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Decolorization of Congo Red dye by bacterial isolates

M. A. M. Abo-State[1,*], *Y. E. Saleh* [2] *and H. A. Hazaa* [2]

[1] Department of Radiation Microbiology, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt.

[2] Department of Botany and Microbiology, Faculty of Science, Cairo University, El- Giza, Egypt.

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Abstract: Congo Red (CR) is one of the azo dyes which is widely used in textile industries and has carcinogenic properties. Large amount of wastewater containing these dyes is discharged from the printing units causing an environmental pollution. So, it is very important to degrade these dyes before discharging it. Several bacterial strains isolated from textile wastewater were tested to study its potential to decolorize CR dye. The decolorization of Congo Red dye by the bacterial strains was observed in various concentrations (25 to 400 mg/L) of CR dye. The most potent bacterial strains were *B. cereus* MAM-B22, *Ochrobactrum* sp. MAM-C9, *Achromobacter xylosoxidans* MAM-29 and *B. cereus* MAM-B11. The maximum decolorization was observed by *B. cereus* MAM-B22 which removed 96.92%, 93.34%, 89.28%, 82.51%, 75.57%, 69.18% and 66.73% of 25, 50, 100, 150, 200, 300 and 400 mg/L respectively after 3 days of incubation at 37°C. When different pH values were used, the maximum decolorization was observed at pH 7.0 which was 90.21%, 90.03%, 89.62% and 85.84% by *Ochrobacterum* sp. MAM-C9, *B. cereus* MAM-B22, *A. xylosoxidans* MAM-29 and *B. cereus* MAM-B11 respectively. The effect of temperature was studied at a range of 25-60°C and the optimum temperature was 37°C. The percentage of decolorization of CR was 92.56%, 91.37%, 89.29% and 88.69% by *B. cereus* MAM-B22, *Ochrobacterum* sp. MAM-C9, *A. xylosoxidans* MAM-29 and *B. cereus* MAM-B11 respectively. The decolorization percentage was bacterial strain, concentration and time dependent.

Keywords: Decolorization, Congo Red, Bacterial strains, Factors, Dye concentrations.

1 Introduction

Dyes are widely used in printing, colour, textile, rubber product, paper and pharmaceutical industries [1]. Synthetic dyes have complex chemical structure so they are highly resistant to degradation [2]**.** Textile industrial effluent contains about 280,000 tons of textile dyes which is discharged every year worldwide [3]**.**

Azo dyes are characterized by the presence of one or more azo bond (-N=N-) and they account for up to 70% of dyestuffs applied in textile processing, due to the ease and cost-effectiveness in their synthesis, stability and availability of variety of colors compared to natural dyes. At least 10 – 15 % of the used dye is discharged into the open streams as effluent causing ecotoxic danger [4-8]**.**

In addition to the mutagenic, carcinogenic and genotoxic properties of the dyes, their presence with intense coloration in aqueous ecosystems reduces sunlight penetration into deep layers which affects photosynthetic activity, deteriorates water quality and also lowers the gas solubility, causing acute toxic effects on aquatic flora and fauna [9-10]. The studies done on *Gambusia affinis*, freshwater fish, revealed the toxicity of dyestuff and wastewater through induced mortality and erythrocyte count [11]. These dyes are resistant to degradation by native microflora, causing their bioaccumulation and biomagnification [12].

Chemical and physical methods such as membrane filtration, ion exchange, adsorption, coagulation, flocculation, irradiation ozonization, precipitation and Fenton's oxidation are usually used for the treatment of dye wastewater [13-14]. The physical and chemical methods have many disadvantages as they are expensive, commercially unattractive and generate complex sludge (secondary pollution), and are not suitable to degrade all dyes [12, 15].

*Corresponding author e-mail: abostatem@yahoo.com

Decolorization and degradation by biological methods have appeared to have many advantages compared to chemical and physical methods as it is an environmentally friendly, cost-competitive, generate less amount of sludge and the operation is simpler and more attractive to degrade all dyes [12, 16-21].

Fungi and algae have been used in dye decolorization using adsorption rather than degradation, as a result, the dye remains in the environment [21-23]. On the other hand, bacteria can degrade and even completely mineralize many dyes under certain conditions and the products of intermediate metabolism during the decolorization process, such as aromatic amines, can be degraded by the enzymes produced by bacteria [16, 24-26].

