Decolorization of textile dye direct yellow 12 using bacteria isolated from soil contaminated with textile industry effluents

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Abstract

This investigation was taken up to study the decolorization of the textile dye, Direct Yellow 12 by making use of free and immobilized bacterial cells isolated from soil contaminated with textile industry effluents. A total of 12 isolates capable of decolorizing Direct yellow 12 were obtained. With screening, 2 isolates namely DY9 and DY 10 were selected for further decolorization studies of the dye. Biochemical characterization of both the isolates was carried and both were tentatively identified as Bacillus species. Optimization of decolorization the dye with respect to various parameters was carried out with one factor at a time approach. The optimum pH for both cultures was found to be 9. A temperature of 37ºC, a Shaking speed of 150 rpm and Bushnell Haas medium supplemented with 100 mg/L Starch and a Dye concentration of 2% were optimum for both cultures. Optimum decolorization with DY9 was obtained with 100 mg/L KNO_3 and that with DY10 was with 100 mg/L Yeast extract. A comparative study on the decolorization of Direct Yellow 12 under unoptimized and optimized conditions using both isolates was carried out. The results showed a marked increase in decolorization with both isolates under optimized conditions. The two cultures, individually and as a consortium were immobilized in Calcium alginate. Batch decolorization of the dye using free and immobilized cultures of DY9 and DY10 was carried out. The immobilized cultures showed an increase in decolorization compared to free cells. At a lower

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dye concentration, the immobilized consortium showed a higher decolorization. However, with increase in concentration of the dye, individual immobilized cultures proved better for decolorization. Repeated batch decolorization was carried out to check the stability of the calcium alginate beads. The current investigation showed that free and immobilized cells of both the isolates could be used in the water and soil bioremediation.

Keywords: Decolorization, Direct Yellow 12, Optimization, Free cells, Immobilization, Calcium alginate.

1 INTRODUCTION

Textile dyes have a strong demand worldwide due to the emerging economics. Based on the Compound Annual Growth Rate (CAGR) with 4.6% approximately during the forecast period indicates the high growth rate in textile dye manufacturing. According to Neha D et al, 2018, the textile industry utilizes large amounts of potable water and also causes environmental problem. According to Nikolaos Voulvoulis, 2018, dyes are stable and can remain in the environment for a long time if not treated adequately. Therefore, this effluent must be treated before discharge into natural water streams. According to Azmi et al., 1998; Moreria et al., 2000; Rajesh Kannan et al., 2011, the processes used to treat wastewater from textile industries could be chemical, biological and physical. Krull et al., 1998 and Verma and Madamwar, 2003 stated that physical and chemical methods are mostly expensive and ineffective. These methods generate produce high sludge, side reactions, and by-products, which are not suitable for decolorization of all dyes.

Biodecolorization of textile dyes has been studies by researchers using Bacteria like Serratia marcescens (15), Micrococcus luteus, Listeria denitrificans and Nocardia atlantica (12).

Biodecolorization of textile dyes has also been reported by immobilized cells of Alcaligenes latus (Usha MS et al., 2010).

2 MATERIALS AND METHODOLOGY

2.1 Dye and other chemicals

Commercial-grade dye Direct Yellow 12, were procured from a textile industry in Bangalore, India.

Absorbance measurements were performed using Colorimetric analysis. Wave length, C.I. name and class of the used textiles dyes are listed in the table.

TABLE 1

Classification of Direct Yellow G based on its characteristics

Dyes	Wave Length	Class
Crysophenine G (Direct Yellow)	540nm	Azo Dyes

The other solvents and chemicals utilized in the current investigation were procured from Himedia Laboratories Pvt. Ltd., Mumbai, and Maharashtra, India. These chemicals were of analytical grade.

Bushnell Hass agar/broth which is a mineral salt medium was used in this investigation. This medium contains Potassium dihydrogen phosphate (1g), Potassium hydrogen phosphate (1g), Magnesium sulfate (0.2g), Calcium chloride (0.02g), Ammonium chloride (1g), Ammonium nitrate (1g), Sodium chloride (0.1g), Ferric chloride (0.05g), Glucose (4g), Yeast extract (4g), Dye (0.1g/mL), Agar (25g), Distilled water (1000mL) and pH was adjusted to 6.8.

2.2 Isolation and screening of bacteria involved in dye decolorization

A total of 7 samples were collected in sterile polythene bags from 7 textile industry effluent contaminated soils in and around Bangalore. Samples were refrigerated at 4°C until further use.

For isolating bacteria capable of decolorizing Direct Yellow 12, One gram of soil sample was inoculated into 100 ml of Bushnell Hass medium with 1% glucose and by dispensing Direct Yellow 12 (azo dye).

The Broth Media was incubated for 3 days aerobically at 37°C under static condition with intermittent addition of 1% dye for enrichment of cultures as first round. Bacteria capable of decolorizing dye were isolated and pure cultures were obtained on mineral salts agar medium. Pure cultures of dye decolorizing bacteria were maintained further on respective agar media with 1% dye.

Loopful of culture from culture media was added to 100 mL of fresh Bushnell Hass medium with 1% Glucose as the sole source of carbon for screening dye decolorizing bacteria. The inoculated flasks were incubated in static condition at 37°C till the cultures reach log phase. Five ml ($4x10^2$ CFU/ml) of inoculum from each flask was added to 100 ml of fresh mineral salts medium with pH 7 containing 2% dye and the flasks were stored under static conditions at 37°C. To monitor the growth of cultures optimal density (OD) under 540 nm wavelength was observed every 24 hours. Culture showed higher optimal density by the end of 7 days at 540nm, therefore selected for further studies.

Loopful of the screened cultures were inoculated onto mineral salts agar medium containing 2% dye. The isolates showing a maximum growth with zone formation indicating the decolourization of the dye were selected for further screening. Totally 12 isolates were obtained from Direct Yellow 12, and 2 isolates DY9 and DY10 were selected based on decolorization percentage that are very specific and clear.

The formula used for the calculation :

Percentage decolorization = [(Initial Concentration-Final Concentration)/Initial concentration] x 100

The concentration of the dye was determined by finding the absorbance of the solution at 540 nm using a UV-visible spectrophotometer.

2.3 Taxonomic identification of bacteria

Preliminary identification of the cultures showing higher OD values and zone formation on agar media was done by Gram's staining. The isolates DY-9, DY-10 of Direct Yellow 12 show highest decolorization rate based on spectrophotometric analysis. The species level identification was performed by Gram staining and biochemical tests following Bergey's Manual of Systematic Bacteriology (Krieg and Staley, 2010). Quadrant streaking was done on the Nutrient Agar plates from respective plates of Bushnell Hass broth and Agar.

2.4 Optimization of process parameters using one-factor-at-a-time experiment

Optimization of decolorization of Direct Yellow 12 was carried out based on one-factor-at-a-time by various parameters for 24 hours and both DY9 and DY10 decolorization efficiency was monitored for 7 days.

2.4.1 *Effect of pH*

Decolorization studies were carried out at different pH ranging from 3 to 11 with an interval of 2. The pH of the medium was adjusted by adding 0.1 N Hydrochloric acid or 0.1N Sodium hydroxide. The flasks were kept in static conditions at 37°C with the respective isolates of the dye and dye concentration of 2%.

2.4.2 *Effect of temperature*

Decolorization studies were carried out at different temperatures ranging from 5°C to37°C with an interval of 5°C-7°C. The different temperatures were maintained by using bacteriological incubator. The pH of the medium was maintained at 7 with a loop full of selected isolates and with the dye concentration of (2%). The flasks were incubated under static condition.

2.4.3 *Effect of dye concentration*

Decolorization studies were carried out by adding different dye concentrations ranging from 2% to 8% with an interval of 2%. The remaining parameters like pH (7.0), cultures inoculated, temperature (37°C) and incubation condition (static) were kept constant.

2.4.4 *Effect of shaking speed*

To determine the effect of aeration or shaking on dye decolorization, the experimental flasks were incubated in an incubator shaker at different shaking speed ranging from 50 rpm to 200 rpm with an interval of 50 rpm. The other parameters like pH (7.0), temperature (30°C), dye concentration (2%) and culture inoculated were constant.

2.4.5 *Effect of carbon sources*

To study the effect of additional carbon sources on dye decolorization, starch, sucrose, glucose and maltose were used as carbon sources at 100 mg/L concentration. The other parameters like pH (7.0), temperature (30°C), dye concentration (2%), culture inoculation and incubation condition (static) were constant.

2.4.6 *Effect of nitrogen sources*

Influence of additional sources of nitrogen on dye decolorization was determined by adding potassium nitrate, peptone, sodium nitrate and yeast extract at 100 mg/L concentration. The other parameters like pH (7.0), temperature (30°C), dye concentration (2%), culture inoculation and incubation condition (static) were constant.

2.5 Studies on the efficiency of immobilized bacteria in Direct Yellow 12 decolorization

Efficiencies of immobilized cells were studied under batch, repeated batch.

