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Received: October 24, 2012 / Accepted: November 16, 2012 / Published: January 25, 2013.

Abstract: The adsorption of *Escherichia coli* and *Staphylococcus aureus* on chaff and WMDP (waste of molasses dates production) has been studied. FTIR spectra were employed to investigate the adsorption of *Escherichia coli* and *Staphylococcus aureus* on surfaces. Adsorption of bacteria resulted in obvious shifts of some infrared bands of adsorbents. The adsorption isotherms of *Escherichia coli* on two surfaces and *Staphylococcus aureus* on WMDP, are of L-curve type according to Giles classification. However, type H isotherm was observed in the adsorption of S. aureus on chaff. The adsorption isotherms of bacteria on the examined adsorbents conformed to the Langmuir and Temkin equations. The adsorption of bacteria was studied at different temperatures (10, 25 and 40 °C), the thermodynamic parameters (ΔH , ΔS and ΔG) have also been calculated and it has been found that the adsorption process of bacteria was exothermic in nature. The number of bacteria adsorbed on surfaces was decreased with the increase of sodium chloride concentration. The amount of bacteria cells adsorbed was increased in the presence of different cations and followed the sequence: FeCl₃ > CaCl₂ > KCl > NaCl. The pseudo-first order and pseudo-second order models for describing the kinetic data were applied and it was found that the process was well described by pseudo-second order model. The desorption studies indicated that the bacteria were strongly retained by two adsorbents.

Key words: Chaff, WMDP, bacteria, adsorption, water treatment.

1. Introduction

Water is essential to life. An adequate, safe and accessible supply must be available to all. Improving access to safe drinking water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible [1]. In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal feces. Wastewater discharge in fresh waters and costal seawaters are the major source of fecal microorganisms, including pathogens [2]. The water contaminated with pathological bacteria, such as *Escherichia coli*, for washing foods and drinking water in aircraft contaminated by bacteria became topics in connection with the generation of health problems [3].

Most people are concerned about the risk that bacteria may pose to human health. The presence of pathological bacteria in drinking water is known to cause various types of health problems such as, fever, diarrhea and abdominal cramps, chest pain, or hepatitis. *E.coli*, one of the most world know pathogens cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and gram-negative pneumonia [4].

The disinfection of bacteria is a major challenge for environmentalists. Generally chlorine has been widely

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used for the treatment of water disinfection throughout the world. But chlorine when react with organic material will generate chloroorganic compounds which are highly carcinogenic [5]. New antibacterial treatments have been demand and studied. The adsorption of bacteria on activated carbon (or single-walled carbon nanotube) has been pointed out and much attention has been directed towards these as a new technique for water treatment [3, 6, 7].

Few studies focus on wastewater treatment and removal of micro-pollutants from wastewater using adsorption technique [8-11]. It was reported that activated carbon was excellent in drinking water treatment to remove micro-pollutants [12-14]. The objective of the current work was to investigate the efficiency of two available natural surfaces (wheat chaff and WMDP) for the removal of biological pollutants. The antibacterial activity of the adsorbents was studied by measuring their ability to adsorbed two kinds of pathogenic bacteria (E. coli and S. aureus). The effect of different experimental conditions (temperature, contact time and electrolyte concentration) on the adsorption process was investigated to establish which parameters determine the process. The study was aimed to obtain the thermodynamic and kinetic data as well as the interacting forces controlling the adsorption of bacteria.

2. Materials and Methods

2.1 Preparation of Biosorbents

The surfaces of chaff WMDP in powder forms were washed with excessive amounts of distilled water; several washings were performed to remove dust and soluble materials. Washed surfaces were then dried under sunlight and in an oven at 120 °C for a period of 1.5 h and kept in airtight containers. The particle size of 150 μ m was used for the two surfaces in all experiments of this work.

2.2 Elemental Microanalyses (CHNS-Analyzer)

The amount of organics present in the adsorbents

was determined by using CHNS-Analyzer (Elemental microanalyzer, EA3000A, Italy).

2.3 Fourier Transform Infrared (FTIR) Analysis

FTIR was done to specify the chemical functional groups present in the adsorbents and the bacteria-loaded sorbent. IR spectra data were obtained for wavenumbers in the range 400-4,000 cm⁻¹ and the spectra of the adsorbents were acquired using the potassium bromide disc (KBr) technique by a Fourier transform infrared spectrophotometer (Shimadzu. 8500, Japan).

