

# Response of bacterial biosorbents to chemical treatment as influenced by cell membrane structure and impact on the adsorption behaviour of dyes

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**The impact of cell membrane structure and adsorption capacity of dyes due to chemical treatment on Gram-positive and Gram-negative bacteria was studied. The adsorption was found to occur through a chemisorption mechanism. The adsorption capacity of treated bacteria was higher (68.49–161.29 mg/g) than untreated bacteria (9.37–29.11 mg/g) during the removal of methylene blue. Furthermore, the treatment allowed bacteria to adsorb methyl orange, which was not removed by untreated bacteria. The applied chemical treatment is therefore influenced by the cell membrane structure and could be considered to improve the adsorption capacity of bacteria for the removal of dyes from polluted water.**

**Keywords:** Adsorption affinity, bacteria, bioremediation, chemical treatment, dyes, water pollution.

SOCIAL and economic growth worldwide is directly related to exponential development of the industrial sector, which contributes significantly to environmental pollution. Of particular concern is the release of various types of dyes in the rivers around the world, affecting the colour of water as well as distribution of sunlight with impact on the aquatic ecosystem. Most dyes are particularly resistant to natural degradation and can persist in the environment for a long time. Their negative impact to human and aquatic life is linked to their chemical structure; dyes are reported to be teratogenic, carcinogenic and mutagenic<sup>1–3</sup>. Conventional methods based on chemical and physical interactions are known to effectively remove some dyes from wastewater. However, most of these methods require the use of large amount of chemicals, are expensive and likely to generate toxic by-products that are difficult to handle<sup>4</sup>. Alternative methods which are eco-friendly, comparatively cheaper and with high re-

moval yield are required for effective treatment of industrial effluents and restoration of usable water<sup>1,5</sup>. The biological approach has been considered for remediation of water pollution because of the availability of biological matter, which is cheaper and has lower environmental impact. Microorganisms can remove dyes from water through two main mechanisms, namely degradation and adsorption. The second mechanism is favoured because it is passive and does not require living cells<sup>6</sup>. The adsorption of dyes on microorganisms is influenced by the binding groups on the surface of the bacterial membranes; the functional groups present on the cell wall are carboxylate, amine, sulphhydryl, sulphate, hydroxide and imidazole. The teichoic acids, lipo-teichoic acids, phospholipids, lipopolysaccharides and peptidoglycan account for the functional groups in the active sites<sup>7</sup>. Two main groups of bacteria, namely Gram-positive and Gram-negative, have been distinguished by the nature of their cell membrane. Hence the adsorption capacity of these bacteria may vary. Therefore, optimization of yield of wastewater treatment will require proper screening of suitable biosorbents with high adsorption capacity or adequate response to chemical or physical pretreatment enhancing their adsorption capacity.

The present study examines the impact of chemical pretreatment of bacteria on the enhancement of bacterial binding affinity and adsorption capacity; this will allow the selection of a suitable bacterial sorbent for removal of dyes from solution. The response to chemical treatment based on cell wall structure and impact on the adsorption capacity of bacteria are also considered in the study.

## Methodology

### Materials

Nutrient broth (Lab-Lemco powder: 1.0 g/l; yeast extract 2.0 g/l; peptone 5.0 g/l; sodium chloride 5.0 g/l; pH

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$7.4 \pm 0.2$  at  $25^\circ\text{C}$ ; Merck Chemicals, South Africa); spectrophotometer (Hexose Spectrophotometer, Helios Epsilon, USA).

### Bacterial growth

*Escherichia coli*, *Bacillus subtilis*, Bacillaceae bacterium and *Pseudomonas aeruginosa* were cultured in nutrient broth for 20 h in an incubator with a shaker at 160 rpm.

### Preparation of dye solutions

Methylene blue and methyl orange stock solutions were prepared by measuring and dissolving appropriate amounts in 1000 ml of distilled water. Working solutions in the concentration range 5, 15, 35, 50, 65 and 90 ppm (mg/l) were prepared through dilution.

### Physical and chemical treatment of bacterial cells

Upon growth the cells were exposed to physical treatment using an autoclave (15 min at  $121^\circ\text{C}$ ); thereafter, the cells were dried in an oven at  $60^\circ\text{C}$  for 24 h to obtain dry mass and ground using a mortar. After physical treatment, the cells were chemically treated with 25 mm of ammonia solution, calcium chloride, nitric acid and propylamine. After chemical treatment, dry mass was obtained by incubating the treated biomass in the oven at  $60^\circ\text{C}$  for 24 h.

### Biosorption experiment

The dye removal experiments were carried out by exposing 0.1 g of dry mass to 50 mg/l of dye in 20 ml solution for 2 h at  $35^\circ\text{C}$  in a shaking incubator (150 rpm). Thereafter, 5 ml aliquots of the aqueous solution was collected and centrifuged at 13,000 rpm for 5 min at  $4^\circ\text{C}$ .

