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Research Article

STANDARDIZATION OF DIFFERENT METHODS OF MICROBIAL IMMOBILIZATION FOR DYE DECOLOURIZATION

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ABSTRACT

The Textile industry in India is the only industry that has generated huge employment for both skilled and unskilled labour. Among the 10,000 dyes used in the textile industries, about 10% are discharged into the wastes. Two-third of the dye industry is dominated by textile industry and discharge large volume of chemical dyes into the environment. The immobilization of microorganisms on preferred adsorbents instigates protects cells from unfavourable agents, microbial metabolism, and preserves their physiological activity. It provides a direct contact between nutrients and the immobilized cells. Cell immobilization technique encompasses the transportation of the cells from the bulk phase to the surface of support, followed by the adhesion of cells and successive colonization of the support surface. The cell-support adhesion is governed by both electrostatic and hydrophobic interactions, which is the key step in controlling the cell immobilization on the support. A Bacillus bacterial strain isolated from the sludge collected from the textile dye effluent at Tirupur, Tamil Nadu. It is characterized and found to decolourize the textile dye colour from water body. The bacterial cells were mass cultivated and harvested by centrifugation. These cells were immobilized by different methods such as sodium alginate, polyacrylamide, carrageenan, agar. The immobilized bacterial cells were characterized using optical microscopy. They are uniformly embedded in the gel matrix and were compared for their decolourization potential of synthetic dye.

Keywords: Cell immobilization, Decolourization, Sodium alginate, Polyacrylamide, Carrageenan, Agar.

INTRODUCTION

The Textile industry in India is the only industry that has generated huge employment for both skilled and unskilled labour. It continues to be the second largest employment generating sector in India. Textile Engineering has direct connection with environmental aspects to be explicitly and abundantly considered. It accounts for around one third of total export of effluent. Out of various activities in textile industry, chemical processing contributes about 70% of pollution. Cotton mills consume large volume of water for various processes such as sizing, desizing, and scouring, bleaching, mercerization, dyeing, printing, finishing, and washing is well known[1].Due to the nature of various chemical processing of textiles, large volumes of wastewater with numerous pollutants are discharged. Since the stream of water affect the aquatic eco-system in number of ways such as depleting the dissolved oxygen content of suspended substances in anaerobic condition, a special attention needs to be demanded[2].The various dyes employed for these purposes are chemically classified as azo, polymeric, anthraquinone, triphenylmethane and hetrocyclic dyes. Among the 10,000 dyes used in the textile industries, about 10% are discharged into the wastes[3].Two-third of the dye industry is dominated by textile industry and discharge large volume of chemical dyes into the environment. Dyes contain chromophores, delocalized electron system with conjugated double bonds, and auxochromes, electron-withdrawing or electron-receiving substituent that cause or intensify the color of the chromophore by altering the overall energy of the electron system. Usual chromophores are -C=C-, -C=N-, -N=N- and the auxochromes are $-NH_3$, -COOH, $-SO_3H$, and -OH. Many of these dyes find their way into the environment via wastewater facilities. Because these compounds retain their color and structural integrity under exposure to sunlight, soil, bacteria and sweat, they also exhibit a high resistance to microbial degradation in wastewater treatment system[4].The anaerobic reduction of azo dyes to simpler compounds has been well researched[5,6]. The problem of aesthetic pollution is solved, by reducing the dye compounds to their intermediates, but a larger and more deleterious problem may be created. Most azo dyes are non-toxic, but a higher percentage of their intermediates have been identified as carcinogens[7].Because of the toxic potential of many aromatic amines, further degradation of the dye compound is necessary if toxicity is to be eliminated or reduced[8,9]. It interferes with the transmission of sunlight into the streams and thereby reducing the photosynthetic activity. The color of effluent is more harmful for agricultural use and rearing of fish[2].Further, the disposal of sludge produced from these processes is again a problem. As the increase of contaminated sites poses a major environmental and human health problem, it appears mandatory to decontaminate the environment and to implement efficient decontamination strategies.

