Kinetic Study of Methylene Blue Removal by Gram-Negative and Gram-Positive Bacteria

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Abstract—Adsorption of methylene blue was carried out in a batch experiment using Gram-positive and Gram-negative bacteria. Parameters such as initial concentration of the dye and pH were investigated to determine optimum conditions for high removal rate. The adsorption behaviour of the bacteria was studied using the pseudo second-order kinetic model.

Overall, it was observed that the biomass adsorption capacity decreased with increased initial concentration of dye. Maximum removal of dye was achieved at pH 8, while acidic and basic conditions were not suitable for dye removal. The pseudo second-order kinetic model fitted well the adsorption of dye by E. coli and B. subtilis, and therefore allowed to determine their adsorption capacities which were 12.56 and 9.37 mg/g, respectively.

It is observed that Gram-positive and –negative bacteria exhibit almost a similar behaviour during removal of methylene blue and are good adsorbents performing well at pH.

Keywords—Dye, water pollution, bioremediation, isotherm, adsorption affinity, methylene blue, methyl orange

I. INTRODUCTION

Only a small fraction of water existing in the world is available to human beings as fresh water; however the shortage of water is predicted to worsen in the future because of natural causes such as climate change and anthropogenic actions. One of the main negative effects of human action on water sources is the dispersion of synthetic dyes in the environment. Different types of synthetic dyes are available on the market and are mainly used in the textile industry. The presence of these dyes in surface waters poses aesthetic and ecological problems, as well as health risks to people who may ingest contaminated water [1], [2]. Among the techniques available for removal of dyes from water, biological techniques are more attractive because of their low cost as well as the environmental advantages. Biosorption is one of the common biological techniques using biosorbents including natural polymers and microorganisms. Bacteria have large surface area to volume ratio and have been extensively used for removal of pollutants from water [3], [4]. However, the adsorption potential of bacteria varies among species and the identification of effective biosorbent is very important for implementation of the technique at large scale. The cell membrane of bacteria is the part in contact with the environment and is therefore susceptible to play a significant role on bacteria adsorption capacity. Gram-positive and –negative bacteria have be shown to differ considerably in the structure of their cell membrane [5]. Gram-negative bacterial cell walls consist of a thin layer of peptidoglycan lipopolysaccharide and lipotechoic acids, while the cell wall of Gram-positive bacteria consists of thick layer of peptidoglycan, external to the plasma membrane, techoic acids and cell wall membrane [6], [7]. The net charge of the cell wall of microorganisms is determined by the functional groups present and affect their binding capacity and affinity [8], [9].

To investigate the impact of cell wall structure on the adsorption capacity of bacteria, Gram-positive and –negative cells were used in this study for the adsorption of methylene blue from aqueous solutions.

II. METHODOLOGY

A. Growth of the bacteria

The bacteria were grown on nutrient agar to obtain single colonies. Pure cultures of B. Subtilis, E.coli, Bacillaece bacterium and P. Aeruginosa was inoculated in nutrient broth contained in sterile Erlenmeyer flasks and incubated in an incubator with shaker at a temperature of 30°C. The bacteria were grown to early stationary phase (20 hours). Thereafter the culture was transferred into the 50ml falcon tubes and centrifuged at 8000rpm for ten minutes, the supernatant was discarded; and the pellet was washed in pure water.

B. Preparation of dye solution

Methylene blue (100mg) was dissolved in distilled water (100ml) for the formation of a solution of 1000ppm. From this solution aliquots of the methylene blue stock solution was mixed with the microbial solution to make up 50ml solutions containing 5ppm, 30ppm, 50ppm and 75ppm of methylene blue, respectively.

C. Dye removal experiment

The dye solutions (5, 30, 50 and 75ppm) with 0.3g/l cells was mixed in a 250 ml Erlenmeyer flask to a total volume of 100ml solution. These solutions were incubated in an incubator with shaker (150 rpm) at 37°C. Samples of 5ml of each of the mixtures were collected at 30, 120, 210 and 300 minutes. The samples were then centrifuged at 13000 rpm for five minutes and the supernatant used to quantify the residual dye.
D. Impact of pH on dye removal

The adsorption experiment as described above was conducted at different pH conditions to examine the effect of pH on dye absorption. Experiments were carried out from acidic to basic pH range (3, 5, 7, 8, 10). This investigation allowed to determine the optimum pH for bacterial adsorption capacity.