### Kinetics experiment

Kinetics experiments were carried out at  $35^\circ\text{C}$  and 150 rpm by exposing 0.1 g of dry mass to 50 mg/l of dye in 20 ml solution at different time intervals – 30, 120, 180 and 240 min. Thereafter, 5 ml aliquots of the aqueous solution was collected and centrifuged at 13,000 rpm for 5 min at  $4^\circ\text{C}$ .

### Characterization of bacterial adsorbent

The cell wall structure of the bacteria was examined using scanning electron microscopy (SEM), to reveal changes on the cell surface before and after treatment. Fourier transform infrared spectroscopy (FTIR) was used to determine the functional groups present on the cell sur-

face; measurement was done within the  $400\text{--}4000\text{ cm}^{-1}$  range.

### Analytical method

The absorbance of methylene blue and methyl orange was measured at wavelength 663 and 470 nm respectively, using an ultraviolet–visible spectrometer (Hexiose Spectrometer, Helios Epsilon, USA). The adsorption capacity was measured by calculating the difference between adsorbance of the abiotic control and sample replicates. A standard curve drawn with the adsorbance of dye solutions of known concentration allowed us to determine the corresponding concentration related to the adsorbance.

The adsorption capacity  $q$  was expressed as

$$q_e = \frac{(C_0 - C_e)V}{m}, \quad (1)$$

where  $q_e$  is the adsorption capacity (mg/g),  $C_0$  the initial concentration of dye in solution (mg/l),  $C_e$  the equilibrium concentration of dye in solution (mg/l),  $m$  the biomass (g) and  $V$  is the volume of the solution (l).

## Results and discussion

### Spectroscopy studies

**Gram-positive bacteria:** FTIR spectrum of *B. subtilis* showed peaks at  $3500\text{ cm}^{-1}$  (N–H stretch),  $2969\text{ cm}^{-1}$  (C–H stretch),  $2109\text{ cm}^{-1}$  (C=C stretch),  $1740\text{ cm}^{-1}$  (C=O stretch),  $1566\text{ cm}^{-1}$  (C–C stretch),  $1371\text{ cm}^{-1}$  (C–H rock),  $1218$  and  $1070\text{ cm}^{-1}$  (C–N stretch),  $928\text{ cm}^{-1}$  (O–H bend),  $849$  and  $715\text{ cm}^{-1}$  (C–Cl stretch),  $654$  and  $611\text{ cm}^{-1}$  (C–Br); while the FTIR spectrum of Bacillaceae bacterium revealed the presence of a number of functional groups on the surface (Figure 1). The  $3500\text{ cm}^{-1}$  band was indicative of the N–H stretch,  $2969\text{ cm}^{-1}$  band the C–H stretch and O–H stretch; bands at  $1745$  and  $1566\text{ cm}^{-1}$  the C=O stretch; the band at  $1212\text{ cm}^{-1}$  the C–N stretch,  $1081\text{ cm}^{-1}$  band the C–O stretch and the bands at  $928$ ,  $775$ ,  $701$  and  $654\text{ cm}^{-1}$  revealed the presence of O–H bend, N–H, C–H and C–Cl stretch respectively.

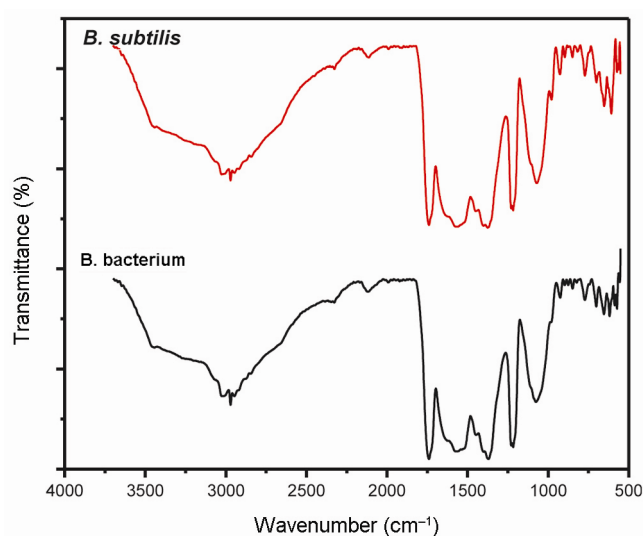
**Gram-negative bacteria:** The FTIR spectrum of *E. coli* showed bands at  $3500\text{ cm}^{-1}$  (N–H stretch)  $3280\text{ cm}^{-1}$  (O–H stretch),  $3032\text{ cm}^{-1}$  (=C–H stretch),  $2917\text{ cm}^{-1}$  (C–H stretch),  $1740\text{ cm}^{-1}$  (C=O stretch),  $1630\text{ cm}^{-1}$  (N–H bend),  $1524\text{ cm}^{-1}$  (N–O asymmetric stretch),  $1223\text{ cm}^{-1}$  (C–N stretch),  $1039\text{ cm}^{-1}$  (C–N stretch),  $827\text{ cm}^{-1}$  (C–Cl stretch),  $701\text{ cm}^{-1}$  (=C–H bend) and  $611\text{ cm}^{-1}$  (C–Br stretch), while the FTIR spectrum of *P. aeruginosa* revealed peaks at  $3500\text{ cm}^{-1}$  (N–H stretch),  $2974\text{ cm}^{-1}$  (C–H stretch),  $1739\text{ cm}^{-1}$  (C=O stretch),  $1576\text{ cm}^{-1}$  (N–H bend),  $1376\text{ cm}^{-1}$  ( $\text{CH}_3\text{C–H}$  bend),  $1223\text{ cm}^{-1}$  (C–O