2.5.1 *Immobilization in Ca-alginate*

The cultures of DY-9, 10 and Red 10B-6, 12 were entrapped in Ca-alginate based on the method proposed by Bettman and Rehm (1984).Distilled water was used to dissolve sodium alginate, 3% w/v. This mixture was then autoclaved.

3% w/v of fresh pellets of the bacterial cultures, DY-9, 10 and equal quantities of these two cultures as a consortium was added to sterile sodium alginate solution. Using a syringe, this mixture of the cultures and sodium alginate was added drop wise into a previously sterilized

0.2 M cold Calcium chloridesolution. Calcium alginate gel beads were thus obtained. These were approximately 2 mm in diameter. Into a fresh 0.2 M solution of Calcium chloride, the beads were suspended for hardening. This process was carried out with gentle agitation for 2 hours. The washing of beads was carried out with sterile distilled water followed by storage in a solution of 0.2 M Calcium chloride until further use at 4°C

2.6 Batch decolorization of Direct Yellow

Batch decolorization of Direct yellow 12 was performed with free cells and cells of DY-9 and DY-10 entrapped in Calcium-alginate.

2.6.1 *Batch decolorization using free cells*

Batch decolorization of Direct yellow with free cells of Dy-9, 10 was carried out using 100 ml of mineral salts medium with pH 7, 150 mg/L of glucose and 150 mg/L of yeast extract. Flasks were incubated at 37°C for 24 hours at 150 rpm shaking speed. Decolorization efficiency of Dy-9,10 was checked at varying concentration of direct yellow ranging from 2% to 8%.

2.6.2 *Repeated batch decolorization of Direct yellow*

To observe long term stability of the beads, repeated batch decolorization of Direct yellow by respective cultures immobilized in Calcium-alginate was performed. Each cycle of incubation of decolorization was carried out at 37°C, with a shaking speed of 150 rpm for a duration of 24 hours. Decantation of the spent medium was carried out after every cycle. Sterile distilled water was used to wash the beads. Minimal mineral salts medium (Manohar and Karegoudar, 1998),with a dye concentration of 2% was freshly prepared and sterilized. The beads were then transferred to this freshly prepared medium. The quantity of Direct yellow in media was estimated by spectrophotometric analysis after every cycle. Cell leakage was monitored after an

interval of 5 cycles. One mL of spent medium was inoculated on plates containing nutrient agar and CFU/mL was checked.

2.6.3 *Decolorization of individual and combination of Direct yellow by individual cultures and consortium*

Decolorization of Direct yellow and Red10B was carried out separately and combining the two using immobilized DY-9, 10 as individual cultures and consortium

3 RESULTS

3.1 Isolation and screening of Direct yellow 12 decolorizing bacteria

Out of the seven samples collected from various soils contaminated with textile industry effluents in and around Bangalore, 12 isolates capable of decolorizing Direct yellow 12 were obtained. Based on growth in mineral salts medium, 2 isolates were screened out for Direct yellow 12 decolorization. On gram staining, it was found that other than gram positive and gram-negative bacteria, a few isolates were yeasts. Based on their growth and zone formation on the agar medium DY-9, 10 were selected and analysed based percent decolorization. Quadrant streaking was done on the NA plates from respective plates of BH broth and Agar. The isolates decolorizing Direct Yellow-12 are shown in Figure 1.

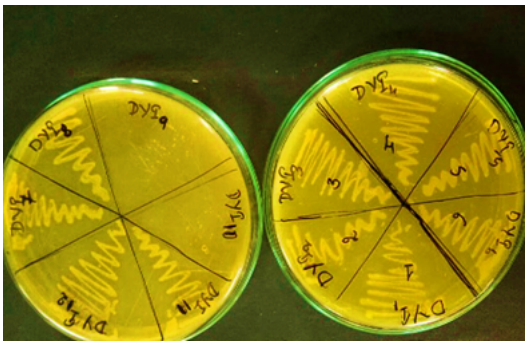

FIGURE 1

Direct Yellow Decolorizing Bacteria

The gram character and percentage decolorization results of the 12 isolates screened for Direct Yellow-12 is listed in table 2.

Selected isolates were separately streaked on Bushnell Haas agar and were subsequently sub cultured to avoid contamination.

The results of the biochemical tests of the isolates DY-9 and DY-10 are listed in table 3.

TABLE 2

A list of isolates screened for direct yellow decolorization based on growth in mineral salts medium with0.02gm, 0.05gm- Dye concentration

PERCENTAGE DECOLORIZATION				
SL.NO	**ISO-LATE**	**R0UND- 1 (0.02gm)**	**ROUND-2 (0.05gm)**	**GRAM CHARACTER**
1	DY-1	11.11	28.57	Yeast
2	DY-2	33.33	64.28	Yeast
3	DY-3	44.44	46.42	Yeast
4	DY-4	44.44	64.28	Gram negative rods
5	DY-5	44.44	32.14	Yeast
6	DY-6	22.22	32.14	Gram negative rods
7	DY-7	22.22	67.85	Gram negative rods
8	DY-8	55.55	46.42	Gram positive rods
9	**DY-9**	**88.88**	**89.28**	**Gram positive long rods**
10	**DY-10**	**77.77**	**82.14**	**Gram positive endospore forming long rods**
11	DY-11	11.11	14.28	Gram positive rods
12	DY-12	11.11	50	Yeast

The results of gram staining of the isolates DY-9 and DY-10 are shown in Figure 2.

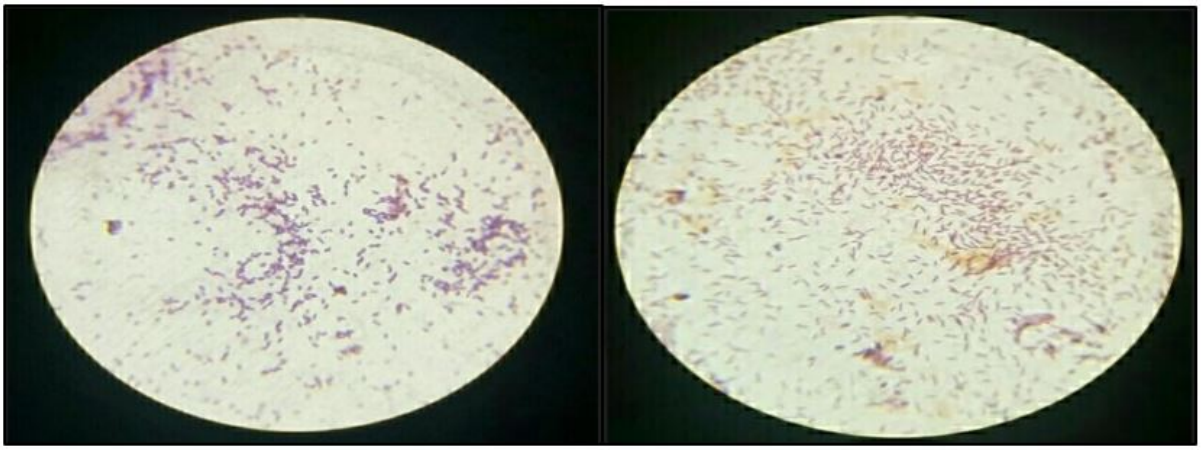

FIGURE 2

Results of Gram staining Tentative identification of DY 9 and DY10: Bacillus spp.

TABLE 3

Biochemical characterization of DY-9, DY-10

SL.No.	Test	Result			
		DY9		DY10	
1	Catalase	+ve		+ve	
2	Oxidase	-ve		-ve	
3	Methyl Red	-ve		-ve	
4	Voges Proskauer test	+ve		+ve	
5	Indole	-ve		-ve	
6	Citrate utilization test	-ve		-ve	
7	Antagonism	-ve		-ve	
8	Starch Hydrolysis	+ve		+ve	
9	Gelatin Liquefaction	-ve		-ve	
		Acid	Gas	Acid	Gas
10	Sucrose fermentation	-ve	-ve	+ve	+ve
11	Glucose fermentation	-ve	+ve	-ve	+ve
12	Lactose fermentation	+ve	+ve	+ve	+ve
13	Motility	Non motile		Non motile	

3.2 Process parameter Optimization using One-factor-at-a-time experiment

DY9 and DY10 showed almost same response towards effect of pH, temperature, dye concentration and shaking speed. A different response was shown towards effect of additional carbon and nitrogen sources.

3.2.1 *Effect of pH by Direct yellow 12 isolates:*

The effect of various hydrogen ion concentrations on decolorization of Direct yellow and by DY-9, 10 are presented in Figure 3. DY-9 and DY-10 preferred alkaline pH for Direct yellow decolorization. By DY-9, Lowest of 24% decolorization was recorded at pH 3 and highest of decolorization was recorded at PH 9 as there was an increase in pH Decolorization of Direct yellow was also increased, reaching a maximum of 76.6% at pH 9. Further increase in pH resulted in decreased Direct yellow decolorization. By DY-10, Lowest of 33% was seen at pH 3 and maximum decolorization was recorded as 79% at PH 9. With further alkaline pH values,

a decreased Direct yellow decolorization was observed. At pH 11 both the isolates showed a decrease of decolorization up to 31% by DY-9 and 42% by DY-10.

