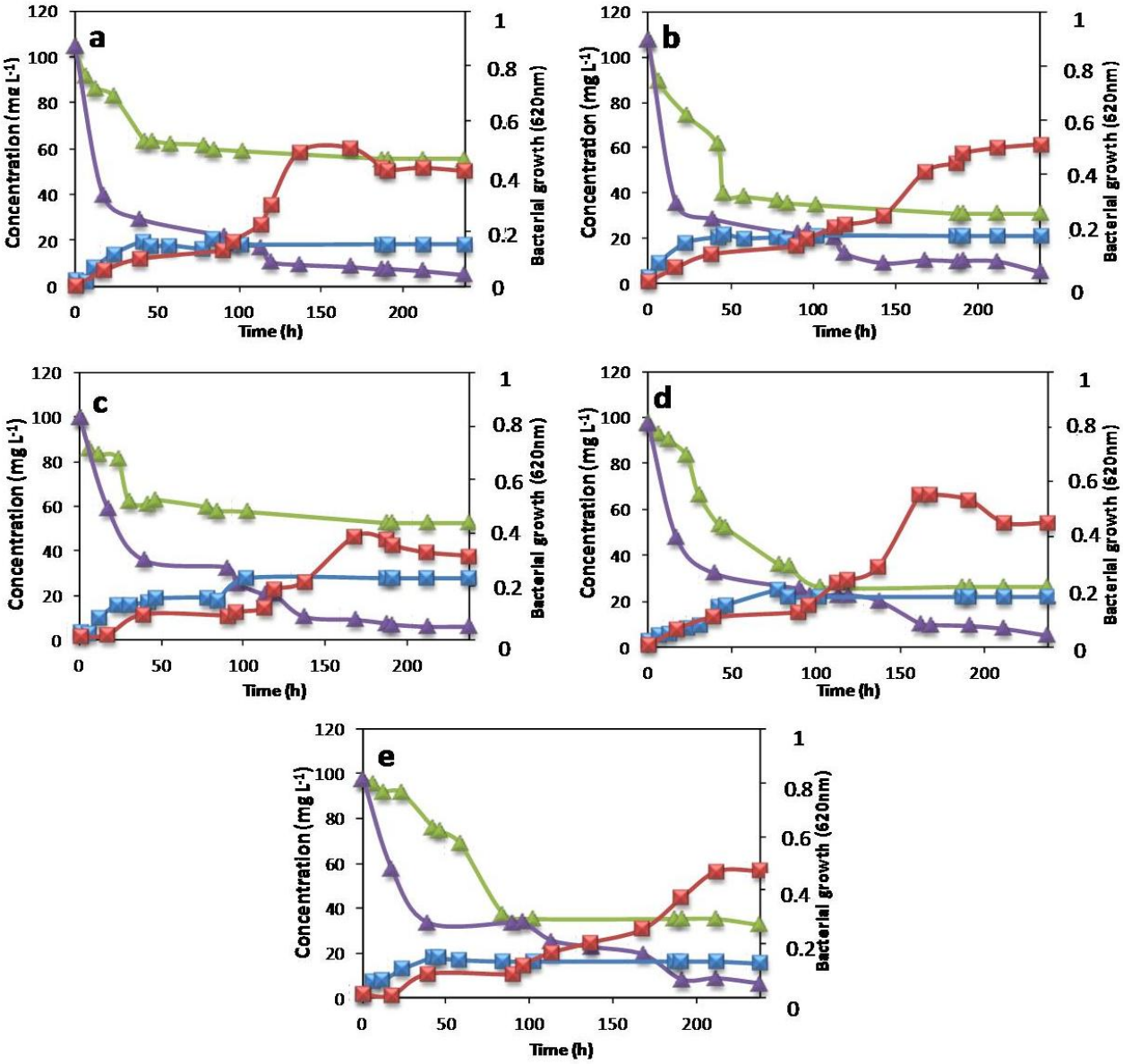



**Figure 1:** Effect of temperature on the bacterial growth ( - ) and remaining concentration of MO ( - ) by S3 (a), S4(b), S6(c), S7(d), S8(e), incubated at 37°C ( - ) and 44 °C ( - ).