Bioremediation is a waste management technique that involves the use of microorganisms or their enzymes to remove or neutralize pollutants from a contaminated site. Bioremediation is short for two words - bio for biological and remediation for to make a remedy. Microorganisms used for bioremediation are called as biomediators[10].The bacterial species employed are *Pseudomonas*, *Bacillus, Aeromonas, Staphylococcus* etc[8].The limiting factor in selecting the microbe is their maintenance at the

site and the extent of decolorization they can offer. The most representative enzymatic classes in the remediation of polluted environments are: hydrolases, dehalogenases, transferases and oxidoreductases[9].Their main producers are bacteria, fungi (mainly white-rot fungi), plants and microbe-plant associations. The NADPH dependent azoreductase prefer NADPH as electron donor, on the other hand NADH dependent azoreductase require FMN as a prosthetic group[11].Obviously, when legal limits exist (not in all the countries) these should be taken as justification. Studies concerning the feasibility of treating dyeing wastewater are very important[10].In the past several decades, many techniques have been developed to find an economic and efficient way to treat the textile dyeing wastewater, Immobilization in general describes a wide diversity of the cell or the particle attachment or entrapment [11]. It can be functional to all types of biocatalysts including enzymes, cellular organelles, animal and plant cells. Currently, different types of immobilization have found wide applications not only in the field of biotechnology, but also in pharmaceutical, environmental, food and biosensor industries[12].Thus cell immobilization arose as a substitute for enzyme immobilization.

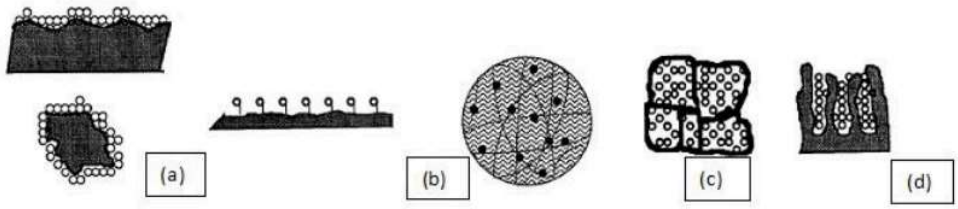


Figure 1: Methods for immobilization of viable microbial cells – (i) Adsorption to surfaces, (ii) Covalent bonding to carrier, (iii) Encapsulation in polymer gel, (iv) Entrapment in matrix

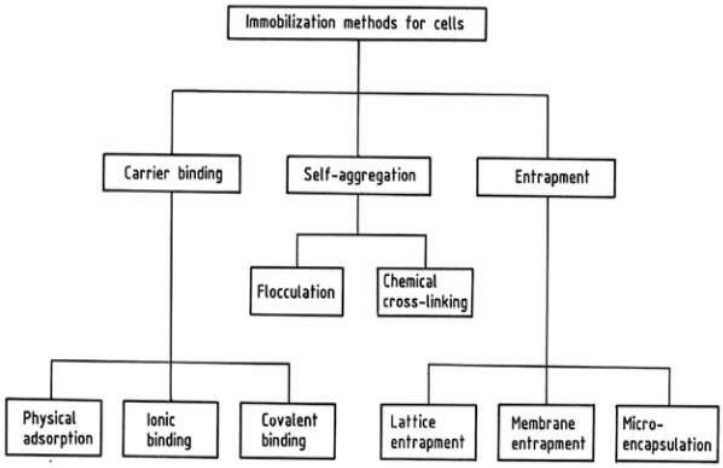


Figure 2: Classification of immobilization methods

The recent reports on enhanced plasmid stability of genetically engineered microorganisms[11]under immobilized conditions, and the viability of microbial cells over a period of 18 months[12,13]under entrapped conditions, are few of the many potential new applications of immobilized cells [14].

MATERIALS AND METHODS

Textile Dyes

Two azo dyes namely Orange M2R and Red RR were collected from a textile industry at Tirupur. Synthetic dye solutions were prepared and used for analysis.

Preparation of Dye Solution

Two different concentrations (20ppm and 100ppm) were prepared for both the dyes and used for analysis.

Determination of Absorption Maxima

The absorption maxima were determined by scanning in the UV-visible spectrophotometer. 2ml of the sample solution was taken in a cuvette and measured the absorbance at different wavelengths in the UV visible region (300-700nm). The respective absorption maxima were used for further degradation studies.

Culture Used

The bacterial strain used was isolated from a textile dye effluent at Tirupur enriching azo and reactive dyes.

Revival of the Cells

Frozen culture was taken from deep freezer and thawed. Small amount of Nutrient broth was added to the cryovial, mixed well and transferred to 100ml of nutrient broth. The flask was incubated at 37°C overnight.

Sub-Cultivation and Mass Cultivation of Dye Degrading Microbes

Sub cultivation is done by transferring cells from mother culture to fresh growth medium. 15ml of cell suspension was inoculated in 1200 liter of nutrient broth for mass culturing.