stretch),  $1075\text{ cm}^{-1}$  (C–N stretch) and  $610\text{ cm}^{-1}$  (C–Br stretch) (Figure 2).

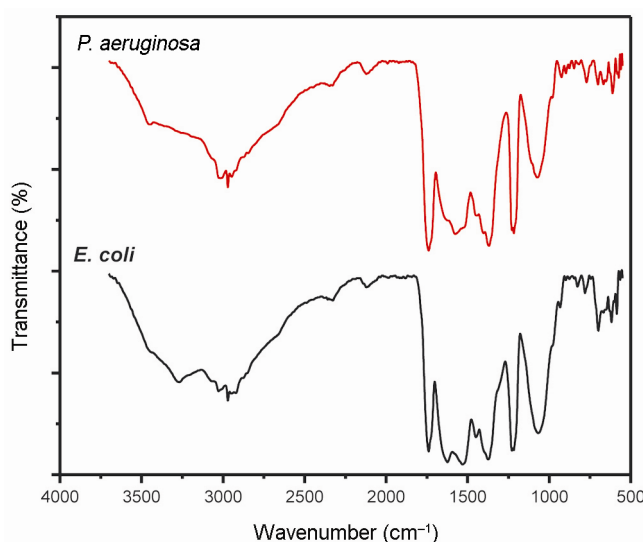
Overall the same major functional groups (O–H, =C–H, C=O, N–H, N–O and C–N) were found at the surface of the membranes of Gram-negative and Gram-positive bacteria, these are typical binding groups reported to be involved in the binding of dyes and metals<sup>8–10</sup>.

### Morphological studies

The SEM images of the treated and the untreated bacteria showed a clear difference (Figure 3 a–d); the untreated



**Figure 1.** FTIR spectra of *Bacillus subtilis* and Bacillaceae bacterium.



**Figure 2.** FTIR spectra of *Escherichia coli* and *Pseudomonas aeruginosa*.

bacteria exhibited rod-like structures as in earlier studies<sup>8,11</sup>. The untreated bacteria on the other hand exhibited finer grains or homogeneous surfaces, indicating that the grinding of cells during treatment reduced their size and therefore increased the surface area necessary for effective binding of dyes.

### Kinetic studies

According to Kumar<sup>12</sup>, chemical kinetics helps explain the rate of adsorption; the study of dye adsorption kinetics is therefore essential for the design of a system that works efficiently. In this study, pseudo first-order and second-order kinetics models were used to determine the rate of dye removal from the solution onto the bacterial cell wall. Biosorption depends on the chemical and physical properties of the adsorbent and adsorbate.

#### Pseudo first-order and pseudo second-order models

The mathematical equations of the pseudo first-order and second-order models are given below:

