Physico-Chemical Treatment Influenced by Bacterial Membrane and Impact on Dye Adsorption Capacity

Lwandle P. Simelane, Elvis Fosso-Kankeu, Frans Waanders, Patrick Njobeh and Sadanand Pandey

Abstract—Bacterial cell walls contain functional groups which are likely to interact with the environment and therefore have the ability to adsorb ions; the abundance and exposure of these groups is influenced by the type of bacteria membrane. In this study untreated and treated Gram-positive (*B. bacterium* and *B. subtilis*) and Gram-negative (*B. bacterium* and *P. aeruginosa)* bacteria were investigated to establish the effect of pre-treatment on the adsorption of Methylene blue (cationic) and methyl orange (anionic). Physical pre-treatment consisted to autoclave the bacteria prior to grinding and chemical treatment was achieved by immersing grinded cells in solutions of C_3H_9N and NH_3. The treated and untreated bacteria were then characterized using SEM and FTIR, prior to the investigation of their adsorption capacities.

FTIR results showed the presence of functional groups namely amines, carboxylic acids, alcohols, aldehyde and amides, while SEM images revealed homogeneous and finer surfaces following physical treatment; it was observed that bacteria response to chemical treatment was significantly influenced by their cell membrane structure. Both untreated as well as treated cells exhibited higher adsorption capacity for the removal of MB from solutions; however, the removal of MO from solutions was favourable when using treated bacteria as untreated bacteria exhibited poor performance in this case.

The pre-treatment increases the efficiency of dye removal in solutions; and has the potential to overcome the limitations encountered in using live bacteria**.**

Index Terms--Adsorption Kinetics, Cell Wall, Functional Groups, Methyl Orange, Methylene Blue.

I. Introduction

Industrialization is essential for economic growth; on the contrary it has a negative impact on environment. Water pollution is of grave concern, because water is an elementary need. The textile industry is one of the causative of water pollution[1]. In addition, dyes are used in different industries namely; pharmaceutical industries, pulp industries, and the primary source being the textile industry. About 10-25% of the dyes used during the dyeing process is lost into the environment [2].

The textile has been in continuance for more than 4000 years; natural dyes were used such as clay, earth, and malachite. The first synthetic dye was discovered by William Henry Perkin in 1856[3]. Approximately 600 thousand tons of dyes are produced per year [4]. Dye is made up of two structures namely chromophore and auxochromes. Chromophore absorbs light and auxochromes are responsible for the increase in colour intensity and are electron donors; they are also essential for affinity to the fibres. Synthetic dyes vary in structure resulting in the different chemical and physical structures. There are different types of dyes used in the textile namely; anionic dye (these types of dyes form covalent bonds between the oxygen and carbon atoms in alkali surroundings); cationic dyes (consist of xanthenes, anthraquinone, cyanine, these types of dye are difficult to decolorize); sulphur dyes (are primarily used on cotton, are insoluble in water; solvents dyes (are insoluble in water and soluble in solvents); mordant dyes (able to create covalent bonds with metals when a chelating compound is present) [5][6].

Dyes are teratogenic, carcinogenic and mutagenic due to the chemicals with which they are made up of[7]. When industrial effluents are leaked into the environment the ecological state is negatively affected. Wastewater constitutes of detergents, softening agents, inorganic substances, metals and surfactants etc. A small amount of dye can be visible when present in water. In addition, the presence of colour in water bodies prevents the sunlight from entering into the water preventing the process of photosynthesis from taking place; the latter has a negative effect on aquatic life and its growth [3]. Some dyes are affiliated with bladder cancer, splenic and hepatocarcinomas[8].

Bioremediation is the application of microorganisms such as bacteria, fungi, and algae in the removal of pollutants and breaking toxic substances using biochemicals reactions into non-toxic products [3]. Furthermore, biodegradation is an environmentally friendly way to eradicate synthetic dyes that have the characteristic of being resistant to microbial degradation in certain conditions. Bio decolourisation is possible through sorption[9]. The process of biosorption has no effect on the dye structure but instead the dye particles are engulfed or trapped in the biomass[10]. On the other hand biodegradation is whereby the dye structure is dismantled into other products this process is also known as mineralization.

