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Decolouration of Crystal Violet by Immobilized and Free Cells of *Pseudomonas aeruginosa*

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Authors' contributions

This work was carried out in collaboration between all authors. Author OBA was involved in the conceptualization of the study, designed the study, interpreted the results, performed the statistical analysis and the writing of the first draft of the manuscript. Author SCO carried out the experimental analysis, interpreted the results and contributed to the first draft of the manuscript and author COA interpreted the results and contributed to the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This investigation was aimed at ascertaining the effects of pH, effect of temperature and effect of sodium acetate concentration on crystal violet decolouration by immobilized and free cells of *Pseudomonas aeruginosa* in a nutrient. The immobilized cells were immobilized sodium alginate solution, agarose and agar in a shaking flask at a speed of 120 revolution/second. Although four dyes were initial selected for the preliminary stage of the study, detailed investigation was carried out using the crystal violet. Prior to inoculation with the respective cells and every 24 h for 120 h, aliquot samples were taken for the estimation of dye using standard procedures. The results revealed highest decrease in the concentration of crystal violet in the presence of the cells was observed at incubation temperature of 35°C. The optimum pH for crystal violet decolouration was shown to be between 8 and 10. Despite the highest decrease in crystal violet concentration in presence of the cells being observed at pH 8 and 10, the decreases did not differ significantly at the different pH. At the different sodium acetate concentrations used in the media, highest decreases in crystal violet concentration in presence of the respective cells was observed at

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20 g/L. The present study was able to provide information on the role of the immobilized and free cells of the test bacterial in crystal violet decolouration under the conditions investigated.

Keywords: Decolouration; Pseudomonas aeruginosa; immobilized cells; dye; wastewater.

1. INTRODUCTION

Most dyes are composed of a complex molecular structure comprising of aromatic rings, azo-bonds and sulphonic acid which increases their capacity to prevent elimination from the surrounding. These complex molecules are aromatic molecular structures which originated from coal-tar based hydrocarbons, such as naphthalene, toluene, benzene, anthracene, and xylene [1,2]. Irrespective of their concentrations, most dyes are harmful and destructive to water. In industrial effluents, the concentration of dye present is said to range between 10 mg/L and 25 mg/L, with concentrations of less than 1.0 mg/L indicated to lead to obvious water coloration [3-5].

Based on their source and molecular structures, dyes are grouped as either anionic or cationic. Anionic dyes, also referred to as acidic dyes, carry net negative charges due to the presence of sulphonate groups while cationic dyes, also known as basic dyes, carry net positives charges when ionized [6,7]. The structural diversity of dyes comes from the use of different chromophoric groups such as azo, anthraquinone, triarylmethane and phthalocyanine groups and different application technologies such as reactive, direct, disperse and vat dyeing [8].

It is opined that textile industries have the largest amount of aqueous waste and dye effluents, which when discharged into receiving water bodies could cause persistent colour and high biological oxygen demand on such waters and making them aesthetically and environmentally unacceptable Dyes in wastewater are highly persistent toxigenic, mutagenic and also carcinogenic. They disrupt the endocrine systems and are not completely removed during treatment processes or might be degraded into other by-products that are sometimes more toxic than the parent compounds. Therefore, it is very important to explore new treatment technologies and/or combine the present treatment processes that will ensure effluent discharge standards and total removal of organic dyes is achieved [9-11]. The aim of this study was to investigate the role of immobilized and free cells of *Pseudomonas aeruginosa* in crystal decolouration in nutrient media.

2. MATERIALS AND METHODS

2.1 Test Dye and Medium Composition

The dye used for this study was crystal violet. Before usage, a stock solution of the crystal violet (0.1%) was prepared and stored at room temperature until when needed.

The media used for the study had the following composition: sodium acetate (5 g/L), magnesium sulphate (0.5 g/L), potassium dihydrogen phosphate (0.5 g/L), potassium nitrate (0.5 g/L), peptone (5 g/L), yeast extract (5 g/L) and a known quantity of the required dye. Before usage, the media was sterilized in an autoclave at 121°C and 1.05 kg/cm^3 for 15 min. To ascertain the sterility of the media, an aliquot of the cooled media was plated in sterile nutrient agar and incubated for 24 h. Only media which showed no growth in the nutrient agar were used for the study.

2.2 Cell Immobilization

The test bacteria used for the study was *Pseudomonas aeruginosa* ATCC 9027 P-1. The bacteria were first cultured in sterile nutrient broth before streaking on sterile nutrient agar plate to ascertain that it was a pure isolate. Both the free and immobilized cells of the bacteria were used for the study. Sodium alginate with calcium chloride, agarose and agar-agar powders were used as respective matrices for immobilization of the cells.

