Research Journal of Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Environmental Sciences

ISSN 1819-3412
DOI: 10.3923/rjes.2018.33.40

Research Article
Dye Decolouration by Immobilized and Free Bacterial Cells at Different Glucose Concentration

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Abstract

Background and Objective: Although dyes are widely used, their presence in receiving water bodies, could cause several environmental and health impacts. The study, which was carried out under batch experimental conditions was aimed at investigating the relationship between glucose concentration and decolouration of bromothymol blue, crystal violet and methylene by free and immobilized cells of *Bacillus subtilis* and *Pseudomonas aeruginosa* in wastewater. **Methodology:** Glucose concentrations that were used in the study were 5, 10, 15 and 20 g L^{-1} and a control that contained no glucose (0 g L^{-1}). For the experimental setup, to a 200 mL quantity of sterile media that contained the respective concentration of the glucose, the respective bacterial cells were inoculated in 250 mL capacity flask. **Results:** Throughout the study, decolouration rate in the media without glucose was observed to be remarkably lower than those with added glucose. Optimum glucose concentrations of 5 and 10 g L^{-1} was observed for decolouration of the bromothymol blue and crystal violet in presence of the free cells of the *Bacillus subtilis* while in presence of the immobilized cells, concentration of 10 and 5-10 g L^{-1} was observed. In presence of the *Pseudomonas aeruginosa,* glucose concentrations of 20 and 10 g L^{-1} were observed to be optimum for decolouration of the bromothymol blue and crystal violet, respectively. **Conclusion:** The presence of carbon source at the optimum concentration is vital for effective decolouration by the test bacterial cells.

Key words: Glucose concentration, dye decolouration, wastewater, immobilized bacterial cells, free bacterial cells

Citation: Oghenerobor Benjamin Akpor, 2018. Dye decolouration by immobilized and free bacterial cells at different glucose concentration. Res. J. Environ. Sci., 12: 33-40.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Although the textile in industry is known to contribute substantially to the GDP and economy of a number of countries, textile fibers and raw material used for making textiles are known to have negative impacts on receiving water bodies and the environment[1]. It is reported that worldwide, an average of 10,000 different dyes and pigments are used industrially, with over 7×10^5 t of synthetic dyes produce annually[2].

Extreme concentrations of dyes in water bodies are known to impact the re-oxygenation ability of the water bodies and obstruct sunlight, thus disrupting the photosynthetic activity and other biological processes of the aquatic algae and plants. Some dyes are indicated to trigger DNA destruction, which may lead to benign tumors and cancer[3]. The increased likelihood of health hazard of dyes results from their uptake through the skin, lungs and the gastrointestinal tract[4].

Although several processes (such as electrocoagulation, electrochemical oxidation, photo assisted electrochemical methods, chemical oxidation and biological) for dye removal from water currently exists, biological processes are advocated in recent years because of their numerous advantages. Biological methods are advocated in recent years because of their environmental friendliness and cost effectiveness[5-8].

Bacterial breakdown and decolouration of dyes, either as single or mixed cultures have been reported in the past. Although mixed cultures have been reported to degrade azo dyes better than singles, it is opined that mixed cultures provide a general description of what is going on within the system without pointing out the individual action of the microorganisms thus producing results that are ambiguous. Several individual bacteria (*Pseudomonas* sp.; *P. mirabilis*, *P. luteola*, *Bacillus* sp. (*Exiguobacterium* sp., *Rhizobium radiobacter*) have however been implicated in dye decolouration[8,9].