The bacterial cell wall has direct contact with the environment. It consists of functional groups namely carboxylic acids, amines, hydroxyl, sulphate and imidazole. Bacterial cell walls are classified into two: Gram-positive and Gram-negative.

Lwandle Simelane is with the Department of Biotechnology, Faculty of Sciences, University of Johannesburg, Doornfontein, Johannesburg, South Africa

Elvis Fosso-Kankeu is with the School of Chemical and Minerals Engineering, North-West University, South Africa,.

Frans Waanders is with the School of Chemical and Minerals Engineering, North-West University, South Africa

Patrick Njobeh is with the Department of Biotechnology, Faculty of Sciences, University of Johannesburg, Doornfontein, Johannesburg, South Africa

Sadanand Pandey is with the Department of Applied Chemistry, Faculty of Sciences, University of Johannesburg, Doornfontein, Johannesburg, South Africa

Gram positive bacteria cell walls are made up of a thick peptidoglycan, techoic acid; the overall charge for Gram-positive cell wall is a negative[11]. The Gram-negative have a thin cell wall, outer membrane giving rise to the anionic nature. The structural difference among these two groups of bacteria is likely to affect the pretreatment efficiency and therefore the adsorption capacity, hence the need to comparatively investigate the adsorption potential of bacteria from the two groups.

II. METHODOLOGY

2.1 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 7.4 ± 0.2 at 25°C; Merck Chemicals, SA); spectrophotometer (hexose spectrophotometer heliose Epsilon made in the USA)

2.2 Bacterial growth

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

2.3 Preparations of dye solutions

Methylene blue and methyl orange stock solutions were prepared by measuring and dissolving the appropriate amounts in the 1000 mL of distilled water. The working solutions that were prepared were 5, 15, 35, 50, 65 and 90 ppm (mg/L).

2.4 Physical and chemical treatment of bacterial cells

Upon growth the cells were exposed to physical treatment using the autoclave (15 minutes at 121 ^{o}C); thereafter the cells were dried in an oven at 60^{o}C for 24 hours to obtain dry mass and ground using a mortar. After the physical treatment the cells were chemically treated with ammonia solution and propyl amine; in the following concentrations; 1, 25, 50, and 100 mM. After chemical treatment, dry mass was obtained by using the oven at 60^{o}C for 24 hours.

2.5 Biosorption experiment

The dye removal experiments were done at 35^{o}C, 150 rpm (rotating incubator), with 0.1 gram of dry mass in 20 mL solutions of dye at different concentrations (5, 15, 35, 50, 65 and 90 ppm) for 2 hours. Thereafter 5 mL aliquots of the aqueous solution were collected and centrifuged at 13000 rpm for 5 minutes at 4^{o}C.

2.6 Kinetic experiment

The kinetic experiments were done at 35^{0}C, 150 rpm (rotating incubator) with 0.1 gram of dry mass in 20 mL of 50 ppm dye; at different time intervals 30, 120, 180 and 240 minutes. Thereafter 5 mL aliquots of the aqueous solution were collected and centrifuged at 13000 rpm for 5 minutes at 4^{o}C.

2.7 Characterization of bacterial adsorbent

The cell wall structure of the bacteria was examined using the Scanning Electron Microscope (SEM), to reveal changes on the cell surface before and after the treatment. The Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the functional groups present on the cell surface; the measurement occurred within a range of 400-4000 cm^{-1}.

2.8 Analytical method

The absorbance of methylene blue and methyl orange was measured at wavelength 663 nm and 470 nm respectively, using an ultraviolet-visible spectrometer (Hexiose Spectrometer, HelioseEpsilon, made in USA). The adsorption capacity was measured by calculating the difference between the adsorbance of the abiotic control and the sample replicates. A standard curve drawn with the adsorbance of dyes solutions of known concentrations allowed to determine the corresponding concentrations of adsorbances.