Bacteria generally are easier to culture and they grow more quickly than fungi. They are able to metabolize chlorinated and other organic contaminants such as oil and mineralize chemicals using them as carbon or energy source [27-29].

A number of bacterial strains including *Shewanella onediensis* MR-1 [30], *Bacillus cereus* MAM-B11, *Bacillus cereus* MAM-B22 [21], *Bacillus* sp. MZS10 [31], *Acinetobacter baumannii* YNWH 226 [32] and others have been reported to decolorize textile dyes efficiently under controlled conditions [21].

Recently, it was found that *Bacillus amyloliquefaciens* degraded Congo Red [8], the newly isolated *Bacillus* sp. MZS-10 was used for decolorizing Azure B dye [33], while azo dye reactive black B was decolorized by *Bacillus cereus* HJ-1 [5]. Another *Bacillus* strain YZU-1 had the ability to decolorize Reactive black B [34].

This study aims to investigate the potential of some bacterial strains isolated from textile wastewater and soil and water contaminated by petroleum oil for decolorizing a solution containing an azo dye, Congo Red. In addition, the effects of various factors affecting the decolorization process were studied.

2 Materials and Methods

2.1 Bacterial strains

The bacterial strains were kindly provided by Dr. Abo-State. Two of these isolates (MAM-B11 and MAM-B22) were isolated from textile wastewater of El-Mahalla El-Kubra textile Company, Egypt. These two strains were identified as *Bacillus cereus* MAM-B11 and *Bacillus cereus* MAM-B22 by 16 S rRNA [21]. The eight bacterial strains were isolated from soil and wastewater polluted with petroleum oil from Cairo Refining Company, Qalyubia, Egypt. One of these isolates (MAM-24) was identified by 16S rRNA as *Bacillus mucilaginosus* with accession No. HQ 013329 [29]. While another one (MAM-29) was identified as *Achromobacter xylosoxidans* with accession No. JN 038055 [28]. However isolate MAM-C9 was identified as *Ochrobactrum* sp. MAM-C9. The other five isolates were MAM-5, MAM-12, MAM-21, MAM-26 and MAM-68. All these isolates were able to degrade chloroaromatic and polycyclic aromatic hydrocarbons (PAHs) and isolated from water and soil contaminated by petroleum oil.

2.2 Dye

Congo Red (CR) ($C_{32}H_{22}N_6O_6S_2Na_2$, C.I. No. 22120) (Fig. 1) was purchased from Sigma-Aldrich Co., 3050 Spruce street, St. Louis, MO 63103 USA.

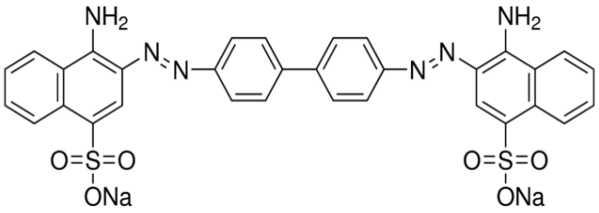

Fig. (1) Chemical structure of Congo Red (λmax (nm) = 492)

2.3 Medium for decolorization of CR

Basal salt medium (BSM) composed of the following ingredients: KH_2PO_4 0.5; K_2HPO_4, 1.5; NaCl, 0.5; $(NH_4)_2PO_4$, 0.5; Na_2SO_4, 3.0; Glucose, 0.5; Yeast extract, 2.0; Calcium chloride, 0.002 and Ferrous Sulphate, 0.002 g/L [35] was used for the decolorization experiments. The pH was adjusted to 7.0.

2.4 Screening of dye degrading microorganisms

Decolorization experiments were conducted in Erlenmeyer flasks (capacity 250 ml) containing 50 ml BSM. The dye (CR) was added at concentration of 100 mg/L. The flasks were inoculated with 5.0 ml (2.0×10^7 CFU/ml) of each strain which represent (10% v/v in BSM). Three replicates were used for each strain. Aliquot (5.0 ml) was withdrawn, centrifuged at 8000 rpm for 10 min to separate the bacterial cells and supernatant. A standard curve for CR at λmax has been determined. The decolorization percentage had been determined after three days of incubation at 37°C.