*Effect of dye Concentration*

The dye concentration is a critical factor that influences microbial textile dye decolorization [9]. The decolorization performance of MO by the strains S3, S4, S6, S7 and S8 was studied by increasing initial dye concentration (50, 100,150 and 200 mg $L^{-1}$) (Figure 3). In general, the maximum growth (OD 0.387- 0.880) was noted in the samples containing 50 mg and 100 mg dye $L^{-1}$, which was directly correlated with the extent of dye decolorization of MO (more than 90% at 100 mg $L^{-1}$). The growth response was very poor at 150 and 200 mg$L^{-1}$ dye, which corresponded with lower decolorization throughout the incubation period. The results further revealed that the increasing dye concentration (50-200 mg $L^{-1}$) was inhibitory not only for the bacterial growth but also for the extent of dye decolorization, irrespective of incubation time. The inhibitory effect could be attributed to toxicity of dye through blockage of enzyme active sites involved in dye decolorization [20, 21]. Similar to our findings, other researchers also reported inverse relationship between dye concentration and extent of decolorization in simulated MM and the textile effluent [22, 23, 24].

Khehra [25] and Kalme [26] also observed that dye decolorization was strongly inhibited when a high concentration dyestuff was used, and it was due to toxic effect of the dye on the degrading microorganisms.

Maximum dye decolorization was achieved with 100 mg $L^{-1}$ dye, and was therefore selected for further experiments.

