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Decolouration of laboratory dyes by immobilized cells of *Pseudomonas aeruginosa* and *Bacillus subtilis* at different carbon sources

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Abstract

Dyes are indicated as one of the most problematic compounds in industrial effluents. This is due to their high solubility and low degradability. The aim of the study was to evaluate the effect of external carbon source on decolouration of bromothymol blue, crystal violet and methylene blue by alginate immobilized cells of *Bacillus subtilis* and *Pseudomonas aeruginosa*. A total of five carbon sources (fructose, sucrose, glucose, methanol, lactose and sodium acetate) were used for the study. The study revealed highest decolouration of the bromothymol blue occurring in media that contained sodium acetate as external carbon source. Decolouration rate of bromothymol blue was observed to increase from 25.49 % and 18.17 % (at 24 h incubation) to 51.49 % and 31.94 % (at 144 h incubation), in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* immobilized cells. For decolouration of the crystal violet, glucose and sodium acetate were observed to be the most appropriate carbon sources. With glucose as carbon source in the media, decolouration rates of 57.48 % and 41.23 % at the end of incubation were observed for the crystal violet dyes in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* cells, respectively. None of the carbon sources used for the investigation was found to enhance decolouration of the methylene blue by the test bacteria species. The study revealed the possible application of the immobilized cells in scale up studies for the remediation of textile effluents.

Keywords: Decolouration, Dye, Immobilized bacteria, Wastewater

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Introduction

It is estimated that over ten thousand various dyes are prepared all over the world and about 8×10^5 tons of artificial dyes are used in textile, paper and leather industries globally (Walker and Weatherley, 1997). Usually, a lot of wastewater containing dyes and other pollutants are discharged by these industries. Most of these dyes are not affected by factors like temperature, pH, light and attack from microbes thus contributing to organic load and toxicity of the wastewater (Nigam et. al., 2000). The non-biodegradable characteristic of the dyes poses a serious environmental threat. The colour of wastewater is aesthetically unpleasant to aquatic bodies which hinders gas solubility in water, hinder photosynthesis and generally disrupt the whole of the aquatic ecosystem and food chain (Xu et. al., 2004).

Dyes have been identified as one of the most problematic compounds in industrial effluents due to their higher water solubility and lower degradability. The removal of dyes before discharging them into natural water streams is essential. Numerous studies have shown that bacteria can completely degrade and mineralize various dyes under suitable conditions and considering the cost-effectiveness, ease and eco-friendliness of this method in comparison to conventional methods, biological methods of removing dyes from wastewater are very efficient (Kapdan and Erten, 2007; Darwesh et. al., 2008; Moawad et. al., 2010). Many bacterial

strains that have the ability to decolorize a wide-range of dyes have been identified and characterized (Deng et. al., 2008).

Several chemical, physicochemical and electrochemical methods, such as oxidation, electrochemical coagulation, floatation, ozonation, membrane filtration and ion exchange have been used for decolorization of dyes in wastewater (Robinson et. al., 2001, Gupta and Rastog, 2008). These methods are less effective, expensive, of limited use, and generate wastes, which are difficult to dispose. On the other hand, biological methods provide a cheaper and affordable, environmentally safe, and effective substitute for the removal of dye from wastewater (Ali et. al., 2009).

Numerous microbial strains having the ability to decolorize a large variety of dyes belonging to different groups have been isolated and studied by many researchers and scientists (Sushama et. al., 2009). These biological methods are eco-safe and affordable thus serving as a healthy substitute to other conventional means (Verma et. al., 2003). This study was therefore aimed at evaluating the effect of external carbon sources on decolouration of laboratory dyes by immobilized bacterial cells under batch experimental conditions.

Materials and Methods

A total of three laboratory dyes were used for this study. The dyes were bromothymol blue, crystal violet and methylene blue. Prior to use, 0.1 % of each of the dyes was prepared as stock solution by dissolving 0.1 g in 100 mL of distilled water.

Before the study, the optimum wavelength for maximum absorbance of each of the dyes was determined as follows. To a clean and dry test tube, 0.5 ml of the stock solution of a respective dye was dispensed and diluted with distilled water. This diluted solution was then dispensed into a cuvette and the absorbance read at different wavelengths starting from 400 nm until no further increase in absorbance was observed with increase in wavelength. The wavelength that gave maximum absorbance reading for each dye was taken as the ideal wavelength for that particular dye. Wavelengths of 430 nm, 520 nm, and 600 nm were obtained as optimum for the bromothymol blue, crystal violet and methylene blue, respectively. A calibration curve for each dye was determined and standard curve and equation of a line obtained. All calibration curves had regression coefficients (R) values of not less than 0.9

The media used for the study was wastewater obtained within the environ of Landmark University, Omu-Aran, Kwara State, Nigeria. The wastewater was first filtered, using Whatman No 1 filter paper before adding 5 g/l of sodium nitrate (as nitrogen source), 5 g/l of the respective carbon source and 0.5 % of the respective dye.