The pseudo first-order kinetics model is given by

$$\log(q_e - q_t) = \log q_e - k_1 \cdot \frac{t}{2.303} \quad (2)$$

The pseudo second-order kinetics model is given by

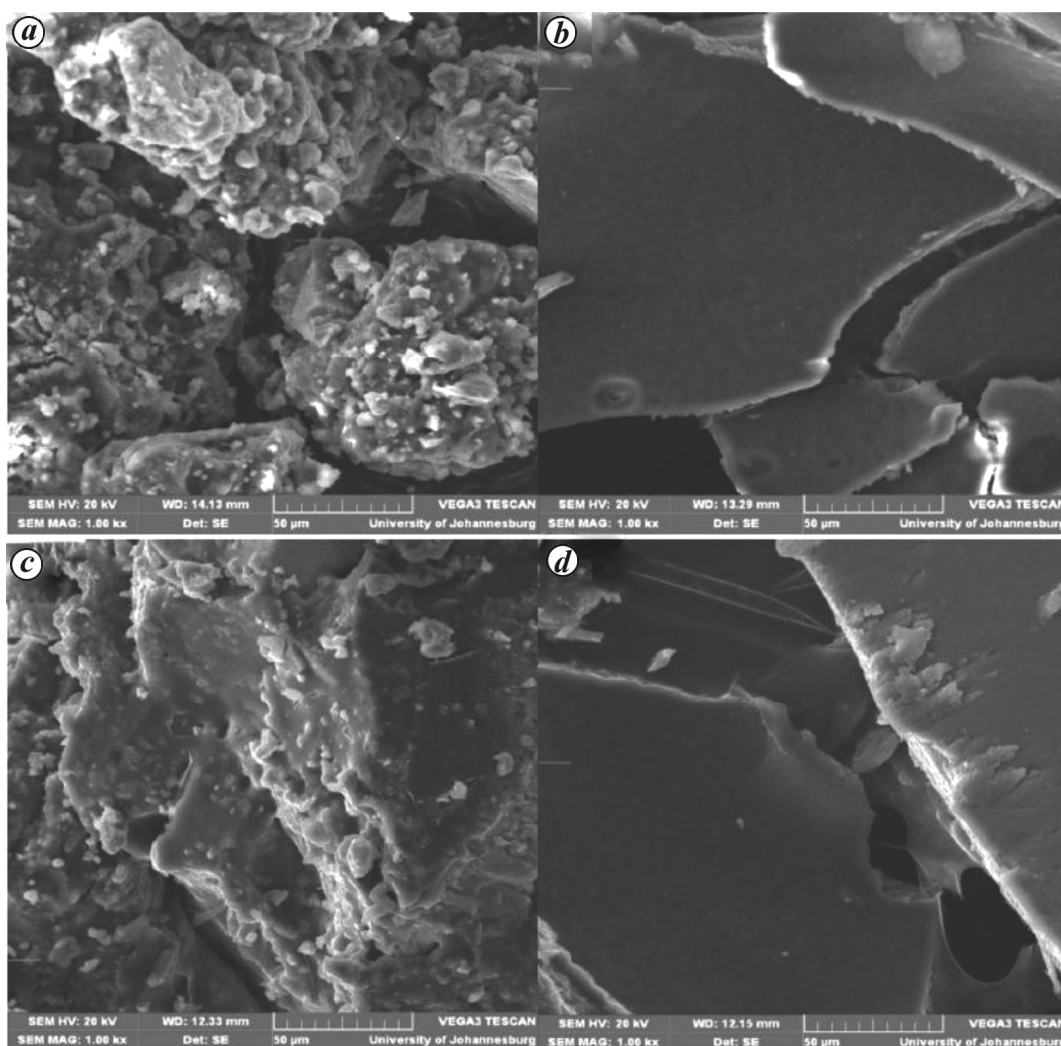
$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} \cdot t \quad (3)$$

In the above equations,  $q_e$  is the amount of dye adsorbed per unit mass of adsorbent at equilibrium (mg/g),  $q_t$  the amount of dye adsorbed per unit mass of adsorbent at time  $t$  (mg/g),  $k_1$  the first-order rate constant ( $\text{min}^{-1}$ ),  $k_2$  the second-order rate constant ( $\text{g/mg min}^{-1}$ ) and  $t$  is the time (min).

When a sorption system is favourable towards the pseudo first-order model, it indicates that adsorption occurs through a physisorption mechanism. When a system is favourable towards the pseudo second-order, it indicates a chemisorption mechanism. The coefficient of determination ( $R^2$ ) is used to determine a suitable kinetic sorption model; a coefficient of determination close to 1 shows that the model is suitable for prediction of adsorption process<sup>12,13</sup>.

The values of  $k_1$  and  $k_2$  were obtained from the intercepts of the plots of  $\log(q_e - q_t)$  versus  $t$  and  $t/q$  versus  $t$  respectively, and listed in Table 1 along with the values of  $R^2$  and  $q_e$ .

The pseudo first-order kinetics parameters poorly fit the biosorption of dyes in comparison to the pseudo



**Figure 3.** SEM images of (a) untreated *P. aeruginosa*, (b) treated *P. aeruginosa*; (c) untreated *B. bacterium* and (d) treated *B. bacterium*.

**Table 1.** Pseudo second-order kinetics parameters for methylene blue removal by untreated Gram-positive and Gram-negative bacteria

Biosorbents	Parameters		
	$k_2$	$R^2$	$q_e$
<i>Escherichia coli</i>	$1.2 \times 10^{-3}$	0.9846	12.56
<i>Pseudomonas aeruginosa</i>	$4 \times 10^{-4}$	0.8833	19.61
<i>Bacillus subtilis</i>	$1.2 \times 10^{-3}$	0.9736	9.37
Bacillaceae bacterium	$7.9 \times 10^{-5}$	0.7984	29.11

second-order, implying that adsorption occurred through a physisorption mechanism.

