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Full Length Research Paper

Biomodification of palm shell activated carbon using *Aspergillus niger* and *Bacillus subtilis* and its effect on the adsorption of lead ions from aqueous solutions

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Adsorption of lead ions from aqueous solutions using commercial untreated granular palm shell activated carbon (PSAC) and PSAC biomodified with *Bacillus subtilis* and *Aspergillus niger* biomass, respectively, was studied. The adsorption capacity of the three biosorbents was evaluated in batch adsorption experiments at pH 3 to 6; lead concentration varied between 20 to 300 mg/L. The PSAC-*B. subtilis* showed the highest uptake of lead at pH 6; it was followed by PSAC-*A. niger* and the original PSAC comprising 74, 72 and 65 mg Pb/g, respectively. The lowest removal of lead in the reversed order was observed at pH 3: 34, 37 and 40 mg Pb/g, respectively. The adsorption followed the pseudo-second reaction order for all biosorbents. High desorption efficiency (90%) was maintained in three consecutive cycles. The results show that the introduction of microbial biomass into the palm shell activated carbon matrix has potential to improve carbon' sorption capacity towards lead ions.

Key words: Adsorption, lead, activated carbon, fungi, microorganisms, biosorbent.

INTRODUCTION

Mass utilization of various electronic devices has led to the increasing volumes of wastes containing metals and problems of their safe disposal mostly due to the toxicity of metals. The removal of heavy metals from wastewater can be achieved by abiotic means, such as activated carbon (Hansen et al., 2010) or with live (Perez-Rama et al., 2010) or dead biomass (Saha and Orvig, 2010). Heavy metals can be bound to surface structures or microorganisms (Mishra et al., 2010) and/or be converted enzymatically (Hancock et al., 1999). For instance, *Bacillus* species biomass (dead and live) showed considerable capacity to uptake lead, copper, zinc, chromium and other metal ions (Wang and Chen, 2009).

Activated carbon is known for its high adsorption capacity in general; and microorganisms are known for their omnipresence and fast growth rates. Combination of low-cost granular activated carbon and accommodation on its surface microbial biomass offers advantages in terms of cost and process operation, especially for the treatment of diluted wastewaters (<100 mg/L) bearing metal ions from various electronics manufacturing industries.

Our research objective was to investigate the performance of hybrid adsorption system whereby untreated palm shell activated carbon (PSAC) is used as an adsorbent for lead and as a matrix for growth of *A. niger* (PSAC-AN) and *B. subtilis* (PSAC-BS) to increase overall removal of lead ions from aqueous solutions. Evaluation of adsorption capacities of these three biosorbents, their adsorption isotherms data, kinetics analysis, desorption efficiencies and surface characterization data are discussed in this paper.

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MATERIALS AND METHODS

Adsorbents

Granular palm shell activated carbon (PSAC) was supplied by Bravo Green (Sarawak, Malaysia). The material is normally produced using steam oxidizing atmosphere in the physical activation process. Palm shell carbon particle size between 1.6 and 2.0 mm was used in all experiments. The material was rinsed once with deionized water to remove dust, and then it was dried at 60°C and kept in a closed plastic container. *A. niger* (Biological science laboratory, UTAR) was cultured in a liquid medium (pH ~5) containing 20 g of sucrose (R and M, Inida), 10 g of peptone (Difco 211677, USA) and 3 g of yeast extract (Difco 212750, USA) mixed in 1 L of distilled water (Mungasavalli et al., 2007). The culture was incubated at 30°C and 250 rpm for a week. The obtained mycelia mass was filtered through a mesh strainer (mesh size <1 mm). *B. subtilis* (Biological science laboratory, UTAR) was cultured in commercial nutrient broth (Merck KGaA, Darmstadt, Germany) at 30°C and 220 rpm for 24 h. The bacterial biomass was harvested by centrifugation at 5000 rpm for 15 min. Separated fungal and bacterial biomasses were rinsed with deionized water before their use in the biomodification procedure.