Standardization of Different Immobilization Methods

Immobilization of Microbes By Sodium Alginate Method

Equal volumes of 2% sodium alginate solution and of cell slurry were gently mixed together and the mixture was added drop wise via a 10 ml syringe into 4% calcium chloride solution. The formed

beads of sodium alginate were left in calcium chloride solution upto 20 minutes for hardening[15].

Immobilization of Microbes In Polyacrylamide Gel

The polyacrylamide gel entrapment procedure was performed [Jonathan W, 1988]. Wet cells were suspended in distilled water and chilled in ice. 0.2M potassium phosphate buffer, pH 7.0 was also chilled in ice. To this buffer add Acrylamide, bisacrylamide and ammonium persulphate were added and mixed. Immediately, the chilled buffer solution was mixed with the chilled cell suspension followed by the addition of TEMED and poured into 2 or 3 glass petri dishes. There after allowed for polymerization to proceed for 1hr. Then the sieved gels were suspended in 0.2M potassium phosphate buffer pH 7.0 and allowed to settle. Then were decanted and the gel was used for degradation studies.

Immobilization of Microbes in Agar

Agar was dissolved in 0.9% (w/v) sodium chloride by heating at 100°C and then cooled to 40°C. Cell slurry was suspended in 0.9% (w/v) sodium chloride solution. Cell slurry was added to agar solution and mixed. Immediately, the mixture was poured onto petridish and cooled to 5°C. The gel was stored in 0.1M phosphate buffer, pH 7.0 until required[15].

Immobilization of Microbes in Carrageenan

Carrageenan was completely dissolved in distilled water at desired concentration at 50°C, and then the solution was sterilized at 110°C -121°c for 1hr. The solution was cooled to 38°C - 40°C with stirring. The cell suspension was added to molten Carrageenan maintained at 40°C, mixed well and poured into sterile flat bottomed petriplate. After solidification it was cut into cubes and added to sterile 2% potassium chloride solution and kept in the refrigerator for 1hr for curing. The cubes were washed 3 – 4 times with sterile distilled water[15].

Comparison of Decolourization Efficiency of Synthetic Dye Solution Using Different Immobilization Methods

Two different concentrations such as 20ppm and 100ppm of 10ml dye solutions were taken. Equal weight of immobilized microbial cells with matrices were suspended in 10ml of solution and kept overnight to determine the percentage decolourization. The experiments were done in duplicates at 24 hours and 48 hours.

Percentage decolourization = 1-(AT/Ao)*100

Where A_T is absorbance at time T, A_o is absorbance at time Zero

Results were compared with control which had only the beads or gel blocks without microbe.

Characterization Using Optical Microscope

The immobilized microbes in different matrices were sliced with scalpel and fine section was observed under optical microscope without staining. The images were stored.

RESULTS AND DISCUSSION

Characterization of Dyes for Absorption Maxima

Table 1: Spectrophotometric analysis of absorbance maxima

Wavelength (nm)	Absorbance of orangeM_2R	Absorbance of Red RR
530	1.580	0.553
535	1.203	0.543
540	0.843	0.520
545	0.569	0.481
550	0.361	0.424
555	0.220	0.353
560	0.133	0.278
565	0.080	0.206
570	0.047	0.143
575	0.029	0.095
580	0.020	0.062
585	0.014	0.039
590	0.010	0.023
595	0.008	0.013
600	0.008	0.007
605	0.007	0.003
610	0.006	0.001
615	0.006	0.000
620	0.006	-0.001
625	0.005	-0.001
630	0.005	-0.002
635	0.005	-0.001
640	0.005	-0.002
645	0.005	-0.002
650	0.005	-0.002
655	0.005	-0.002
660	0.004	-0.002
665	0.004	-0.002
670	0.004	-0.003
675	0.004	-0.003
680	0.004	-0.003
685	0.004	-0.003
690	0.004	-0.003
695	0.004	-0.003
700	0.004	-0.003

TABLE 1.1 and TABLE 1.2 Spectrophotometric readings in the maximum absorbed range for Orange M_2R and Red RR

Wavelength(nm)	Absorbance of orangeM_2R
486	2.456
487	2.461
488	2.469
489	2.474
491	2.478
492	2.483
493	2.487
494	2.491
Wavelength(nm)	**Absorbance of Red RR**
516	0.558
517	0.560
518	0.559
519	0.559
521	0.559
522	0.559
523	0.558
524	0.558

The optical density of dyes at wavelengths 340-700nm were tabulated in Table: 1. From Table:1.1 the absorbance maxima was found to be 494nm for Orange M_2 as tabulated in Table 1.2, the absorbance maxima was found to be 517nm for Red RR.