Because one of the disadvantages associated with bioremediation is interruption of growth of microorganism due to dye product or the activity of other microorganisms, the use of immobilized cells in bioremediation studies are encouraged[10]. This study was therefore, aimed at assessing the relationship of glucose concentration to dye decolouration by free and immobilized cells of *Bacillus subtilis* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

The study was carried out between January and June, 2017 in Landmark University, Nigeria. Three laboratory dyes, (bromothymol blue, crystal violet and methylene blue) were used for the study. Before usage of the respective dyes for the study, wavelength that gave maximum absorbance was determined by scanning dilute solutions of the dyes at increasing wavelengths, starting from 400 nm until obtaining reaching a wavelength that gave no further increase in absorbance. The minimum wavelength that gave highest absorbance reading was regarded as the optimum. In this study, optimum wavelengths of 430, 520 and 600 nm were obtained for the bromothymol blue, crystal violet and methylene blue, respectively. In addition, calibration curves for the respective dyes were determined and equation of lines were obtained. Calibrations curves that were used had regression coefficients (R) values that were greater than 0.9.

The composition of the media used for the study was wastewater supplemented with 5 g L^{-1} sodium nitrate (to serve as nitrogen source), a known quantity of the respective dye and the respective concentrations of glucose (to serve as carbon source). The concentrations of glucose used for the study were 5, 10, 15 and 20 g L^{-1}. A control that contained no glucose (0 g L^{-1}) was also used.

During preparation, each of the media composition was first weighed and dissolved separately in little portions of the wastewater before combining all the constituents and making up to the required mark with the wastewater. The prepared media was first dispensed in 200 mL quantities in 250 mL capacity conical flasks before sterilizing at standard conditions in an autoclave.

Both free and immobilized cells of two bacteria species (*Bacillus subtilis* and *Pseudomonas aeruginosa*) were used for the study. The free cells were prepared by centrifuging nutrient broth for 24 h old cultures of the bacteria and suspending in sterile normal saline solution (0.85% NaCl w/v).

Immobilization of the cells was carried by mixing 100 mL of normal saline suspended free cells of the respective bacteria with 200 mL of sterile 2.5% sodium alginate. The mixture was allowed to react with constant agitation and for 2 h, after which the bacterial-alginate mixture was added dropwise to a flask that contained calcium chloride solution (2.5%) for bead formation and allowed to stand for 2 h at room temperature to allow the beads to harden. After t he beads had hardened,

they were washed several times with sterile distilled water to remove excess solution. The viability of the formed beads was ascertained by culturing selected representative beads in sterile nutrient agar plates and incubated for 24 h to check for growth. Only viable beads were used as immobilized cells for the study. All free and immobilized cells were kept in the refrigerator at 4±2°C until when needed.

For the experimental setup, to a 200 mL quantity of sterile media that contained the respective concentration of the glucose, a test bacterial cell (free or immobilized) was inoculated. Immediately after inoculation and every 24 h for a 144 h duration of incubation, aliquot samples were withdrawn from each flask and absorbance read at the optimum wavelength for the respective dye, after centrifuging at 5000 rpm for 10 min. All absorbance values were converted to concentrations (mg L^{-1}) and decolouration rates calculated. Decolouration rate was calculated as:

$$\text{Decolouration rate } (\%) = \frac{A - B}{A} \times 100$$

where, 'A' and 'B' represent initial absorbance and absorbance after incubation, respectively.

In all experimental setups, uninoculated controls were ran alongside the inoculated. All analyses were carried out in duplicate.

RESULTS

Generally, the decolouration of the bromothymol blue in presence of the free cells of the test bacterial species at the different concentrations of glucose showed a steady increase in decolouration rate with time. This trend was irrespective of the glucose concentration used for investigation. In presence of the *Bacillus subtilis* cells, decolouration rate was observed to be remarkably lowest in the media that contained no glucose while it was highest in the media that contained 5 g L^{-1} of glucose. From decolouration rates of -0.33, -0.53, 2.36, 6.69 and 5.53% at 24 h incubation, there were increases at the end of incubation to 14.52, 56.96, 30.48, 32.18 and 33.20% at glucose concentrations of 0, 5, 10, 15 and 20 g L^{-1}, respectively (Fig. 1).