Biomodification procedure

2 ml (1.9 g) of wet microorganism's biomass (fungal or bacterial) were resuspended in 40 ml of Mili-Q water. The wet microorganisms biomass was mixed with 1.2 g (dry mass) of PSAC in a 50 ml centrifuge tube and left on an orbital shaker at 27°C and 45 rpm for 72 h (Rivera-Utrilla et al., 2001). The resulted biosorbent mass was rinsed, filtered and dried in an incubator at 80°C overnight. The obtained dry mass of original PSAC, PSAC-AN, and PSAC-BS was kept in separate plastic containers at room temperature (27°C).

Lead adsorption

0.15 M sodium nitrate, $NaNO_3$, (Merck KGaA, Darmstadt, Germany) was used as the background electrolyte solution. 0.1 M lead nitrate, $Pb(NO_3)_2$, (Merck KGaA, Darmstadt, Germany) stock solution was used to prepare a range of concentrations from 20 to 300 mg/L in 100 ml volume flasks. A dose of a biosorbent in all experiments was 250 mg per 100 ml solution volume. The prepared solutions with lead and respective biosorbent were placed onto a shaker (SSL1; Stuart®, Korea) at 150 rpm and 27°C overnight. The measurements of the initial and equilibrium lead ions concentrations were carried out using ICP-OES (Optima 7000DV, Perkin Elmer, USA). pH range from 3 to 6 was investigated. The desired pH was adjusted by addition of 0.1 M HNO_3 or 0.1 M NaOH.

Adsorbent characterization

The Brunauer emmett teller (BET) surface area was measured by N_2 adsorption (77 K) using a Thermo Finnigan Sorptomatic 1990 series surface analyzer (ThermoFisher Scientific, USA). The Boehm titration method was used to determine the concentrations of the adsorbents' acidic surface functional groups (Boehm, 1994). pH_{pzc} was determined by using the drifting method (Khormaei et al., 2007). Energy dispersive X-ray spectroscopy (EDX) and SEM (scanning electron microscopy) analysis were carried out using Ametek EDAX and Hitachi S-3200N instruments, respectively. Also, Fourier transform infrared (FTIR) spectroscopy analysis (Perkin-Elmer Inc., USA) was carried out for all biosorbents.

Evaluation of adsorption isotherms and kinetics

Langmuir (1918) and Freundlich (1906) adsorption isotherm models were used to evaluate the experimental data and determine maximum adsorption capacities of the three biosorbents. The evaluation of kinetics was based on the application of the pseudo-first and pseudo-second reaction models (Lagergren, 1898; Ho and McKay, 1999).

Desorption procedure

100 mg of original PSAC was placed into a solution of 100 mg Pb^{2+}/L for 8 h at 150 rpm. The adsorbent was removed from the solution through filtration (0.4 ηm pore size filter paper), rinsed with deionized water and placed in a 100 ml of 0.1 M HCl solution for 6 h at 180 rpm. ICP concentration measurements were used to estimate lead desorption efficiencies. The procedure was repeated three times for the same sample.

RESULTS AND DISCUSSION

Biomodification and characterization of the biosorbents

The biomodification procedure is a crucial step of the hybrid adsorbent preparation. It was initially attempted to co-cultivate microorganisms cells together with the activated carbon in a suspension (1 g of PSAC in a 100 mL of nutrient medium shaken at 150 rpm) but the results of the followed adsorption experiments showed extremely low lead uptake, reaching estimated adsorption capacity of only 15 mg Pb^{2+}/g. Figure 1 shows Langmuir isotherm for lead removal in a suspension with *A. niger* with PSAC. It is believed that high liquid to solid ratio, and continuous stirring conditions, are the main factors influencing poor attachment of microorganisms cells and biofilm growth on the carbon surface. Consequently, after several modified trials, it was decided to use the separated wet microbial biomass to modify the original PSAC. Table 1 shows surface characterization data for original and biomodified PSACs. A decrease in the total surface area of the biomodified activated carbon was observed along with the decrease in the micropore volumes, which was indicative of the microbial biomass presence in the PSAC matrix. However, the decrease in surface area was more noticeable for PSAC-BS, and it probably related to the bacteria cell' size which are much smaller compared to the fungal cells and therefore they are able to infiltrate deeper into the carbon matrix, reducing the micro and mesopore volume as well.

