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Adsorption and Biodegradation of 1-Methyl Naphthalene Using Immobilized *Pseudomonas macerans and Bacillus subtilis* on Burnt Kaolin

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ABSTRACT: This work is aimed at assessing the effect of incorporating *Pseudomonas macerans and Bacillus subtilis* on burnt kaolin (BK) during the biodegradation of 1-methyl naphthalene. The biodegradation was monitored by determining the concentration of CO_2 released. Immobilized *Pseudomonas macerans* on BK released CO_2 in the range of 0.72 - 0.83 mg/L, while this was 0.68 - 0.78 mg/L with *Bacillus subtilis*; for the degradation alone the range was 0.39 - 0.46 mg/L after 72 h. Generally, the concentration of carbon (IV) oxide released by the immobilized *Pseudomonas macerans* was more than that by *Bacillus subtilis.* Therefore, immobilization using BK resulted to better removal of the organic pollutant. The FTIR indicated presence of new peaks within the regions 3272-3265cm^{-1} and 1647-1640cm^{-1} attributed to overlapping of hydroxyl (-OH) and carbonyl (C=O) stretching in carboxylic acid. The absorptions within 1114-1088 cm^{-1}, and at 1408cm^{-1} are due to C-O stretching and O-H in plane bending of carboxylic acid respectively. The use of kaolin for environmental clean-up of organic pollutants will enhance the value chain of solid minerals in Nigeria.

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Petroleum is a complex of different chemicals that primarily comprises hydrocarbons: namely aromatic, aliphatic and resins (API, 2014; Chuwudi, 2014). The aromatics are the dominant fractions of petroleum, together with its derivatives with most important members of the group given the acronym BTEX (benzene, toluene, ethylbenzene, xylene) and PAHs (Polyaromatics hydrocarbons) (Garapati, 2012; API, 2014). PAHs are important hydrocarbon pollutants, classified among the priority pollutant by United State Environmental Protection Agency (USEPA) and European Commission (Nasseri *et al*., 2010). This group of organic compounds is considered to be acutely toxic to the ecological system (Garapati, 2012), carcinogenic, mutagenic, terogenic (Bojes and Pope, 2007; Nasseri *et al*., 2010; Sihag *et al.,* 2014). The toxicity of PAHs depends on the nature of the individual exposed and the duration of exposure.

PAHs are reported to hinder growth and retard mental development in children, thereby affecting the verbal IQ of children (Jedreychowski *et al.,* 2015). However, clean-up exercise of hydrocarbon spill is difficult and conventional methods adopted are very expensive (Garapati, 2012). Also, most methods are not suitable for industrial scale (Chen *et al*., 2016). So, microbial degradation of pollutants is very effective because it is environmental friendly, less expensive and do not produce secondary waste (Benchouk and Chibani, 2017; Chen *et al*., 2016; Chukwudi, 2014). But the success of biological method depends on the affinity of the microorganism for the hydrocarbon and optimization of the biological activity for biodegrability of the hydrocarbon (Chen *et al*., 2016). Among other factors, the incorporation of inert solid media support is advantageous over the use of freely suspended microorganisms (Yuan, 2016). Bioremediation of hydrocarbon pollution through immobilization of microorganisms on natural solid supports (bagasse, chitin, kaolin, orange peels and pineapple peels) have been reported (Annadurai *et al*., 2007; Quitelas, 2009; Agarry and Aremu, 2012a; Agarry and Aremu, 2012b).Therefore, this work is aimed at studying the potential of burnt kaolin (BK), as a solid support for Pseudomonas *macerans* and *Bacillus subtilis* in the biodegradation of 1-Methyl naphthalene.

MATERIALS AND METHODS

Collection and Preparation of Kaolin: The kaolin samples used were obtained in November, 2015 from Birjin in Darazo Local Government Area of Bauchi State, Nigeria located on 10° 59' 43" North, 10° 24' 48" East. The kaolin samples collected was crushed into small size and ground into powder with an agate mortar and pestle. This was then sieved with a 2 µm stainless steel sieve, autoclaved at 20^0C for 21 min, and stored in sterile sample bottles prior to analysis (DIRD, 2009).

