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Studies on sorption properties of pathogens on natural materials

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Abstract: *Presence of pathogens in high numbers in waste water is a cause of concern. Techno economic feasibility has restricted the conventional and non conventional treatment approaches for pathogen removal. Despite prolific use, carbon adsorption technology remains an expensive treatment process. The present study investigates the use of rice husk (RH), saw dust (SD), groundnut shells (GS) as natural agro-residues and partially weathered deccan trap basalt (PWDTB) for their sorption capacities and desorption pattern for two indicator organisms viz. Escherichia coli K12 and Staphylococcus aureus. Sorption experiments were carried out at flow-rate of 1.5 bed volumes per hour (bv hr^{-1}) for cell suspension volume of 4, 8, 16 and 32 bed volumes. PWDTB have shown high sorption coefficient and log removal for E. coli K12 whereas GS have shown high sorption coefficient and log removal for S. aureus. PWDTB have shown maximum desorption constant and log retention for E. coli K12 whereas GS have shown maximum desorption constant and log retention for S. aureus during desorption experiment. Retention pattern suggest that adsorption is partially irreversible for almost all the materials used. It suggest that PWDTB in combination with RH and / GS could help in removal of pathogens from waste water.*

Key words: *Sorption, Desorption, Agro residues, Partially weathered deccan trap basalt, Pathogens*
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Introduction

There are various technological options available for wastewater treatment. The conventional methods used are activated sludge process, trickling filter, lagoon, ozone oxidation while non conventional approach includes wetland, aquaculture and land treatment system. But none of them cater to pathogen removal explicitly and pathogen counts in wastewater remains a problem. Many studies with activated carbon as an adsorbent for the reclamation of municipal and industrial water have been reported (Cheremisinoff and Ellerbusch, 1979; Weber and Van Vliet, 1980). Despite its prolific use, carbon adsorption remains an expensive treatment process and hence a range of low cost carbonaceous and mineral precursors have been tried (Pollard *et al*., 1992). Many of these starting compounds are either agro by products or industrial by products. Their use as secondary adsorbents contributes to waste minimization, recovery and reuse (Patterson, 1989). Attachment of bacteria to the various solid components is an important factor influencing the microbial physiology in these systems, subsequently their removal from wastewater (Lyklema *et al.,* 1989).

Rice cultivation is practiced in nearly 75 countries across the globe feeding nearly half of the population of the world and generates 80 million tones of rice husk (Shafey, 2007). Rice production is expected to be 118.8 Mt yr^{-1} with rice husk generation of 213.9 Mt yr^{-1} by 2010 in India. Rice husk is an important agricultural crop residue and rice processing generates roughly 23% rice husk as by-product during dehusking at rice mills (Kumar and Bandyopadhyay, 2006). It contains about 20% silica, and has been reported as a good sorbent for metals and basic dyes. India is the second largest producer of groundnuts in the world with 8 million ha of cultivation producing 8,004,000 Mt yr^{-1}. Groundnut shell production is expected to be 12.2 Mt yr^{-1} with husk generation of 28.1 Mt yr^{-1} by 2010 in India (Ravindranath *et al*., 1997). Groundnut shells are carbonaceous, fibrous solid waste, and known to be the precursor for making activated carbon. These by-products offer the advantage of having a greater percentage of non-carbon constituents in their composition compared to coal or peat and therefore afford a greater chance of retaining functional groups, especially oxygenated groups, in the carbonized product.

This paper presents biosorption properties of partially weathered deccan trap basalt (PWDTB) and some agro-residues *viz*. rice husk (RH), saw dust (SD), groundnut shells (GS) along with Seralite, a weak cation exchange resin (as control) in a glass column reactor with the aim of using them for pathogen removal from wastewater.