However, the total surface area of the support adsorbent showed no strong influence over the amount of accommodated microbial cells. The concentrations of surface acidic groups of the biomodified PSACs were lower compared to the original PSAC, indicating microorganism biomass effect; and probably is a result of interaction between the extracellular polymeric substances (EPS) of microorganisms cell walls and

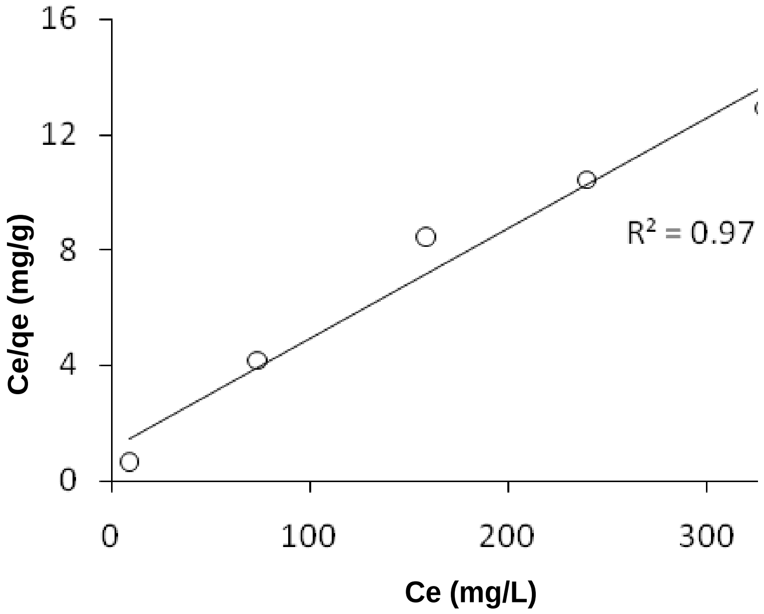


Figure 1. Langmuir isotherm for original PSAC in liquid culture with *A. niger* (pH 5).

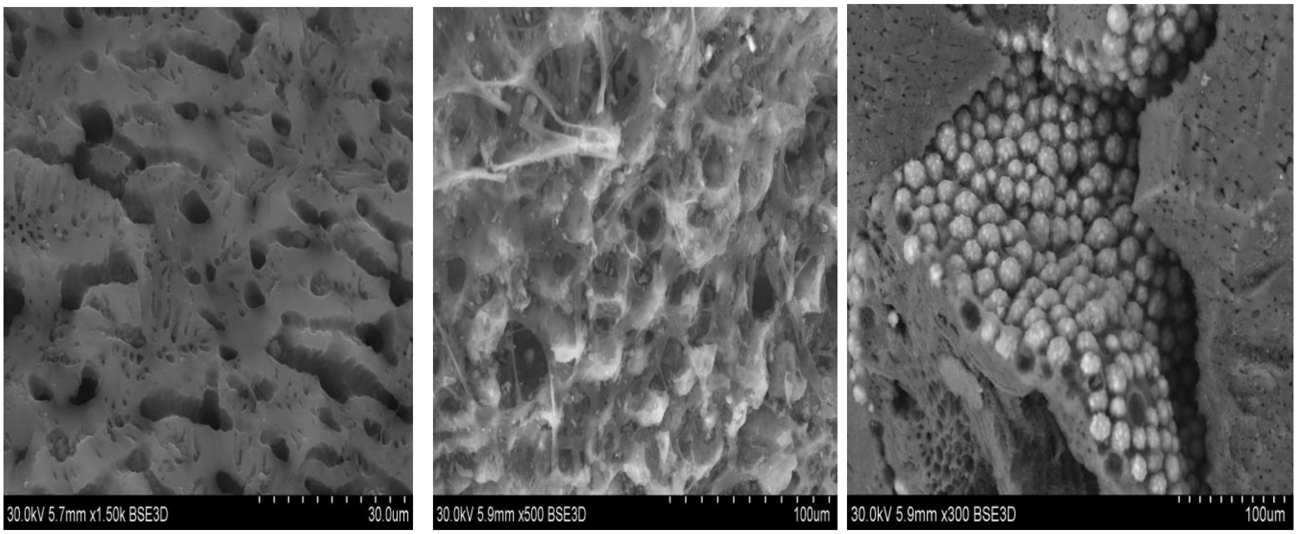
Table 1. Surface characterization data for original and biomodified PSACs.