2.4 Biochemical Test of Bacteria

E. coli and *S. aureus* were as test bacteria, EMB (eosin methylene blue) and MS (mannitol salt) agars were used for the isolation, cultivation and differentiation of *E. coli* and *S. aureus*, respectively [15]. The diagnosis of bacteria was also carried out by using microscope method (Gram's Method) [16].

2.5 Preparation of Bacteria Solution

A stock solution of bacteria was prepared by adding 1.0 mL of bacteria to nutrient broth media. Solution was incubated at 37 °C for 24 h in EHCT incubator (Triup International Corp., Italy). The method of SPC (standard plate count) [17] was used to determine the number of bacteria in the stock solution. The number of colonies recorded in stock solution of *E. coli* and *S. aureus* was found 6.0×10^8 and 1.7×10^8 CFU/mL, respectively. The number of bacteria cells in the stock solution was also determined spectrophotometry, measured and compared with the corresponding value of McFarland standards [18].

2.6 Adsorption Isotherm

The adsorption isotherms were determined by shaking 0.2 g of adsorbent into 10 mL bacteria solutions, having different numbers $(1.2 \times 10^7 - 1.2 \times 10^8 \text{ for } E. \ coli$ and $4.4 \times 10^6 - 1.8 \times 10^7 \text{ for } S. \ aureus)$.

The flasks were shaken in a thermostatically controlled water bath at a speed of 150 rpm for 60 min. The absorbance of bacteria was analyzed at a wavelength of 600 nm by UV-Vis spectrophotometer (Double beam, Shimadzu. PC 1650, Japan), and concentration of bacteria cells was calculated using bacterial calibration curve. The quantity of bacteria cells adsorbed was calculated according to equation:

$$q_e$$
 or $\frac{x}{m} = \frac{V(C_o - C_e)}{m}$

q_e: sorption capacity (mg/g), *x*: the quantity adsorbed (mg), *m*: weight of adsorbent (g);

 $C_{o:}$ initial concentration (mg/L), C_e : equilibrium concentration (mg/L) and *V*: volume of solution (L).

2.7 Effect of Ionic Strength

To determine the factors affecting bacterial adsorption, the adsorption experiments were carried out in the presence of different chloride salt solutions, viz: NaCl, KCl, CaCl₂ and FeCl₃, as well as in the presences of different concentrations of NaCl solutions (0.01-0.2 w/v).

2.8 Effect of Temperature

Adsorption experiment was repeated in the same manner at temperatures of 10, 25 and 40 °C to estimate the basic thermodynamic functions for adsorption of bacteria.

2.9 Kinetic Studies

In order to determine the adsorption kinetics of

bacteria on the adsorbents, 10 mL of bacteria solution with an initial concentration of bacterial cells 7.3×10^7 for *E. coli* and 1.1×10^7 for *S. aureus*, was introduced into the flask and mixed with 0.2 g of sterilized solution at 25 °C and contact time 1-180 min. following the procedure of adsorption experiment.

2.10 Desorption Experiments

The desorption experiments were performed by resuspending the bacteria loaded sorbents in 10 mL of sterile distilled water. The suspension was shaken for 60 min at 25 °C. The spectrophotometric and standard plate count methods were used to calculate the amount of bacteria desorbed in the solution.

3. Results and Discussion

3.1 CHNS Analysis of Surfaces

The CHNS percentage analyses of adsorbents are shown in Table 1. The results indicate high elemental carbon with significant values for hydrogen and nitrogen for both surfaces, which may be due to its plant origin.

3.2 Adsorption of Bacteria on Surfaces

The adsorption isotherms of *E. coli* and *S. aureus* bacteria on adsorbents at 25 $^{\circ}$ C are shown in Fig. 1.

Table 1CHNS analysis of the surfaces.

Element	Chaff	WMDP	
С	42.8	50	
Н	6.5	5.8	
N	1.2	1.6	
S	0	0	



Fig. 1 Adsorption isotherms of (a) E. coli and (b) S. aureus on adsorbents at 25 °C.

The adsorption capacity of chaff surface for both bacteria was considerably larger than that of WMDP surface. The greater amount of bacteria adsorption on chaff may be attributed to the positive charge on its surface which attracts the negative charges of bacteria cells. The physical characterization of chaff as a kind of food plant contains abundant floristic fiber, functional groups and protein which make biosorptive process possible [19].