E. Analytical method

The adsorbance of methylene blue was measured at wavelength 663 nm 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 methylene blue solutions of known concentrations allowed to determined corresponding concentrations of adsorbances.

The adsorption capacity q was expressed as follows:

\[ q_e = \frac{(C_0 - C_e)V}{m} \]

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)

III. RESULTS AND DISCUSSION

A. Effect of initial concentration of methylene blue removal

The effect of initial concentration of methylene blue on its removal by microbial sorbents was studied and the results are shown in Fig 1. It was observed in general that the removal rate increased with the initial concentration till a maximum, and then decreased at very high concentrations. This can be explained by the fact that the available binding sites on the surface of bacteria quickly reached saturation at very high initial concentration of the dye.

B. Effect of pH on methylene blue removal

The pH of the solution is a very important parameter in adsorption experiment, as it affects the electrostatic state of both the sorbate and the sorbent. In this study, the effect of pH on the adsorption of dye was investigated at acidic, neutral and basic conditions. A similar trend of the behaviour of bacteria was observed as maximum removal was achieved at pH 8 and they showed poor adsorption capacity at very low and high pH. This can be explained by the fact that at low pH, protons in solution compete with the cationic dye for the binding sites on the surface of bacteria [10], [11]. At higher pH, hydroxyl groups in solution complex with cationic dye preventing their adsorption by microbial sorbents [12]. However the Gram-positive and –negative bacteria behave differently at lower and higher pH, as the adsorption capacity of the previous at pH 3 and 10 is zero, while Gram-negative bacteria can still remove methylene blue at such pH.

C. Adsorption kinetics of methylene blue removal

Kinetic studies are often carried out to better understand the adsorption process and determine the capacity of adsorbents as well as their adsorption behaviour; information received from such investigation can facilitate the design of the process for a given application.

Pseudo-second order kinetic

The pseudo second-order adsorption kinetic rate equation is expressed as:

\[ \frac{t}{q_t} = \frac{1}{k_2q_e^2} + \frac{1}{q_e}.t \]

Where \( q_e \) and \( q_t \) are the adsorption capacity at equilibrium and at a time t, respectively (mg/g)

\( k_2 \) is the pseudo second-order rate constant (g mg\(^{-1}\) min\(^{-1}\))

A plot of \( t/q_t \) versus t must give a linear relationship for the applicability of the second-order kinetic. Data plotted in Fig 3 and 4 represent the pseudo second-order kinetic trends for Gram-positive and –negative bacteria respectively. They all follow a similar pattern which is an increase of the \( t/q_t \) value...
Table 1 shows the rate constant, the coefficient of determination as well as the adsorption capacity of the second-order kinetic model. The values of $R^2$ were higher ($R^2 > 0.95$) for E. coli and B. subtilis, indicating that the pseudo second-order kinetic gave a better correlation for the 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 the biochemical activities involved the biodegradation and decolourization of dye equally contribute to the removal of dye [13], [14], [15].

IV. CONCLUSION

The adsorption of methylene blue by Gram-positive and –negative bacteria was investigated in this study. It was observed that both bacteria groups have the potential to remove methylene blue from aqueous solutions. The difference in cell wall structure did not significantly affect the adsorption capacity of microbial sorbents, however a difference in the adsorption behaviour was observed at extreme pH, condition at which the adsorption capacity of Gram-positive bacteria was reduced to zero. It is therefore suggested that the behaviour of Gram-positive bacteria in wide pH range being further investigated in order to improve their capacity as biosorbents.

REFERENCES


Born in Cameroon, the author completed the high school’s certificate at Bafoussam-Cameroon and then completed a BSc in Biochemistry at the University of Dschang in Cameroon. He then traveled to South Africa where he continued his studies and completed a B-Tech and M-Tech in Biotechnology; after a year in the consulting sector, he went back in academia to complete a D-Tech in Extraction Metallurgy focusing on Bioprocessing.


Dr Elvis Fosso-Kankeu has been a recipient of several merit awards, the more recent is the best paper award received at the International Mine Water Conference in Bunbury-Australia, October 2012.