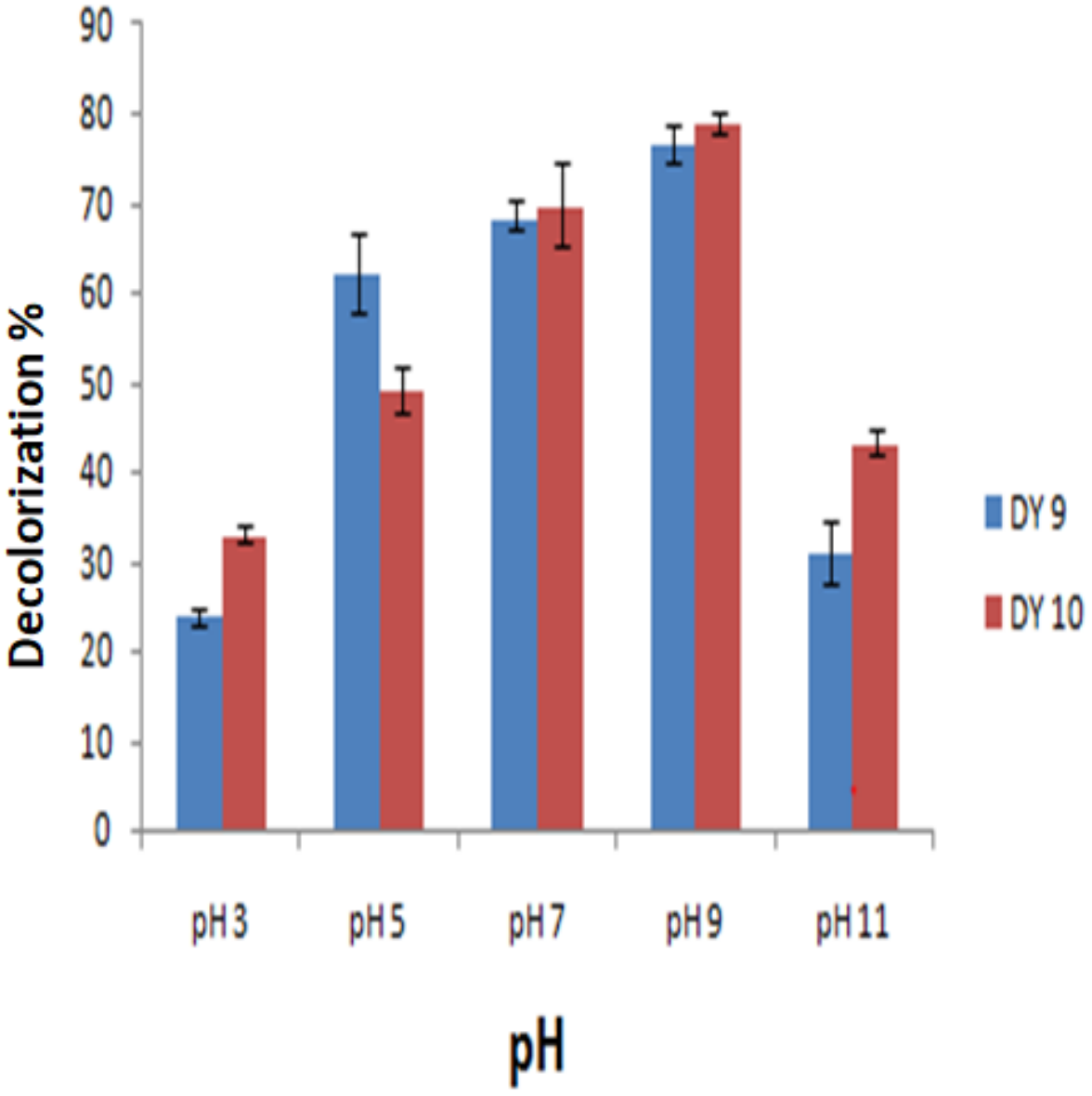

FIGURE 3

Effect of pH on decolorization of Direct yellow by DY-9 and DY-10 Isolates. Note: Error bars indicate ± SD.Note: Error bars indicate ± SD

3.2.2 *Effect of Temperature by Direct yellow 12 isolates:*

The influence of varying temperatures ranging from 5°C to 37°C on Direct yellow decolorization by DY-9 and DY-10 are presented in figure 4. Both DY-9 and DY-10 showed maximum

decolorization in the temperature range of 30°C to 37°C. Increase in temperature above 37°C and below 30°C resulted in decreased efficiencies of both the isolates. A lowest of 15.66% and 10% decolorization of DY9 and DY10 respectively were shown by the respective isolates at 5°C. A highest of 74% and 61.33% decolorization of DY9 and DY10 respectively were shown by the isolates at 37^0C and 30^0C respectively.

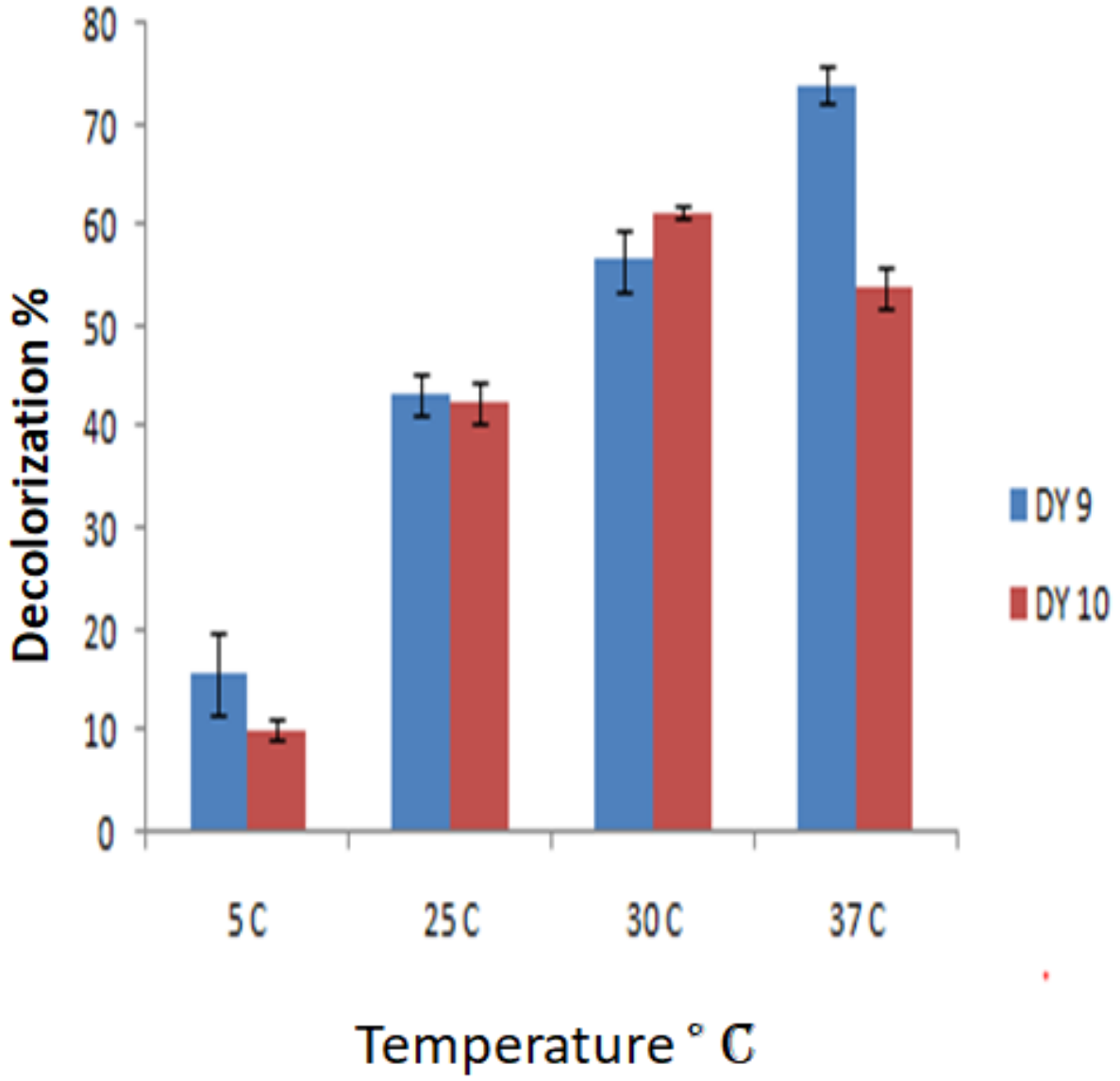

FIGURE 4

Effect of temperature on decolorization of Direct yellow by DY-9 and DY-10 Isolates. Note: Error bars indicate ± SD

3.2.3 *Effect of Dye Concentration by Direct yellow 12 isolates:*

The effect of different concentrations of dye ranging from 2% to 8% on Direct yellow decolorization by DY-9 and DY-10 are presented in figure 5.