III. EQUATIONS

The adsorption capacity q was expressed as follows:

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

Where:
q_e is the adsorption capacity in mg/g
C_0 is the initial concentration of dye in solution (mg/L)
C_e is the equilibrium concentration of dye in solution (mg/L)
m is the biomass (g)
V is the volume of the solution (L)

IV. RESULTS AND DISCUSSION

Surface structure characterization analysis

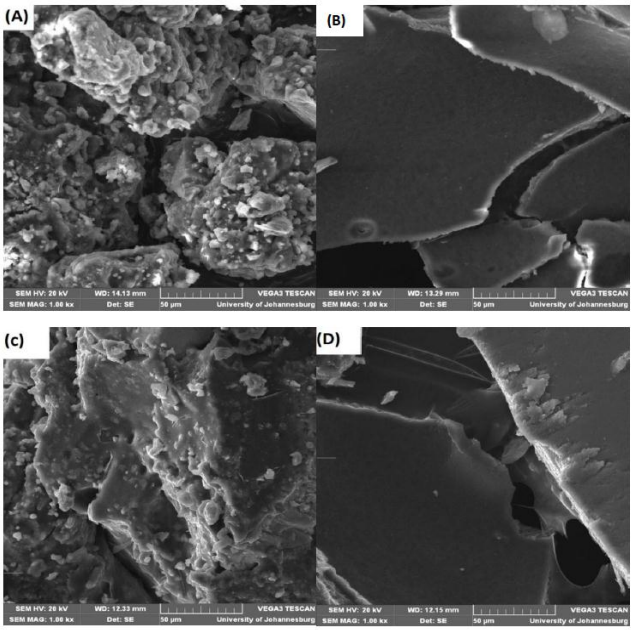


Fig. 1 SEM images of (A) Untreated *P. aeruginosa*, (B) treated *P. aeruginosa*; (C) untreated *B. bacterium* and (D) treated *B. bacterium*

The Scanning Electron Microscope is used to investigate the morphological change in the untreated and treated bacteria. Fine, homogenous particles were observed in the treated bacteria. The fine particles demonstrate the increase in the surface area, which enables an increase in the adsorption capacity of bacterial adsorbents.

Fourier Transform Infrared

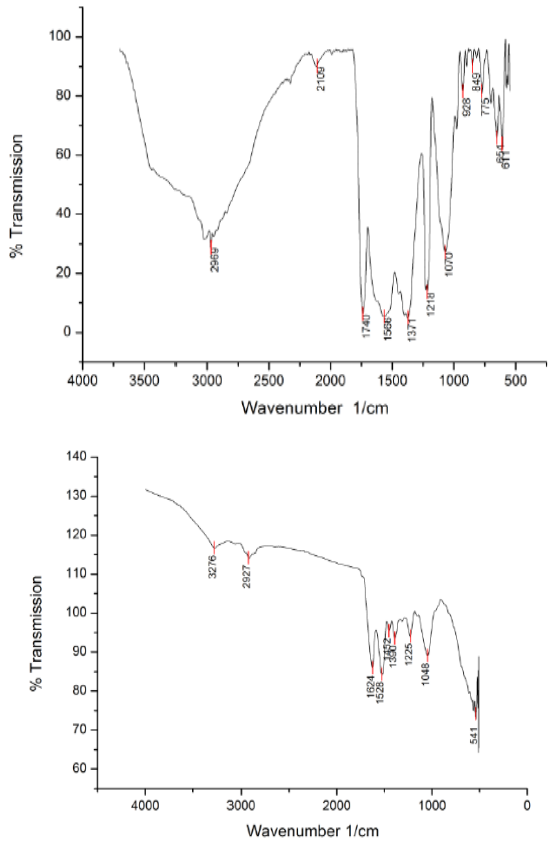
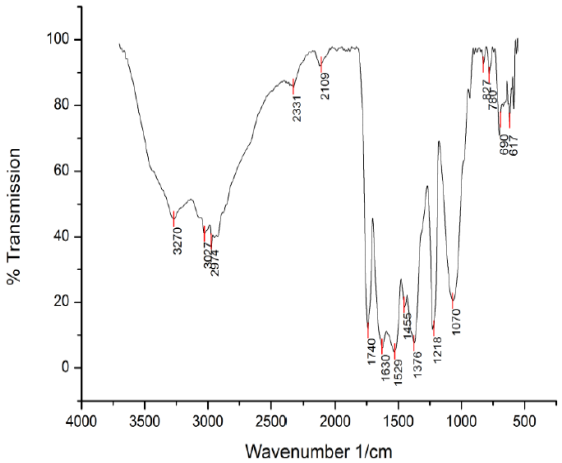