In preparation of the media, each of the constituents was weighed and diluted separately in little portion of the wastewater before making up to the required mark. Before sterilization, the media was dispensed in 200 ml quantity in 250 ml Erlenmeyer's flasks and plugged with cotton wool. Sterilization was carried out using an autoclave at 121 °C for 15 min at 15 psi.

The test bacteria used for this experiment were immobilized cells of *Bacillus subtilis* and *Pseudomonas aeruginosa*. Immobilized cells were prepared by mixing 100 ml of cells suspended in normal saline with 200 ml of sterile 2.5 % sodium alginate and allowed to react in a shaker at a shaking speed of 100 rpm for 2 h. At the end of the shaking, the microbe-alginate mixture was dropped (using a burette) into a flask containing sterile 2.5 % calcium chloride solution for bead formation. The formed beads were allowed to harden for 2 h, at room temperature, before washing out the excess solution with sterile distilled water. To ascertain the viability of the formed beads, aliquot samples were cultured on sterile nutrient agar plates and incubated overnight to check for growth. The presence of growth confirms the viability of the immobilized cells. The viable beads were kept refrigerated at 4 ± 2 °C until when needed.

For the decolouration study, to a 200 ml quantity of sterile media containing a known carbon source, the respective immobilized cells were inoculated..

A total of five carbon sources (fructose, sucrose, glucose, methanol, lactose and sodium acetate) were used for the study. Prior to inoculation and every 24 h for 144 h, aliquot samples were withdrawn from each flask, centrifuged at 5000 rpm for 10 min before taking the absorbance readings with a spectrophotometer at the optimum wavelength for the respective dye. The absorbance values were converted to concentrations (mg/l), using the equation of the line obtained from the calibration curve for each dye.

Decolouration rate was calculated as:

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

Where 'A' represents initial absorbance, 'B' represents absorbance after reaction.

Results

When sodium acetate was used as carbon source in the media, decolouration rates of the dyes in presence of the immobilized cells showed consistent increases with time of incubation. Remarkable decolouration of the bromothymol blue and crystal violet dyes was observed only in media inoculated with the *Bacillus subtilis*. Decolouration rate of bromothymol blue was observed to increase from 25.49 % and 18.17 % (at 24 h incubation) to 51.49 % and 31.94 % (at 144 h incubation), in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* immobilized cells, respectively (Fig. 1).

Similarly, in media containing the crystal violet dye, decolouration rates of 12.17 % and 15.13 % were observed after 24 h incubation, in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* immobilized cells, respectively. At the expiration of the 144 h incubation, decolouration rates showed increases to 59.64 % and 29.80 %, respectively (Fig. 1). For media containing the methylene blue dye, only slight decolouration was observed throughout the period of incubation. This trend was irrespective of the immobilized cells used for inoculation. Decolouration rate was however observed to show increase in presence of the *Bacillus subtilis* from 4.51 % to 13.96 % at 24 h and 144 h, respectively. In presence of the Pseudomonas aeruginosa, an increase in decolouration rate from 8.96 % (at 24 incubation) to 17.09 % (at 144 h incubation) was observed (Fig. 1).