When the free cells of the *Pseudomonas aeruginosa* were used for inoculation, highest and lowest decolouration rates at the end of incubation were observed in media that contained 20 g L^{-1} of glucose and the one that contained no glucose, respectively. Throughout the period of incubation,

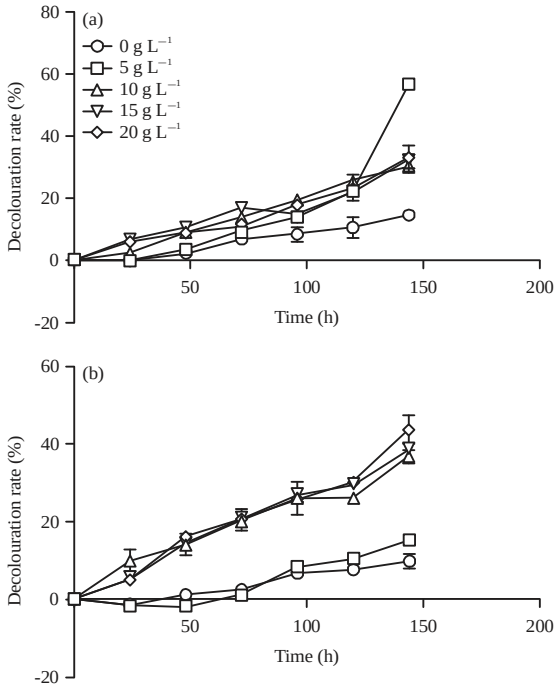

Fig. 1(a-b): Decolouration of the bromothymol blue by free cells of the test bacterial species (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa* at the respective glucose concentrations

decolouration rates at the different glucose concentrations ranged from -1.32 to 9.69, -1.69 to 15.26, 9.86-36.88, 5.39-38.60 and 4.99-43.76%, at 0, 5, 10, 15 and 20 g L^{-1} of glucose, respectively (Fig. 1).

In media that were inoculated with the immobilized cells of the bacterial species, bromothymol blue decolouration rate was also observed to show consistent increase with time of incubation, an observation that was irrespective of the glucose concentration used. Throughout the period of incubation, decolouration rate of the blue in presence of the *Bacillus subtilis* showed ranges from 0.20-24.11, 0.62-36.42, 6.77-51.96, 11.93-41.48, 4.96-33.29 and 8.61-43.74%, at glucose concentrations of 0, 5, 10, 15 and 20 g L^{-1}, respectively (Fig. 2).

Similarly, in presence of the *Pseudomonas aeruginosa* immobilized cells, decolouration rates of the bromothymol blue ranged from 2.79-21.20, 6.67-36.79, 1.76-42.78 and 8.61-43.74%, at glucose concentrations of 0, 5, 10, 15 and 20 g L^{-1}, respectively. The highest and lowest were observed at glucose concentrations of 20 g L^{-1} and media without added glucose (0 g L^{-1}), respectively (Fig. 2).

Decolouration of the crystal violet dye in the presence of free cells of the test bacterial species showed steady

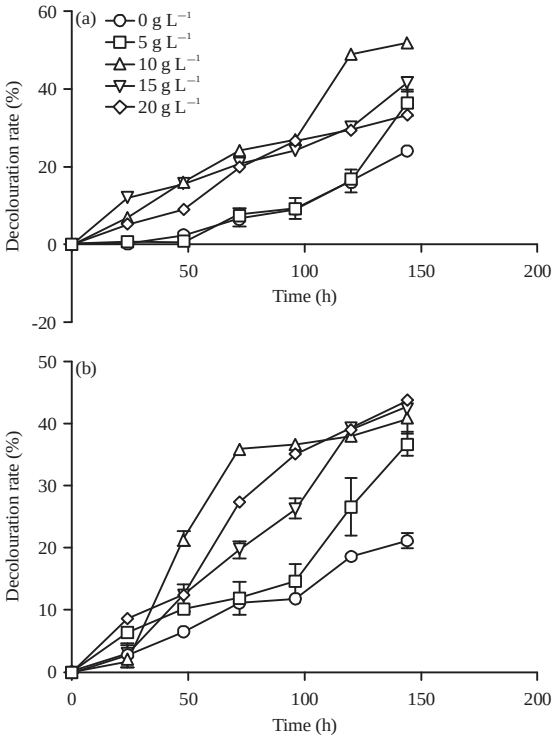

Fig. 2(a-b): Decolouration of the bromothymol blue by immobilized cells of the test bacterial species (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa* at the respective glucose concentrations