A 1.0 g sieved kaolin was treated in a muffle furnace (RHF15/3, United Kingdom) at 400^0C for 2 h, and then

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autoclaved at 21^0C for 20 min. The prepared kaolin sample was then stored prior to analysis.

Preparation of inoculums in synthetic Waste water: The synthetic wastewater used is composed of: K_2HPO_4 (1.0 g), KH_2PO_4 (0.5 g), $(NH4)_2SO_4$ (0.5 g), NaCl (0.5 g), $CaCl_2$ (0.02 g), $MnSO_4$ (0.02 g), $CuSO_4.5H_2O$ (0.02 g), H_3BO_3 (0.01 g), $MgSO_4.7H_2O$ (0.5 g), $FeSO_4$ (0.02 g), Molybdenum powder (0.02 g), deionised water 1000 cm^3. A primary culture was prepared by transferring two loops full of microorganism from an agar slant culture into 100 cm^3 of feed medium containing 20 cm^3 of minerals salt medium and 80 cm^3 of 500 mg/L of 1-methyl naphthalene solution in a 250 cm^3 Erlenmeyer conical flask. The flask was incubated in a rotary shaker for 48 hours at a temperature of 30^0C and agitated with a speed of 120 rpm. After the incubation period, about 10 cm^3 of the primary culture was transferred into another 100 cm^3 of feed medium in a conical flask and the incubation process was repeated as above. This secondary culture was used in the biodegradation process of 1-Methyl naphthalene (Agarry and Aremu, (2012b).

Isolation of Hydrocarbon Degrading Bacteria: Pseudomonas macerans and Bacillus subtilis were isolated based on protocol of Collin and Lyne (1976). The bacterial isolates were considered hydrocarbon degraders and thus were used for the biodegradation studies. Pure cultures were prepared by inoculation on nutrients agar and subsequently sub-cultured to agar slant in bottles for identification.

Biodegradation of 1-Methyl naphthalene study: Nine experimental set-up were used to test the biodegradation of two different bacteria *Pseudomonas macerans* and *Bacillus subtilis* immobilized on BK (Agarry and Aremu, 2012b). A 100 cm^3 of each organism inoculums (*Pseudomonas macerans* and *Bacillus subtilis*) was added into nine sets of 250 cm^3 Erlenmeyer conical flasks, each set contain varied masses (1.0 g, 2.0 g, 3.0 g) of burnt Kaolin, (BK) followed by the addition of 150 cm^3 of synthetic wastewater containing 1-Methyl naphthalene of initial concentration 500 mg/L into each of the flask.

Each flask was then covered with a rubber cork and placed in a rotary shaker for 72 h at a speed of 180 rpm and temperature 30^0C, so as to reach equilibrium. For the adsorption study, 150 cm^3 of the synthetic water containing 500 mg/L of the 1-Methyl naphthalene was added into each set of Erlenmeyer conical flask containing varied masses (1.0 g, 2.0g, 3.0 g) of BK, the flask was placed in a shaker for 72 hours at speed of 180 rpm and temperature 30^0C. The maximum amount of 1-Methyl naphthalene adsorbed at equilibrium (Qe) was calculated according to the equation (1)

$$Qe = \frac{(Co - Ce)V}{W} \qquad 1$$

The ability of the microorganism to mineralize the hydrocarbon was monitored by determining the pH level, carbon (IV) oxide (Titration to find out carbonic acid concentration) after 36 hours, 72 hours, and by FITR and UV instrumentation techniques.