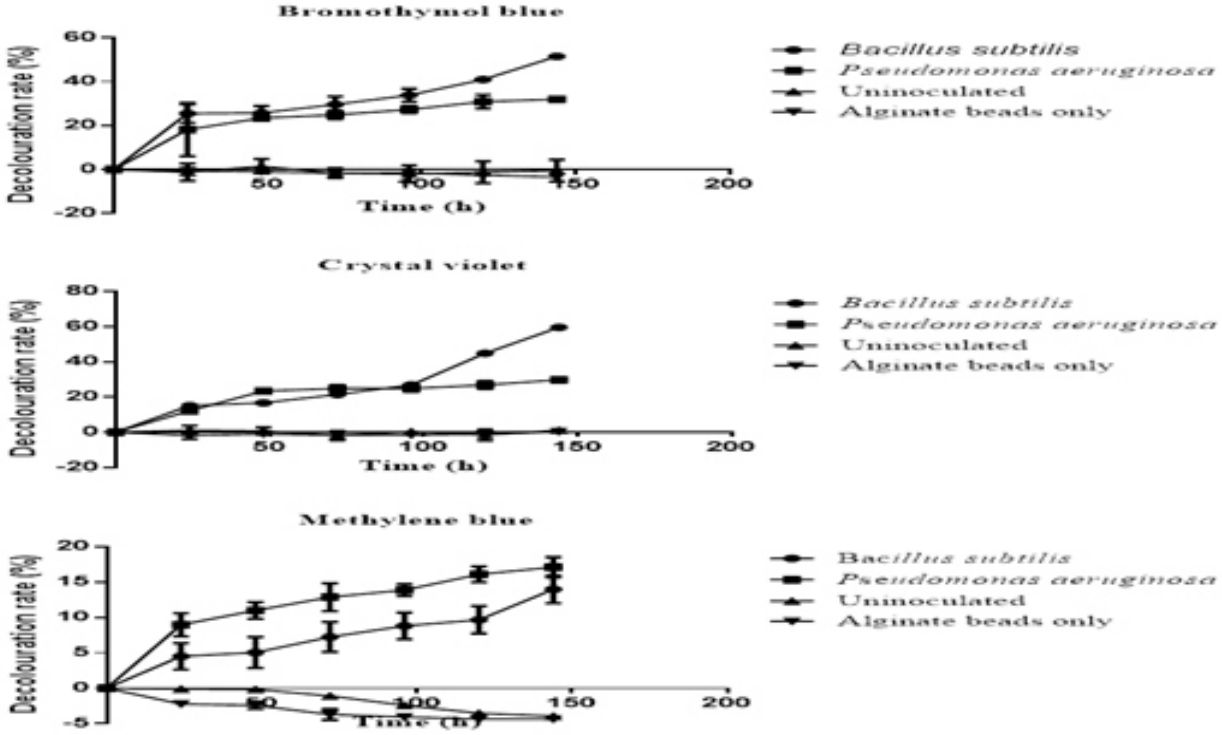

Fig. 1: Decolouration rates of the dyes in presence of immobilized cells, when sodium acetate was used as carbon source

With glucose as carbon source in the media, remarkable decolouration rates of 57.48 % and 41.23 % at the end of incubation were observed for the crystal violet dyes in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* cells, respectively. Only minute decolouration was observed for the bromothymol blue and methylene blue dyes in presence of the cells. In presence of the immobilized cells, decolouration rates of the dyes showed consistent increases with time of incubation (Fig. 2).

In presence of the *Bacillus subtilis*, decolouration rates of the dyes after 24 h incubation was observed to be 0.53 %, 2.12 % and 0.37 %, for the bromothymol blue, crystal violet and methylene blue, respectively. At the end of the 144 h incubation period, decolouration rates of 29.98 %, 57.48 % and 19.73 %, were observed, respectively (Fig. 2).

When *Pseudomonas aeruginosa* cells were used for inoculation, decolouration rates after 24 h period of incubation were 6.46 %, 1.02 % and 3.21 %, for the bromothymol blue, crystal violet and methylene blue, respectively. At the expiration of the 144 h incubation time, decolouration rates of 36.79 %, 41.23 % and 11.80 % were observed, respectively (Fig. 2).