For the preparation of the free cells, a 24 h broth culture of the bacteria centrifuged using a centrifuged (ANKE TDL- 5000B) at 5000 rpm for 30 min after which the supernatant was discarded while the cell pellets were suspended in 100 mL normal saline solution (0.9% NaCl). Before usage, a known dilute quantity of the suspended cells was plated in sterile agar in petri dishes, using the standard pour plating technique. The plates were then incubated for 24 h at 37°C ± 2°C. At the expiration of incubation, the number of colonies growing in each plate were counted and expressed as colony-forming

unit per millitre (cfu/mL) of suspended solution. The suspended cells were stored in the refrigerator at 4°C ± 2°C until when needed.

In the preparation of the alginate-immobilized cells, to a sterile 150 mL quantity of 5% sodium alginate solution, 20 mL of the normal saline suspended cells were added and allowed to stand for 2 h. The alginate cell mixture was then pipetted dropwise into a flask containing 2.5% calcium chloride solution to serve as a crosslinking solution for the formation of beads. After the formation of beads, the solution was allowed to stand for 3 h to allow the beads to harden before washing out the sodium alginate-calcium chloride solution from the beads, using sterile distilled water that have been previously allowed to cool. The washed beads were then stored in sterile bottles in a refrigerator at 4°C ± 2°C. The approximate number of cells in a bead was estimated by suspending known number of beads in 20 mL of sterile nutrient agar that have been cooled to 45°C. The agar containing the beads was then poured in a petri dish and incubated for 24 h at 37°C for growth. At the end of incubation, the number of colony-forming units were counted and estimated as colony-forming units per bead.

For the agarose and agar immobilized cells, 6% and 3% solutions were used, respectively. To 150 mL quantities of the respective sterile agarose and agar solutions, 20 mL of the normal saline suspended cells were added. Before adding the cell suspensions, the agarose and agar solutions were allowed to cool to about 45°C. The respective agarose or agar-agar cell mixture were vortexed and poured in petri dishes, then allowed to solidify. After solidifying, the respective beads were made using sterile cork borers and stored in sterile bottles before incubating at 4°C ± 2°C in a refrigerator. Viability of the beads were determined by suspending known number of beads in 20 mL of sterile nutrient agar that have been cooled to 45°C. The agar containing the beads was then poured in a petri dish and incubated for 24 h at 37°C for growth.

2.3 Dye Removal Study

For the experimental setup, to each 250 mL capacity conical flask containing 200 mL of the media, a known inoculum of the respective free and immobilized cells was inoculated under aseptic conditions before incubating at a known temperature in orbital shakers (STUART: S1500). Just before inoculation and every 24 h, for the 120 h, aliquot samples were aseptically withdrawn from each flask for the estimation of residual dye in the media.

The study was carried out in batches by varying incubation temperatures (25°C, 35°C and 45°C), pH (6, 8 and 10) and sodium acetate concentrations (10 g/L, 15 g/L and 20 g/L). All experimental setups were carried out in duplicate. For each batch experiment, uninoculated control setups were run concurrently with inoculated setup.

Dye concentration in the media was determined by taking the absorbance reading of the media after first centrifuging at 5000 rpm for 30 min. Aliquots of the homogenized supernatant was placed in a cuvette and absorbance read in a spectrophotometer (Jenway 6705 UV/VIS) at a wavelength of 500 nm. The ideal wavelength was determined by first scanning samples of the dye at different wavelengths. The wavelength that produced the highest absorbance was used for the study.

In all, dye concentration was estimated from a calibration curve that was obtained for a stock solution of the dye, from which different concentrations were prepared. Absorbance values obtained for samples were converted to concentrations using the equation of the line obtained for a dye.

3. RESULTS AND DISCUSSION

3.1 Decolouration Studies at the Different pH

Fig. 1, shows the trend in crystal violet concentration in the media at different pH in presence of the alginate-immobilized cells. As shown in the figure, significant decrease in the concentration of crystal violet was observed between 48 h and 72 h, after which increases were observed with time. After 48 h incubation period, crystal violet concentration decreased from initial concentrations of 63.36 mg/L to 0.65 mg/L, from 67.46 mg/L to 2.82 mg/L and from 61.23 mg/L to 12.16 mg/L, at pH 6, 8 and 10, respectively. At the conclusion of 120 h incubation period, crystal violet levels in the media were 25.6 mg/L, 50.79 mg/L and 64.90 mg/L, at pH 6, 8 and 10, respectively (Fig. 1). Although the concentrations of crystal violet in the media were observed to differ throughout the period of incubation, these differences were not observed to vary significantly between the different pH ($p \leq 0.05$).