The optimum decolorization of 42.6% was observed 2% dye concentration with DY-9. Similarly, the optimum decolorization of 33% was observed at 2% dye concentration with DY-10. Further increase in dye concentration resulted in decreased decolorization efficiency of both the isolates. A least decolorization of 22% and 13.3% was recorded with 8% concentration of direct yellow by the DY-9 and DY-10 isolates.

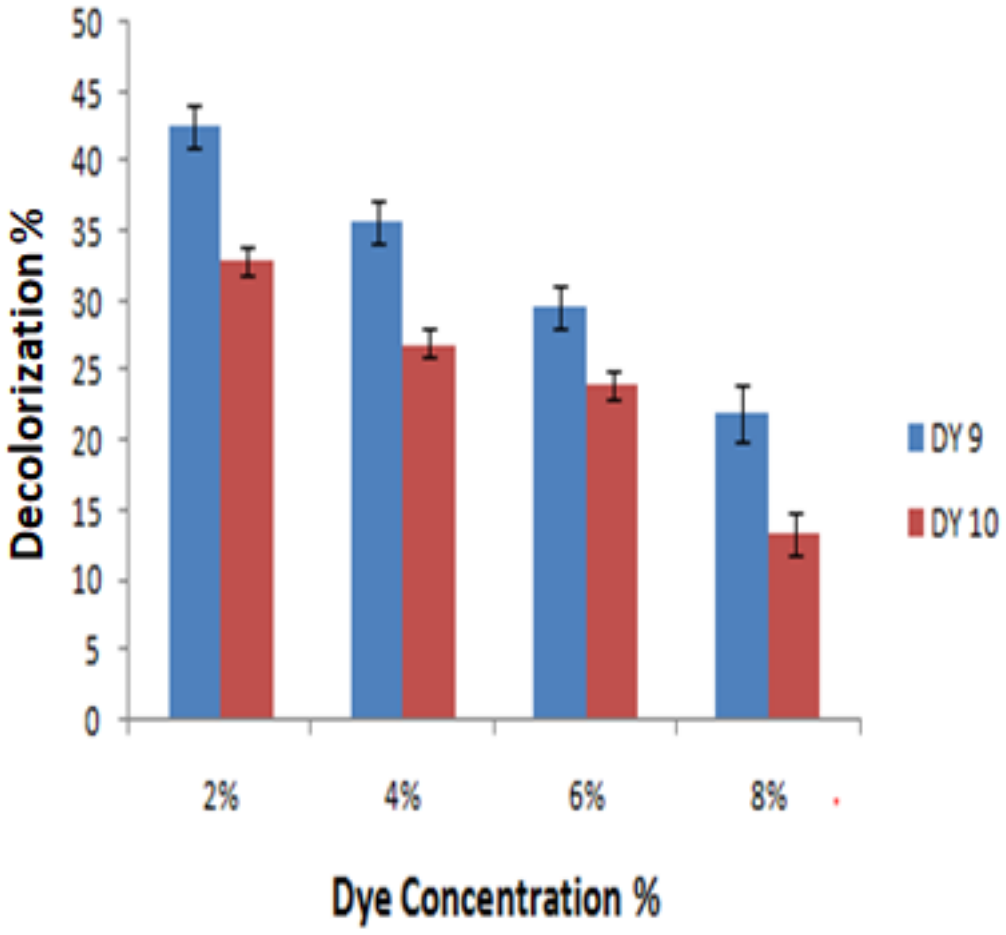

FIGURE 5

Effect of dye concentration on decolorization of Direct yellow by DY-9 and DY-10. Note: Error bars indicate ± SD

3.2.4 *Effect of Carbon Source by Direct yellow 12 isolates: -*

The results of effect of additional carbon sources on Direct yellow decolorization by DY-9 and DY-10 isolates are presented in figure 6.

Glucose and Maltose had a negative influence on Direct yellow decolorization by DY-9 and DY-10 respectively, whereas the other two carbon sources namely, starch and sucrose increased the percent decolorization of Direct yellow byDY-9 and DY-10. A least of 42.33% decolorization and maximum of 71% decolorization of Direct yellow was recorded in presence of Glucose and Starch respectively by DY-9.

Similarly, sucrose and maltose decreased the efficiency of DY-10 in Direct yellow decolorization, whereas, starch and glucose had a positive influence on Direct yellow decolorization by DY-10. DY-10 showed 35.33% decolorization of Direct yellow with Maltose as a carbon source and 39% decolorization of Direct yellow with sucrose as a carbon source. A maximum of 61% decolorization of Direct yellow was shown by DY-10 in presence of Starch.

3.2.5 *Effect of Nitrogen Source by Direct yellow 12 isolates:*

The influence of sources of nitrogen on Direct yellow decolorization by DY-9 and DY-10 are presented in figure 7. DY-9 could show a least of 42% decolorization of Direct yellow in presence of Yeast extract. A highest of 70% decolorization of Direct yellow was recorded in presence of Potassium nitrate, whereas, with DY-10 a least of 33% and a highest of 68% decolorization of Direct yellow was recorded in presence of Peptone and Yeast extract respectively.

3.2.6 *Effect of shaking speed by Direct yellow 12 Isolates*

The effects of different shaking speeds (rpm) ranging from 50 to 200 on Direct yellow decolorization by DY-9 and DY-10 are presented in figure 8.

Both DY-9 and DY-10 showed increase in percent decolorization of respective dye with increase in shaking speed upto 150 rpm. Further increase in shaking speed resulted in drastic decrease in percent decolorization with both the isolates. A maximum of 76.6% decolorization by DY-9 and 80% decolorization by DY-10 isolates was recorded at 150 rpm. A least decolorization of 47% by DY-9 at 50rpm and 50.33% by DY-10 at 200 rpm was recorded with Direct yellow.