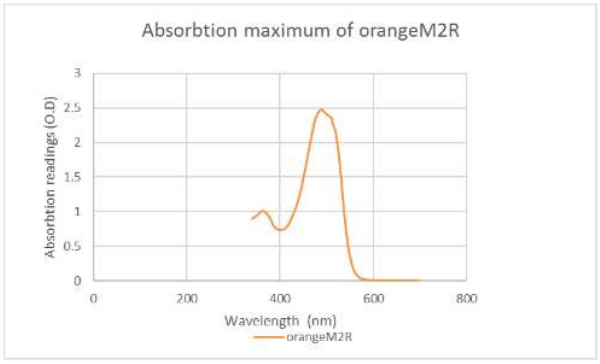


Figure 3: Absorption maximum of Orange M_2R

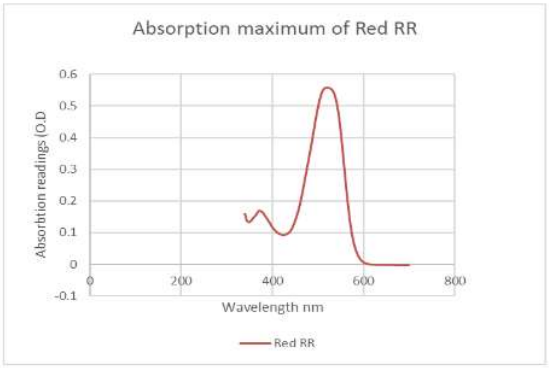


Figure 4: Absorption maximum of Red RR

Immobilization by different methods

Immobilization of microbes by Sodium alginate method

Beads were formed when sodium alginate is dropped into calcium chloride. The beads left in calcium chloride solution were hardened after about 20 minutes.

Immobilization of microbes by Polyacrylamide

The gel in the petriplate was cut into smaller pieces of uniform length using a sterile blade. These were suspended in 0.2M potassium phosphate buffer until use.

Immobilization of microbes by Agar

Cell slurry was added to agar which is dissolved in sodium chloride solution and this mixture was poured onto petridish and cooled. The resulting gel was stored in 0.1M phosphate buffer until required.

Immobilization of microbes by Carrageenan

Cell suspension was added to molten Carrageenan and was poured onto petriplate. It was cut into cubes after solidification. The cubes were stored in 2% potassium chloride solution and kept in refrigerator for curing.