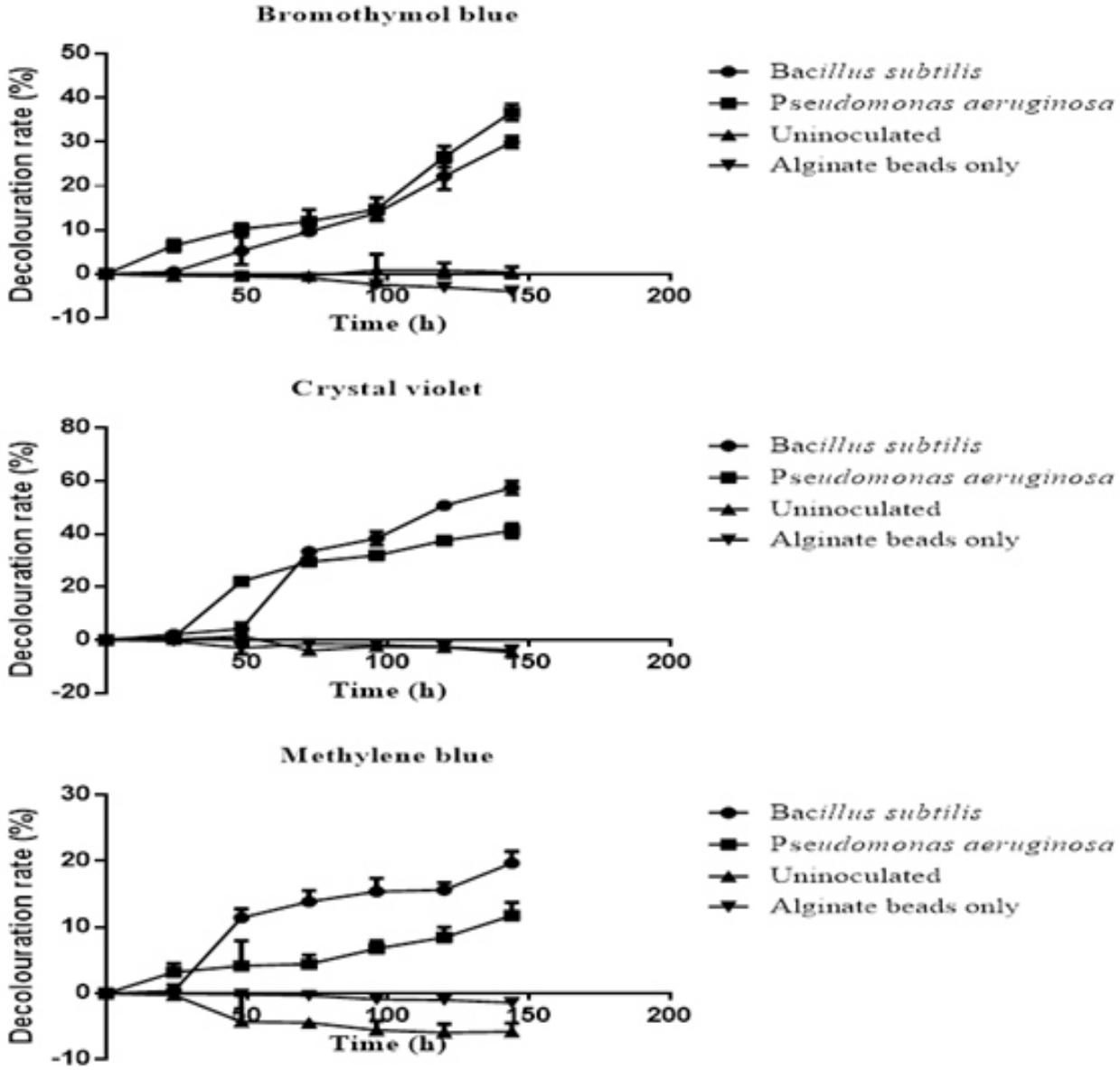

Fig. 2: Decolouration rates of the dyes in presence of immobilized cells, when glucose was used as carbon source

In media that contained fructose as carbon source, although decolouration was observed to show increase with time of incubation in presence of the immobilized cells, remarkable decolouration at the end of incubation was only observed in presence of the *Bacillus subtilis* cells for the bromothymol blue dye. Only slight decolouration was observed for the crystal violet and methylene blue throughout the period of incubation. This trend was irrespective of the cells used for inoculation (Fig. 3).

For the bromothymol blue dye, decolouration rates of 65.17 % and 32.02 % were observed at the end of incubation in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* cells, respectively. In media that contained the crystal violet dye, rates showed increases from 1.15 % and 10.28 % after 24 h period of incubation to 30.35 % and 29.17 % after 144 h incubation in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* cells, respectively (Fig. 3).

Decolouration rates of the methylene blue dye was observed to increase from 2.46 % and 4.37 %, after 24 h incubation to 8.14 % and 13.78 % in media inoculated with the *Bacillus subtilis* and *Pseudomonas aeruginosa* cells, respectively (Fig. 3).