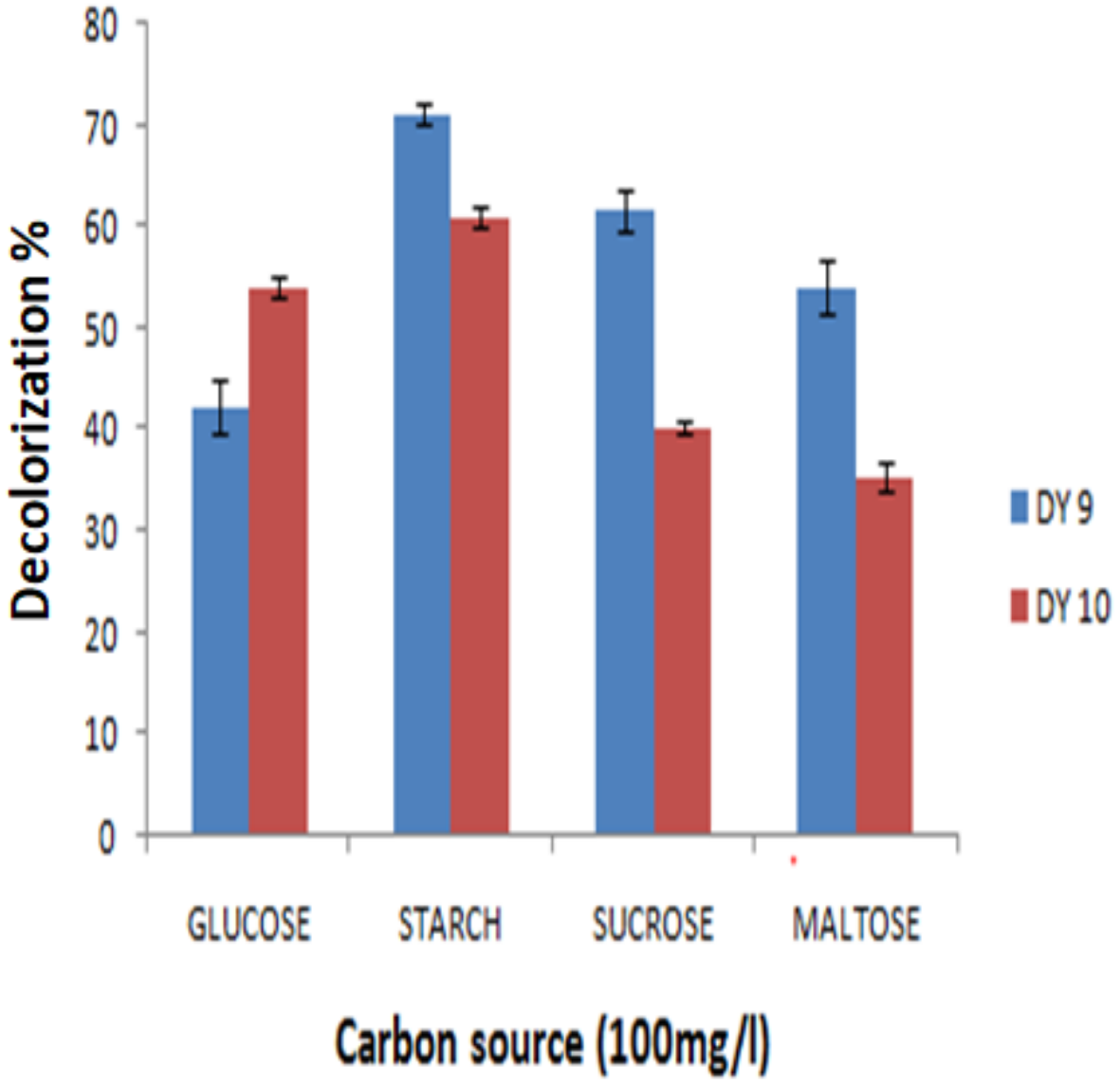

FIGURE 6

6 Effect of carbon sources on decolorization of Direct yellow by DY-9 and DY-10 Isolates. Note: Error bars indicate ± SD

3.3 Batch decolorization of Direct yellow Isolates

Decolorization efficiencies of free and immobilized cells of DY-9, 10 have been shown in figures 9 and 10. It can be observed from the results that free cells of DY-9 and DY-10 could show 42.66% and 33% decolorization of Direct yellow with 2% dye concentration respectively. When the concentration of Direct yellow was increased further, it was seen that efficiency of decolorization of DY-9 and DY-10 decreased. A least decolorization of 22% and 13.33% was recorded with 8% concentration by DY-9 and DY-10 respectively by Direct yellow, whereas immobilized cells could show 55.66% by DY-9 and 50.33% by DY-10 decolorization of Direct yellow at 2%

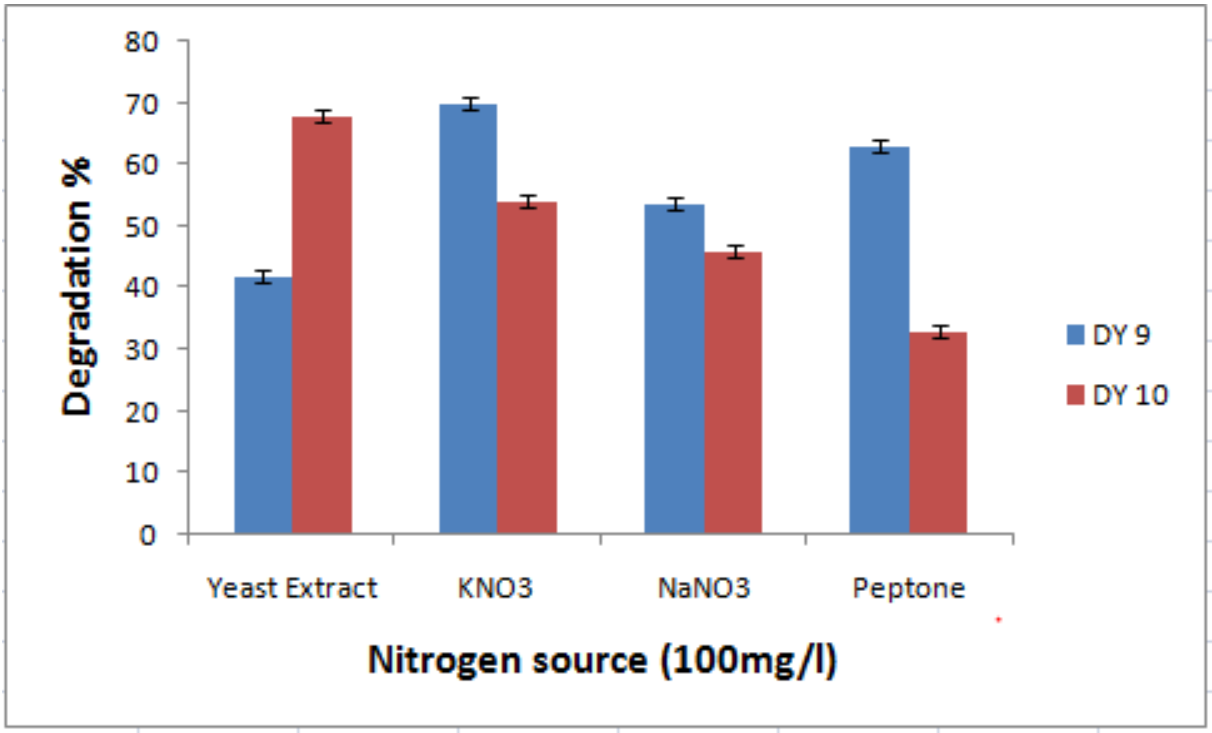

FIGURE 7

Effects of nitrogen sources on the decolorization of Direct yellow by DY-9 and DY-10 Isolates. Note: Error bars indicate ± SD

concentration and with 8% concentration of Direct yellow immobilized cells could still show 66.33% by DY-9 and 42.66% by DY-10 of decolorization of the dye.

3.4 Repeated batch decolorization of Direct yellow Isolates

Ca-alginate immobilized cells of both DY9 and DY 10 isolates were stable upto some cycles after which the stability was lost. The results of repeated batch decolorization are presented. Figure 11 shows that Calcium-alginate entrapped cells of the isolate DY-9 can be reused without impacting the efficiency of decolorization of 73% till 5 cycles. A reduction in efficiency of decolorization to 68% was observed at the end of 10^{th} cycle. As the number of cycles further increased, a gradual decline in efficiency of decolorization was recorded. A 17% decolorization potential was retained by the calcium-alginate entrapped DY-9 isolate after the 30^{th} cycle. At this stage, 120x10^3 CFU/mL cell leakage was observed.

Figure 11 shows that Calcium-alginate entrapped cells of the isolate DY-9 can be reused without impacting the efficiency of decolorization of 65% till 5 cycles. A reduction in efficiency of decolorization to 45.66% was observed at the end of 10^{th} cycle. At the end of cycle 25, 13%

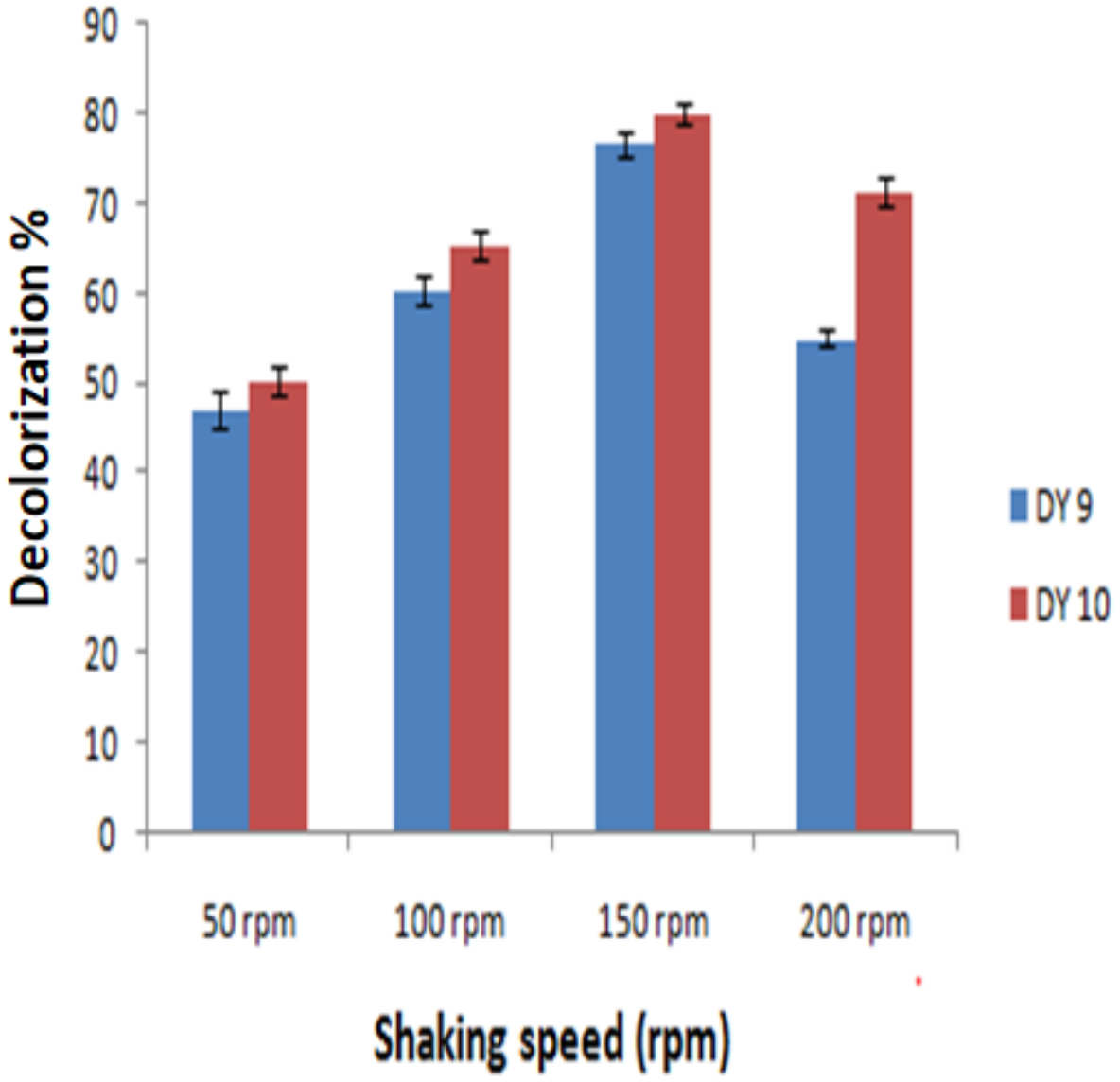

FIGURE 8

Effect of shaking speed on decolorization of direct yellow by DY-9 and DY-10 Isolates. Note: Error bars indicate $\pm$ SD

decolorization potential was retained. The beads lost the stability at this stage of 25^{th} cycle with a 140x10^4CFU/mL cell leakage.