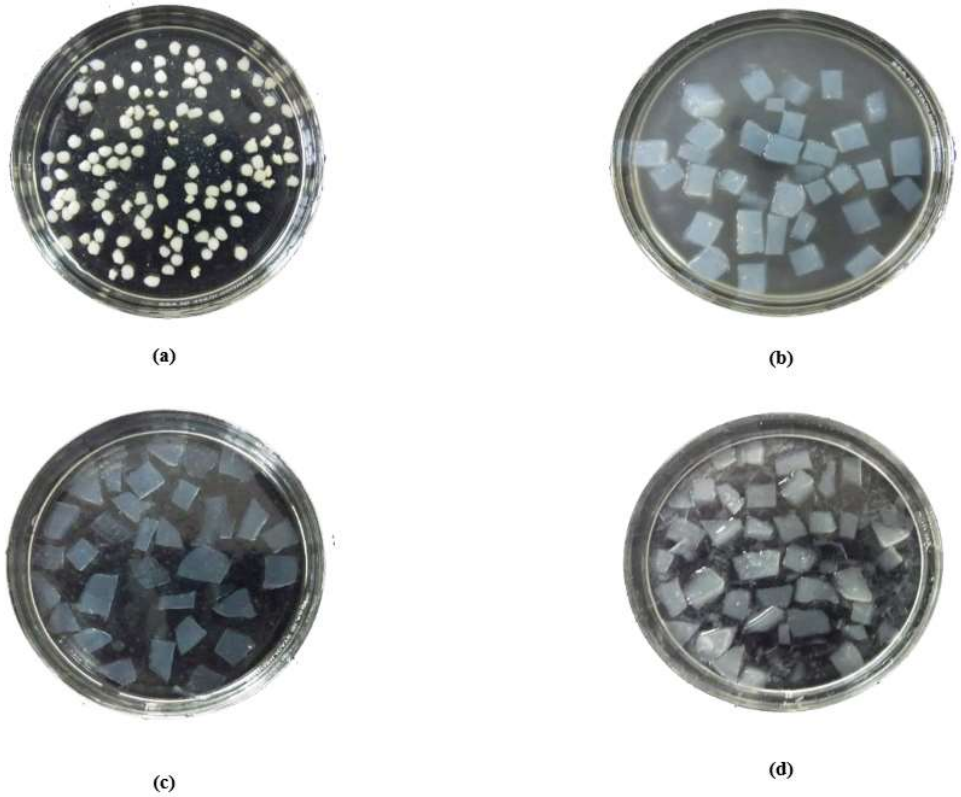


Figure 5: Different methods of immobilization (a) sodium alginate (b) polyacrylamide (c) agar (d) carrageenan

Characterization of immobilized microbes

Optical microscopic image showed microbes entrapped within the matrix.

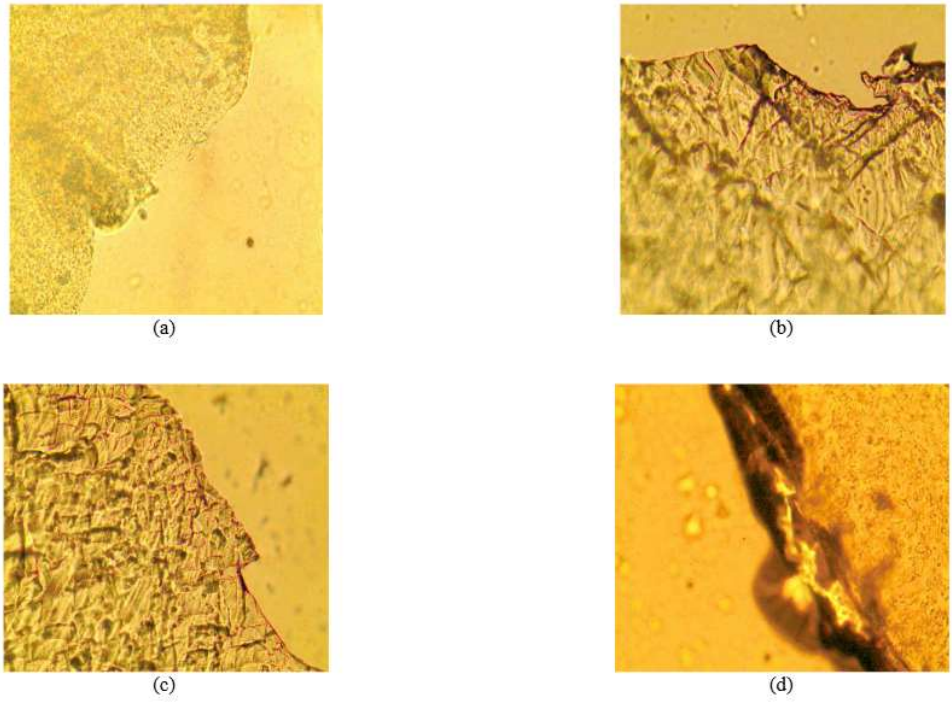


Figure 6: Optical microscopic image of immobilized microbes (a) sodium alginate (b) polyacrylamide (c) carrageenan (d) agar

Decolourization studies

Degradation efficiency of each of the methods was analyzed for 24 hrs and 48 hrs culture. Sodium alginate and polyacrylamide methods showed higher degradation potential when compared with the other methods. The decolorization percentage for individual immobilization methods are tabulated in Table 3 and Table 4. The readings are represented in a graphical format in Figure 15 and Graph 16. The decolorization of dyes using different immobilization methods are shown in Figure 7 to Figure 14.



Figure 7(a)



Figure 8(b)



Figure 9(a)



Figure 10(b)



Figure 11(a)



Figure 12(b)



Figure 13(a)

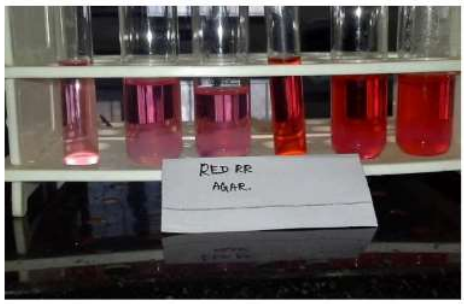


Figure 14(b)