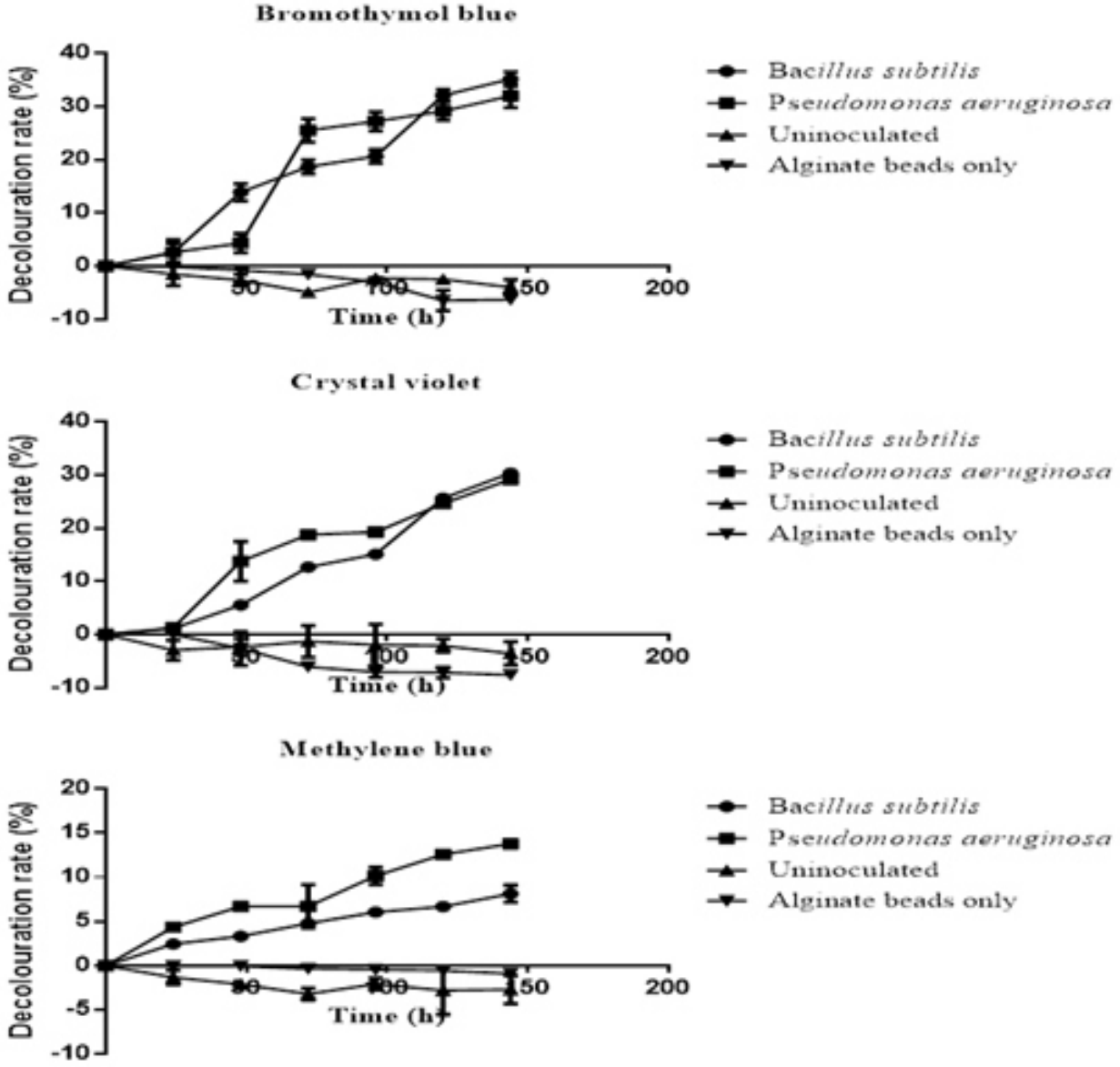

Fig. 3: Decolouration rates of the dyes in presence of immobilized cells, when fructose was used as carbon source

When lactose was used as carbon source in the media, decolouration rate of the dyes was observed to be minute throughout the incubation period, except for crystal violet in presence of the *Bacillus subtilis* cells. This observation was irrespective of the dyes and the cells used for inoculation. The decolouration rates of the bromothymol blue in presence of the *Bacillus subtilis* was observed to be 3.85 % and 35.75 % at 24 h and 144 h, respectively. In presence of the *Pseudomonas aeruginosa* cells, decolouration rates of 0.99 % and 20.43 % were observed, respectively (Fig. 4).

For the crystal violet dye, decolouration rates of 4.87 % and 2.0 % were observed after 24 h of incubation in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* cells, respectively. At the expiration of incubation, decolouration was observed to increase to 47.81 % and 22.72 %, respectively (Fig. 4). In media that contained the methylene blue, decolouration rate was observed to increase from 3.45 % and 7.20 %, after 24 h incubation to 17.97 % and 11.80 % at the end of the 144 h incubation period, in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa* cells, respectively (Fig. 4).