Estimation of Carbon dioxide (CO_2) and pH: Biodegradation converts hydrocarbon completely to carbon (IV) oxide and water. The concentration of carbon (IV) oxide in each degradation was estimated by titration as follows. A 10.0 cm^3 portion of each medium was collected at the middle of the biodegradation period, and at the end of the treatment, and was titrated against 0.005M NaOH solution, using phenolphthalein as an indicator. The end point was determined when the pink colour was stable (Darsa *et al*., 2014), and the amount of carbon (IV) oxide was calculated by using the equation:

$$Free\ CO_2\ (mg/L) = \frac{Titre\ x\ Normality\ of\ NaOH}{Volume\ of\ the\ bioreactor}\ x\ 44 \qquad 2$$

Effect of Dosage of Solid Media on Biodegradation and Adsorption: The effect of dosage of solid media on the adsorbent were studied in the -biodegradation and adsorption of 1-methylnaphthalene using fixed concentration of hydrocarbon (500 mg/L) and fixed adsorbent particle size (2 µm), the effect of the dosage treatment was presented in form of the percentage of hydrocarbon mineralized at equilibrium. The percentage removal of the hydrocarbon was obtained using the equation (3) below.

$$\%\ Removal = \frac{Co-Ce}{C0}\ x\ 100\ x\ 100 \qquad 3$$

Adsorption Isotherms: The extent of the adsorption/ biodegradation of 500 mg/L 1-methylnaphthalene of fixed size 2 µm was studied. The maximum adsorption at equilibrium (Q_e) obtained in the adsorption and biodegradation was used to explain the extent of the adsorption, using Langmuir isotherm adsorption model

$$\frac{Ce}{Qe} = \frac{1}{Qob} + \frac{Ce}{Qo} \qquad 4$$

Where C_e is the equilibrium concentration (mg/L), Q_e is the amount of hydrocarbon adsorbed at equilibrium (mg/g), and Q_0 and b is Langmuir constants related to adsorption capacity and energy of adsorption. A plot of $1/c_e$ against $1/Q_e$ was then obtained. The R_L values signals the type of adsorption, R_L is given by the following equation

$$RL = \frac{1}{1+bCo} \qquad 5$$

Irreversible (RL = 0), favourable (0 < RL < 1), linear (RL = 1) or unfavourable (RL > 1) (Agarry and Aremu, 2012a).

Statistical Analysis: Students t-test was used to compare the effect of microbes and dosage of the solid media on the removal of the hydrocarbon from wastewater at 95% confidence limit.

RESULTS AND DISCUSSION

The isolates were identified to be *Pseudomonas macerans* and *Bacillus subtilis* based on colour change using microgen identification test media. For the biodegradation of 1-methylnaphthalene by *Pseudomonas macerans* immobilized on BK (Table 1), the concentration of carbon (IV) oxide determined after 36 h of incubation of the reactors at dosage of 1.0 g, 2.0 g and 3.0 g, was found to be 0.50 mg/L, 0.50 mg/L and 0.50 mg/L respectively. However, after 72 h of incubation this was found to be 0.72 mg/L, 0.78 mg/L and 0.83 mg/L respectively. The result showed that, for each dosage there was increase in the concentration of carbon (IV) oxide as the period of incubation increases from 36 to 72 hours. Similarly, as the reaction period increased the pH value of the systems decreased from 8.27-8.19, 8.36-8.25 and 8.44-8.32 for the 1.0 g, 2.0 g and 3.0 g dosage respectively (Table 1).

The increase in the release of the carbon (IV) oxide confirms that *Pseudomonas macerans* immobilized on burnt kaolin (BK) is capable of breaking down the hydrocarbon. Also the decrease in pH is due to the neutralizing effect of carboxylic acid formed during degradation (Wu *et al*., 2010). In addition, the FTIR result also show new peaks within the regions 3272-3265cm^{-1} and 1647-1640cm^{-1}, these bands are attributed to overlapping of hydroxyl (-OH) and carbonyl (C=O) stretching in carboxylic acid respectively, the peaks within the regions 2959-2952 cm^{-1} and 2877- 2840 cm^{-1} are due to C-H. The absorptions within 1114-1088 cm^{-1}, and at 1408cm^{-1} are due to C-O stretching and O-H in plane bending of carboxylic acid respectively (Figure 1); which represents the metabolites of the hydrocarbon degradation.