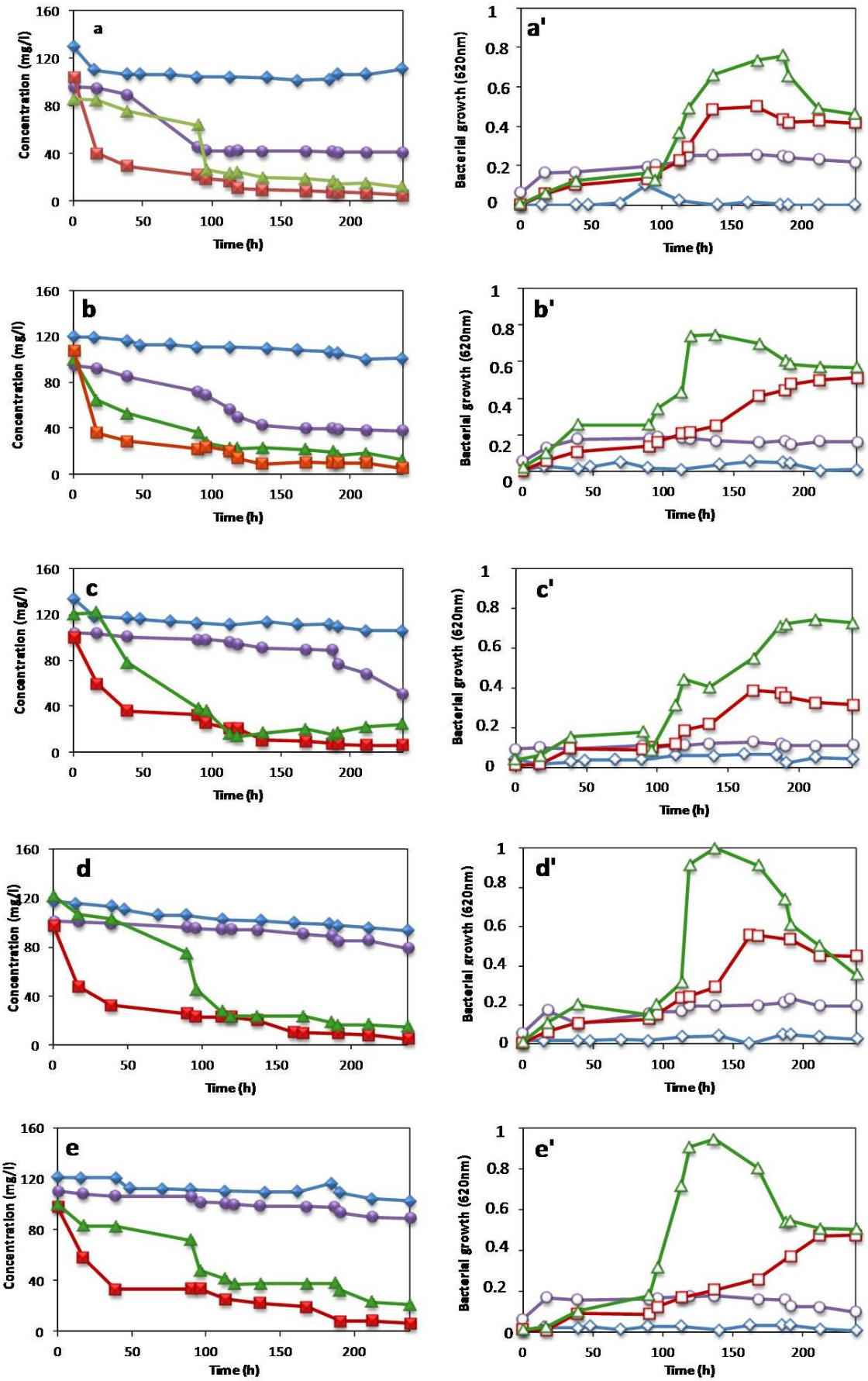



**Figure 2:** Effect of pH on the MO decolorization (a, b, c, d and e) and on the bacterial growth (a', b', c', d' and e') of S3, S4, S6, S7 and S8 respectively in MM at pH 4 ( - ), pH6 ( - ), pH7 ( - ) and pH9 ( - ).