Materials and Methods

Preparation of adsorbents: The rice husk (RH) used in the present work was obtained from a rice mill located at Kalyan near Mumbai, India. Sawdust (SD) was collected from a local sawmill. It was of teakwood origin. Groundnut shells was collected from local market. Seralite (SERALITE-WRC-50) a weak cation exchanger was supplied by SISCO Research Laboratory, Mumbai. PWDTB was obtained from a quarry site at Vikhroli, Mumbai. The materials (except for saw dust) were washed with pure water several times to remove dust and fines. The washing process was repeated until the color of the wash water was transparent. The washed materials

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were then dried in a hot air oven at 60°C for 24 hr. The saw dust was washed thoroughly with double distilled water and then with 0.1 N NaOH to remove lignin based color materials. It was then washed with 0.1 N H_2SO_4. Finally, it was washed with double distilled water several times and dried in an oven at 105±5°C for 6 hr. All the dried materials were sieved into the different sizes ranging from 250 to 780 µm.

Preparation of column: The adsorption experiments were carried out by filling the adsorbents (0.5-7 g) with particle size (250-780 µm) in glass columns (2 cm x 100 cm) that were equipped with a stopper for controlling the column elute flow rate as shown in Fig. 1. Bed volume (bv) for all the adsorbents was kept constant as 314 cm^3. Glass wool was placed at the bottom of the column to prevent the loss of adsorbent during the experiment. The flow rate was kept constant with the help of peristaltic pump at the feed end and by controlling stopper valve at the column end. The process was carried out at ambient temperature.

Experimental set-up: *Escherichia coli K12* (MTCC 1302) and *Staphylococcus aureus* (MTCC 3160) were obtained from microbial type culture collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH) Chandigarh, India. These bacterial species were at first analyzed for growth curve with the aim of getting cells in the mid-log phase. Growth curve was performed by overnight inoculation of the cultures in 25 ml of the nutrient broth and then re-inoculating after 16 hr in the fresh 100 ml of nutrient broth. The optical density was checked using UV-VIS spectrophotometer at 520 nm. Simultaneously viable count was also carried out to find out the initial number of cells present. After specific intervals spectrophotometer analysis and viable count were repeated. The mid-log phase culture suspension was harvested and centrifuged at 10000 rpm for 10 min (Fig. 2).

Centrifuged biomass was washed repeatedly with sterilized physiological saline and re-centrifuged. Cell pellets were then mixed with physiological saline to have required cell suspension volume (4, 8, 16 and 32 bed volume). The adsorbent materials were sterilized, cooled and incubated overnight. This cycle was repeated at least three times to ensure complete sterilization. Glass columns were then filled with pre-weighed sterilized adsorbent and were given washing with physiological saline (0.85% w/v NaCl) before starting the column experiment. The sorption experiment aimed at examining the change in viable cell-counts after contacting with adsorbent materials in the column at a flow-rate of 1.5 bed volumes per hour (bv hr^{-1}). Fixed flow of 1.5 bv hr^{-1} was maintained with the help of peristaltic pump and measured at the outlet of the column towards steady state flow. Volume of cell suspension varied as mentioned earlier in the range of 4-32 bed volume (1.25-10.0 ltrs). Accordingly, the contact time required for sorption also varied (2.56, 5.2, 10.4 and 20.8 hr respectively). Samples were collected from collection tank at regular interval of time as well as at the end of the experiment for final viable cell count. Sorption coefficient in term of unit mass of adsorbent was calculated as per Kawabata *et al.* (1983).

$$\text{Removal coefficient (ml g}^{-1}\text{hr}^{-1}) = \log\left(\frac{N_0}{N_t}\right) x \frac{V}{wt} \quad (1)$$

Similarly, a sorption coefficient in terms of unit volume of adsorbent was calculated as per Kawabata *et al.* (1983).

$$\text{Removal coefficient (ml cm}^{-3}\text{hr}^{-1}) = \log\left(\frac{N_0}{N_t}\right) x \frac{V}{vt} \quad (2)$$

where, N_0 is the initial number of viable cells (cells ml^{-1}), N_t the final number of viable cells (cells ml^{-1}), V is the volume of influent cell suspension (ltr), w is the weight of the adsorbent (g), v is the volume of the column (ltr) and t is the time of contact in hr.