2.5 Factors affecting decolorization of CR

2.5.1 Decolorization at different concentration of CR and incubation periods

The decolorization of CR was tested at different concentrations (25, 50, 100, 150, 200, 300 and 400 mg/L). The dye was added to 250 ml Erlenmeyer flasks containing 50 ml of BSM. The flask containing dye was inoculated with (10% v/v) of bacterial strain suspension. The flasks

were incubated at 37°C on shaking incubator (150 rpm). The decolorization percentage had been determined after 3, 7 and 14 days of incubation.

2.5.2 Effect of pH

The decolorization studies of CR (100 mg/L) were carried out at pH values 2, 3, 4, 5, 6, 7 and 8 by adjusting pH of the medium using McIlvaine buffer for acidic pH and phosphate buffer for alkaline pH. The dye was added to 250 ml Erlenmeyer flasks containing 50 ml of BSM and The flask containing dye was inoculated with (10% v/v) of bacterial strain suspension. The decolorization was determined after 3 days of incubation at 37°C on shaking incubator (150 rpm).

2.5.3 Effect of temperature

The decolorization of CR (100 mg/L) was tested at different temperatures 25, 30, 33, 37, 40, 50 and 60°C by inoculating BSM with 5.0 ml (2.0×10^7 CFU/ml) which represent (10% v/v) of each bacterium. The decolorization was determined after 3 days of incubation.

2.6 Decolorization assay

According to [36] and [37] the decolorization percentage was calculated by measuring the absorbance of the supernatant at the respective wavelength by using Spectrophotometer (LW-V-200 RS, UV/VIS, Germany).

The following equation was used to calculate the decolorization percentage

$$\% \text{ Decolorization} = (A_0 - A)/A_0 \times 100$$

A_0= the initial absorbance of dye concentration (Control)

A= the absorbance of the residual of dye that was treated with bacterial cells (Supernatants).

2.7. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons test. Readings were considered significant when P was ≤ 0.05.

3 Results

Total 30 bacterial isolates were screened for Congo red dye decolorization. Among all isolates, the best 10 strains were chosen to study the decolorization ratio and the other factors affecting CR decolorization.

The decolorizing activity of the best 10 isolates was studied using CR at different initial concentrations varying from 25 to 400 mg/L. All the ten bacterial strains used in the present study having the potential of decolorizing CR but with variation in their capacities. Decolorization of CR after different incubation periods also have been determined. The maximum decolorization was observed at low concentrations and the rate of the decolorization decreases with increasing dye concentration as indicated in Fig. (2-11). This means that the decolorization was concentration dependent.

The decolorization percentage of *Bacillus cereus* MAM-B11 was 93.30±0.90% of 25 mg/L and 53.29±0.16% of 400 mg/L of CR after 3 days of incubation at 37°C, while it was 97.00±0.90% of 25 mg/L and 62.44±0.08% of 400 mg/L after 14 days as indicated in Fig. (2).

Fig. (3) showed that the highest removal of CR dye was observed at 25 mg/L concentration, pH 7 and temperature 37°C with the inoculation of strain *Bacillus cereus* MAM-B22 which removed 96.92±1.62% after 3 days and 98.33±0.32% after 14 days, and the removal decreased to 66.73±0.20% at 400 mg/L of CR after 3 days of inoculation.

The *Ochrobactrum* sp. MAM-C9 removed CR dye at a range from 62.08±0.56% to 93.62±0.69% of 25 mg/L to 400 mg/L after 3 days of incubation at 37°C as indicated in Fig. (4). These results were close to that were observed by *Achromobacter xylosoxidans* MAM-29 which removed 94.62±1.45% of 25 mg/L and 58.51±0.69% of 400 mg/L after 3 days of incubation at 37°C (Fig. 5).

Fig. (6) showed that the decolorization percentage of MAM-26 was 89.25±0.92% after 3 days, 91.62±1.51% after 7 days and 92.75±0.74% after 14 days with the concentration of 25 mg/L. When a concentration of 400 mg/L was used, the percentage of dye decolorization was dropped to 50.34±1.08%, 52.63±0.22% and 53.82±2.33% after 3, 7 and 14 days respectively.