Parameter	Original PSAC	PSAC-BS	PSAC-AN
Surface area, m^2/g	936.29	773.40	809.70
Total pore volume cm^3/g	0.49	0.42	0.42
Micropore volume, cm^3/g	0.17	0.16	0.17
Micropore area, m^2/g	432.50	381.30	414.70
Surface groups, meq/g	3.20	3.00	3.03
pHpzc (in 0.15 M $NaNO_3$)	9.25	8.50	8.90

PSAC' surface groups during the period of biomodification. pH_{pzc} slightly decreased after biomodification, more noticeably for PSAC-BS, which is associated with a displacement of the charge towards the charge of the microorganism's biomass (Dinamarca et al., 2010). Overall, factors such as size of microbial cells, ionic strength (Yee et al., 2007) and support-material's structure (Jeyachandran et al., 2006) affect the process of integration of the microorganisms into carbon matrix; therefore the resulting adsorption capacity may differ. Figure 2 shows scanning electron microscopy (SEM) images of the three biosorbents; presence of the fungal and bacterial biomass in the carbon matrix and differences in the surface appearances can be clearly seen. Figure 3 shows the FTIR spectra obtained for all biosorbents where a few bands were identified: >3400 cm^{-1}, O-H stretching in alcohols; >1600 cm^{-1}, C=O stretching in quinones; and >1100 cm^{-1}, stretching in aromatic rings due to probable presence of phenols and ether bridges (Guo and Lua, 2000).

Effect of pH and initial concentration on lead adsorption

Figures 4, 5 and 6 present data on the removal of lead onto the three biosorbents at different pHs and initial concentrations. Comparison of adsorption performance between the biosorbents showed that PSAC-AN showed higher lead removal rates at pH above 4. The behaviour of the three biosorbents towards pH changes was very similar: as the solution's pH increased from pH 4 to 6, lead uptake also increased, however, with slight differences between the biosorbents. More obvious decrease in lead uptake was observed at pH 3. PSAC-BS and PSAC-AN showed sharper decrease in lead uptake as compared to the original PSAC, which is associated with generally higher sensitivity of the microbial biomass towards acidic pH. Figure 4 shows removal of lead by PSAC-BS in solutions of different pHs. Strong dependency of metal ions adsorption on the solution' pH is explained by the increasing competitiveness of proton



(a) Original PSAC (b) PSAC-AN (c) PSAC-BS

Figure 2. SEM images of the biosorbents. (a) Original palm shell activated carbon; (b) PSAC biomodified with *A. niger*. (c) PSAC biomodified with *B. subtilis.*