Table 1: Biodegradation of 1-Methylnaphthalene by immobilized *Pseudomonas macerans* on burnt kaolin (BK)

Mass of immobilized organism	pH after 36 h	pH after 72 h	Conc. of CO_2 (mg/L) after 36 h	Conc. of CO_2 (mg/L) after 72 h
1.0 g	8.27	8.19	0.50	0.72
2.0 g	8.36	8.25	0.50	0.78
3.0 g	8.44	8.32	0.50	0.83

A similar trend was observed with respect to changes in concentration of carbon (IV) oxide generated, as well as the pH change during the process of biodegradation of the hydrocarbon by the *Bacillus subtilis* immobilized on burnt kaolin (BK). As the incubation period increases from 36-72 h with increased dosage from 1.0 g - 3.0 g; there was noticeable increase in the concentration of carbon (IV) oxide produced. At the dosage of 1.0 g, 2.0 g and 3.0 g, the concentrations of carbon (IV) oxide generated by *Bacillus subtilis* immobilized on burnt kaolin (BK) were 0.50 mg/L, 0.47 mg/L and 0.50 mg/L respectively; while after 72 h the concentrations of carbon (IV) oxide generated at dosage of 1.0 g, 2.0 g and 3.0 g were 0.68 mg/L, 0.74 mg/L and 0.78 mg/L (Table 2). Also, the concentration of carbon (IV) oxide generated using BK was 0.37 mg/L, 0.43 mg/L and 0. 44 mg/L, after 72 h however, the concentration was observed to be 0. 39 mg/L, 0.46 mg/L and 0.46 mg/L (Table 3).Furthermore, since biodegradation resulted to the production of carbon (IV) oxide, this amount increases lineally as the dosages of *Pseudomonas macerans* immobilized on burnt kaolin (BK) and *Bacillus subtilis* immobilized on burnt kaolin (BK) increases (Table 1 and Table 2). Several factors responsible for this trend include, the immobilized cells are at highest dosage, thereby bring about high number of biofilm, this led to much quorum sensing and better coordination in converting more of the hydrocarbon to basic level of carbon (IV) oxide. Secondly, increase in adsorbent dose results in increased mineralization, so the maximum production of carbon (IV) is at 1.0 g treatment dose.

In contrast to the biodegradation of 1-methyl naphthalene by burnt kaolin (BK), degradation of the hydrocarbon by BK alone showed that the immobilized cells have advantage in mineralizing the hydrocarbon better (Table 1-3). Immobilized *Pseudomonas macerans* on burnt kaolin (BK) released CO_2 in the range of 0.72 - 0.83 mg/L, while this was 0.68 - 0.78 mg/L when *Bacillus subtilis* was immobilized on BK; for the degradation using only burnt kaolin (BK) the range was 0.39 - 0.46 mg/L after 72 h.

This implies that, in the immobilized cells a combination of adsorption and biodegradation processes occur, making the CO_2 released to be higher. Generally, the concentration of carbon (IV) oxide released by the immobilized *Pseudomonas macerans* on burnt kaolin (BK) was more than the amount released by *Bacillus subtilis* on BK (Table 3). This indicates that the nature of the organism, substratum and concentration of substrate dictate biodegradation of 1-methyl naphthalene.