Fig. 2 FTIR spectrum of (a) *B. subtilis* and (b) *B. bacterium*

The FTIR spectrum (Fig 1a) of *Bacillus subtilis* showed the following peaks 2969cm^{-1} (C-H stretch), 2109 cm^{-1} (C=C stretch) , 1740 cm^{-1} (C=O stretch), 1566 cm^{-1} (C-C stretch), 1371 cm^{-1} (C-H rock), 1218 and 1070 cm^{-1} (C-N stretch), 928 cm^{-1} (O-H bend), 849 and 715 cm^{-1} (C-Cl stretch), 654 and 611 cm^{-1} (C-Br); while the FTIR spectrum (Fig 1b) revealed the presence of a number of functional groups present on the surface of *B. bacterium*. The 2969 cm^{-1} band indicates the presence of C-H stretch, O-H stretch; the 1745 and 1566 cm^{-1} bands indicate the presence of C=O stretch; the 1212 cm^{-1} shows the presence of C-N stretch, 1081 cm^{-1} C-O stretch; 928, 775, 701 and 654 cm^{-1} revealed the presence of O-H bend, N-H wag, C-H "oop" and C-Cl stretch respectively.



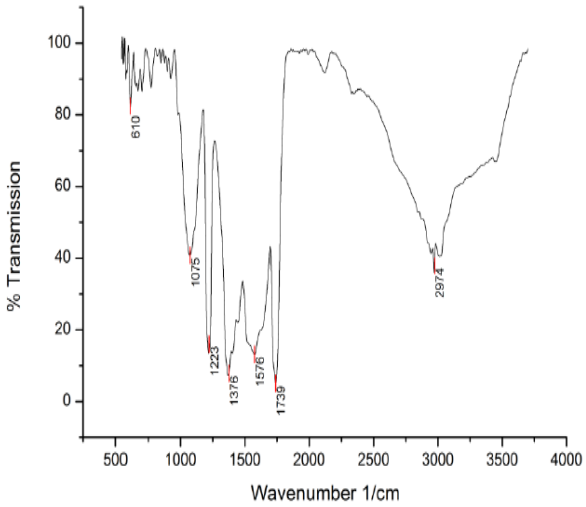


Fig. 3 FTIR spectrum of (a) *E.coli* and (b*) P.aeruginosa*

The FTIR performed on the *E. coli* showed the following bands; 3280 cm^{-1} (O-H stretch), 3032 cm^{-1} (=C-H stretch), 2917 cm^{-1} (C-H stretch), 1740 cm^{-1} (C=O stretch) , 1630 cm^{-1} (N-H bend), 1524 cm^{-1} (N-O asymmetric stretch), 1223 cm^{-1} (C-N stretch), 1039 cm-^{1} (C-N stretch), 827 cm^{-1} (C-Cl stretch), 701 cm^{-1} (=C-H bend) and 611 cm^{-1} (C-Br stretch); while the FTIR spectrum of *P. aeruginosa* revealed the peaks at 2974 cm^{-1} (C-H stretch), 1739 cm^{-1} (C=O stretch), 1576 cm^{-1} (N-H bend), 1376 cm^{-1} (CH_3C-H bend), 1223 cm^{-1} (C-O stretch), 1075 cm^{-1} (C-N stretch) and 610 cm^{-1} (C-Br stretch).