3.5 Decolorization of Direct yellow 12 using immobilized consortium of DY-9+ DY-10

As shown in figure 13, Ca-alginate immobilized DY-9+ Red10B-6 was able to show 98.66 % decolorization of Direct yellow, whereas 71.33% decolorization of direct yellow was recorded with Ca-alginate immobilized DY-10+ Red10B-12 and 62.66% decolorization of direct yellow was recorded with Ca-alginate immobilized DY-9+ DY-10.

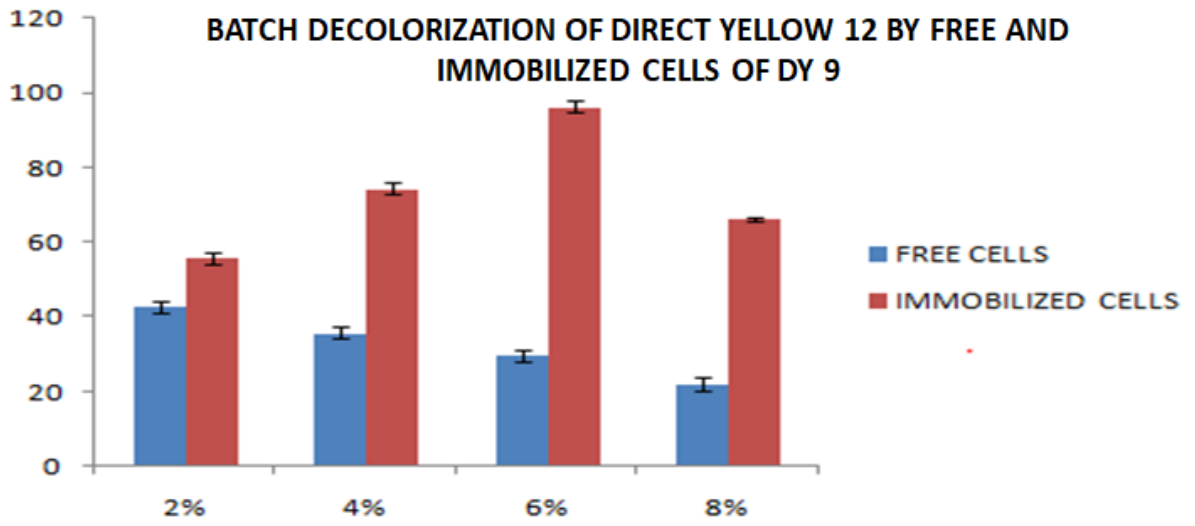

FIGURE 9

Batch decolorization of Direct yellow by free cells of DY-9 and Ca-alginate entrapped cells of DY-9.Note: Error bars indicate ± SD

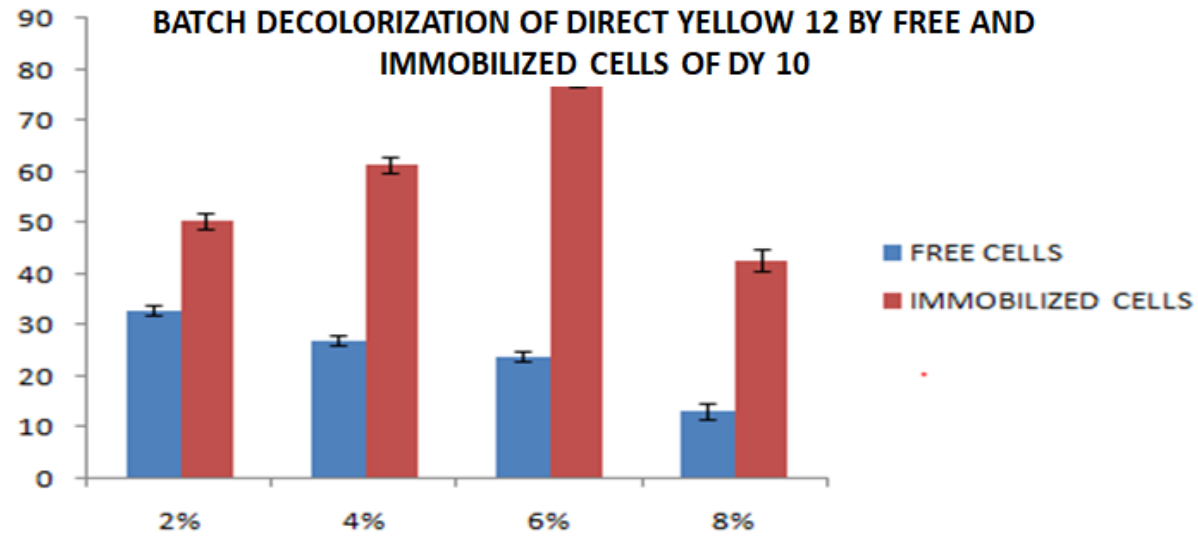

FIGURE 10

Batch decolorization of Direct yellow by free and Ca- alginate immobilized cells of DY-10 Isolate.Note: Error bars indicate ± SD

3.6 Comparison of decolorization in optimized and un-optimized conditions: DY 9 and DY 10

The results of the comparative analysis of decolorization of Direct Yellow by the isolate DY9 and DY10 are presented in figures 14 and 15 respectively. In both the cases, the efficiency of decolorization increased significantly in optimized conditions, compared to unoptimized conditions.

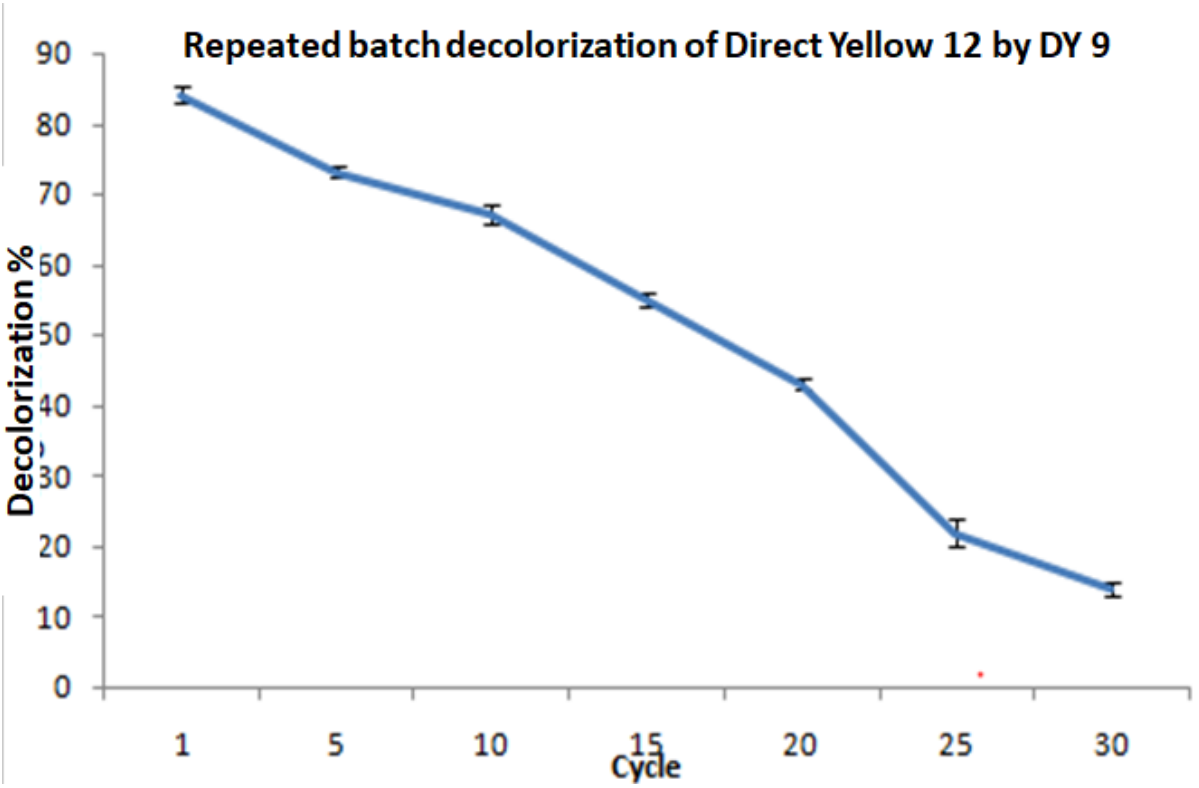

FIGURE 11

Repeated batch decolorization of Direct yellow Ca-alginate immobilized DY-9 Isolate.Note: Error bars indicate ± SD