The bacterial strain MAM-12 removed more than 80% of CR dye till the concentration of 100 mg/L after 3 days, but when the concentration increased, the decolorization percentage decreased to 49.22±0.19% at 400 mg/L after 3 days as indicated in Fig. (7).

The bacterial strain MAM-21 removed CR dye at a range from 50.44±0.19% to 86.63±2.22% of 25 mg/L to 400 mg/L after 3 days of incubation at 37°C as indicated in Fig. (8). While the strain *Bacillus mucilaginosus* MAM-24 removed the dye at a range from 50.62±0.22% to 87.33±0.33% of 25 mg/L to 400 mg/L after 3 days of incubation at 37°C as indicated in Fig. (9).

The decolorization percentage of MAM-68 was 88.62±1.33% of 25 mg/L and 38.12±0.62% of 400 mg/L of CR after 3 days of incubation at 37°C, while it was 93.62±0.53% of 25 mg/L and 42.22±0.22% of 400 mg/L after 14 days as indicated in Fig. (10).

The least activity was observed by the strain MAM-5 which removed 85.62±2.22% of 25 mg/L and the removal decreased to 33.81±0.54% at 400 mg/L after 3 days of

incubation as indicated in Fig. (11).

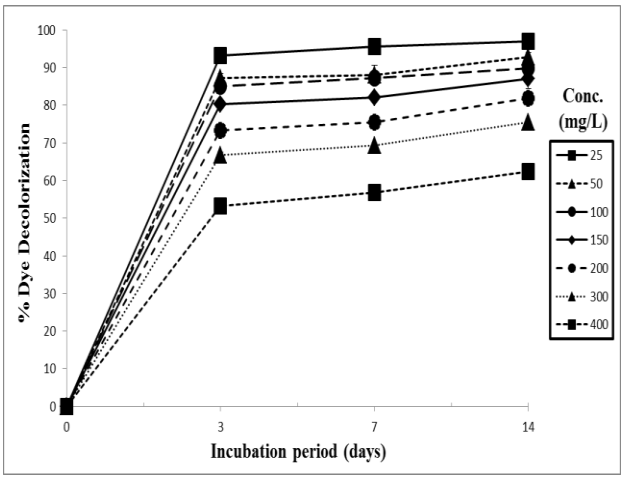

Fig. (2): Decolorization of CR by the bacterial strain *B.* cereus MAM-B11 at different concentraions after different incubation periods

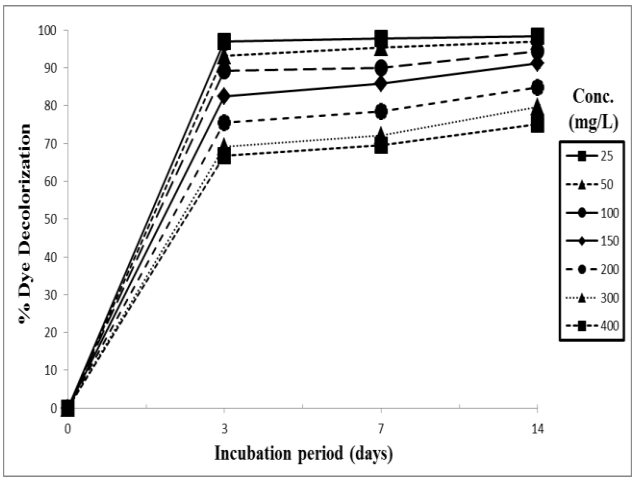

Fig. (3): Decolorization of CR by the bacterial strain *B.* cereus MAM-B22 at different concentraions after different incubation periods

The results revealed that decolorization was also strain dependent *i.e.* decolorization percentage depends on bacterial strain. The most potent strains for decolorization of Congo Red dye were *B. cereus* MAM-B11, *B. cereus* MAM-B22, *Ochrobacterum* sp. MAM-C9 and *Achromobacter xylosoxidans* MAM-29.

At pH 7.0, 90.21±0.66% CR dye removal was observed by *Ochrobacterum* sp. MAM-C9 followed by 90.03±0.22%, 89.62±0.25% and 85.84±0.69% by strains *B. cereus* MAM-B22, *Achromobacter xylosoxidans* MAM-29 and *B. cereus* MAM-B11 respectively. At pH 6, the decolorization of dye was 87.36±0.55% by strain *Ochrobacterum* sp. MAM-C9 and at pH 8; the decolorization was 87.07±1.26% by the same strain, which give an indication that the maximum decolorization was observed in neutral pH as indicated in Fig. (12).