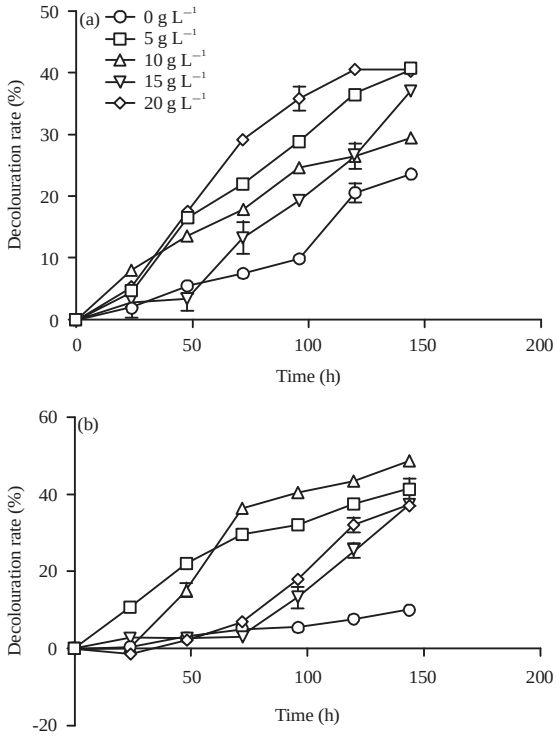

Fig. 3(a-b): Decolouration of the crystal violet by free cells of the test bacterial species (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa* at the respective glucose concentrations

decolouration rates irrespective of the isolate used for inoculation. In media that were inoculated with the *Bacillus subtilis*, the lowest and highest decolouration rates were observed in the media without glucose and at glucose concentration of 5 g L^{-1}, respectively. The decolouration rates of the crystal violet were observed to range from 2.06-23.51%, 4.64-40.73 g L^{-1}, 7.97-29.38 mg L^{-1}, 2.88-36.98 g L^{-1} and 15.26-40.35 g L^{-1}, at glucose concentrations of 0, 5, 10, 15 and 20 g L^{-1}, respectively (Fig. 3).

When inoculated with the free cells of the *Pseudomonas aeruginosa*, the decolouration rates of the crystal violet at glucose concentrations of 0, 5, 10, 15 and 10 g L^{-1} were observed to range from 0.45-9.90, 10.93-41.23, 002-48.87, 2.69-37.18 and -1.41 to 36.91%, respectively. The lowest and highest decolouration rates were observed in media without added glucose and 10 g L^{-1}, respectively (Fig. 3).

In the presence of the immobilized cells of the *Bacillus subtilis,* decolouration of the crystal violet dye was highest in media that contained 5 g L^{-1} of glucose. Remarkably high decolouration was however observed in media with 5 and 10 g L^{-1} glucose. At the end of the period of incubation, crystal violet decolouration rates of 17.69, 65.61, 52.86, 38.56 and 34.09% were recorded in media that contained 0, 5, 10, 15 and 20 g L^{-1} glucose, respectively (Fig. 4).

At the different concentrations of glucose in the media, decolouration rates of the crystal violet in presence of the immobilized cells of the *Pseudomonas aeruginosa* ranged between 3.54 and 18.10, 10.93 and 41.23, 0.02 and 48.77, 2.69 and 37.18 and between -1.41 and 36.91%, at 0, 5, 10, 15 and 20 g L^{-1}, respectively. The highest decolouration rate was however observed at glucose concentration of 10 g L^{-1} (Fig. 4).