The structure and the chemical composition of *E. coli* (gram-negative) used in this study is different from that of *S. aureus* (gram-positive), the major difference between cell wall type is in polymer composition. The thin layer of lipid A, lipopolysaccharides and peptidoglycan exist in the cell surface of *E. coli*, and there is only a peptidoglycan layer for *S. aureus*. Imbedded in the Gram-positive cell wall are polyalcohols, known as teichoic acids, some of which are lipid-linked to form lipoteichoic acids. These acids give the cell wall an overall negative charge, due to the presence of phosphodiester bonds between the teichoic acid monomers. On the contrary, the highly charged nature of lipopolysaccharides confers an overall negative charge on the gram-negative cell wall [5, 20].

Bacteria adsorption and attachment to a surface is a two-step process. The first step, reversible adsorption, takes place when bacteria located in the secondary minimum overcome the secondary repulsive force between themselves and the surface, and adsorb to the surface. Reversible adsorption is a weak interaction between the bacteria and the porous materials. The primary forces responsible for this first phase are electrostatic forces and van der waals forces. The second step is the irreversible adsorption, sometimes referred to as adhesion. Adhesion is a permanent interaction involving a large amount of energy [21]. Many researches suggested that different forces play a major role in the mechanism of bacteria adsorption, such as static force, hydrophobic attraction and hydrogen bonding [22-24].

The FTIR spectra before and after bacteria adsorption were investigated (Figs. 2 and 3) and the results indicated that the wide absorption bands at 3,355 and 3,386 cm⁻¹ for chaff and WMDP, respectively, corresponding to the stretching vibration of O-H and N-H groups, shifted and reduced after adsorption of bacteria. This result implied that non-electrostatic force may also involved in bacteria adsorption. Among non-electrostatic forces, hydrogen bonding may contribute significantly to the adsorption process. The absorption bands at 1,738 and 1,728 cm⁻¹ for chaff and WMDP, respectively, shifted to lower wavenumber for most adsorption systems. In the WMDP spectrum, the peak at 1,635 cm⁻¹ shifted to 1,627 and 1,620 cm⁻¹ after adsorption of *E. coli* and *S.* aureus, respectively.



Fig. 2 FTIR-spectra of (a) chaff, (b) chaff-S. aureus, (c) chaff-E. coli.



3.3 Adsorption Isotherms

The adsorption isotherms of *E. coli* on two surfaces and *S. aureus* on WMDP comport with a type L Giles classification (Fig. 1). On the other hand, the adsorption isotherm of *S. aureus* on chaff is of H-type feature in Giles classification. The equilibrium data presented were applied to Langmuir, Freundlich and Temkin isotherm equations (Figs. 4 and 5) and Table 2.

By comparing the results presented in Table 2, it can be observed that the Langmuir isotherm can accurately



Fig. 4 Langmuir isotherms of (a) E. coli, (b) S. aureus on adsorbents at 25 °C.



Fig. 5 Temkin isotherms of (a) E. coli, (b) S. aureus on adsorbents at 25 °C.

The Temkin equation was also fitted to the experimental data ($r^2 > 0.85$). The constants K_T and b were determined from the intercept and slope of the plot between q_e and $\ln C_e$ (Fig. 5). Linear plots for Temkin adsorption isotherms, which consider

chemisorption of an adsorbate on the adsorbent [25]. This further supports the findings that the adsorption of bacteria on adsorbent particles may be occur by chemisorption process.

3.4 Thermodynamic Parameters of Adsorption

The effect of incubation temperature on bacteria adsorption is shown in Figs. 6 and 7. The amount of *E. coli* and *S. aureus* uptake by adsorbents decreased with the increase of temperature indicating that the adsorption

 Table 2
 Freundlich, Langmuir and Temkin isotherm constants for bacteria uptake by adsorbents.