At the close of the 120 h of incubation, reduction in the crystal violet concentration in the media in presence of the agarose-immobilized cells, was observed after 48 h incubation to vary from the initial concentration of 63.36 mg/L, 67.46 mg/L and 61.23 mg/L to concentrations of, 33.85 mg/L, 6.73 mg/L and 6.29 mg/L, at pH 6, 8 and 10, respectively, after 48 h of incubation. After the 120 h incubation period, crystal violet concentration in the media was observed to be 97.03 mg/L, 33.43 mg/L and 69.89 mg/L, at pH 6, 8 and 10, respectively (Fig. 1). Despite the differences in crystal violet concentrations at the different pH, these differences were not observed to be significant ($p \leq 0.05$).

In presence of the agar-immobilized cells, crystal violet concentration after 48 h incubation was observed to decrease significantly from 63.60 mg/ to 1.95 mg/L at pH 6, from 67.46 mg/L to 12.39 mg/L, at pH 8 and from 61.23 mg/L to 12.81 mg/L at pH 10. At the conclusion of the 120 h incubation period, crystal violet levels in the media were 16.5 mg/L, 7.19 mg/L and 0.43 mg/L at pH 6, 8 and 10, respectively (Fig. 1). Generally, despite the difference in crystal violet concentration in the media at the different pH, these differences were not observed to be significant ($p \leq 0.05$).

At the different pH, crystal concentration in the media in presence of the free cells suspended in normal saline showed remarkable decrease after 24 h, at pH 8 and after 48 h, at pH 6 and 10. After 48 h of incubation, concentrations of crystal violet showed significant reduction from 63.36 mg/L to 16.01 mg/L, at pH 6, from 67.46 mg/L to 11.28 mg/L, at pH 8 and from 61.23 mg/L to 14.11 mg/L, at pH 10. At the expiration of incubation, concentrations of the crystal violet were 59.05 mg/L, 67.07 mg/L and 14.54 mg/L, at pH 6, 8 and 10, respectively (Fig. 1). No significant difference was observed between crystal violet concentrations in the media at the different pH ($p \leq 0.05$).

Table 1 shows the variation in pH of the media at the different initial pH investigated. As shown in the Table, at pH 6 and 8, in presence of the respective immobilized and free cells, increases in pH of the media were observed at the expiration of incubation. At pH 10, decreases in pH were observed. These trends were irrespective of the immobilized or free cells used for inoculation.

The use of immobilized and free cells in this investigation was deliberate. It is hypothesized that immobilizing bacteria will increase the density of bacteria within the bioreactor which can in turn increase the rate of degradation in a media. Into diffuse a media that contains immobilized cells, the substrate is indicated to have the ability of diffusing through the gel surface, thereby enabling the organisms to

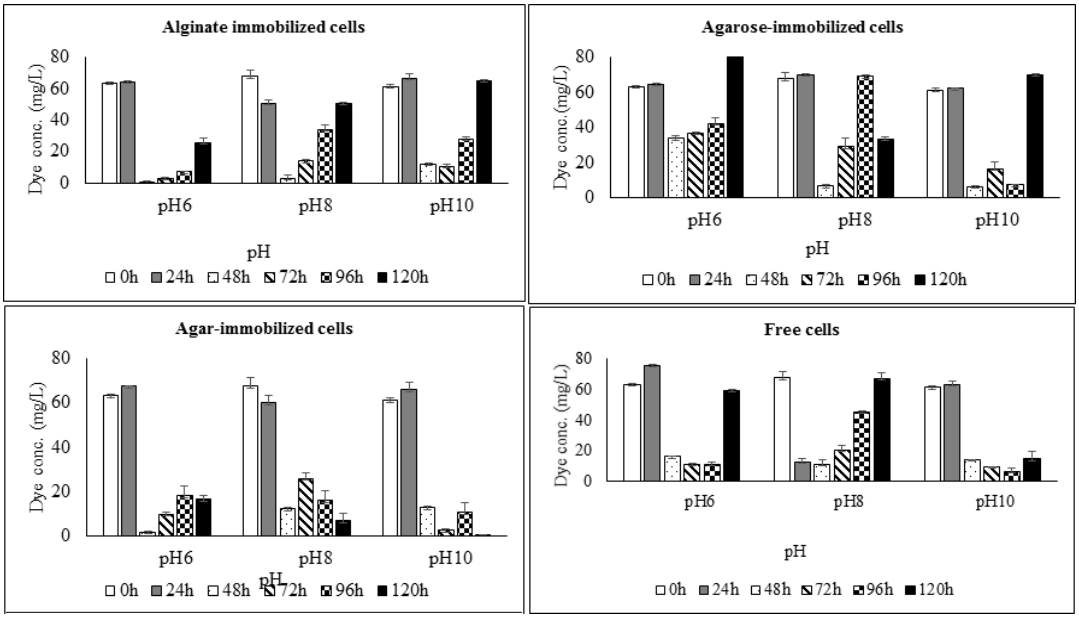

Fig. 1. Effect of pH on crystal violet concentration in the media in presence of the immobilized and free cells of the *Pseudomonas aeruginosa*

degrade the substance in a laminar flowing conditions within the gel bead. Besides, immobilized cells are less possible to wash out from a system, thereby increasing the possibility of reuse [12].