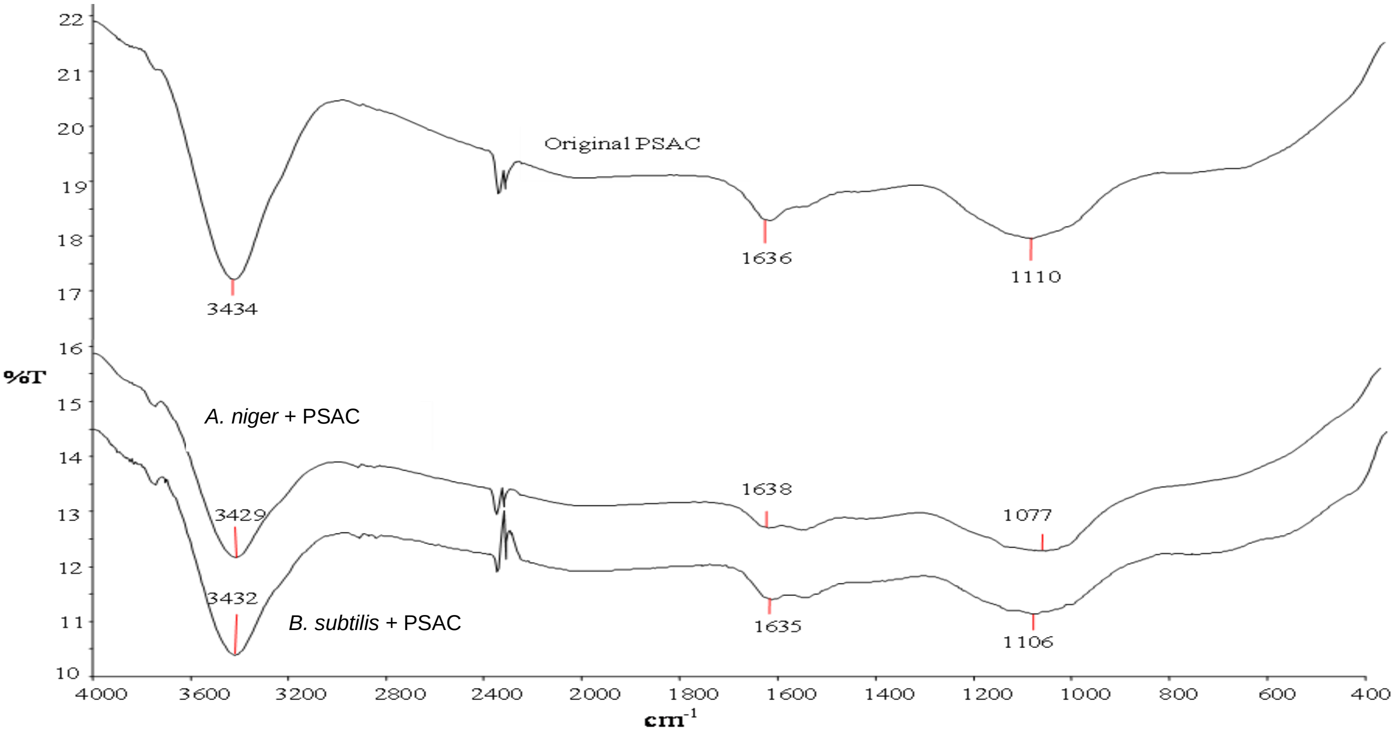


Figure 3. FTIR spectra for original and biomodified PSACs.

ions when pH decreases due to their smaller ionic radius and charge as compared to lead ions, resulting in the decreased removal of the latter. Such observation implies that both types of ions are adsorbed on the same surface sites of an adsorbent.

Figures 4, 5 and 6 also show effect of the initial lead concentration. Almost complete removal of lead from the solutions by all biosorbents was observed within a range