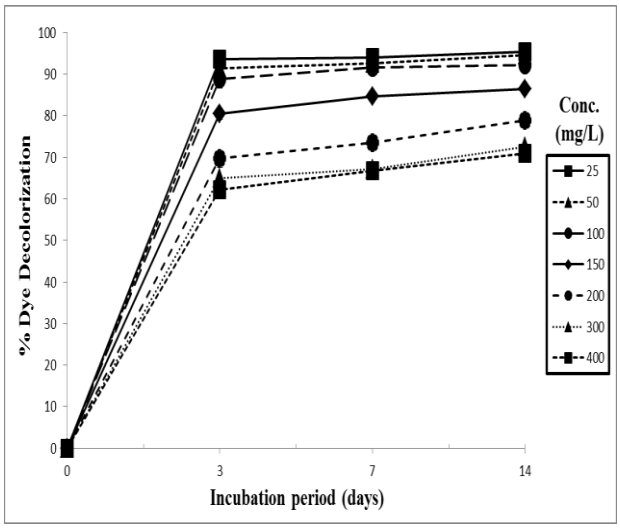

Fig. (4): Decolorization of CR by the bacterial strain *Ochrobacterum* MAM-C9 at different concentraions after different incubation periods

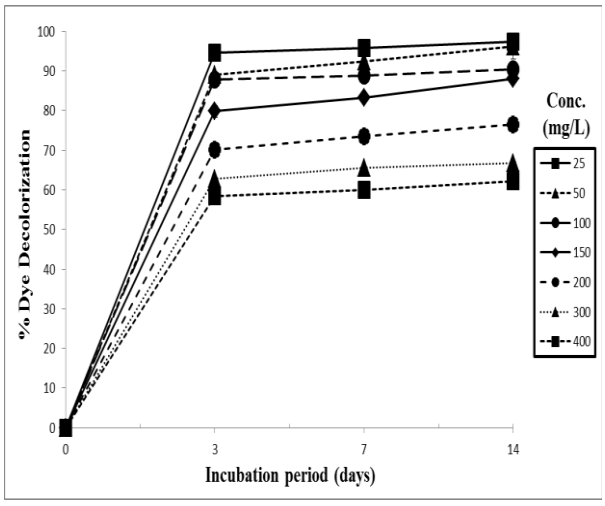

Fig. (5): Decolorization of CR by the bacterial strain *A. xylosoxidans* MAM-29 at different concentraions after different incubation periods

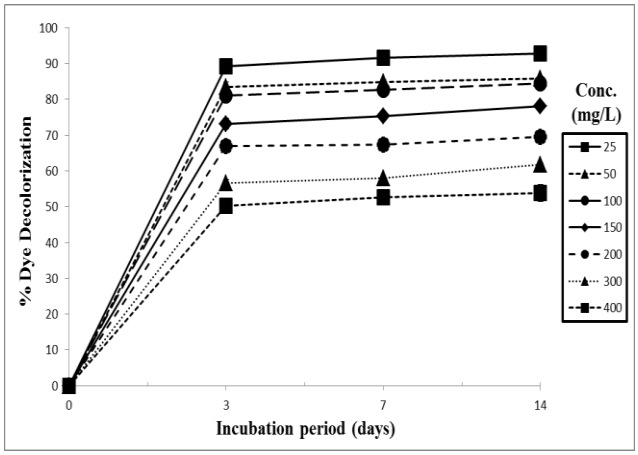

Fig. (6): Decolorization of CR by the bacterial strain MAM-26 at different concentraions after different incubation periods