Desorption studies: Subsequently, desorption studies were also performed by passing physiological saline (0.85 % w/v NaCl) of same volume (4, 8, 16 and 32 bed volume) at a same flow rate through the same sorption column and leachate were checked for viable cell count. Desorption coefficient in terms of unit volume of adsorbent was calculated as per Kawabata *et al.* (1983).

$$\text{Desorption coefficient (ml cm}^{-3}\text{hr}^{-1}) = -\log\left(\frac{N_s}{N_d}\right) x \frac{V}{vt} \quad (3)$$

where, N_s is the sorbed viable cells (cells ml^{-1}) on to the adsorbent during sorption experiment, N_d the eluent number of viable cells (cells ml^{-1}) after elution with normal saline, V is the volume of influent cell suspension (ltr), v is the volume of the column (ltr) and t is the time of contact in hr.

Analysis: Trace element concentrations of the PWDTB and agro residues were determined by XRF spectroscopy on pressed powder pellets using a Philips PW 2404 instrument. Data obtained was processed using Philips SuperQ software. All the analyses were carried out in triplicates and mean with standard deviation was calculated. The surface area of the PWDTB was determined by BET method using a surface area analyzer (Model 1750 SORPTY, Carlo Erba, Italy). The bulk density of PWDTB was determined using specific gravity bottles. The physico-chemical properties of rice husk, ground nut shells and saw dust is described by Shukla *et al.* (2005), Akinyele and Akinyosoye (2005). Viable count for the representative organisms *E. coli K12* and *S. aureus* present in the eluent from sorption and desorption experiment was also carried out. Appropriately diluted (10^{-3}–10^{-7}) sample volume (0.1 ml) was plated out using spread plate method to ensure 20-250 colonies. Media used were HiCrome ECC Selective Agar Base (M1294) for *E. coli K12* and HiCrome UTI Agar/Modified (M1418) for *S. aureus* obtained from Hi Media Laboratory Pvt. Ltd., India. Plates were incubated for 24 hr at 37°C and counts were expressed as cells ml^{-1}.

Results and Discussion

The surface properties in terms of SiO_2, K_2O, Fe_2O_3, Al_2O_3, CaO, MnO and Na_2O for PWDTB, rice husk, saw dust and ground nut shells are shown in Table 1.

Sorption: Sorption and desorption results for PWDTB along with agro-residues *viz.* rice husk (RH), saw dust (SD), groundnut shells (GS) and commercial anion exchanger (Seralite) for different cell suspension volume *viz.* 4, 8, 16 and 32 bed volume are presented in Table 2. Influent concentrations of both the indicator organisms were kept constant in the range of 10^8-10^9 cells ml^{-1}. Effluent concentrations decreased with decrease in bed volume (bv). Sorption coefficients in terms of unit mass and volume of the adsorbent was calculated as per the equation 1 and 2 respectively. Summary of sorption results in terms of log removal and removal coefficient (ml $g^{-1}hr^{-1}$ and ml cm^{-3} hr^{-1}) is presented in Table 3.

Sorption coefficients were expressed in terms of per unit mass (ml $g^{-1}hr^{-1}$) and unit volume (ml $cm^{-3}hr^{-1}$) of the adsorbent. Volume based sorption coefficients for *E. Coli K12* were found to be maximum for PWDTB followed by RH, GS, Seralite and SD for all four suspension volumes (4-32 bv). It was as high as 4.32 ml cm^{-3} hr^{-1} for PWDTB and lowest for SD (2.42 ml $cm^{-3}hr^{-1}$) for 4 bed volume. Weight based sorption coefficient have shown the performance in the order of RH > GS > SD > Seralite > PWDTB. This may be due to relative low bulk densities of agro-residues in comparison to PWDTB. It was as high as 31.1 l $g^{-1}hr^{-1}$ for RH as against 3.6 ml $g^{-1}hr^{-1}$ for PWDTB for 4 bed volume. Log removal for *E. coli K12* was also high for PWDTB followed by RH, GS and SD (for all four suspension volumes). It was as high as 6.4 for PWDTB and lowest for SD (3.9) for 4 bed volume. Seralite used as a control have shown second lowest sorption coefficient (volume and weight base) and log removal for *E. coli K12*. Volume based sorption coefficients for *S. aureus* were found to be maximum for GS followed by RH, PWDTB, SD and Seralite for all four suspension volumes (4-32 bv). It was as high as 3.66 ml cm^{-3} for GS and lowest for SD (2.90 ml $cm^{-3}hr^{-1}$) for 4 bed volume. Weight based sorption coefficient have shown the performance in the order of RH > GS > SD > Seralite > PWDTB. This may be due to relative low bulk densities of agro-residues in comparison to PWDTB. It was as high as 25.9 ml $g^{-1}hr^{-1}$ for RH as against 2.9 ml $g^{-1}hr^{-1}$ for PWDTB for 4 bed volume.