	Destaria	Langmuir equation			Freundlich equation			Temkin equation		
	Dacterra	K_L	q_m	r^2	K_F	п	r^2	K_T	b	r^2
	E. coli	3.0×10^{-8}	16.7×10^{5}	0.9000	-	-	0.8473	1.2×10^{-7}	6.6×10^{3}	0.9530
Chaff	S. aureus	11.6×10^{-5}	$10.0 imes 10^5$	0.9993	0.0002	3.709	0.9661	2.0×10^{-7}	1.3×10^2	0.8800
WMDD	E. coli	1.0×10^{-7}	11.1×10^{5}	0.9893	0.0003	3.153	0.8605	2.4×10^{-5}	7.6×10^{2}	0.8665
WMDP	S. aureus	6.0×10^{-7}	$5.0 imes 10^5$	0.9865	-	-	0.5512	1.7×10^{-5}	3.4×10^2	0.9439



Fig. 6 Adsorption isotherms of (a) E. coli, (b) S. aureus on chaff at different temperatures.



Fig. 7 Adsorption isotherms of (a) E. coli, (b) S. aureus on WMDP at different temperatures.

process was exothermic in nature. The extent of bacteria adsorption on solid surfaces was affected by bacterial physiological state, and vigorous bacterial metabolism facilitates their adsorbabilities [26]. Therefore, the greater adsorption of bacteria on adsorbents at low temperature may be related to the physiological state of *E. coli* and *S. aureus*.

The values of enthalpy change and other thermodynamic parameters are listed in Table 3. With the exception of the negative ΔG value of *S. aureus* adsorption on chaff, the positive and weak values of ΔG indicate that the process is feasible but non-spontaneous. The positive value of ΔS reveals the increased randomness at the solid-solution interface during the fixation of the *S. aureus* bacteria on the active sites of chaff surface.

3.5 Effect of Ionic Strength

As shown in Fig. 8, the adsorption of *E. coli* and *S. aureus* on adsorbents decreased remarkably in the presence of 0.01%-0.20% of NaCl salt. The decrease in bacteria adsorption with the increase cation concentration could be explained that the sodium ions of electrolyte would adsorb more preferentially on the adsorbents than the negatively surface of bacteria.

When adsorption of bacteria on adsorbents was carried out in the presence of different electrolytes, the adsorption capacity of the bacteria was markedly increased (Table 4).

These results can be explained if the initial adhesion

Table 3 Thermodynamic parameters of adsorption process of bacteria on the adsorbents at 25 °C.

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Bacteria	Adsorbett	$\Delta H (kJ \cdot mol^{-1})$	$\Delta G (kJ \cdot mol^{-1})$	$\Delta S (J \cdot mol^{-1} \cdot K^{-1})$	Equilibrium Constant (K)
E coli	Chaff	-40.003	+3.059	-144.508	0.291
E. COll	WMDP	-52.719	+3.689	-189.293	0.226
C. automa	Chaff	-5.093	-7.035	+6.518	17.111
S. aureus	WMDP	-22.920	+0.320	-77.9916	0.879



Fig. 8 Effect of sodium chloride on the adsorption of (a) E. coli, (b) S. aureus on adsorbents at 25 °C.

Table 4	Effect of different electrolytes	s (0.05%) on the	e adsorption of bacteri	ia at 25 °C.
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Salt		Е.	coli		S. aureus				
	Chaff		WMDP		Chaff		WMDP		
	$C_e imes 10^8$ (cfu·L ⁻¹)	$q_e(cfu \cdot g^{-1})$	$C_e \times 10^8$ (cfu·L ⁻¹)	$q_e (\mathrm{cfu} \cdot \mathrm{g}^{-1})$	$\frac{C_e \times 10^7}{(\text{cfu} \cdot \text{L}^{-1})}$	$q_e (\mathrm{cfu} \cdot \mathrm{g}^{-1})$	$\frac{C_e \times 10^7}{(\text{cfu} \cdot \text{L}^{-1})}$	$q_e (cfu \cdot g^{-1})$	
NaCl	1.32	4,857,143	1.84	2,214,286	6.22	10,937,500	1.87	10,312,500	
KCl	1.20	5,000,000	1.77	2,357,143	5.91	10,950,000	1.84	10,325,000	
CaCl ₂	1.12	5,857,143	1.61	3,000,000	5.27	86,250,001	1.72	10,387,500	
FeCl ₃	0.94	6,385,714	1.58	3,571,429	4.24	91,250,001	1.57	10,462,500	