Table 2: Biodegradation of 1-Methylnaphthalene by immobilized *Bacillus subtilis* on burnt kaolin (BK)

Mass of immobilized organism	pH after 36 h	pH after 72 h	Conc. of CO_2 (mg/L) after 36 h	Conc. of CO_2 (mg/L) after 72 h
1.0 g	8.40	8.20	0.50	0.68
2.0 g	8.65	8.32	0.47	0.74
3.0 g	8.70	8.27	.50	0.78

Table 3: Degradation of 1-Methyl naphthalene by burnt kaolin (BK)

Mass of solid media	pH after 36 h	pH after 72h	Conc. of CO_2(mg/L) after 36 h	Conc. of CO_2(mg/L) after 72 h
1.0 g	7.87	7.86	0.37	0.39
2.0 g	7.81	7.99	0.43	0.46
3.0 g	8.49	8.48	0.44	0.46

Effect of Dosage Treatment of Immobilized Microbes/solid media on Percentage Recovery: From the result of the effect of dosage of solid media (BK) used for immobilization of *Pseudomonas macerans* and *Bacillus subtillis* in the simultaneous adsorption-biodegradation process, and the adsorption process using BK only. As presented in Table 4, the removal of 1-methylnaphthalene by *Pseudomonas macerans* and *Bacillus subtilis* immobilized on burnt kaolin (BK), well as the burnt kaolin (BK) alone as adsorbent, indicated that the percentage removal of the hydrocarbon with regard to the use of *Pseudomonas macerans* (Table 4) immobilized on BK at dosage of 1.0 g, 2.0 g and 3.0 g was in the range of 79 - 89%; while the use of *Bacillus subtilis* immobilized on 1.0 g, 2.0 g and 3.0 g of BK had 79 - 88% removal. The lowest was with the use of BK alone with 69 - 77% removal. This implies that the immobization using BK resulted to better removal of the organic pollutant. There was a significant difference in the percentage removal of 1-Methyl naphthalene from wastewater by biodegradation compared to by adsorption alone onto BK ($P < 0.05$).

Table 4: Effect of dosage of burnt kaolin on the percentage removal of 1-methyl Naphthalene by biodegradation and adsorption

Treatment	Dosage (g)	% Removal
BK	1.0	69
BK	2.0	76
BK	3.0	77
Pseudomonas macerans immobilized on BK	1.0	79
Pseudomonas macerans immobiilized on BK	2.0	83
Pseudomonas macerans immobilized on BK	3.0	89
Bacillus subtilis immobilized on BK	1.0	79
Bacillus subtilis immobilized on BK	2.0	80
Bacillus subtilis immobilized on BK	3.0	88

Adsorption Isotherms: Adsorption is usually described through *isotherms*, that is, the amount of adsorbate on the adsorbent as a function of its pressure (if gas) or concentration (if liquid) at constant temperature. The quantity *adsorbed* is nearly always normalized by the mass of the adsorbent to allow comparison of different materials. In this study, the Langmuir adsorption isotherm was employed and a linear plot of $\frac{1}{Ce}$ vs $\frac{1}{Qe}$ indicates that the adsorption obeys lanmuir isotherm model , the values of Q_0 and b derived from the slope and intercept of the plot as shown in the Table 5. The R_L values obtained for this study implies that the adsorption is favourable with regard to the immobilized microbes as well as the burnt kaolin, as presented in Table 5, since $0 < RL < 1$).

Table 5: Langmuir Isothermal model adsorption data for the mineralization of 1-methyl Naphthalene

Treatment	Langmuir constants	R_L values
BK	$Q_o = 400$ b= 0.0011567	0.6337
Pseudomonas macerans immobilized on BK	$Q_o = 222.2$ b=0.0070093	0.2220
Bacillus subtilis immobilized on BK	$Q_o = 142.85714$ b=0.0121	0.1419

Conclusion: From the study, the use of solid media (burnt kaolin) during biodegradation have more advantages, resulting to higher percentage removal of 1-Methyl naphthalene compared to the use of adsorbent alone. The concentration of carbon (IV) oxide produced by immobilizing Pseudomonas *macerans* and *Bacillus subtillis* on burnt kaolin increased as the mass of burnt kaolin increases. Also the biodegradation of 1-methyl naphthalene, resulted to mineralization of the hydrocarbon as evidenced in the Fourier Transform-infrared result. The use of solid mineral in the biodegradation of hydrocarbons is a good dimension to be earnessed in environmental clean-up, and a source of foreign exchange earning to developing economies.

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