Table - 1: Surface property of the materials

Name of adsorbent	Surface metal oxides (%)						
	SiO_2	K_2O	Fe_2O_3	Al_2O_3	CaO	MnO	Na_2O
PWDTB	44.8	0.6	13.1	10.8	8.4	0.2	1.2
Rice husk	96.3	2.3	0.5	0.2	0.4	—	—
Saw dust	0.5	0.9	0.4	0.1	1.7	—	0.04
Groundnut shells	3.6	1.8	4.1	1.2	0.01	0.1	0.2

Table - 2: Sorption and desorption counts obtained for Agro-residues, PWDTB and seralite

Suspension volume (bed volume ltr)	Materials	*E. coli K12**			*S. aureus**		
		Sorption		Desorption	Sorption		Desorption
		Influent	Effluent	Effluent	Influent	Effluent	Effluent
32	RH	1.6×10^9	1.5×10^6	8.0×10^5	7.0×10^9	1.3×10^7	1.3×10^7
	SD	1.5×10^9	5.6×10^7	$6.7x\ 10^6$	5.2×10^8	1.0×10^7	1.0×10^7
	GS	2.1×10^9	1.1×10^7	1.1×10^6	1.7×10^9	2.1×10^6	3.1×10^5
	PWDTB	5.3×10^9	9.8×10^5	7.7×10^5	1.5×10^{10}	8.8×10^7	1.3×10^8
	Seralite	2.5×10^9	1.7×10^7	5.1×10^6	3.4×10^9	5.1×10^8	2.6×10^9
16	RH	2.1×10^{10}	2.7×10^6	5.7×10^5	2.1×10^{10}	4.7×10^5	2.4×10^6
	SD	9.2×10^9	9.0×10^6	6.6×10^7	2.1×10^{10}	1.2×10^6	2.1×10^7
	GS	1.1×10^9	2.2×10^6	2.3×10^6	9.1×10^9	4.7×10^4	8.2×10^5
	PWDTB	2.0×10^{10}	1.9×10^5	4.8×10^5	7.1×10^9	7.1×10^5	2.1×10^6
	Seralite	5.7×10^9	9.4×10^5	2.4×10^6	2.1×10^{10}	2.9×10^7	1.7×10^{10}
8	RH	1.3×10^9	7.5×10^4	5.9×10^4	1.3×10^9	1.8×10^4	2.5×10^4
	SD	7.0×10^9	6.3×10^6	6.9×10^7	1.3×10^{10}	2.9×10^5	1.5×10^7
	GS	8.0×10^8	6.3×10^4	2.6×10^5	1.3×10^{10}	1.9×10^4	2.9×10^5
	PWDTB	3.0×10^{10}	5.1×10^4	8.8×10^5	1.3×10^9	4.9×10^4	2.6×10^5
	Seralite	3.0×10^{10}	2.6×10^6	7.6×10^6	1.3×10^9	2.1×10^5	3.4×10^8
4	RH	2.1×10^8	8.2×10^3	2.1×10^4	2.1×10^{10}	2.8×10^5	2.5×10^5
	SD	4.2×10^{10}	5.2×10^6	2.4×10^4	2.1×10^{10}	2.1×10^4	4.1×10^5
	GS	4.2×10^9	2.1×10^4	1.6×10^3	2.1×10^{10}	2.1×10^4	2.2×10^5
	PWDTB	8.3×10^9	3.3×10^3	2.8×10^3	2.1×10^9	2.3×10^4	2.9×10^4
	Seralite	4.2×10^{10}	8.4×10^5	9.3×10^4	2.1×10^{10}	1.0×10^5	2.1×10^8