Table 1. pH profile of the media at the different initial pH

pH	Initial	Final	% change
Alginate immobilized cells			
pH 6	6.09	8.23	-35.14
pH 8	8.04	8.73	-8.58
pH 10	9.96	8.98	9.84
Agarose immobilized cells			
pH 6	6.09	9.04	-48.44
pH 8	8.04	9.24	-14.93
pH 10	9.96	9.27	6.93
Agar immobilized cells			
pH 6	6.09	6.47	-6.24
pH 8	8.04	8.77	-9.08
pH 10	9.96	8.76	12.05
Free cells			
pH 6	6.09	6.47	-5.25
pH 8	8.04	8.77	-12.56
pH 10	9.96	8.76	3.31
Uninoculated control			
pH 6	6.09	6.41	2.14
pH 8	8.04	8.03	2.12
pH 10	9.96	9.63	2.12

Initial and final represent pH at 0 h and 120 h, respectively. All % values were increases. All values were averages of duplicate analysis. Positive and negative values represent decreases and increases, respectively

The choice of *Pseudomonas aeruginosa* as the test isolate in this investigation was deliberate. Several species of *Pseudomonas* have been implicated in dye decolouration. Ponraj et al. [13] have indicated *Bacillu*s sp., *Klebsiella* sp. *Salmonella* sp. and *Pseudomonas* sp. as dye decolourizing bacterial isolates. In their study, on the decolourization of Orange 3R. *Pseudomonas* sp. and *Bacillus* sp. showed maximum dye decolourization of 89% at the end of 144h under optimum condition, although the *Bacillus* sp. was found to be more efficient in dye decolourization. Decolorization of azo dye by immobilized and free cells of *Pseudomonas luteola* have been reported by earlier workers [14]. In that study, after five repeated batch cycles, it was reported that the decolourization rate of the free cells showed a decrease of nearly 54%, while that of the immobilized cells still retained 82% of their original activity. Also, when compared with the free cells, the immobilized cells were observed to exhibit better thermal stability during storage and reaction.

In this study, in presence of alginate and agar-immobilized cells, crystal violet decolourization was observed only at 35°C, between 48 h and 72 h of incubation. For the agarose-immobilized cells, decreases occurred but were not significant.

Also, decolouration of the crystal violet occurred in presence of the agarose-immobilized cells after 48 h for only pH 8 and pH 10; although this was not significant. In presence of the agar-immobilized cells, decolouration of the crystal violet occurred at pH 6, pH 8 and pH 10 while in presence of the free cells, decolouration was only observed at pH 8, after 24 h incubation. The intense colour of triphenylmethane dyes, of which crystal violet is an example is said to be due to the extended conjugated system of alternate double and single bonds. In a kinetic study of the discolouration of crystal violet dyes, it was observed that the reaction of the OH^- ion in a basic medium with containing the dye resulted in the disruption of conjugation and loss of colour with time while in intermediate pH, the reaction occurred via nucleophilic attack by water at very slower rate. In acidic medium however, crystal violet was observed to be unstable, thus leading to the formation of a colourless compound, hence increase in discolouration was observed to be proportional to decrease in pH [15].

In a study on spectrophotometric determination of reactive dyes after pre-concentration using activated carbon at different conditions of pH and ionic strength, the results revealed that at 200 mg/L the *Kd* value was decreased from 38.0 to 33.2 dm^3/g when the pH was increased from 1 to 3 with the distribution value remaining unchanged up to pH 8. A similar behavior for red dye was also noted [16]. The decolourization abilities of *Pseudomonas aeruginosa, Pseudomonas putida* and *Bacillus cereus* have been investigated by earlier workers. The study reported that the ability of bacterial isolates for decolourization of textile wastewater effluents was optimum at incubation conditions and pH of 7 [17]. Some other studies have observed that the highest percent decolouration rate occurred between pH 4.5 to 5.5 in similar investigations [18,19]. Also, Tripathi and Srivastava, [20] have reported a pH of 7 at optimum for maximum decolouration of acid orange 10 azo dye, when they used *Pseudomonas putida* and *Bacillus cereus* for their study.