Using the pseudo second-order rate equation, it was found that  $R^2$  values were relatively high. Furthermore, the calculated ( $q_e$  (cal.)) and experimental ( $q_e$  (exp.)) values of adsorption capacity were closer. This clearly implies that the pseudo second-order model is more appropriate for prediction of dye biosorption process<sup>14-17</sup>.

#### Adsorption behaviour of untreated bacteria

The untreated bacteria showed poor performance in the removal of methyl orange; no removal was achieved after exposure of untreated bacteria to aqueous solution containing methyl orange. This implies that they are unable to remove methyl orange from solution. The adsorption behaviour of untreated bacteria was estimated for the removal of methylene blue from solution. Figure 4 a and b represents the pseudo second-order kinetics trends for the Gram-positive and Gram-negative bacteria respectively. They followed a similar pattern, i.e. increase in the  $t/q_t$  overtime. However, linearity of the points varied among bacteria and was not dependent on the bacteria group; Table 1 illustrates the linear relationship. It also shows the rate constant, coefficient of determination as well as adsorption capacity of the pseudo second-order kinetics model. The values of  $R^2$  were higher ( $R^2 > 0.95$ ) for *E. coli* and *B. subtilis*, indicating that the pseudo

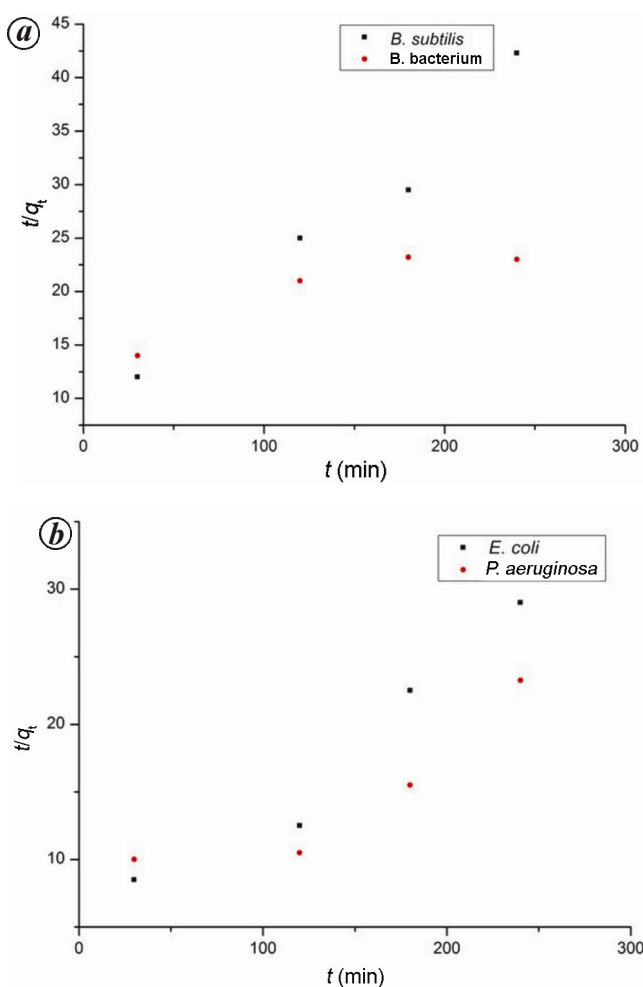
second-order kinetics gave better correlation for dye adsorption only by these two microbial sorbents. These results imply that the adsorption behaviour of bacteria in this case could not be related to their cell wall structure. It is therefore possible that biochemical activities involved in the biodegradation and decolourization of dyes equally contribute to their removal<sup>18–20</sup>.

#### Adsorption behaviour of treated bacteria

Following the limitation of untreated bacteria in the adsorption of methyl orange which is an anionic dye, the bacteria were predicted using physico-chemical methods to improve their adsorption capacity.

#### Calcium chloride

Figure 5 *a* and *b* shows the trend of adsorption based on pseudo second-order model. It can be observed that for



**Figure 4.** Pseudo second-order kinetics for methylene blue adsorption by Gram-positive bacteria (*a*) and Gram-negative bacteria (*b*).

methylene blue (Figure 5 *a*), only adsorption by Gram-positive bacteria (*B. subtilis* and Bacillaceae bacterium) follows a linear pattern; for methyl orange (Figure 5 *b*), the adsorption by *P. aeruginosa* and *B. subtilis* follows a linear pattern.

#### Ammonia

Figure 6 *a* and *b* shows the linear relationship between adsorption capacity of bacteria treated with ammonia and time during the removal of methylene blue and methyl orange respectively. It can be observed that irrespective of the type of bacterial adsorbent, the adsorption of methylene blue follows a linear trend compared to that of methyl orange.