Figure 7 – 14: Dye decolourization of (a) Orange M_2R (b) Red RR by microbes immobilized on different matrices

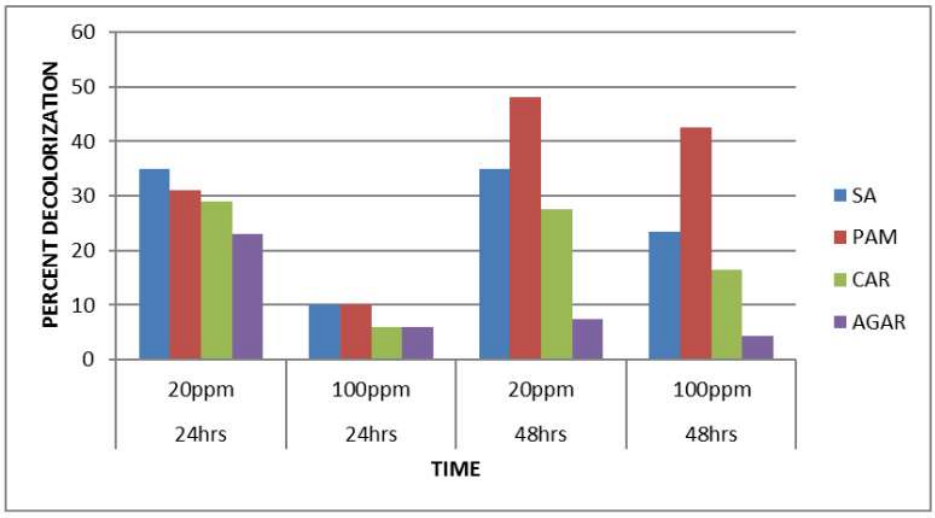


Figure 15: Comparison of the dye decolorization percentage for Orange M_2R at 24 and 48 hours

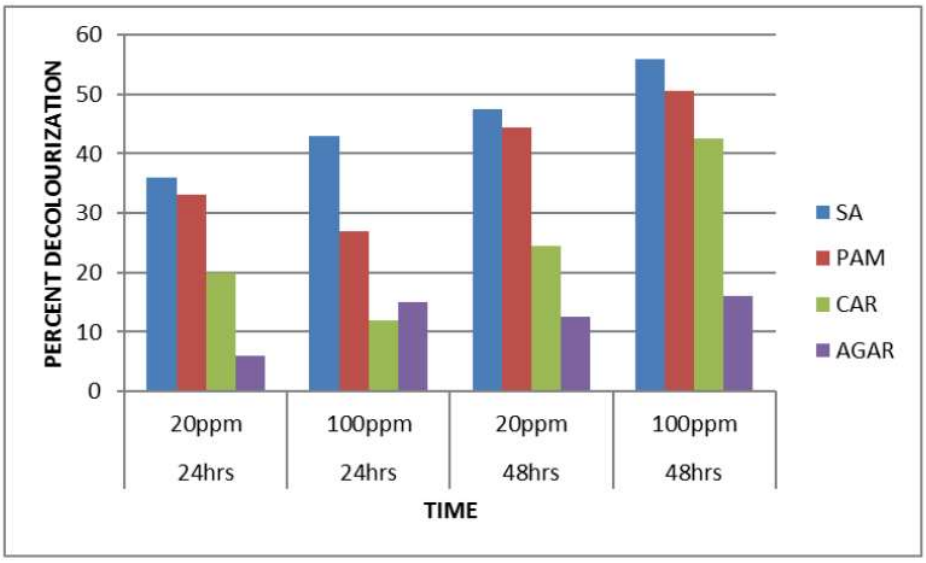


Figure 16: Comparison of the dye decolorization percentage for Red RR at 24 and 48 hours

Immobilization of cells have more advantages such as elimination of long and expensive procedures for enzymes separation and purification[14,15]. In comparison with immobilized enzymes, immobilized cells provide new promises as they can be used as natural, water-insoluble carriers of required enzyme activities [16].

CONCLUSION

A Bacillus bacterial strain isolated from the sludge collected from the textile dye effluent at Tirupur, Tamil Nadu. It is characterized and found to decolourize the textile dye colour from water body. The bacterial cells were mass cultivated and harvested by centrifugation. These cells were immobilized by different methods such as sodium alginate, polyacrylamide, carrageenan, agar. The immobilized bacterial cells were characterized using optical microscopy. They are uniformly embedded in the gel matrix and were compared for their decolourization potential of synthetic dye.

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