3.2 Decolouration Studies at the Different Incubation Temperatures

At the different incubation temperatures, crystal violet concentration in presence of the alginate-immobilized cells revealed decrease between 48 h and 72 h at 35°C. At 25°C and 45°C, no decrease in crystal violet concentration was observed in presence of the alginate-immobilized cells. This trend was irrespective of the time of sampling. Although only slight changes in concentration were observed at 45°C, at 25°C, significant increase in concentration was observed at the expiration of incubation. After 72 h incubation time, crystal violet concentration in the media in presence of the alginate-immobilized cells showed variation from initial level 26.92 mg/L to 26.61 mg/L, 16.71 mg/L and 25.64 mg/L, at incubation temperatures of 25°C, 35°C and 45°C, respectively (Fig. 2). The decrease in the concentration of crystal violet in the presence of the alginate-immobilized cells was observed to be significantly higher at 35°C than at the other incubation temperatures ($p \leq 0.05$).

Crystal violet concentration in media inoculated with the agarose-immobilized cells was observed to show no remarkable decrease throughout the period of incubation. This trend was irrespective of the incubation temperatures. After the 120 h incubation period, concentration of crystal violet in the media in presence of the agarose-immobilized cells showed a variation from 26.92 mg/L to 35.26 mg/L, from 26.78 mg/L to 29.69 mg/L and from 31.46 mg/L to 30.39 mg/L at incubation temperatures of 25°C, 35°C and 45°C, respectively (Fig. 2). Generally, there was no observed significant difference between the concentrations of crystal violet in presence of the agarose-immobilized at the different incubation temperatures ($p \leq 0.05$).

At incubation temperature of 35°C, in presence of the agar-immobilized cells, crystal violet concentration in the media showed significant reduction at 72 h of sampling, after which increases were observed with time. When incubated at either 25°C or 45°C, no remarkable decrease in concentration of the crystal violet was observed with time. After the 120 h incubation period, crystal concentration in the media showed an increase from the initial concentrations of 26.92 mg/L, 26.92 mg/L and 31.46 mg/L, to final concentrations of 25.12 mg/L, 35.12 mg/L and 30.17 mg/L at incubation temperatures of 25°C, 35°C and 45°C respectively (Fig. 2). In presence of the agar-immobilized cells, no significant difference in the crystal violet concentration in the media was observed between the different incubation temperatures ($p \leq 0.05$).

As shown in Fig. 2, in presence of the free cells suspended in normal saline solution, no remarkable decrease in the concentration of crystal violet in the media was observed throughout the period of sampling. This observation was irrespective of the temperature of incubation. After the 120 h incubation period, crystal violet concentration in the media showed a rise from the initial concentration of 26.92 mg/L to 29.48 mg/L at 25°C, from 26.78 mg/L to 25.98 mg/L at 35°C and from 26.46 mg/L to 30.82 mg/L at 45°C. (Fig. 2). No significant difference was observed between crystal violet concentrations in the media at the different incubation temperatures in presence of the free cells ($p \leq 0.05$).

As shown in Table 2, pH of the media inoculated with the respective cells showed consistent increase with time. No change in pH was observed in the uninoculated control setups. This observation was irrespective of the incubation temperatures. The highest increase in pH was observed at incubation temperature of 35°C, with an increase of 31.40%, 34.14%, 33.84% and 37.18% in presence of the alginate-immobilized, agarose-immobilized, agar-immobilized and free cells, respectively (Table 2).

The temperature and pH of solution are known to affect the biosorption capacity of microorganisms in dye. In a report, the biosorption isotherms derived at different pH and temperatures revealed that a low pH and high temperature (35°C) favoured biosorption. In a batch series study, the suitable conditions for Reactive Black 5 decolourization were reported to be pH less than 10, light presence, glutamine or lactate as external carbon source with concentration greater than 500 mg/L when lactate is selected, NH_4Cl as a nitrogen source (100 mg/L), sodium chloride (less than 5%) and Reactive Black 5 concentration less than 700 mg/L [21]. During investigation on the enzymatic discoloration of the diazo dye, Congo red (CR), by immobilized plant peroxidase from turnip "*Brassica rapa*", using a partially purified turnip peroxidase that was immobilized by entrapment in spherical particles of calcium alginate, results revealed that during discoloration of the aqueous Congo red, pH, reaction time, temperature, colorant, and hydrogen peroxide played significant roles. In the

report, highest decolouration was observed at pH 2.0, constant temperature of 40°C in the presence of 10 mM H_2O_2, and 180 mg/L of the Congo red [22].