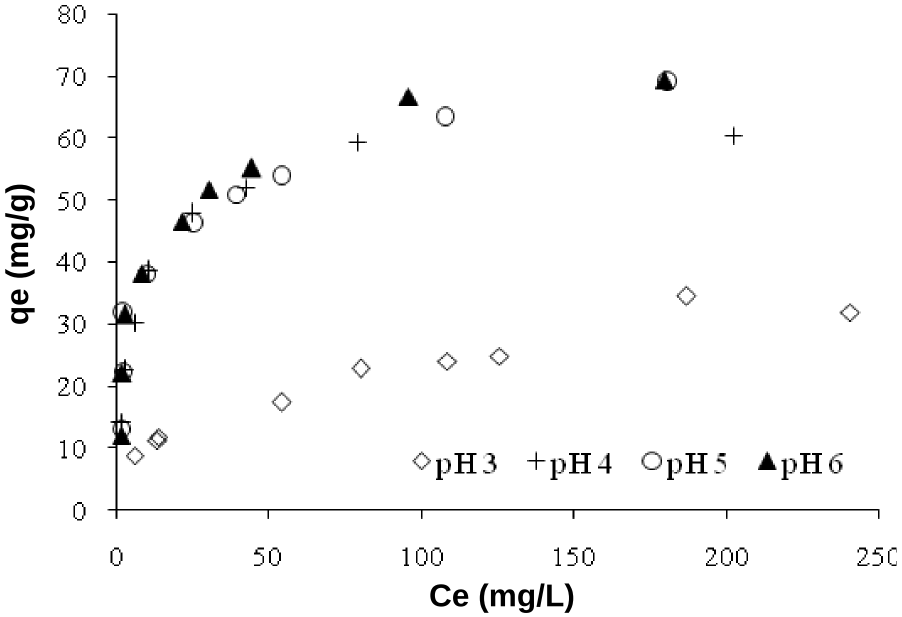


Figure 4. Removal of lead by PSAC-BS at different pHs and initial Pb concentrations.

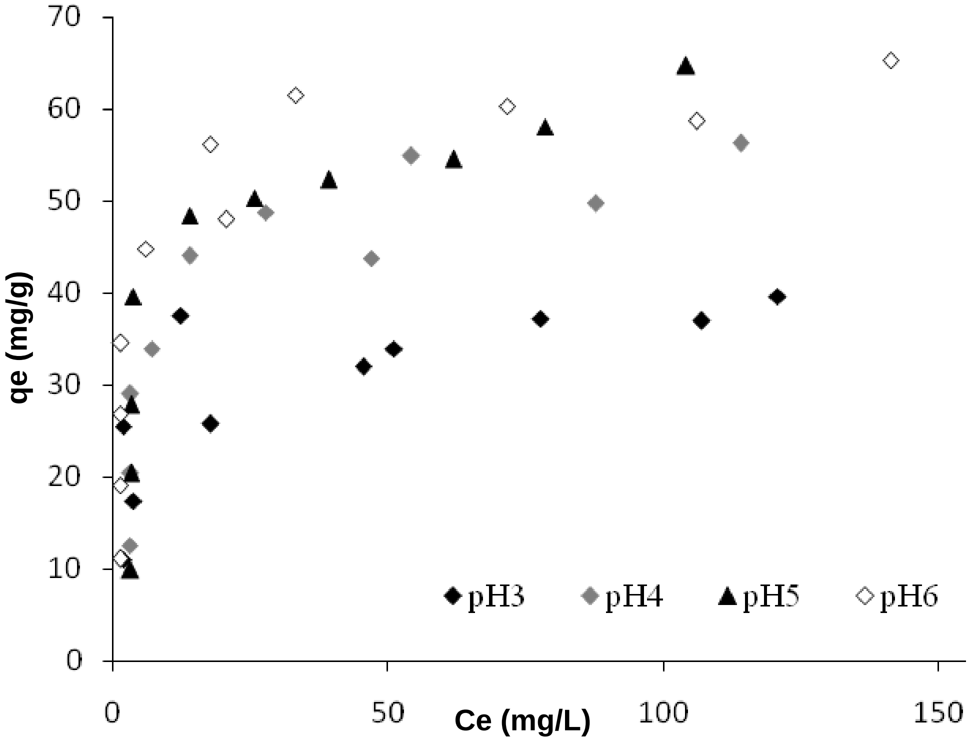


Figure 5. Removal of lead by original PSAC at different pHs and initial Pb concentration.