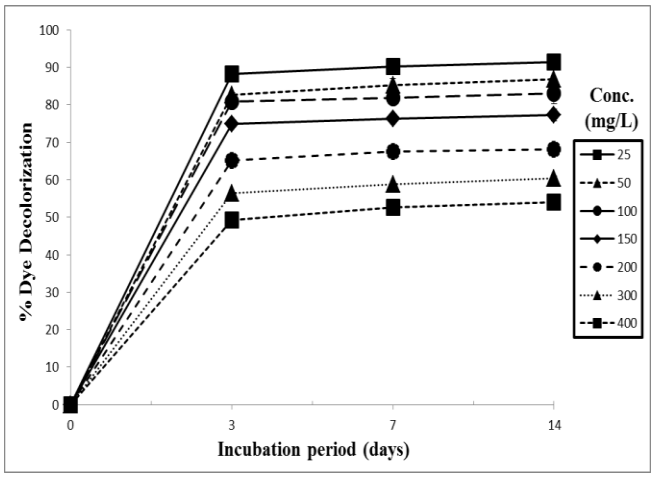

Fig. (7): Decolorization of CR by the bacterial strain MAM-12 at different concentraions after different incubation periods

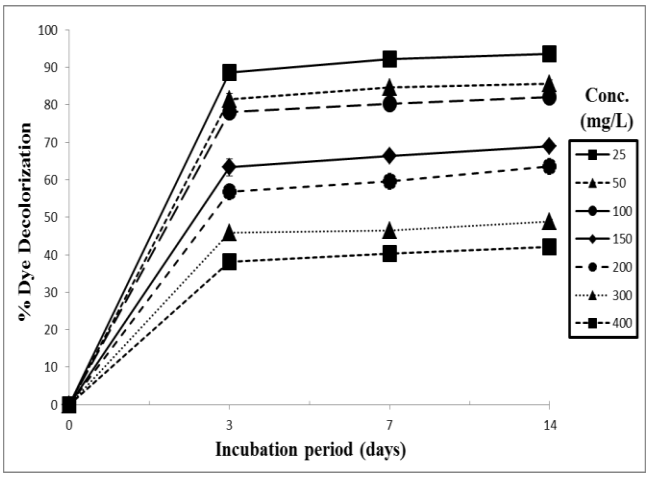

Fig. (10): Decolorization of CR by the bacterial strain MAM-68 at different concentraions after different incubation periods

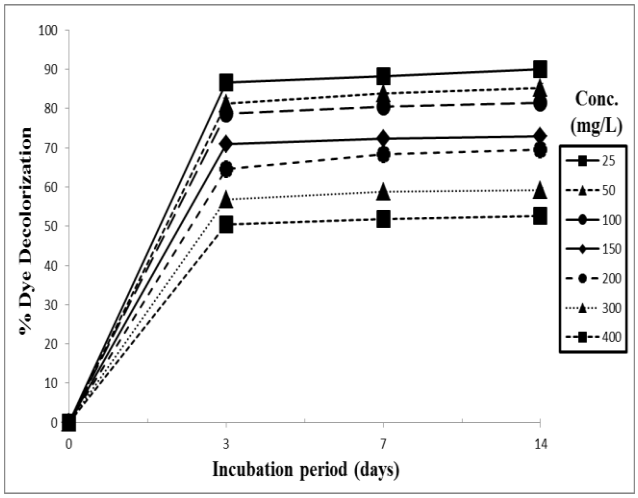

Fig. (8): Decolorization of CR by the bacterial strain MAM-21 at different concentraions after different incubation periods

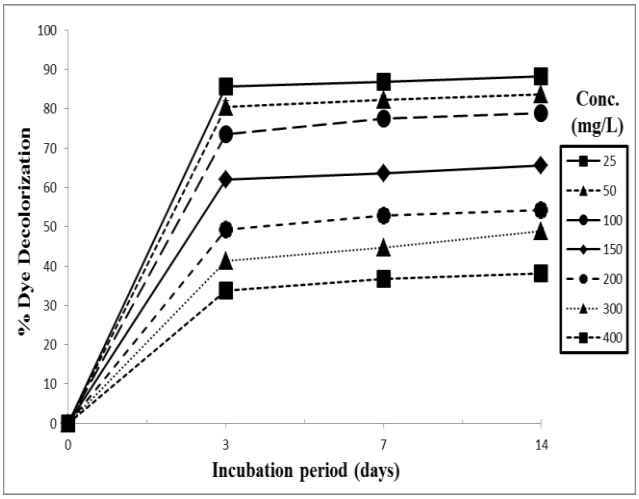

Fig. (11): Decolorization of CR by the bacterial strain MAM-5 at different concentraions after different incubation periods