Table 2. pH profile of the media at the different incubation temperatures

Temperature levels	Initial	Final	% change
Alginate immobilized cells			
25°C	6.70	8.65	29.10
35°C	6.59	8.66	31.40
45°C	6.61	6.81	3.03
Agarose immobilized cells			
25°C	6.70	8.90	32.84
35°C	6.59	8.84	34.14
45°C	6.61	6.74	1.97
Agar immobilized cells			
25°C	6.70	8.84	31.90
35°C	6.59	8.82	33.84
45°C	6.61	6.69	1.21
Free cells			
25°C	6.70	8.92	33.13
35°C	6.59	9.04	37.18
45°C	6.61	6.68	1.06
Uninoculated control			
25°C	6.70	6.63	1.04
35°C	6.59	6.64	0.76
45°C	6.61	6.59	0.30

Initial and final represent pH at 0 h and 120 h, respectively. All % values were increases. All values were averages of duplicate analysis

In the study of Rohilla et al. [19], using white-rot fungi *Perreniporia tephropora* MUCL 47500 isolated in Gabon, it was reported that the study of temperature ranged from 15-45°C, with maximum decolouration observed at 22°C, 30°C and 35°C for both methyl orange and reactive blue 4. The report indicated that decolouration was inhibited at 15°C and 45°C due to loss of cell viability or deactivation of the enzymes. It was also noted that the decolouration of dye can be observed using fungal isolates at optimal temperature and pH. In addition, Hu et al. [23] also observed that obstacles of dye decolouration by fungus could be caused by of higher structural complexity, molecular mass, and the presence of inhibitory groups such as sulphides, chlorides and aromatics in the dyes.

3.3 Decolouration Studies at the Different Sodium Acetate Concentrations

At the respective concentrations of sodium acetate in the media, significant reduction in the concentration of crystal violet was observed after 48 h in presence of the alginate-immobilized cells. After 48 h of incubation, crystal violet levels in the media were observed to vary from 62.29 mg/L to 9.12 mg/L, from 69.25 mg/L to 10.64 mg/L and from 65.55 mg/L to 11.94 mg/L, at sodium acetate concentrations of 10 g/L, 15 g/L and 20 g/L, respectively. The fall in dye concentration was remained consistent for concentration 15 and 20 g/L until incubation was terminated when 12 alginate beads were used for inoculation At the expiration of incubation, crystal violet concentration was found to be 34.95 mg/L, 15.85 mg/L and 13.90 mg/L in media containing 10 g/L, 15 g/L and 20 g/L of sodium acetate, respectively. (Fig. 3). The decrease in crystal violet concentration at sodium acetate concentration of 20 g/L was observed to be significantly lower than the decreases at 10 g/L and 15 g/L ($p \leq 0.05$).

In presence of the agarose-immobilized cells, crystal violet concentration was observed to show consistent decreases with time at sodium acetate concentration of 20 g/L. When either 10 g/L or 15 g/L of sodium acetate was used, remarkable decrease in crystal violet concentration was observed at 48 h, after which there was consistent increase with time till the end of incubation. At the end of the period of incubation, crystal violet concentrations were shown to vary from 62.29 mg/L to 34.03 mg/L, from 69.25 mg/L to 32.34 mg/L, from 65.55 mg/L to 10.69 mg/L, at sodium acetate concentrations of 10 g/L, 15 g/L and 20 g/L, respectively (Fig. 3). Generally, the decrease in crystal violet concentration in the agarose-immobilized cells was significant higher than those at 10 g/L and 15 g/L of sodium acetate ($p \leq 0.05$).

As shown in Fig. 3, in the presence of the agar-immobilized cell, crystal violet concentration in the media at the different sodium acetate concentrations was observed to show significant decreases from 48 h of incubation. This trend was irrespective of the sodium acetate concentration in the media. At the end of the period of incubation, crystal violet concentration in the media showed a variation from 62.29 mg/L to 33.43 mg/L, from 69.25 mg/L to 18.02 mg/L and from 65.55 mg/L to 20.50 mg/L, at sodium acetate concentration of 10 g/L, 15 g/L and 20 g/L, respectively (Fig. 3). Although the crystal violet concentrations in the media at the different sodium acetate concentrations were observed to differ, these variations were not significant ($p \leq 0.05$).