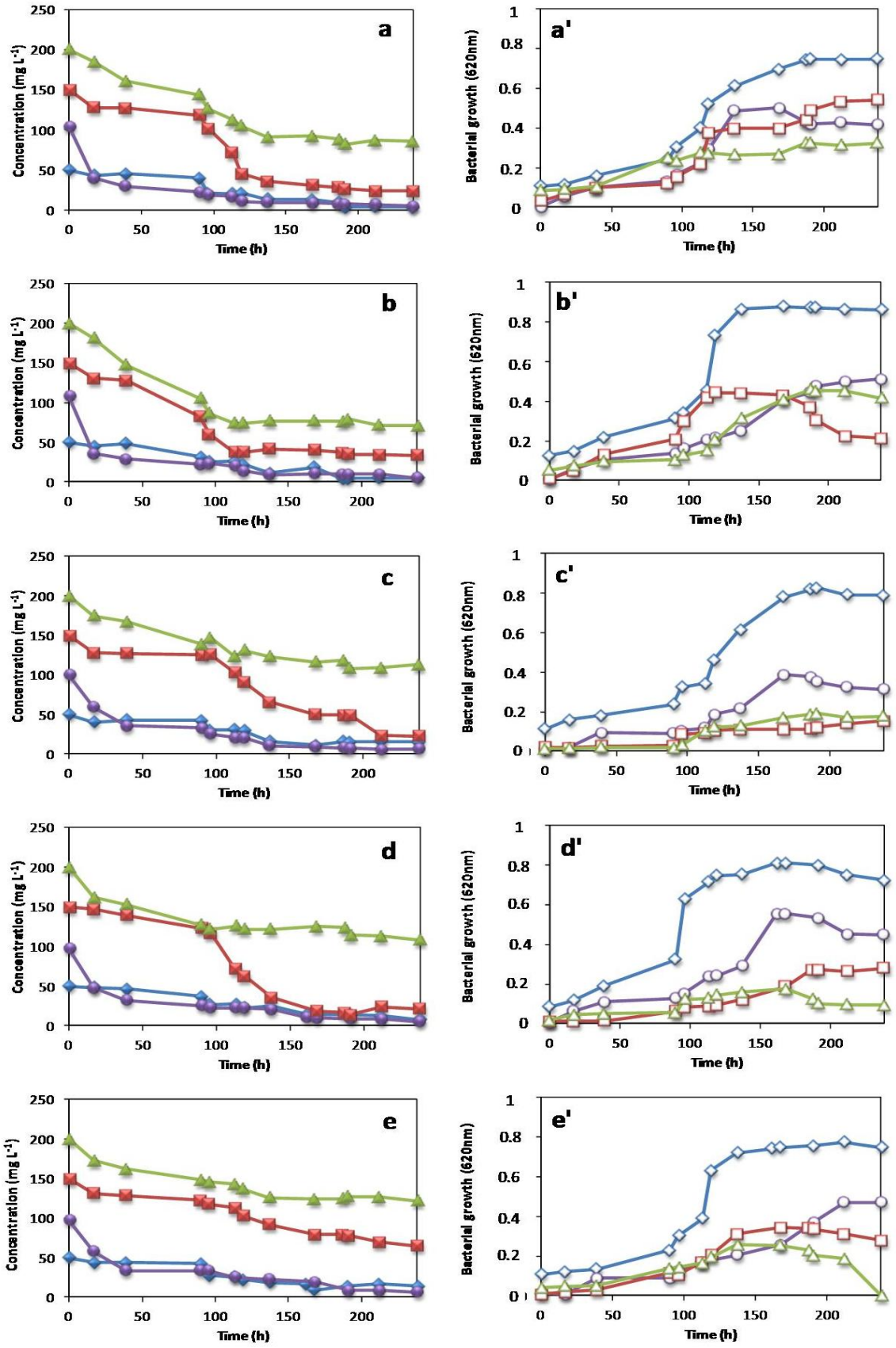



**Figure 3:** Effect of concentration dye, 50 mg $L^{-1}$ ( - ), 100 mg $L^{-1}$ ( - ), 150 $mgL^{-1}$ ( - ) and 200mg $L^{-1}$ ( - ), on the MO decolorization (a, b, c, d and e) and on the bacterial growth (a', b', c', d' and e') of S3, S4, S6, S7 and S8 respectively in MM, pH 7 and 37°C.

*3.2.3. Effect of glucose concentration*

The addition of a carbon source such as glucose at different concentrations has an effect on the percentage of decolorization as showed in Figure 4. Indeed, the percentage of decolorization increases with the increase in concentration of glucose from 0.25 to 1%. As depicted in Figure 4 and 5,both decolorization and bacterial growth are more stimulated with glucose concentration of 1%. This concentration was optimum for growth (OD 0.501; 0.511; 0.387;0.555and 0.472for S3; S4; S6; S7 and S8 respectively) and MO decolorization (90.8; 90.9; 91; 89.5 and 91.3 % for S3; S4; S6; S7 and S8 respectively).Whereas, in lower concentration of glucose (0.25%), the rate of decolorization of MO by S4 and S8 strains was only 40%. For strains S3, S6 and S7, decolorization was slightly low at 0.25% of glucose compared to decolorization at 1%. This can be explained by the very weak growth of bacteria in lower concentration of glucose.Whereas in high concentration of glucose, the bacteria utilized most of the energy generated from the glucose degradation for removal of MO. It should be noted also that in the presence of 1.5% of glucose, bacterial growth and decolorization is slightly slower than in the presence of 1% of glucose. The presence of glucose in excess in the culture medium slows down the bacterial growth [15].