* all units in cells ml^{-1}

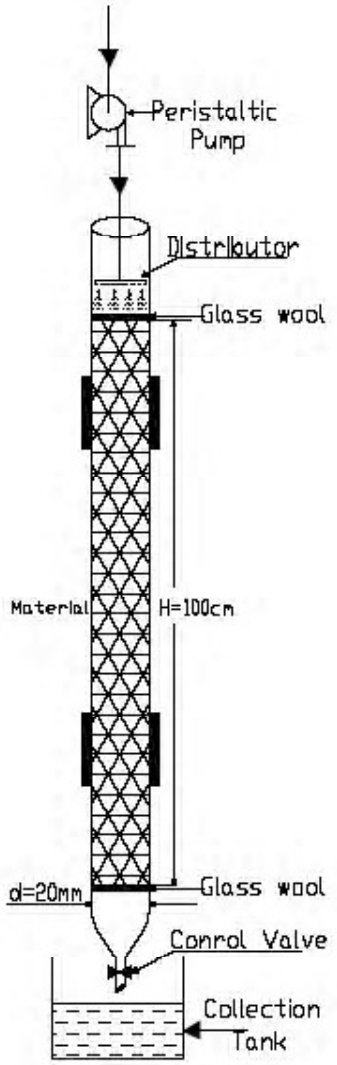

Fig. 1: Glass column used for adhesion studies

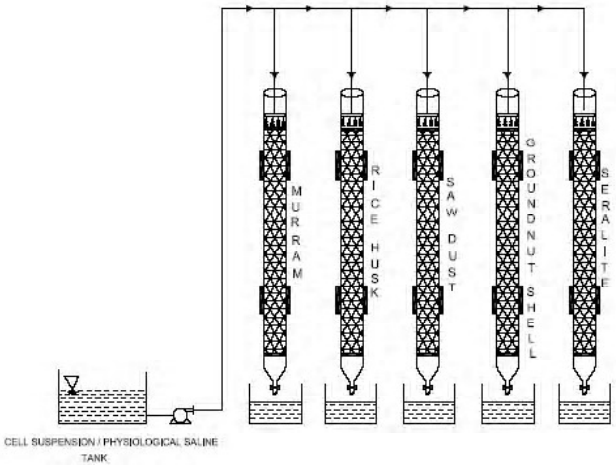

Fig. 2: Experimental set up for sorption and desorption studies using different materials

Log removal was also high for GS followed by RH, SD, PWDTB and Seralite (for all four suspension volumes). It was as high as 6.0 for PWDTB and lowest for SD (4.9) for 4 bed volume. With decrease in cell suspension loading (bed volume), removal coefficient (unit mass and volume based) and log removal were found to be decreasing.

Desorption: It was of interest to know the sorption pattern of microorganisms (reversible or irreversible) used in the study and hence, desorption experiment with normal saline were carried out immediately after sorption experiments. Desorption coefficient in terms of unit volume was calculated as per equation 3. Summary of desorption experiment in terms of desorption constant and log retention on to the media is discussed in Table 7. Desorption coefficients was expressed in terms of per unit volume (ml $cm^{-3}hr^{-1}$) of the adsorbent and termed as desorption rate constant or desorption constant. PWDTB have shown maximum desorption constant and log retention for *E. coli K12* followed by GS, SD, Seralite and RH. Similarly, GS have shown maximum desorption constant and log retention for *S. aureus* followed by RH, PWDTB, SD and Seralite. Seralite as a control even though has shown second maximum sorption for *S. aureus*, desorption pattern however has shown least desorption constant and log retention.

Increasing experimental evidences suggest that bacterial adhesion onto the surfaces is strongly influenced by cell characteristics, external physicochemical environment and surface properties of the materials. Derjaguin-landau-verwey-overbeek (DLVO) theory has been widely used to estimate net interaction between bacterial cells and inert surfaces resulting from the addition of Lifshitz-van der waals and electrostatic interactions. These interactions are also influenced by cell modifications and properties such as physicochemical state, extracellular polymeric substance (EPS), C/N ratio in nutrients, lipopolysaccharides production, motility or shape of bacteria. Among the cell surfaces, hydrophobicity is the driving force for bacterial adhesion. Among other, physical (porous media, temperature, flow velocity) and chemical (ionic strength, ionic species, pH) factors are known to impact bacterial adhesion (Jacobs *et al.,* 2007). Variables which could affects the sorption of microorganism on solid particles are grouped as: (i) character of microorganism, (ii) character of adsorbent (type, ionic form, particle size, cross linkage and concentration), (ii) character of environment (hydrogen ion concentration, inorganic salt concentration, organic compounds, agitation, time of contact and temperature).