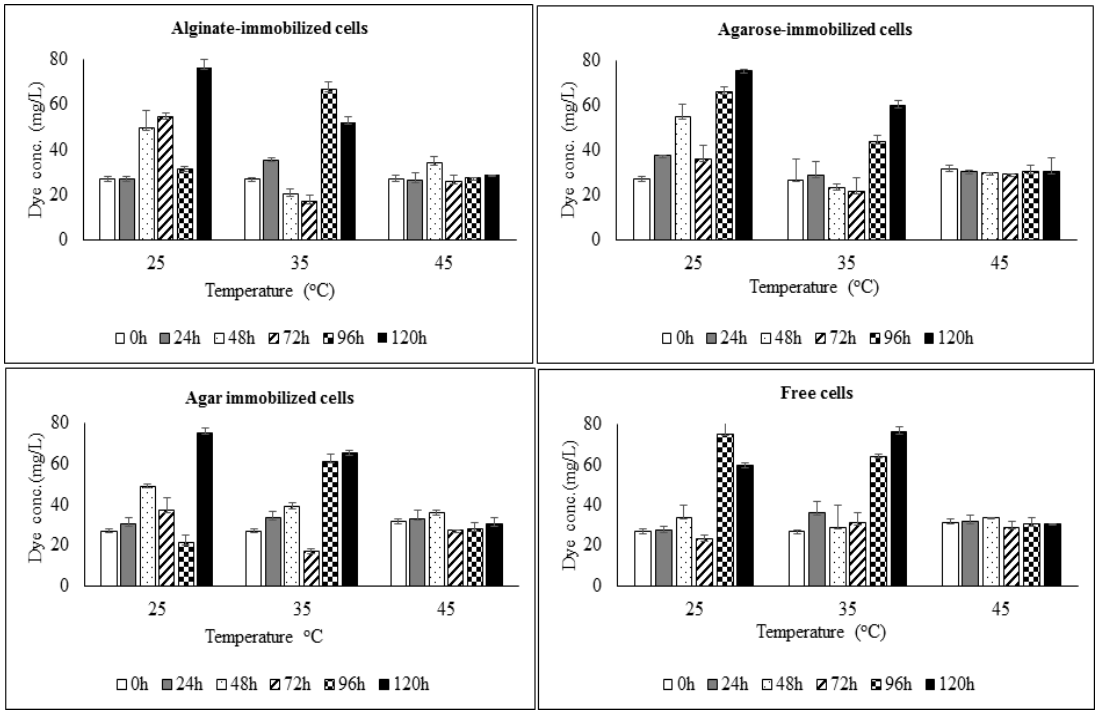

Fig. 2. Effect of temperature on crystal violet concentration in the media in presence of the immobilized and free cells of the *Pseudomonas aeruginosa*

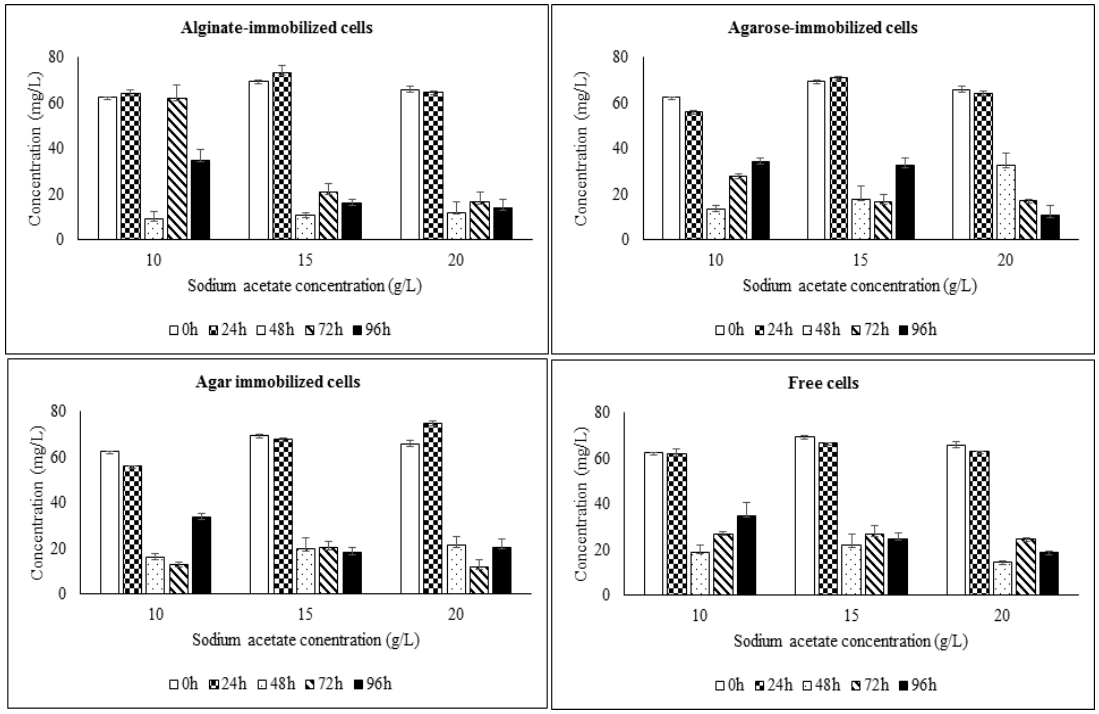

Fig. 3. Effect of sodium acetate concentration on crystal violet concentration in the media in presence of the immobilized and free cells of the *Pseudomonas aeruginosa*

In media inoculated with the free cells, the concentration of crystal violet in the media was observed to show significant decrease after 48 h of incubation from 62.29 mg/L to 18.88 mg/L, from 69.25 mg/L to 21.94 mg/L and from 65.55 mg/L to 14.55 mg/L, at sodium acetate concentration of 10 g/L, 15 g/L and 20 g/L, respectively. Despite the decrease at 48 h, at the end of incubation, concentrations were observed to be 34.95 mg/L, 24.74 mg/L and 18.47 mg/L at 10 g/L, 15 g/L and 20 g/L, respectively (Fig. 3). Despite the variation in crystal violet acetate concentrations in the media at the different sodium acetate concentrations, these differences were not observed to be significant ($p \leq 0.05$).