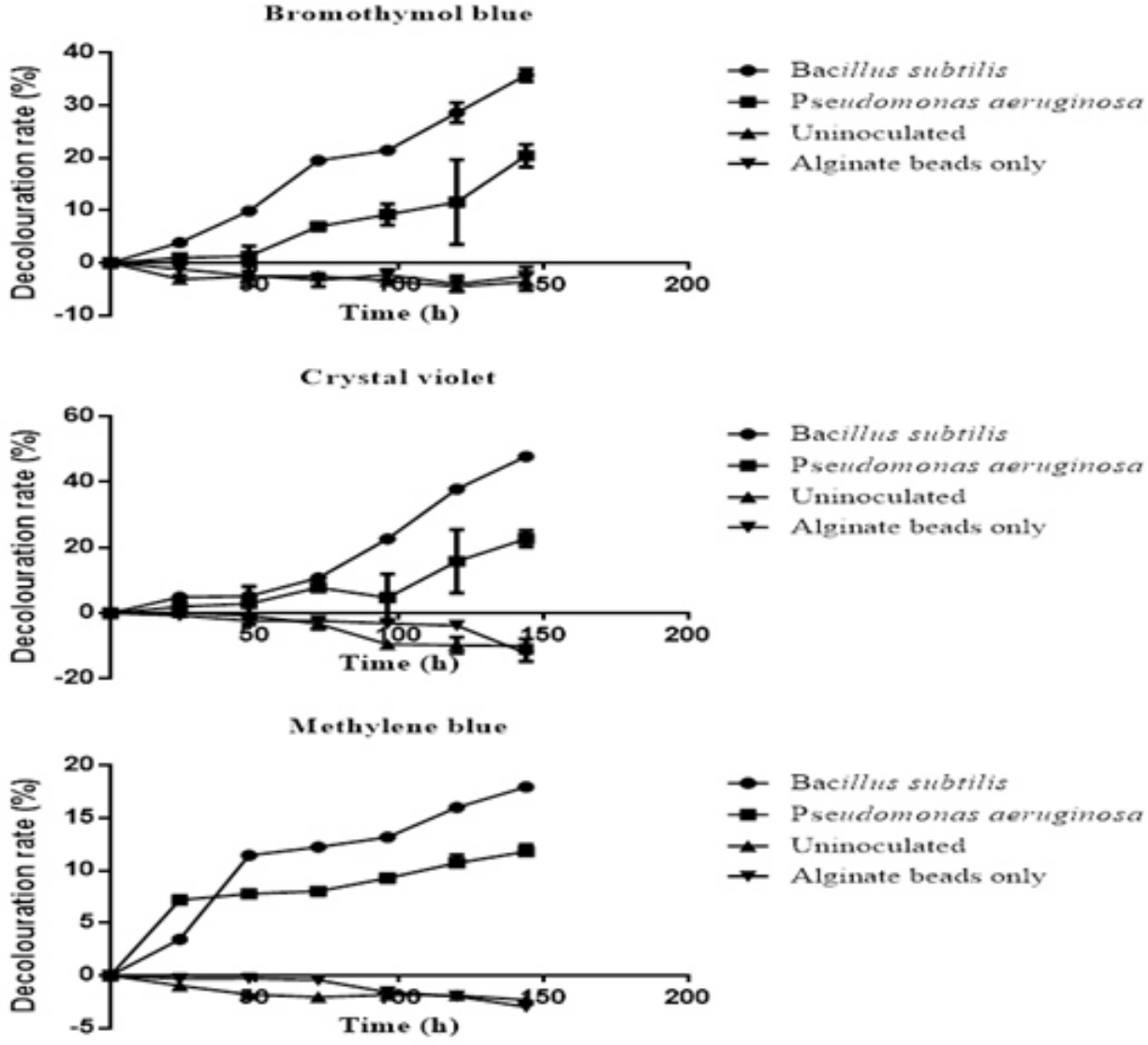

Fig. 4: Decolouration rates of the dyes in presence of immobilized cells, when lactose was used as carbon source

In presence of sucrose as carbon source, despite the fact that decolouration rates of the respective dyes were observed to show increases with time of incubation, no remarkable decolouration was observed throughout the period of incubation. This observation was irrespective of the cells used for inoculation. For the bromothymol blue, decolouration rate of 4.64 % and 4.03 % were observed after 24 h incubation in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively. At the end of the 144 h incubation time, decolouration rate was observed to be 37.19 % and 29.43 %, respectively (Fig. 5).

In the case of the crystal violet dye, decolouration rates of 21.39 % and 32.56 % were observed at the expiration of incubation in presence of the *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively. For the methylene blue dye, decolouration rates increased from 2.64 % and 7.11 % at 24 h incubation to 14.77 % and 17.73 %, for media inoculated with the *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively (Fig. 5).