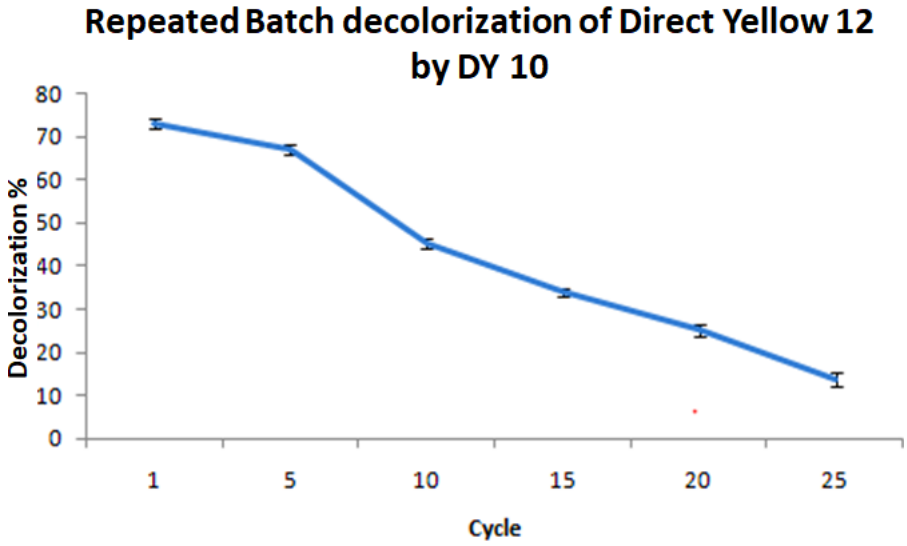

FIGURE 12

Repeated batch decolorization of Direct yellow Ca-alginate immobilized DY-10 Isolate. Note: Error bars indicate ± SD

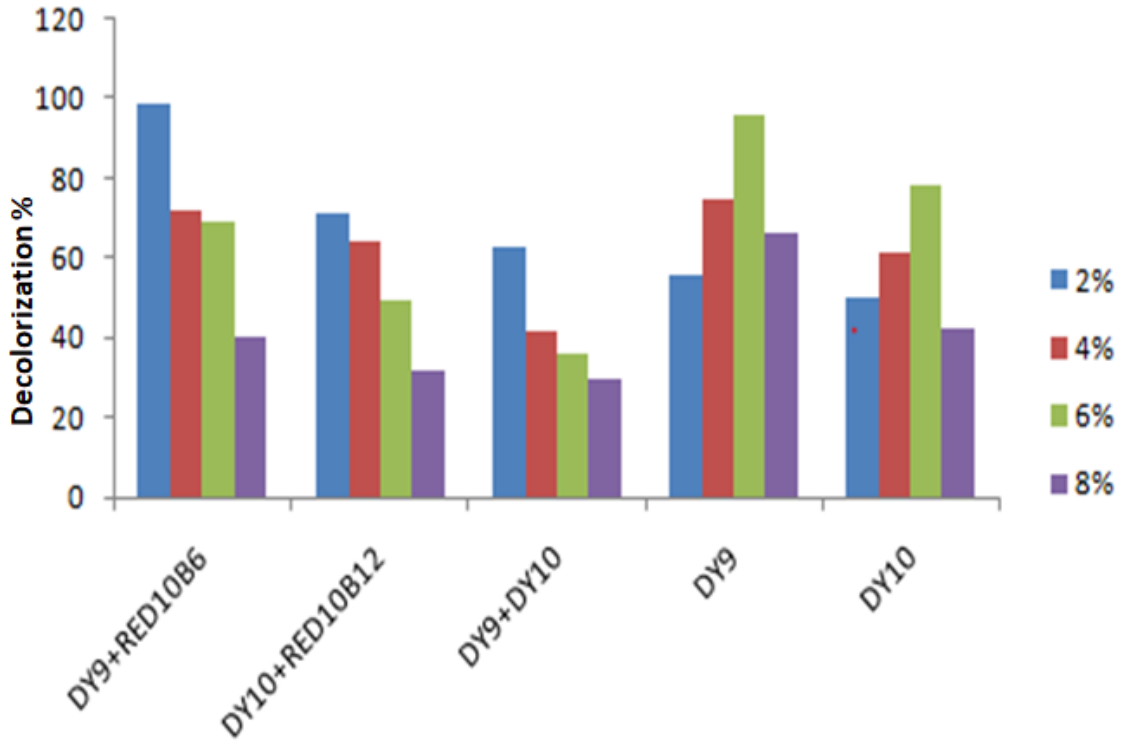

FIGURE 13

Decolorization of Direct yellow 12 using immobilized consortium of DY-9+ DY-10.Note: Error bars indicate ± SD

4 DISCUSSION

In the present study, 12 isolates capable of decolorizing Direct Yellow 12 were obtained by enrichment technique. Further 2 isolates labelled as DY9 and DY10 could show good growth in minimum mineral salts medium and were good decolourizers. The isolates were tentatively identified as Bacillus species.

Decolorization of dye includes different techniques such as physical, chemical, biological treatment in general. Among them it is been noticed that physical and chemical decolorization techniques are not much effective compare to biological treatment, due to the adaptability of the microorganism in textile effluent such as water and soil. The microorganisms tend to utilize carbon and nitrogen source from textile effluent and decolourize accordingly, hence biological technique is said to be more effective, cost efficient and best way to decolourize dye (Jamee R et al, 2019).

Efficient decolorization of Direct yellow 12 using Photocatalytic activity by UV/TiO_2 in shallow pond slurry reactor under acidic medium with 4.5 pH has been documented (10). In the current investigation, the dye was decolorized using free and calcium alginate entrapped cells of the isolates DY-9 and DY-10.