The FTIR readings performed on the Gram- positive and Gram-negative bacteria showed bands which are represented by the following functional groups: carboxylate, hydroxide, amine, imidazole, sulphate and sulfhydryl. In this present study it was noted that there was no significant difference between Gram-positive and Gram-negative functional groups.

Kinetic studies

Adsorption kinetics are essential in designing an efficient system for the removal of dye from water bodies. In this research the Lagergren's pseudo second order was used to study the adsorption of dyes. The process of biosorption is dependent on chemical and physical properties of the cell wall surface and dyes.

Untreated bacteria

The adsorption experiment carried out without pretreatment of adsorbents, revealed that methyl orange was notremoved from solution. Untreated bacteria do not have the ability to remove the anionic dye (MO). The adsorption of methylene blue was also studied using untreated bacteria. Figures 4 and 5 illustrate the adsorption of dye by Gram-positive and Gram-negative bacteria respectively. An increase of t/qt over time was observed. The type of bacteria had no effect on the linearity of the adsorption.

Ammonia

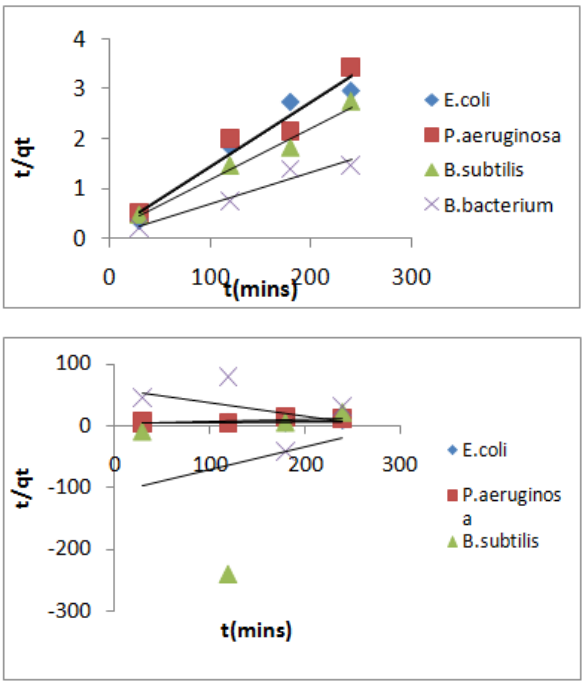


Fig. 4 The pseudo second kinetic order of bacteria treated with ammonia.

Propyl amine

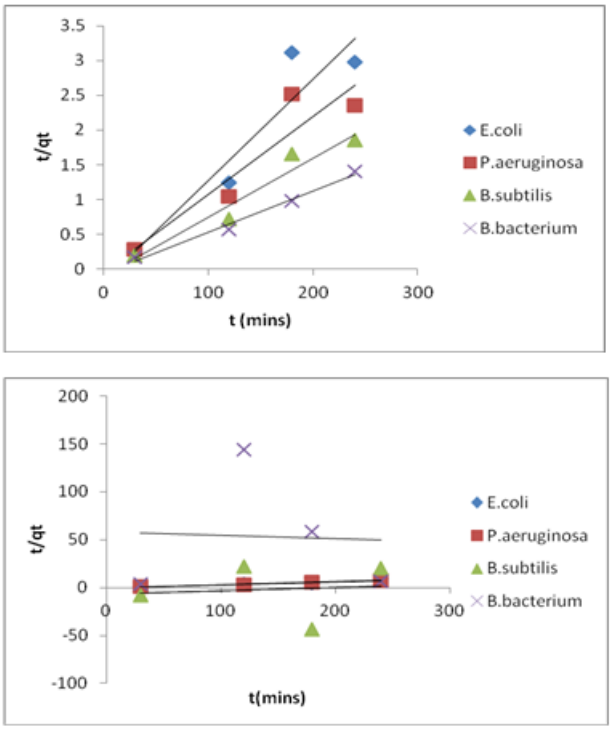


Fig. 5 The pseudo second orderkinetic for the removal of (a) methylene blue and (b) methyl orange by bacteria treated with propyl amine

A linear relationship was observed in the trend lines of bacteria treated with ammonia. Methylene blue was easily removed from solution compared to the methyl orange.