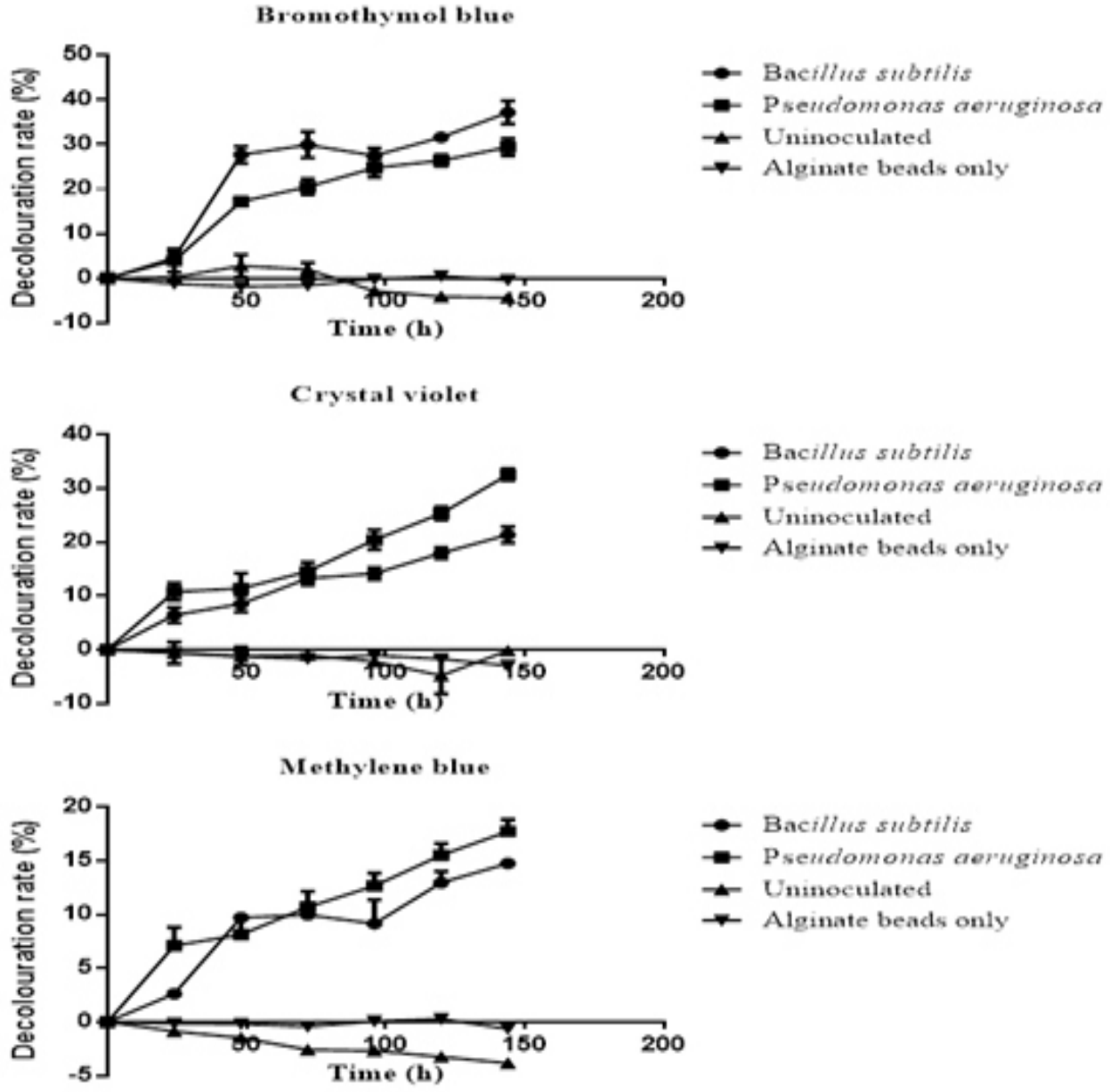

Fig. 5: Decolouration rates of the dyes in presence of immobilized cells, when sucrose was used as carbon source.

When methanol was used as carbon source in the media, as was observed for the other carbon sources, decolouration rate of the dyes showed consistent increases with time. This trend was irrespective of the cells used for inoculation and the test dye used for investigation. In the presence of the *Bacillus subtilis* cells, decolouration rates of the dyes showed increases from 5.20 %, 15.15 % and 1.52 % after 24 h incubation to 38.05 %, 39.53 % and 12.49 % at the expiration of incubation time, for the bromothymol blue, crystal violet and methylene blue, respectively (Fig. 6).

In presence of the *Pseudomonas aeruginosa* cells, decolouration rates of 4.81 %, 8.37 % and 1.37 % were observed after 24 h incubation, for the bromothymol blue, crystal violet and methylene blue, respectively. After the 144 h incubation period, decolouration rates of 17.25 %, 25.96 % and 15.96 %, were observed, respectively (Fig. 6).