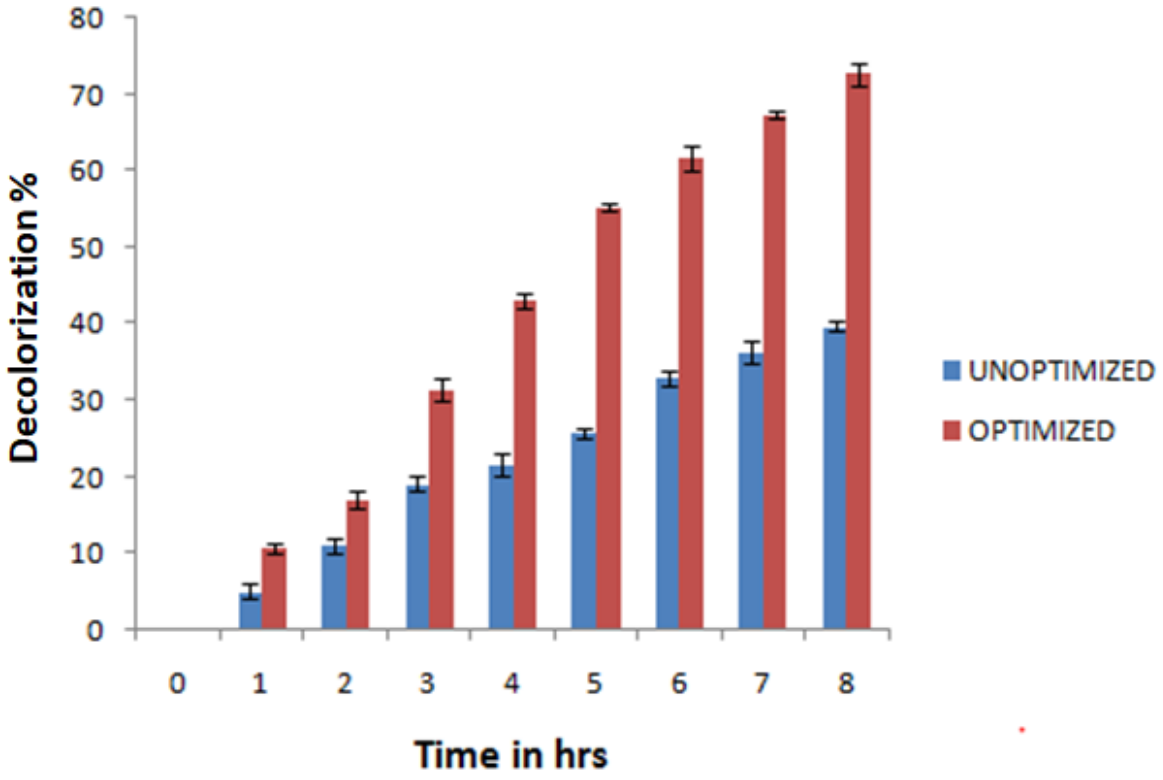

FIGURE 14

14 Comparison of decolorization in optimized and unoptimized conditions: DY 9

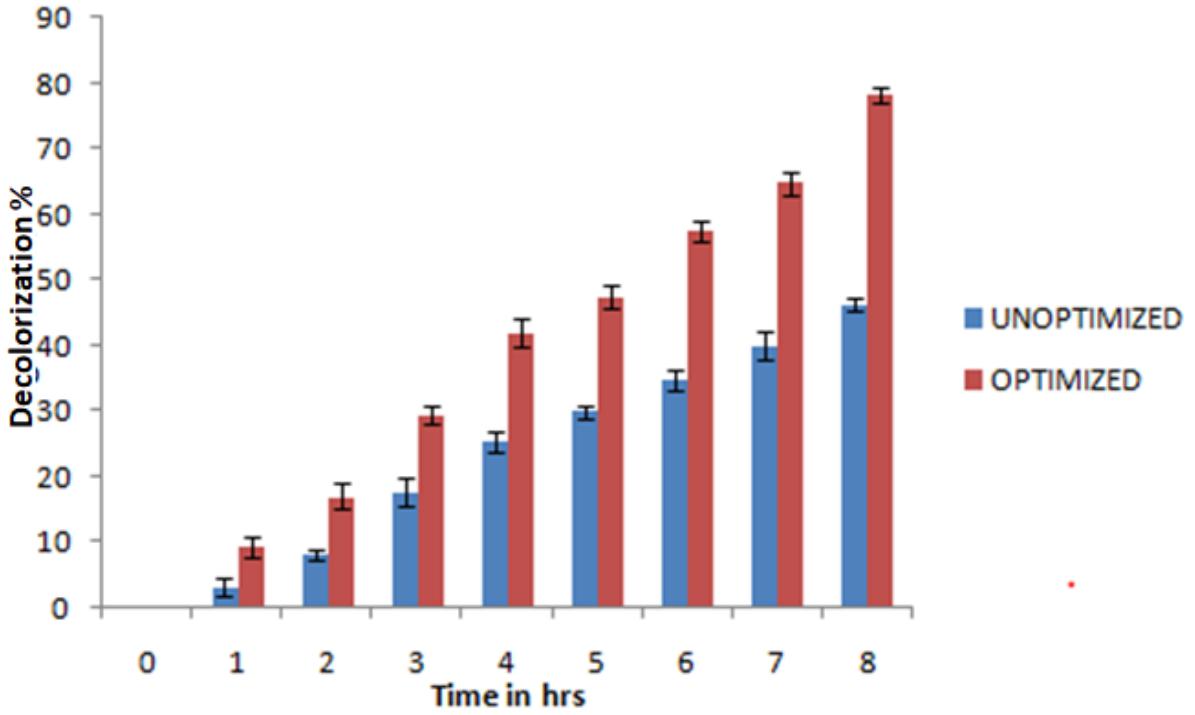

FIGURE 15

15 Comparison of decolorization in optimized and unoptimized conditions: DY 10

Parameters such as carbon and nitrogen source, temperature, pH and inoculum volume were optimized for the decolorization process by Shah MP et al., (2013). Optimal condition for Bacillus cereus were 1% sucrose, 0.25% peptone, pH 7, temperature 37°C. The results obtained in the current investigation using DY-9 and DY-10 isolates, both tentatively identified as Bacillus species showed highest decolorization potential with starch as a source of carbon. Highest decolorization with DY-9 and DY-10 was observed with KNO_3 and Yeast extract respectively. The results obtained in the current investigation showed an optimum pH of 9 for both DY-9 and DY-10. The optimum temperatures of 37°C and 30°C respectively for DY-9 and DY-10 respectively. A few results with respect to temperature and pH for both investigations are found to be similar.

The most suitable pH and temperature for colour removal were 5.5 to 10 and 20 to 35°C respectively under anoxic conditions using Aeromonas hydrophila. (Satyendra Kumar Garg and Manikant Tripathi et al., 2016). The results obtained in the current investigation showed an optimum pH of 9 for both DY-9 and DY-10. The optimum temperatures of 37°C and 30°C respectively for DY-9 and DY-10 respectively.

Bushnell Haas medium supplemented with 100 mg/L Starch and a Dye concentration of 2% were optimum for both cultures. It predicts that at lower the dye concentration better decolorization ability is observed. Optimum decolorization with DY9 was obtained with 100 mg/L KNO_3 and that with DY10 was with 100 mg/L Yeast extract. A comparative study on the decolorization of Direct Yellow 12 under unoptimized and optimized conditions using both isolates was carried out. The results showed a marked increase in decolorization with both isolates under optimized conditions. The two cultures, individually and as a consortium were immobilized in Calcium alginate. Batch decolorization of the dye using free and immobilized cultures of DY9 and DY10 was carried out.

The immobilized cultures show an increase in degradation potential of any complex structure like that of Endosulfan, compared to free cells (6). The Calcium-alginate entrapped cells of DY-9 and DY-10 showed increased decolorization potential compared to free cells.

At a lower dye concentration, the immobilized consortium showed a higher decolorization. However, with increase in concentration of the dye, individual immobilized cultures proved better for decolorization. Repeated batch decolorization was carried out to check the stability of the calcium alginate beads. With this investigation, it was indicated that free and immobilized cells of both the isolates are potential to be used in the bioremediation of water and soil contaminated with Direct Yellow 12.

5 CONCLUSION

Conditions for decolorization of Direct Yellow 12 was optimized using one factor at a time approach. It was demonstrated that DY-9, DY-10 are efficient strains in decolorization of Direct Yellow 12. Further, it was concluded that Immobilized cells are better decolourizers compared to free cells. However, the efficiency of decolorization of dyes using consortia needs to be improved with further studies.

References

[1] D J Brenner, N R Krieg, J T Staley, and G M Garrity. Bergey's Manual of Systematic Bacteriology. *The Proteobacteria), part C (The Alpha-, Beta-, Delta-, and Epsilonproteobacteria)*, 2, 2005.

[2] Usha M S Sanjay, M K Gaddad, S Shivannavar, and C. Degradation of h-acid by free and immobilized cells of Alcaligenes latus. *Brazilian Journal of Microbiology*, (4):931–945, 2010.

[3] W Azmi, R J Sani, and U C Banerjee. Biodegradation of triphenylmethane dyes. *Enzyme Microbial Technology*, pages 185–191, 1998.

[4] Satyendra Kumar Garg and Manikant Tripathi Microbial Strategies for Discoloration and Detoxification of Azo Dyes from Textile Effluents. *Research Journal of Microbiology*, pages 1–19, 2017.

[5] M P Shah, K A Patel, and S S Nair. Darji AM Potential Effect of Two Bacillus spp on Decolorization of Azo dye. *J Bioremediation and Biodegradation*, 4:199–199, 2013.

[6] V Pradeep and . U Subbaiah, Use of Ca-alginate immobilized Pseudomonas aeruginosa for repeated batch and continuous degradation of Endosulfan.3 Biotech 6, 2016.

[7] R Krull, M Hemmi, and P Otto, Hempel DC Combined biological and chemical treatment of highly concentrated residual dyehouse liquors. Water Science and Technology, 1998.

[8] H Bettman and H J Rehm. Degradation of phenol by polymer entrapped microorganisms. *Appl.Microbiol.Biotechnol*, 20:285–290, 1984.

[9] Nikolaos Voulvoulis Water reuse from a circular economy perspective and potential risks from an unregulated approach,Current Opinion in Environmental Science & Health, 2018.

[10] . A P Toor, A Verma, . C. K Jotshi, P K Bajpai, and Singh, Photocatalytic degradation of Direct Yellow 12 dye using UV/TiO2 in a shallow pond slurry reactor. Dyes and pigments, 2006.

[11] D Neha and Parmar. Sanjeev R Shukla decolorization of dye wastewater by microbial methods - Review. *Indian Journal of Chemical Technology*, pages 315–323, 2018.

[12] M M Hassan, M Z Alam, and M Anwar. N Biodegradation of Textile Azo Dyes by Bacteria Isolated from Dyeing Industry Effluent. *International Research Journal of Biological Sciences*, (2):27–31, 2013.

[13] S Manohar, C K Kim, and T B Karegoudar. Enhanced degradation of naphthalene by pseudomonas sp strain NGK1 immobilized in polyurethane. *App. Microbial. Biotechnology*, 55:311–316, 2001.

[14] Jamee Siddique. Biodegradation of Synthetic Dyes of Textile Effluent by Microorganisms: An Environmentally and Economically Sustainable Approach. *European Journal of Microbiology & Immunology.2019*, 9(4):114–118.

[15] P Verma and D Madamwar. Decolorization of synthetic dyes by a newly isolated strain of Serratia marcescens. *World Journal of Microbiology and Biotechnology*, 19(6):15–618, 2003.

[16] R Rajesh Kannan and M Rajasimman, Rajamohan N Decolourization of malachite green using tamarind seed: optimization, isotherm and kinetic studies, 2011.

[17] M T Moreria, I Mielgo, and G Feijoo. Lema JM Evaluation of different fungi strains in the decolourisation of synthetic dyes. *Biotechnology Letters*, pages 499–503, 2000.