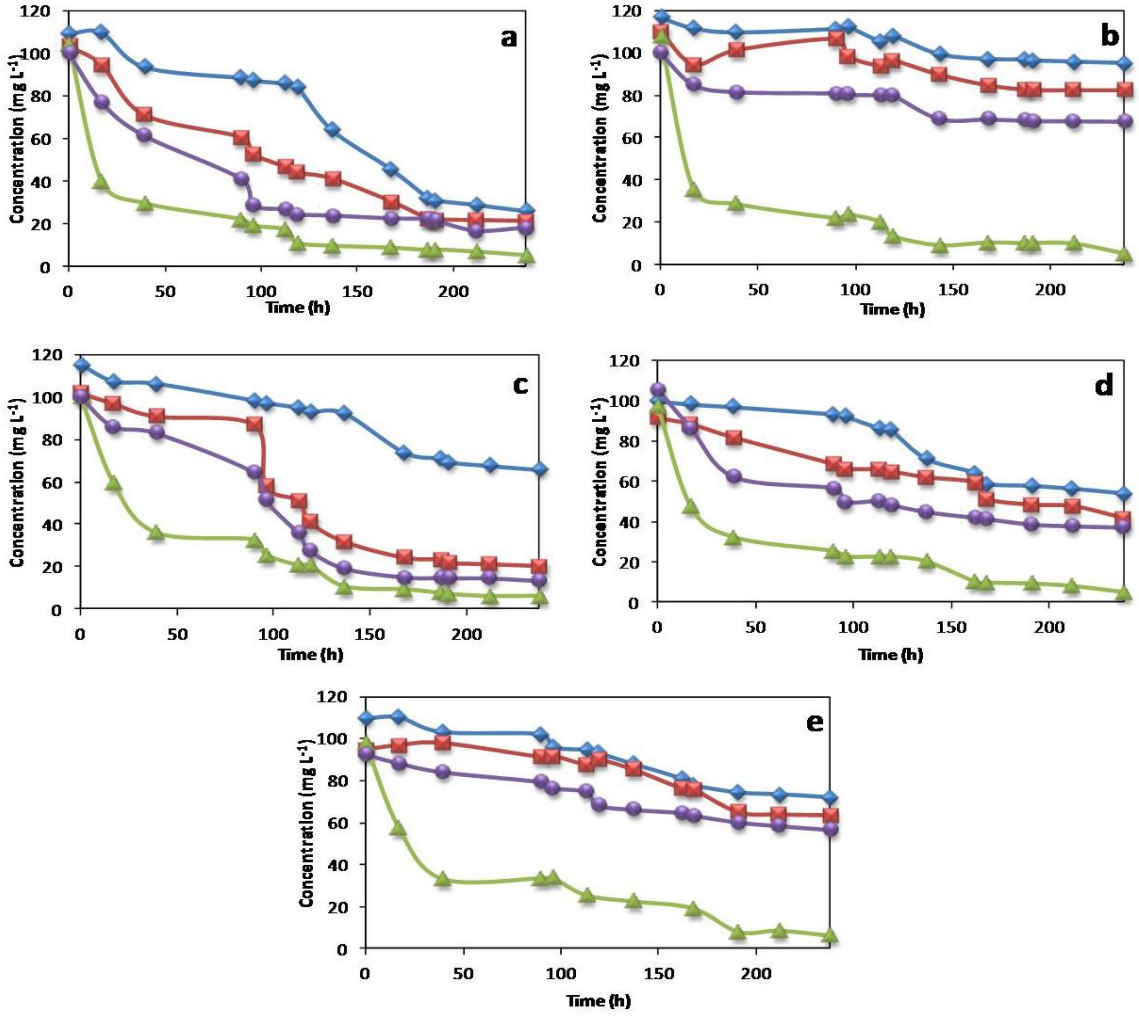



**Figure 4:** Effect of glucose concentration on the MO decolorization (a, b, c, d and e) of S 3, S4, S6, S7 and S8 respectivelyincubated in MM containing 0.25% ( ), 0.5% ( ), 1% ( ) and 1.5% ( ) of glucose.

*Decolorization and Biodegradation Studies*

To disclose the possible mechanism of dye decolorization, we also analyzed the degraded products of MO dye by UV-Vis technique. UV-Vis absorbance of (200-800 nm) of MO in MM showed single peak in visible region at 463 nm (λmax). In present study we investigated the decolorization potential of bacteria towards textile azo dye MO. Bacteria was highly efficient in decolorizing the dye in aerobic and shaking conditions. Figure 6, denotes the absorption spectra of MO before and after its bacterial treatment in visible range. Peak responsible for absorption maxima of parent dye (463nm) almost completely disappeared in the samples obtained after dye decolorization.