In the case of the methylene blue dye, in presence of the free cells of the *Bacillus subtilis*, decolouration rate was observed to only be remarkable in media containing glucose concentrations of 15 and 20 g L^{-1}. At the different glucose concentrations, decolouration rates that ranged between 5.06 and 16.86, 0.99 and 21.22, 8.30 and 18.45, 3.40 and 33.60 and between 5.44 and 33.39% were observed at glucose concentrations of 0, 5, 10, 15 and 20 g L^{-1}, respectively (Fig. 5).

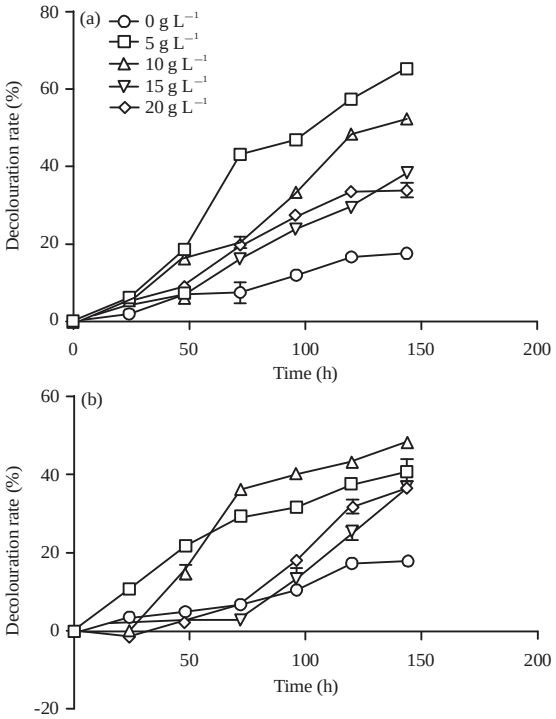

Fig. 4(a-b): Decolouration of the crystal violet by immobilized cells of the test bacterial species (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa* at the respective glucose concentrations

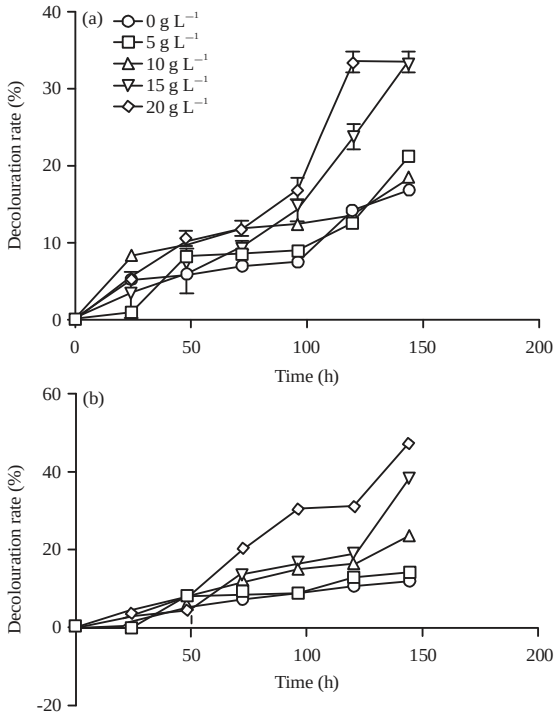

Fig. 5(a-b): Decolouration of the methylene blue by free cells of the test bacterial species (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa* at the respective glucose concentrations

For media inoculated with the free cells of the *Pseudomonas aeruginosa*, decolouration rate of the methylene blue was only remarkable at glucose concentrations of 15 and 20 g L^{-1}. Throughout the incubation period, decolouration rates were observed to range from 3.55-12.04, 0.10-13.93, 4.20-23.83, 2.53-38.42 and 2.82-47.83%, at glucose concentrations of 0, 5, 10, 15 and 20 g L^{-1}, respectively (Fig. 5).