of 20 to 100 mg Pb^{2+}/L. Subsequent increase of lead concentration resulted in a slower sorption of lead ions due to the saturation of the sorption surface, limitation in the number of adsorption sites and decreasing distance between lead ions; thus, restricting charge distribution, ions' mobility and diffusion forces (Quintelas et al., 2009; Fosso-Kankeu et al., 2010). A ratio between a metal loading concentration and surface groups concentration

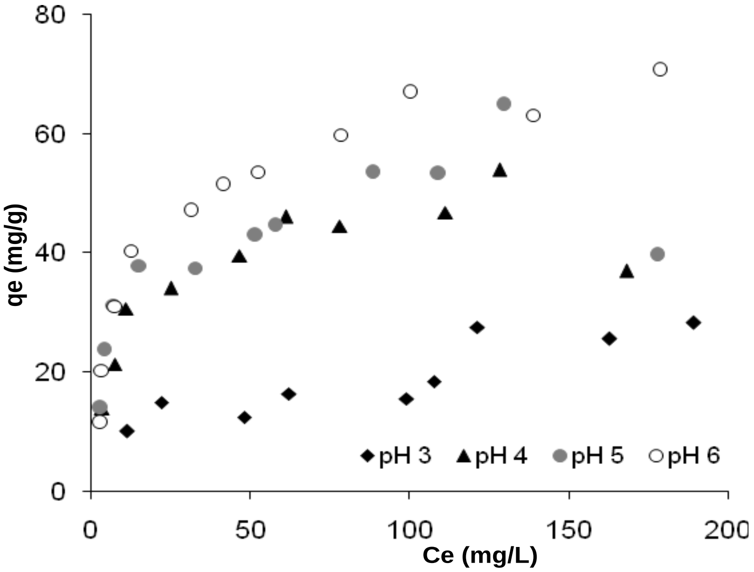


Figure 6. Removal of lead by PSAC-AN at different pHs and initial Pb concentration.

Table 2. Langmuir model parameters for adsorption of lead ions.

pH	Original PSAC				PSAC-BS				PSAC-AN			
	q_{max}, mg/g	*b*, L/mg	R^2	SSE	q_{max}, mg/g	*b*, L/mg	R^2	SSE	q_{max}, mg/g	*b*, L/mg	R^2	SSE
3	39.68	0.04	0.992	0.237	36.5	0.03	0.953	1.984	34.25	0.02	0.998	2.530
4	53.76	0.22	0.991	0.083	62.5	0.16	0.999	0.004	54.95	0.08	0.986	0.079
5	59.88	0.24	0.997	0.006	68.49	0.11	0.992	0.044	63.69	0.07	0.962	0.187
6	64.52	0.28	0.995	0.027	71.94	0.12	0.997	0.018	73.53	0.07	0.992	0.049

Table 3. Freundlich model parameters for adsorption of lead ions.

pH	Original PSAC				PSAC-BS				PSAC-AN			
	K_f	*1/n*	R^2	SSE	K_f	*1/n*	R^2	SSE	K_f	*1/n*	R^2	SSE
3	5.22	0.372	0.980	0.004	4.67	0.35	0.993	0.002	3.72	0.41	0.921	0.024
4	15.51	0.294	0.914	0.011	17.00	0.27	0.868	0.053	10.99	0.33	0.927	0.021
5	21.07	0.238	0.993	0.935	22.88	0.21	0.949	0.011	17.80	0.24	0.927	0.010
6	34.17	0.128	0.976	2.908	25.67	0.19	0.991	0.030	23.14	0.21	0.961	0.002

of the biosorbent is another considerable factor (Mishra et al., 2010), which becomes especially important at very low lead concentrations when carboxyl groups play more significant part (Sarret et al., 1998). As a rule, the initial steps of adsorption are rapid and surface saturation level is proportional to the adsorption capacity.