of the bacteria on adsorbents is considered to be a physicochemical process. In the presence of electrolytes, the repulsive electrostatic interaction would be reduced, so favouring bacterial adsorption [7]. More bacteria were adsorbed in the presence of FeCl₃, than with CaCl₂ or KCl, because of the greater ionic strength of the former. The cell surface hydrophobicity of bacteria is increased in the presence of aqueous metal salt solutions. Cell surface hydrophobicity is known to influence positively cell-substrate adsorption and increases with quantity of metal bound by the outer membrane [27]. Most cations are bound in amounts related to their respective valencies, ionic radii and hydration energies [28]. The above results suggested that the electrostatic forces may play a role in bacteria-sorbent adhesion. The thickness of the

electrostatic double layer surrounding the bacterium or adsorbent was compressed with increase of electrolyte concentration, resulting in the increase of electrostatic attractive force and promotion of bacteria adsorption [29].

3.6 Adsorption Kinetics

The kinetics data for the adsorption of bacteria on adsorbents were correlated with a pseudo-first order and a pseudo-second order kinetic models. Fig. 9 shows the plot of $\ln(q_e-q_t)$ against *t* for the testing of first order model.

Similarly, second order model was tested by the plot of t/q_t versus t (Fig. 10).

The values of two models parameters and the corresponding correlation coefficients are summarized



Fig. 9 The applicability of pseudo-first order kinetic model to bacteria adsorption (a) *E. coli*, (b) *S. aureus* on adsorbents at 25 °C.



Fig. 10 The applicability of pseudo-second order kinetic model to bacteria adsorption (a) *E. coli*, (b) *S. aureus* on adsorbents at 25 °C.

		Chaf	f		WMD	Р	
	k_I	q_e	r^2	k_{I}	q_e	r^2	
E. coli	0.047	2937296	0.8466	0.0462	724328	0.6906	
S. aureus	0.071	571488	0.9968	0.083	295670	0.9838	

Table 5	Adsorption kinetics	parameters of	pseudo-first	t order of	bacteria o	n adsorbents.
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Table 6 Adsorption kinetics parameters of pseudo-second order of bacteria on adsorbents.

	Chaff					WMD	Р	
	k_2	q_e	h	r^2	k_2	q_e	h	r^2
E. coli	1.0×10^{-8}	5,000,000	250,000	0.9574	6.1 × 10 ⁻⁸	1,428,571	125,000	0.9786
S. aureus	2.5×10^{-8}	1,000,000	25,000	0.9058	45.0×10^{-8}	333,333	50,000	0.9864

in Table 5 and 6.

As shown in Fig. 10 and Table 6, the pseudo-second order model correlates the adsorption kinetic data slightly better than the Lagergren first order model. However, the values of r^2 for pseudo-first order for adsorption of *S. aureus* on two adsorbents are relatively high (above 0.98). The diffusion of bacteria into adsorbent was affected by its shape and size. It is generally believed that adsorption of bacteria with size less than 1µm (*S. aureus*) is higher than that with size greater than 1µm (*E. coli*) [16].

3.7 Desorption Studies

To evaluate the binding ability of biomass and the parameters affecting the process of bacteria adsorption, the desorption of both bacteria was performed by using different concentrations of bacteria. The bacteria-sorbent complex was washed by sterile distilled water and the percentage of desorption for bacteria was determined. For 1.76×10^7 cfu only 5.6% and 20% of S. aureus cells released from chaff and WMDP surface respectively, while the desorption percentage of *E. coli* bacteria $(1.12 \times 10^8 \text{ cfu})$ from chaff and WMDP was 20% and 30%, respectively, suggesting that the bacteria were strongly retained by two adsorbents and the adsorption may be resulted from the electrostatic and non-electrostatic interactions.

4. Conclusions

Main conclusions of this work are:

Chaff and WMDP have been found to be

economically viable and potential biosorbents for the removal of pathogenic bacteria from aqueous solution.

Chaff surface appeared of higher activity in the adsorption of bacteria.

The adsorption isotherms of bacteria on chaff and WMDP obeyed the Langmuir and Temkin isotherm.

The bacteria-adsorbent complex exhibited low exothermic enthalpy values.

The amount of bacteria adsorbed on two surfaces was decreased with the increase of ionic strength of solution.

The kinetic studies indicated that the adsorption of bacteria followed pseudo-second order rate expressions.

The adsorption of bacteria on two surfaces was reversible to a limited extent.

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