Bacterial cell surface carries a net negative charge under most physiological conditions (pH 5.0-7.0). As most natural surfaces *viz.* soil, sand and agro residues are negatively charged as well bacteria generally experience electric double layer repulsion when approaching these surfaces. But bacterial cell surface is a highly dynamic surface in response to change in environmental conditions. For example, charged groups may associate or dissociate upon changes in pH or ionic strength of suspending fluid. Bacterial cell surface may be penetrable to solvents and solutes due to the presence of peptidoglycan layer as on gram positive bacteria, presence of surface appendages (*viz.* fibrils, fimbriae or flagellae) or a slime capsule. Charged groups present inside bacterial cell wall influences electric double layer interactions in bacterial adhesion (Poortinga *et al.,* 2002).

Table - 3: Summary of sorption experiments

Suspension volume (bed volume ltr)	Material	*E. coli K12*			*S. aureus*		
		Sorption coefficient		Log removal	Sorption coefficient		Log removal
		ml g^{-1} hr^{-1}	ml cm^{-3} hr^{-1}		ml g^{-1} hr^{-1}	ml cm^{-3} hr^{-1}	
32	RH	22.4	2.28	3.03	19.0	2.12	2.73
	SD	11.9	1.81	1.43	11.5	1.91	1.72
	GS	14.5	2.03	2.28	16.7	2.24	2.91
	PWDTB	2.2	2.49	3.73	1.6	1.96	2.23
	Seralite	3.1	1.99	2.17	2.7	1.68	0.82
16	RH	24.1	2.46	3.89	26.5	2.79	4.65
	SD	13.8	2.19	3.01	16.0	2.60	4.24
	GS	15.2	2.18	2.70	21.4	3.26	5.29
	PWDTB	2.6	2.99	5.02	2.3	2.58	4.00
	Seralite	4.1	2.50	3.78	3.3	2.12	2.86
8	RH	25.0	2.86	4.24	27.1	3.28	4.86
	SD	14.5	2.22	3.05	15.6	2.83	4.65
	GS	19.0	2.84	4.10	22.7	3.62	5.84
	PWDTB	2.9	3.41	5.77	2.5	2.98	4.42
	Seralite	4.0	2.50	4.06	4.3	2.62	3.79
4	RH	31.1	3.26	4.41	25.9	2.97	5.00
	SD	15.2	2.42	3.91	16.6	2.90	4.88
	GS	21.4	3.41	5.30	25.0	3.66	6.00
	PWDTB	3.6	4.32	6.40	2.9	3.27	4.96
	Seralite	4.1	2.75	4.70	5.0	3.16	5.32

Table - 4: Summary of desorption experiments

Suspension volume (bed volume ltr)	Materials	*E. coli K12*		*S. aureus*	
		Desorption constant (ml $cm^{-3}hr^{-1}$)	Log retention	Desorption constant (ml $cm^{-3}hr^{-1}$)	Log retention
32	RH	-5.05	3.30	-4.18	2.73
	SD	-3.57	2.33	-6.04	1.71
	GS	-5.02	3.28	-7.22	3.74
	PWDTB	-5.88	3.84	-7.54	2.07
	Seralite	-4.11	2.69	-7.21	0.05
16	RH	-6.99	4.57	-6.04	3.94
	SD	-3.28	2.14	-4.60	3.01
	GS	-4.10	2.68	-6.19	4.05
	PWDTB	-7.07	4.62	-5.40	3.53
	Seralite	-5.17	3.38	-0.14	0.09
8	RH	-6.65	4.34	-7.22	4.72
	SD	-3.07	2.01	-4.50	2.94
	GS	-5.34	3.49	-7.12	4.65
	PWDTB	-6.94	4.53	-5.66	3.70
	Seralite	-5.51	3.60	-0.89	0.58
4	RH	-6.12	4.00	-7.54	4.92
	SD	-9.56	6.24	-7.21	4.71
	GS	-9.83	6.42	-7.62	4.98
	PWDTB	-9.91	6.47	-7.43	4.85
	Seralite	-8.66	5.65	-3.06	2.00

Gram positive cells are structurally the less complicated of the two principal types and are composed primarily of techoic acids, proteins, polysaccharides and mucopeptides. Gram negative bacterial cell walls are composed of a complex multilayered arrangement of lipo-polysaccharides, phospholipids, proteins and mucopeptides (Poortinga *et al.,* 2002).