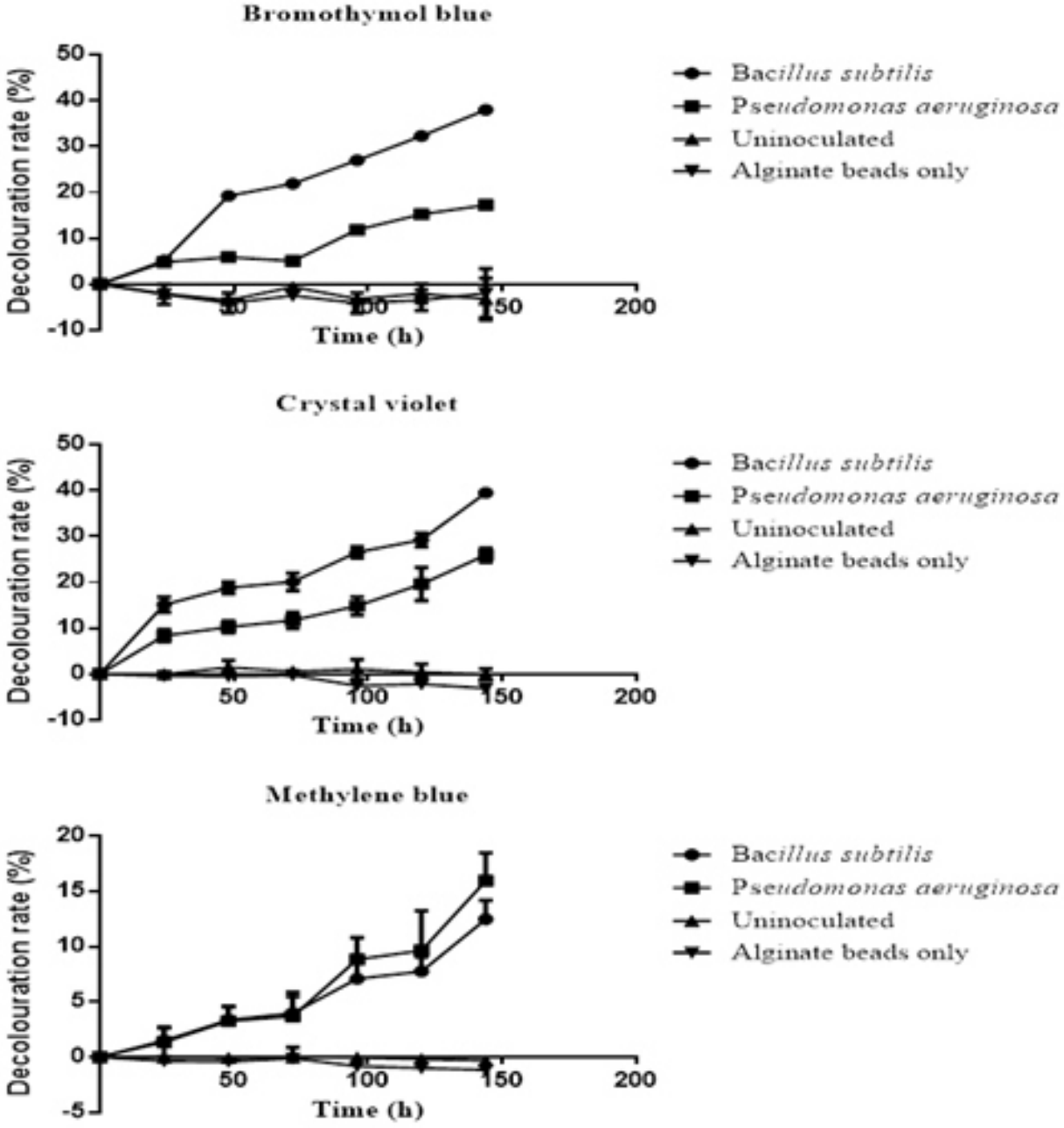

Fig. 6: Decolouration rates of the dyes in presence of immobilized cells, when methanol was used as carbon source

Discussion

The test dyes used in this study (bromothymol blue, crystal violet and methylene blue) are indicated to belong to the triarylmethane group of dye and are artificial dyes used mainly in the textile, cosmetics and food industries and for histological and biological staining in laboratories and their application makes up a large proportion of the total usage of dyes (Gregory, 1993). Their use in this study was because that they form a large representation of dyes in general, which are affordable and easily accessible (Carliell et. al., 1998).

Five different external carbon sources (fructose, glucose, lactose, sucrose, methanol and sodium acetate) were used for the study. Similar carbon sources have been used by earlier workers in related studies (Durve et. al., 2012; Akpor et. al., 2016). External carbon sources are required for metabolic activities of microorganisms to take place (Reddy, 1995). The metabolism of these carbon sources may result in the production of electron donors which cleave to the chromophoric group of the dyes to break them down (Oranusi and Ogugbue, 2005a). It is reported that during dye decolouration under aerobic conditions, the ready cleavage of dyes occurs through four electron reductions at the azo linkage, thus producing colourless aromatic amines. The required electrons are supplied by the presence of carbon source, such as glucose, fructose, sucrose starch, etc., which donate electrons, although the required carbon source defies from microbial species to species (Garg and Tripathi, 2017).

In this study, the decolouration of the bromothymol blue in presence of the immobilized cells of the *Bacillus subtilis* and the *Pseudomonas aeruginosa* occurred more effectively, when sodium acetate was used as the external carbon source. However, decolouration of the crystal violet was more evident in presence of either sodium acetate or glucose, as respective carbon sources. None of the carbon sources was able to enhance the decolouration of the methylene blue in presence of the isolates. Aruna and co-workers (2015) have reported glucose to be a more effective carbon source for the decolouration of the bromothymol blue, when compared to other carbon sources. A similar observation has also been reported by Mohan et. al. (2012). In the study by Oranusi and Ogugbue (2005b), during decolouration of the crystal violet in the presence of glucose and starch as carbon sources, glucose influenced greater decolouration than the starch. Maximum decolouration of 90.2 out% for crystal violet was obtained at glucose concentration than with any other carbon source used.

Furthermore, in a study carried out by Fouda et al. (2016), sucrose and glucose served as the most preferable carbon sources for the highest decolouration of disperse yellow in comparison with other carbon sources like fructose, maltose and starch. Furthermore, decolouration of this dye was actually activated in presence of sucrose or glucose and they provided suitable growth conditions for the organisms, for enzymatic actions and dye decolouration (Fouda et al. 2016). Earlier investigators have found the simplest carbohydrate as the most preferred carbon source among all others for decolouration (Mallikarjun et. al., 2014). Mohan et. al. (2013) have reported that glucose influenced decolouration of azo dyes more by organisms more than those found with addition of other carbon sources

Although some studies have reported the effective decolouration of methylene blue in presence of glucose as carbon source, none of the carbon sources used in this study could enhance decolouration of methylene blue in presence of the test organisms. The finding of this study negates the report of Jennifer and Kavitha (2015) who indicated decolouration of methylene blue occurring best in presence of glucose as a carbon source.

Conclusion

This study which investigated the effect of carbon source on decolouration of dyes by immobilized cells of the test bacterial species used reveal that decolouration of bromothymol blue, crystal violet and methylene blue is dependent on the type of carbon source. Among the different carbon sources used for the study, sodium acetate was observed to enhance decolouration of the bromothymol blue in presence of the test bacterial species.

Glucose and sodium acetate were found to be the ideal carbon source during decolouration of the crystal violet dye in presence of the immobilized cells. However, decolouration of the methylene blue dye in presence of the immobilized cells was not enhanced by any of the carbon sources used in the media

Despite the findings of this study, there is also the need for further studies on the use of the immobilized cells in scale up experimental setup and possible application in situ, which is the focus of our next investigation.

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