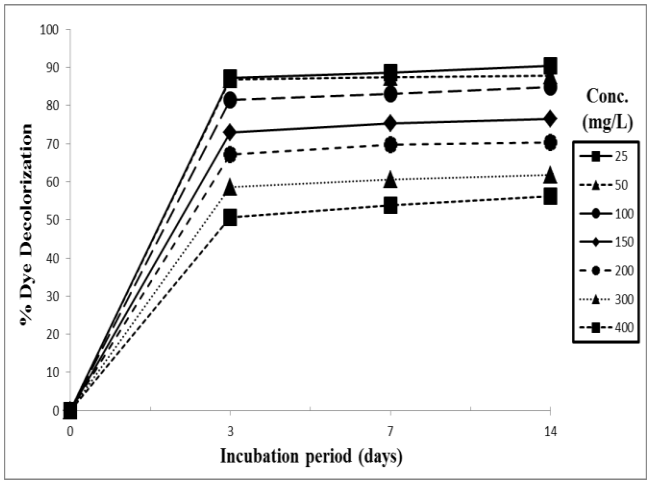

Fig. (9): Decolorization of CR by the bacterial strain *B. mucilaginosus* MAM-24 at different concentraions after different incubation periods

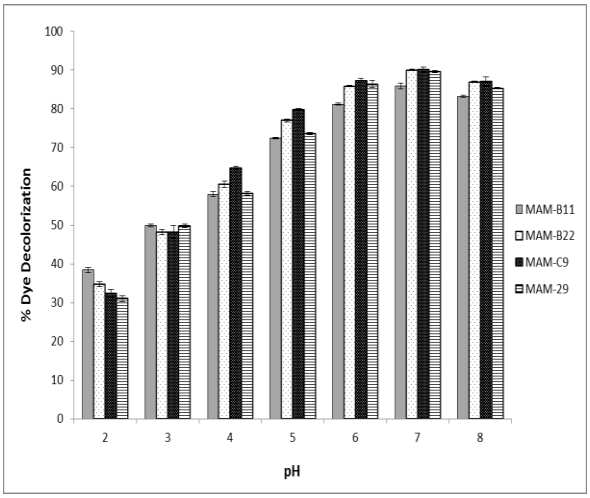

Fig. (12): Effect of pH on CR decolorization (100 mg/L) by the most potent bacterial strains

The effect of temperature was studied at a range of 25-60°C. The temperature 37°C showed maximum decolorization of CR dye that was 88.69±0.60%, 92.56±1.49%, 91.37±2.08% and 89.29±0.60% with the strains *B. cereus* MAM-B11, *B. cereus* MAM-B22, *Ochrobacterum* sp. MAM-C9 and *Achromobacter xylosoxidans* MAM-29 respectively as shown in Fig. (13).

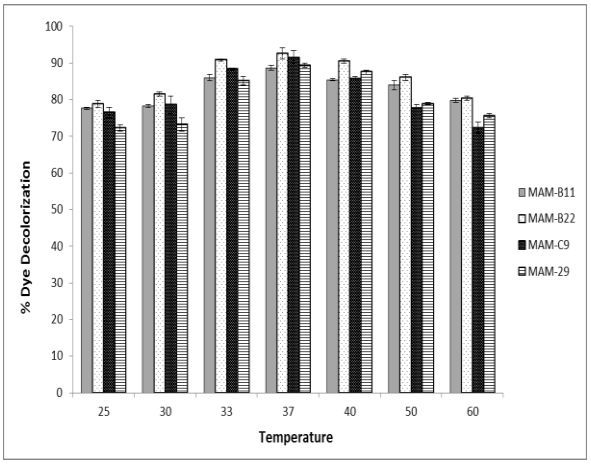

Fig. (13): Effect of temperature on CR decolorization (100 mg/L) by the most potent bacterial strains

4 Discussion

It was observed that the rate of decolorization decreased with increasing dye concentration, because of the toxicity of the dye to the cells and/or higher dye concentrations need more biomass concentration. A previous study of [38] was similar with these findings, when the dye concentration increased, the decolorization efficiency was decreased and the time required for final decolorization was longer.