Propyl amine and ammonia are alkaline. Alkaline pre-treatments have the ability to disrupt the cell wall of the microorganisms redeeming more functional groups (Yu et.al, 2007). Furthermore, purification occurs due to the alkaline pre-treatment whereby lipids, some proteins, autolytic enzymes and other impurities that hinder binding sites are removed to reveal active sites with functional group allowing for more dye adsorption.

E. coli

The biomass pre-treated with ammonia and propyl amine suited the pseudo second order model. The results show the adsorption capacity in the adsorption of methylene blue deduced from the graphs; the adsorption capacity was 77.52 and 89.29 mg/g (propyl amine and ammonia respectively), which surpassed that of untreated bacteria found to be 12.6 mg/g. During the adsorption of methyl orange, the biomass treated with ammonia and propyl amine exhibited adsorption capacities of 25.74 mg/g and 48.78 mg/g.

P. aeruginosa

The biomass treated with ammonia and propyl amine fitted the pseudo second order model. The adsorption capacities were 68.49 mg/g and 76.92 mg/g, respectively; these results were relatively high compared to the adsorption capacity of 19.61 mg/g of untreated bacteria. The adsorption of methyl orange by the biomass treated with ammonia allowed to obtain an adsorption capacity of 32.68 mg/g.

B. subtilis

In the adsorption of methylene blue, the adsorption capacities of biomass treated with ammonia and propyl amine were 117.6 mg/g and 96.15 mg/g respectively; which are higher than the adsorption capacity recorded in untreated bacteria (9.37 mg/g).

B. bacterium

The removal of methylene blue by *B.bacterium* treated with ammonia and propyl amine was well represented by the pseudo second order model with adsorption rates at 10.43 h.mg/g and 153.85 mg/g; untreated bacteria had an adsorption capacity of 29.11 mg/g.

V. Discussion

The FTIR results revealed the presence of functional groups on the bacterial cell wall. The functional groups on the cell wall are responsible for the removal of toxicants in a solution. Gram-negative and Gram-positive bacteria were found to have the same types of functional groups (carboxylate, hydroxide, amine, imidazole, sulphate and sulfhydryl). Jiang et.al (2004), stated that there is a similarity in the functional groups present on the cell surface of both types of bacteria. In the SEM study, the fine, grainy particles were observed on the treated biomass; this shows the increase in surface area upon physical pre-treatment.

The pseudo-second order was used to establish the efficient kinetics parameters and adsorption capacities. In this research the treated bacteria had the ability to adsorb methyl orange which was not adsorbed by untreated bacteria. According to Nadeem et.al.(2008); the pre-treatment of biomass using acidic and alkalis solution results in the removal of proteins and lipids which masks the functional groups on the cell wall. Upon pre-treatment Gram-positive biomass exhibited a higher improvement compared to Gram-negative bacteria. Gram-positive bacteria consist of a thicker peptidoglycan layer

than Gram-negative bacteria, increasing the number of potential functional groups.

The physico pre-treatment of biomass improves the adsorption of dyes, as observed in this research. The adsorption capacities improved tremendously when compared to the adsorption capacities of untreated bacteria.

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The corresponding author is currently anAssociate Professor in the School of Chemical and Minerals Engineering at the North-West University (Potchefstroom). He is an NRF rated researcher who has published journal articles, book chapters and book.

Prof Elvis Fosso-Kankeu has been the recipient of several merit awards