In presence of the immobilized cells of the bacterial cells, decolouration rates of the methylene blue was observed to increase with time of incubation. This observation was irrespective of the cells used for inoculation. In presence of the *Bacillus subtilis*, highest decolouration rate was observed in media with glucose concentration of 5 g L^{-1}. At the expiration of the 144 h period of incubation, the observed decolouration rates were 18.78, 39.62, 18.45, 18.90 and 18.42%, at glucose concentrations of 0, 5, 10, 15 and 20 g L^{-1}, respectively (Fig. 6).

For media that was inoculated with the immobilized cells of the *Pseudomonas aeruginosa*, no remarkable decolouration of the methylene blue was observed at the different concentrations of glucose. Highest decolouration rate was however observed at glucose concentration of 20 g L^{-1}. During the incubation period, decolouration rate of the methylene blue varied from 0.4-9.44, 3.31-13.81, 1.09-14.39, 3.35-12.04 and 4.44-23.04%, at glucose concentrations of 0, 5, 10, 15 and 20 g L^{-1}, respectively (Fig. 6).

DISCUSSION

The external carbon source used for this study was glucose. The choice of glucose was deliberate. During reduction of azo dyes, it is generally reported that the presence of readily available substrates that act as electron donors for azo bond reduction is vital. Although a number of substrates, such as acetate, butyrate, propionate, lactose sucrose and starch have been used, several studies have reported the use of glucose as an ideal source of carbon and energy for azo decolouration of dyes[11,12].

For the decolouration of the bromothymol blue, the study revealed decolouration occurring at the different glucose concentrations used for investigation, although concentration range of 10 -20 g L^{-1} was observed to enhance decolouration

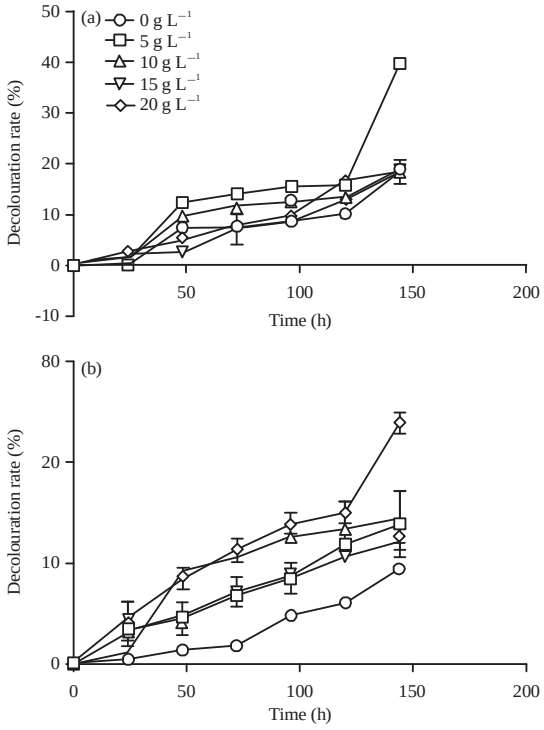

Fig. 6(a-b): Decolouration of the methylene blue by immobilized cells of the test bacterial species (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa* at the respective glucose concentrations

in presence of the free cells of the *Pseudomonas aeruginosa* and the immobilized cells of the *Bacillus subtilis.* For the crystal violet dye, optimum glucose concentration range of between 5 and 10 g L^{-1} was observed to be optimum for decolouration in presence of the free or immobilized cells. In the case of the methylene blue, optimum decolouration was observed in media that contained between 15 and 20 g L^{-1}, except in presence of the immobilized cells of the *Bacillus subtilis,* where maximum decolouration occurred in media that contained 5 g L^{-1} of glucose.