[21] isolated and identified two bacterial strains from textile wastewater I and II. The two strains were identified by 16 S rRNA as *B. cereus* MAM-B11 and *B. cereus* MAM-B22. These two strains were the most potent strains in decolorization of seven textile dyes which used in printing and dyeing in El-Mahalla El-Kubra textile factories, Egypt. MAM-B11 and MAM-B22 were able to decolorize Isma Fast Red (IF), Drimarene Blue (DB) , Dycrofix Red (DR), Fantacell Olive (FO), Jakazol Black (JB), Jakofix Yellow (JY) and Dycrofix Violet (DV) efficiently at a concentration ranges (25 to 400 mg/L) for each dye. The strain MAM-B11 decolored 95.33% of IF, 37.69 of DR, 68.4% of FO, 84.64% of JB, 71.26% of JY and 84.76% of DV, while the strain MAM-B22 decolored 93.3% of IF, 40.65% of DR, 73.4% of FO, 85.93% of JB, 42.11% of JY and 40.29% of DV with concentration of 200 mg/L after 48 h. The strains MAM-B11 and MAM-B22 decolored 71.46% and 61.57% respectively of 200 mg/L of DB after 6 days.

The white rot fungus, *Pleurotus sajor-caju* removed 98.0% of 25 mg/L methelene Blue (MB) after 7 days and 32.0% of 500 mg/L MB after the same period. *P. sajor-caju* could remove more than 92.0% of MB till concentration 150 mg/L, while it could remove 100% of maxilon (Max.) up to 200 mg/L after 7 days [39].

Another white rot fungus, *phanerochaete chrysosporium* removed 88.4 % to 97.2 % of 25 mg/L to 700 mg/L of MB dye. However, *Pleurotus ostreatus* removed 100 % of Max dye till 150 mg/L after 10 days and after 7 days for *P. sajor-caju*. Free enzymes of *P. sajor-caju* were able to decolorize MB and Max completely after 12 h. till 200 mg/L of dyes. Immobilized enzymes of *P. sajor-caju* removed a range of 20 – 46 % of 25 – 75 mg/L Max dye after 2 h. [40].

[32] reported that the bacterial isolate *Acinetobacter baumannii* YNWH 226 had an ability to decolorizede and degrade CR dye, as it showed removal of 98.62% and 96.31% of dye with concentration of 100 mg/L and 200 mg/L respectively after 48 h. and when concentration of 500 mg/L of dye was used, only 80.73% decolorization was observed after 48 h.

The pH and temperature were considered to be important factors during the process of biological decolorization. Many observations were reported that the maximum decolorization was shown around pH 7 and temperature around 37°C.

[1] reported that bacterial consortium showed 98% decolorization at 100 mg/L concentration of Congo Red dye at pH 7 and temperature 37°C.

[30] reported that the optimum temperature for Congo Red decolorization by *Shewanella onediensis* MR-1 was 35°C. It was found that the decolorization percentage increased from 85.7% to 95.7% when the temperature increased from 28°C to 35°C, but with further increased in the temperature to 40°C, the decolorization percentage decreased to 84.5%.

[41] found that the temperature 36°C enhanced the growth of the bacterial consortium used in the study and showed maximum decolorization of mixed dyes (DB 151 and DR 31) that was 87.49% after 5 days.

[42] reported that the microbial community exhibits the optimal degradation efficiency with a pH of 5.0 - 7.0 and a temperature range of 30-35°C.

Citrobacter sp. CK3 achieved the best decolorization of reactive red 180 (96%) at pH 6.0 – 7.0 after 48 h and show strong decolorizing activity at temperature from 27°C to 37°C [34].

Bacillus sp. showed 90% decolorization of crystal violet after 2.5 h at pH 7.0 and 92% decolorization after 3 h at temperature 30°C [43], while *B. subtilis* HM cells was found to have desirable characteristic; removing Fast Red color over a wide range of pH´s (5.0 – 9.0) with optimum at pH 7.0 [44].

5 Conclusions

The present study showed the ability of several bacterial strains to decolorize Congo Red dye. The results showed that the decolorization depends on the bacterial strains, dye concentration, pH and temperature. Over the range of 25-60°C the optimum temperature was 37°C. The decolorization was the maximum at pH 7.0. High decolorization efficiency was observed after 3 days. As the concentration of dye increased, the decolorization percentage decreased for all the studied bacterial strains. The most efficient bacterial strain was *B. cereus* MAM-B22.

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