Bacterial adsorption onto agro-residues is supposed to occur mainly through affinity adsorption, anion exchange and the carbon surface of the agro residues. Affinity adsorption is associated to the surface behavior of agro-residues, anion exchange relates to the existing forms of the microbial species and carbon in contact with water reduces oxygen to a hydroxyl group, thus the carbon loses electrons

and become positively charged facilitating adhesion and hence pathogen removal (Poortinga *et al.,* 2002). It is assumed that in soil environment, most cells are attached due to the large surface to volume ratio of the inorganic phase rather than due to a strong (specific) interaction between bacteria and soil particles (Jacobs *et al.,* 2007).

BET surface area of the material used in this study was found to be in the order of SD > GS> PWDTB > RH. Surface properties indicate that RH has more negatively charged SiO_2 followed by PWDTB (Table 2, 3). Hence more electrolytic repulsion is expected with RH and PWDTB as compared to SD and GS. PWDTB has AEC of 1.9±0.4 $cmole_c kg^{-1}$.

Sorption coefficient based on volumes has shown maximum removal of *E. coli K12* with PWDTB and least with SD; similarly, maximum removal of *S. aureus* with GS and least with Seralite was observed. Log removal pattern for sorption have shown maximum removal of *E. coli K12* with PWDTB and maximum removal of *S. aureus* with GS. Log removal of as high as 6 log order obtained for both the organisms was comparable with reported value of 3-6 for Fecal coliform by Stevik *et al.* (1999), Ausland *et al.* (2002) working with porous media. Seralite used as a control have shown second lowest sorption coefficient and log removal for *E. coli K12.* It also has shown least desorption constant and log retention for the same. The difference in the results obtained could be due to the difference in the surface, physical and chemical properties of the materials used. PWDTB shows more adhesion for gram positive *E. coli K12* than gram positive *S. aureus* in terms of log removal and both types of sorption coefficient. All agro residues show more adhesion for gram positive *S. aureus* than *E. coli K12* in terms of log removal and both types of sorption coefficient for all.

The relative effectiveness of various anions to elute adsorbed cells has been known. It is generally promoted by high concentration of salts such as NaCl. High salt concentration denatures the cell integrity. But a concentration of 0.85% v/v is very often used for cell suspension and does not show any such effect. Desorption experiment shows lowest desorption as well high log retention with PWDTB for *E. coli K12.* Contact angle measurement shows PWDTB as hydrophilic material. Hence, partly irreversible adhesion of hydrophilic *E. coli K12* can be explained (Loosdrecht *et al.,* 1989). Trend of desorption pattern was not seen for all suspension volumes with agro-residues. Second lowest log retention for *S. aureus* with PWDTB is in line with the fact that PWDTB is hydrophilic whereas *S. aureus* is hydrophobic in nature. Literature suggests that adhesion Gibbs energy value of 10 kT per particle roughly represents the border between reversible and irreversible adhesion. Studies reveal that *S. aureus* represents more negative Gibbs energy for adhesion than *E. coli* (Loosdrecht *et al.,* 1989). Desorption data of this work shows high desorption constant for *S. aureus* than *E. coli K12.* In another word, there was more secondary minimum retention of *E. coliK12* than *S. aureus* on to natural materials.

PWDTB shows more adhesion for gram positive *E. coli K12* than gram positive *S. aureus* in terms of log removal and both types of sorption coefficient. All agro residues show more adhesion for gram positive *S. aureus* than *E. coli K12* in terms of log removal and both types of sorption coefficient for all. Retention pattern in desorption experiment suggest that adsorption is partly irreversible for all the materials used. It can be concluded that PWDTB in combination with RH and / GS could help in removal of pathogens from waste water.

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