#### Propylamine

Figure 7 *a* and *b* shows the adsorption behaviour of bacteria treated with propylamine based on pseudo second order model. As observed for bacterial adsorbent treated with ammonia, the adsorption of methylene blue follows a linear trend, which is not the case for methyl orange.

#### Nitric acid

The adsorption behaviour of bacteria treated with nitric acid does not follow a linear trend in case of both methylene blue and methyl orange based on the pseudo second-order model (Figure 9 *a* and *b*).

High  $R^2$  values were observed for bacteria treated with propylamine and ammonia in the adsorption of methylene blue (Table 2). In addition, adsorption of dyes by bacteria fitted the pseudo second-order method in most cases, implying that the adsorption occurred predominantly through a chemisorption mechanism.

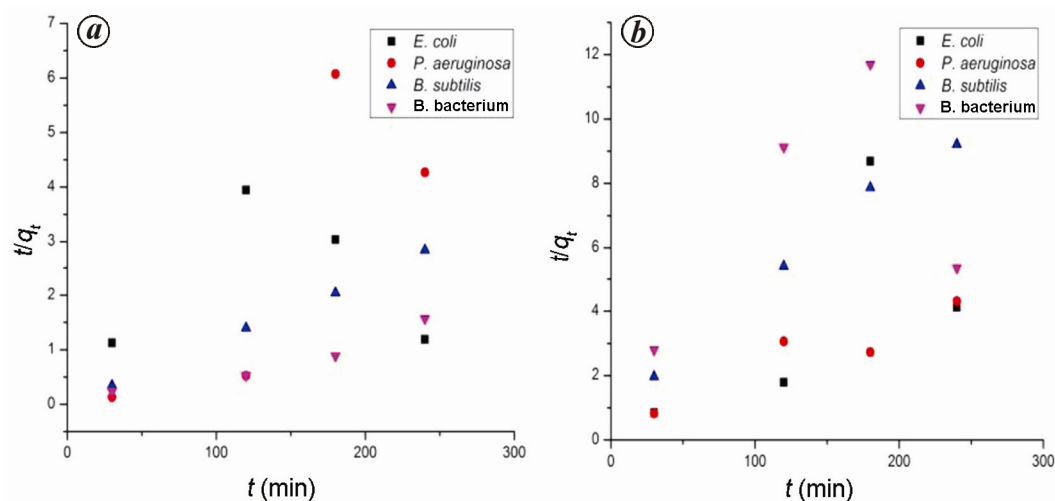
Although the  $R^2$  values observed were relatively low for the adsorption of methyl orange, it is clear that the pretreatment of cells improved their capacity to remove methyl orange from solution compared to the untreated cells.

## Discussion

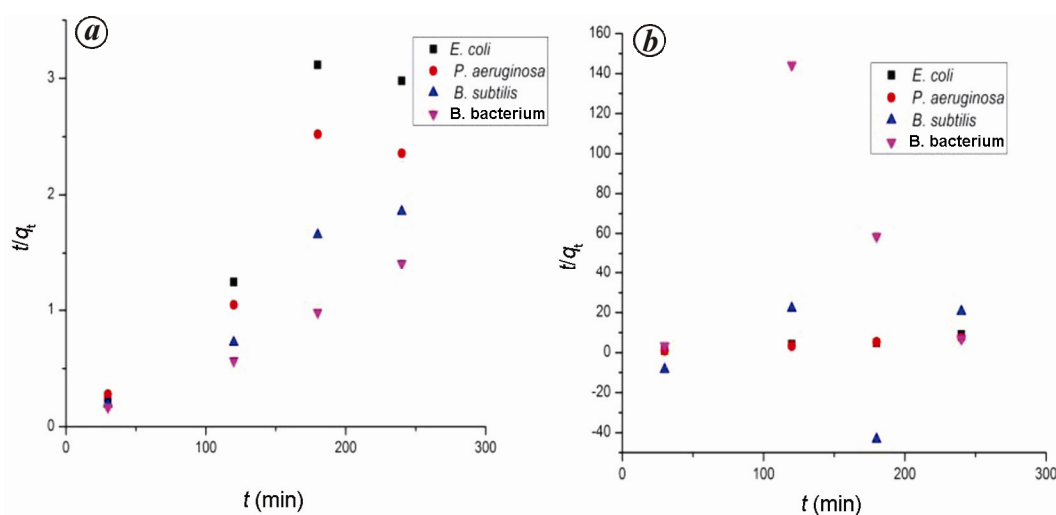
Evaluation of the effect of treatment is based on the adsorption capacity ( $q_e$ ) recorded in Tables 1 and 2 for different bacteria and dyes.

#### Escherichia coli

Only the cells treated with ammonia and propylamine exhibited adsorption behaviour that fitted the pseudo second-order model during the removal of methylene



**Figure 5.** Fit of the kinetic data to pseudo-second order rate model for the adsorption of (a) methylene blue and (b) methyl orange by bacteria treated with calcium chloride.