As presented in Table 3, there was a general increase in pH of the media in presence of the immobilized and free cells. This trend was irrespective of the sodium acetate concentration used for investigation. In presence of the alginate-immobilized and agar-immobilized cells, highest pH increases of 30.89% and 30.44%, respectively were observed at sodium acetate concentration of 10 g/L. In media inoculated with the agarose-immobilized and free cells, highest pH increases of 39.79% and 19.63%, respectively were observed at sodium acetate concentration of 15 g/L (Table 3).

Sodium acetate was used as the carbon source in the media in this study. The choice of sodium acetate was deliberate, for it has been indicated to be an ideal carbon source in biological nutrient removal studies from wastewater [12,24-26]. The study revealed highest decolouration of the crystal violet at sodium acetate concentration of 20 g/L. This trend was irrespective of the cells used for inoculation. In a study on the bacterial removal of orange 3R, using glucose as carbon source, 81.83%, 56.83%, 83.33% and 75.76% in the presence of *Bacillus, Klebsiella*, *Salmonella* and *Pseudomonas* species, respectively. When sucrose was used as the carbon source, 87.80%, 72.36%, 86.18% and 80.49% removal of the orange 3R was observed in presence of the *Bacillus*, *Klebsiella*, *Salmonella* and *Pseudomonas* species, respectively. With mannitol as the carbon source, removal of 87.09%, 64.04%, 86.84% and 73.68% was achieved in the presence of *Bacillus, Klebsiella*, *Salmonella*, *Pseudomonas*, and *Salmonella* species, respectively [13].

A study by Wang et al. [27] on the decolouration of reactive red 180 dye, using *Citrobacter* species, a highest decolouration of 96.2% was achieved with 4 g/L of glucose as carbon source. In a similar study by Embla, [28] on the decolourization of reactive red 180 in the presence of immobilized *Citrobacter* species, using glucose concentrations of 0, 2, 4, 6 and 8 g/L, remarkable decolouration was achieved at glucose concentration of 8 g/L while no decolouration occurred at glucose concentration of 2 g/L.

Table 3. pH profile of the media at the different sodium acetate concentrations

Sodium acetate concentration	Initial	Final	% change
Alginate immobilized cells			
10 g/L	6.57	8.60	-30.89
15 g/L	6.61	6.63	-0.30
20 g/L	6.66	6.63	0.45
Agarose immobilized cells			
10 g/L	6.57	8.95	-36.22
15 g/L	6.61	9.24	-39.79
20 g/L	6.66	7.73	-16.07
Agar immobilized cells			
10 g/L	6.57	8.57	-30.44
15 g/L	6.61	6.98	-5.60
20 g/L	6.66	6.73	-0.01
Free cells			
10 g/L	6.57	7.86	-19.63
15 g/L	6.61	8.09	-22.39
20 g/L	6.66	7.36	-10.51
Uninoculated control			
10 g/L	6.57	6.70	-1.98
15 g/L	6.61	6.68	-1.06
20 g/L	6.66	6.72	-0.90

Initial and final represent pH at 0 h and 120 h, respectively. All % values were increases. All values were averages of duplicate analysis. Positive and negative values represent decreases and increases, respectively

4. CONCLUSION

This study that was aimed at evaluating the effects of temperature, pH and sodium acetate concentration on dye decolouration from nutrient media in presence of the immobilized and free cells of *Pseudomonas aeruginosa* showed that the highest decrease in the concentration of crystal violet in the presence of the cells was observed at incubation temperature of 35°C. The optimum pH for crystal violet decolouration was shown to be between 8 and 10. Despite the highest decrease in crystal violet concentration in presence of the cells being observed at pH 8 and 10, the decreases did not differ significantly at the different pH.

At the different sodium acetate concentrations used in the media, highest decreases in crystal violet concentration in presence of the respective cells was observed at 20 g/L. In presence of the alginate or agarose immobilized cells, the decrease in crystal violet concentration at sodium acetate concentration of 20 g/L was observed to be significantly lower than the decreases at 10 g/L and 15 g/L. For the agar immobilized and free cells, the decrease at sodium acetate concentration of 20 g/L did not differ significantly from those at 15 g/L and 10 g/L.

The present study has been able to provide information of the role of the immobilized and free cells of the test bacterial in crystal violet decolouration under the conditions investigated. However, further studies are recommended to elucidate the mechanisms behind this process. Knowledge of this will help in more efficient treatment of textile effluents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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