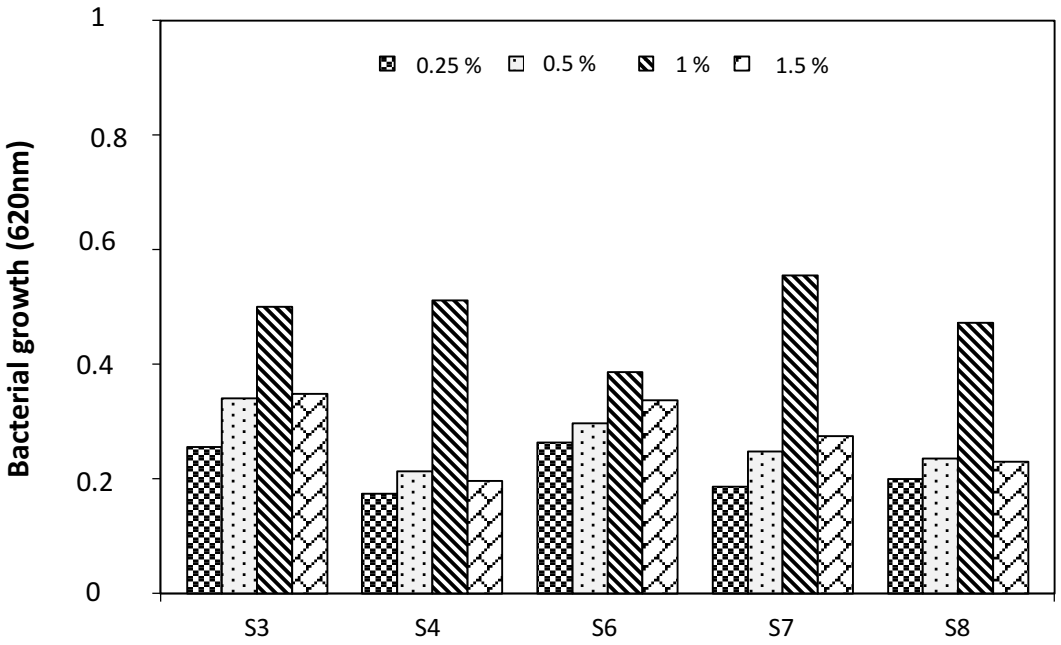



**Figure 5:** Effect of glucose concentration on bacterial growth S3, S4, S6, S7 and S8.

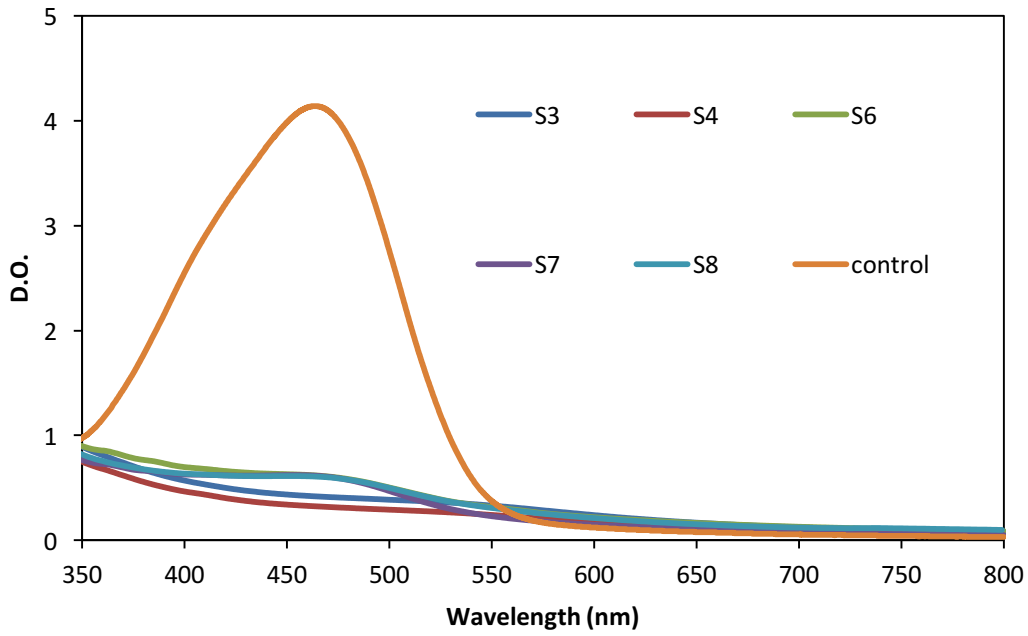



**Figure 6:** UV-vis spectra of methyl orange before (control) and after decolorization by strains (S3, S4, S6, S7 and S8).

According to Asad et al [27], decolorization of dyes by bacteria could be due to adsorption by microbial cells or to biodegradation. In the case of adsorption, the UV-Vis absorption peaks decrease approximately in proportion to each other, whereas in biodegradation, either the major visible light absorbance peak disappears completely, or a new peak appears. The observation of cell mass showed that bacteria strains retained their natural color after decolorization of MO.

## Conclusion

This study reveals that bacterial strains isolated from the local industrial wastewater and sludge are very promising for degradation of textile dyes.The five strains isolated showed more than 90% of biodecolorization of the methyl orange dye effectively under wide range of cultural and nutritional conditions in a synthetic dye

medium. Therefore, under specific range of cultural and nutritional conditions, the strains may be attempted for biodecolorization of Methyl orange containing textile dye effluent.
This work further recommends the identification of strains, purification of enzymes and their kinetics involved in the degradation of dyes by the isolate, and exploitation of potential bacterial consortium from the local industrial wastewater and sludge in the treatment of dye polluted wastewater, which would be cost effective, and can contribute to an effective bioremediation of the dye pollution.

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