**Figure 6.** Fit of the kinetic data to pseudo-second order rate model for the adsorption of (a) methylene blue and (b) methyl orange by bacteria treated with ammonia.

blue. The calculated adsorption capacities were 89.29 and 77.52 mg/g respectively, which were higher than the adsorption capacity (12.56 mg/g) of the untreated cells. For the adsorption of methyl orange, higher  $R^2$  values were also recorded for cells treated with ammonia and propylamine – 28.74 and 48.78 mg/g respectively – showing an improvement as the untreated cells did not remove methyl orange.

#### *Pseudomonas aeruginosa*

Considering the adsorption of methylene blue, the effect of treatment on the adsorption performance of *P. aeruginosa* was similar to that of *E. coli* as the adsorption data fitted the pseudo second-order model for cells treated with ammonia and propylamine. The recorded adsorption capacities were 68.49 and 76.92 mg/g respectively, which

were higher than the value of 19.61 mg/g recorded for the untreated cells. Only the adsorption behaviour of cells treated with ammonia during removal of methyl orange could be predicted by the pseudo second-order model, giving an adsorption capacity of 32.68 mg/g, which is better than the untreated cells.

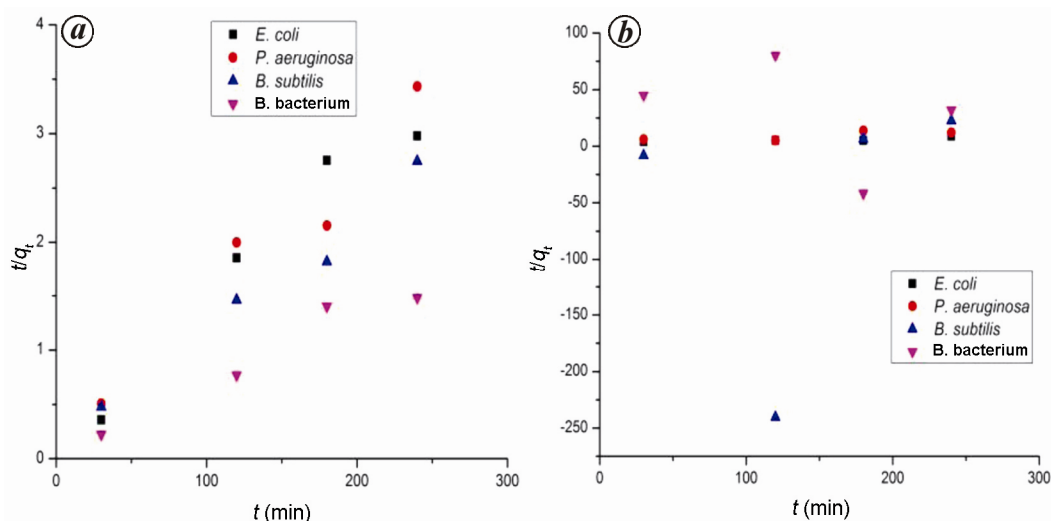
#### *Bacillus subtilis*

The adsorption kinetic data for removal of methylene blue by *B. subtilis* fitted well the pseudo second-order model, irrespective of the type of treatment. The adsorption capacities obtained for treatment with calcium chloride, ammonia, propylamine and nitric acid were 84.74, 117.65, 96.15 and 76.63 mg/g respectively. The treatment of cells with calcium chloride and nitric acid was suitable for expression of the adsorption behaviour during removal

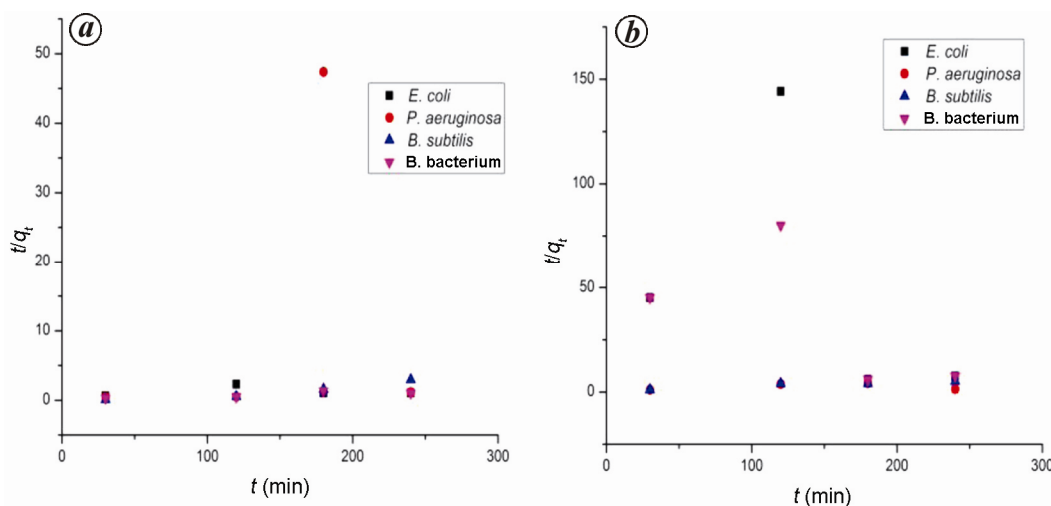
**Table 2.** Pseudo-second order kinetics parameters of different chemically pretreated bacteria for methylene blue and methyl orange dye removal at 35°C