Wang *et al.*[13] have reported decolouration rate of 96.2% of reactive red 180 by *Citrobacter* sp. in presence of glucose of 4 g L^{-1}. In a similar study using *Pseudomonas putida*, a decolouration rate of 89.8% was observed for acid orange 7 in media that was supplemented with 0.4% (w/v) concentration of glucose in minimal salt media[14].

Optimum glucose concentration of 5 g L^{-1} have been reported during microbial decolouration of dye by earlier investigators[15]. According to findings by Singh *et al.*[16], optimum dye decolouration is observed in media with glucose concentration between the range of 5 and 7.5 g L^{-1}. Jain *et al.*[17] have also reported total decolouration at glucose concentration of 5 g L^{-1} glucose, while 85% decolouration occurred at higher glucose concentrations of 15-20 g L^{-1}.

In a study by Bheemaraddi *et al.*[18], it was reported that during the decolouration of a group of dyes, decolouration rate was observed to progressively increase with time, at glucose concentration of between 0.1 and 2.0 g L^{-1}. With the introduction of an extra 1 g L^{-1} of glucose in the media, there was total decolouration of the dyes within 24 h of incubation. It is reported that the reduced decolouration observed in media with high glucose concentrations could be due to the regulation of glucose metabolism, also known as catabolite repression[17]. During catabolite repression, it is indicated that there is a high possibility of ending the transcription of cyclic-AMP-dependent genes as a result of the presence of higher glucose levels[19].

In another related study, Garg and Tripathi[20] have indicated optimum glucose concentration of 4 g L^{-1} during decolouration of acid orange 7 by *Bacillus cereus*, with deviations from the optimum concentration of the glucose leading to inhibition and reduction of growth and extent of reduction, respectively. In a study on the dye decolourising potential of a novel fungus *Coriolus versicolor* ML04, it was reported that medium that contained 0.15% of glucose resulted in 78.32% decolouration, with higher concentration of glucose in the media leading to reduction of the dye within 48 h[21]. In addition, Shah *et al.*[22], when investigating the optimization of environmental parameters on decolorization of Remazol Black B, using mixed culture, they reported maximum decolorization occurring in media with 1% (w/v) glucose concentration.

The present study revealed decolouration in media that contained no glucose as external carbon source, although with reduced rate. In a study on the effect of various carbon and nitrogen sources on decolorization of textile dye remazol golden yellow using bacterial species, Palanivelan *et al.*[23] reported the presence of reduced decolouration rate in media that was supplemented with various carbon and nitrogen sources in the presence of the test bacterial species (*Bacillus*., *Micrococcus*., *Pseudomonas* and *Staphylococcus*) they used for investigation. Several azo-reducing bacteria, such as *Citrobacter*, *Acinetobacter*, *Pseudomonas* have been reportedly implicated to reduce azo compounds with molecular hydrogen or some short-chain fatty acids as electron donors, which may indicate that the coupling of the oxidation of electron donors to the reduction of azo compounds is a likely common biochemical process in nature[24].

CONCLUSION

From the findings of this study, both the free and immobilized cells of the test bacterial species showed remarkable potential in the decolouration of the dyes. A general trend in the study was progressive increases in decolouration of the dyes with time. This observation was irrespective of the dye investigated and the bacterial cells used for inoculation. The observation was also irrespective of the glucose concentration added to the media.

Although the presence of carbon source is vital during microbial decolouration of dyes, from the findings of this investigation, the presence of such carbon source at the optimum concentration is vital.

SIGNIFICANCE STATEMENT

This study discovers the possible role of immobilized and free cells of *Pseudomonas aeruginosa* and *Bacillus subtilis* in the decolouration of laboratory dyes in the presence of glucose as carbon source. The findings of this study could provide vital information in understanding and elucidating the role of the test bacterial in the possible treatment of textile effluents. In addition, based on the result of this investigation, future study can be done for scaling-up reactors and *in situ*. This will help to explore the prospect of creating enabling environment for the proliferation of the test bacterial species in wastewater treatment systems.

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