Dye	Bacteria	Calcium chloride				Ammonia				Propylamine				Nitric acid			
		$q_e$	$k_2$	$R^2$		$q_e$	$k_2$	$r^2$		$q_e$	$k_2$	$r^2$		$q_e$	$k_2$	$r^2$	
Methylene blue	<i>E. coli</i>	-	-	No fit	89.29	-0.0028	0.8708		77.52	0.001162	0.951		-	-	-	No fit	
	<i>P. aeruginosa</i>	39.06	0.000724	0.6291	68.49	-886.65	0.8843		76.92	0.00106	0.9483		-	-	-	No fit	
	<i>B. subtilis</i>	84.74	-0.567	0.9985	117.65	-0.0007	0.9468		96.15	0.000727	0.9795		74.63	0.0003003	0.8988		
	<i>B. bacterium</i>	161.29	0.00053	0.9167	10.43	-0.14	0.9857		153.85	0.00078	0.9494		222.22	0.0001134	0.677		
Methyl orange	<i>E. coli</i>	-	-	No fit	28.74	-189.84	0.9349		48.78	0.000147	0.7386		-	-	-	No fit	
	<i>P. aeruginosa</i>	-	-	No fit	32.68	-0.0058	0.994		26.88	0.000351	0.606		-	-	-	No fit	
	<i>B. subtilis</i>	28.33	0.033	0.9889	-	-	No fit		-	-	No fit		53.76	0.0002843	0.8558		
	<i>B. bacterium</i>	-	-	No fit	-	-	No fit		-	-	No fit		-	-	-	No fit	





**Figure 7.** Fit of the kinetic data to pseudo-second order rate model for the adsorption of (a) methylene blue and (b) methyl orange by bacteria treated with propylamine.



**Figure 8.** Fit of the kinetic data to pseudo-second order rate model for the adsorption of (a) methylene blue and (b) methyl orange by bacteria treated with nitric acid.

of methyl orange. The adsorption capacities obtained were 28.33 and 53.76 mg/g respectively.

### *Bacillaceae bacterium*

The adsorption of methylene blue by *Bacillaceae bacterium* was better expressed by the pseudo second-order model for cells treated with calcium chloride, ammonia and propylamine. These revealed adsorption capacities of 161.29, 10.43 and 153.85 mg/g respectively. The adsorption of methyl orange by *B. bacterium* did not fit the pseudo second-order model.

Overall, it was observed that treatment of cells using physico-chemical methods improved the removal of dyes. Heat treatment, chemical treatment or crushing increase the surface area and expose the intracellular components

and more surface binding sites due to destruction of the cell membrane<sup>21,22</sup>.

Among the chemicals used, ammonia and propylamine contributed the most in enhancing the adsorption capacity of bacteria; this corroborates previous findings which reported that the amine groups play a major role in the binding of adsorbate to the surface of adsorbents<sup>23</sup>.

### *Effect of cell membrane structure on the performance of bacteria*

The adsorption of dyes by untreated cells did not show any difference that could be ascribed to the group of microorganisms (cell membrane structure). However, the adsorption of dyes by treated cells showed a difference in Gram-negative versus Gram-positive bacteria. The latter



responded well to treatment by chemicals and exhibited superior adsorption capacity. The difference could be observed after pretreatment because the binding groups of the cell membrane were exposed following disruption.

## Conclusion

On the basis of the results obtained, it can be concluded that the untreated bacteria used in this study are unable to remove the anionic dyes such as methyl orange from aqueous solution; physico-chemical pretreatment of these bacteria has improved their adsorption capacity for removal of both methylene blue and methyl orange. This has been ascribed to the exposure of internal binding groups of the cell membrane and addition of chemical compounds. Although cell membrane did not play a significant role in the adsorption capacity and affinity, it was observed that the response to chemical pretreatment was influenced by cell membrane structure as the Gram-positive bacteria exhibited better adsorption capacity for methylene blue and methyl orange compared to Gram-negative bacteria. The adsorption of dyes was found to fit the pseudo second-order kinetics model in most cases, implying that the removal of dyes by bacteria took place through a chemisorption mechanism.

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