Adsorption isotherms

Adsorption experimental data were analyzed using the Langmuir and Freundlich isotherm models; the estimated adsorption capacities and models parameters are presented in Tables 2 and 3, respectively. Figure 7 shows the Langmuir adsorption isotherms obtained for the tested biosorbents at pH 6 and 3, at which the highest and lowest lead uptake was determined. As can be noted, all biosorbents behaved very similarly at pH 6. At pH 3, lead removal by the original PSAC was greater in comparison to the biomodified PSACs. For the Freundlich model, parameter, *1/n*, (Table 4) has values lower than unity for all biosorbents at all tested pHs, indicating

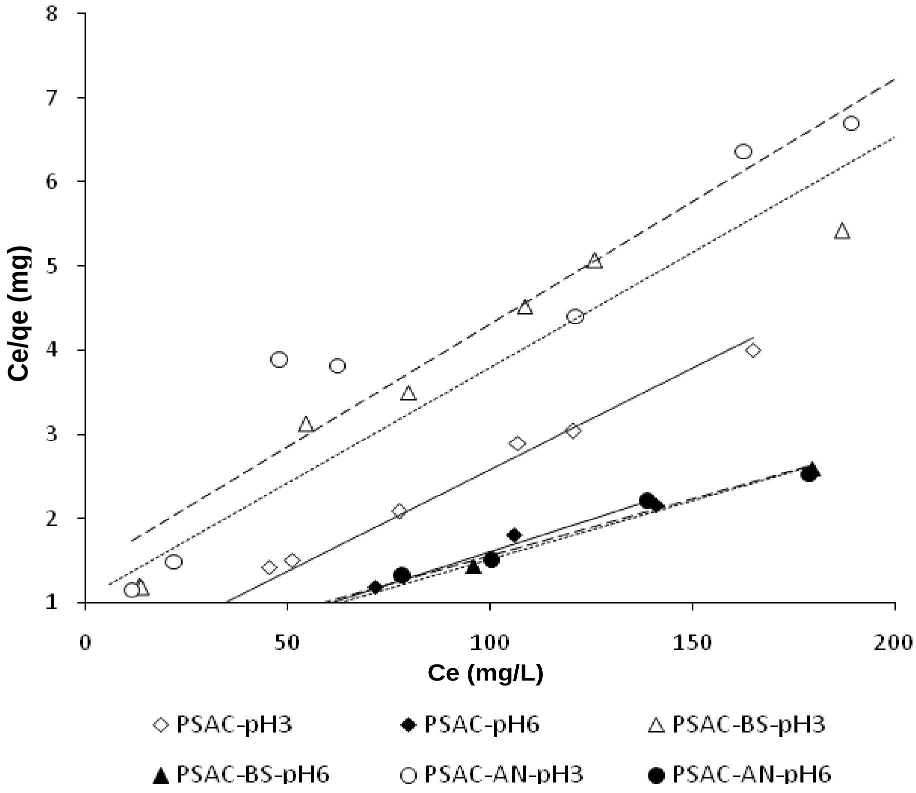


Figure 7. Langmuir adsorption isotherms for original and biomodified PSACs at pH 3 and 6.

Table 4. Comparison of adsorption capacities of lead by various adsorbents.

Adsorbent	Modifying agent	Adsorption capacity (mg/g)		Surface area (m^2/g)		Reference
		Original	Modified	Original	Modified	
Activated carbon	*E. coli*	21.50	26.40	1089		Rivera-Utrilla et al. (2001)
Peanut shell activated carbon	20% HNO_3	24.02	35.46	1019	867	Xu and Liu (2008)
Coconut shell activated carbon	HCl	-	17.19	-	1245	Song et al. (2010)
	HCl+H_2O_2	-	28.46	-	1062	Song et al. (2010)
	HCl+HNO_3	-	40.12	-	1053	Song et al. (2010)
Hazelnut husk activated carbon	-	13.05	-	1092	-	Imamoglu and Tekir (2008)
Aspergillus niger	NaOH	-	32.60			Dursun (2006)
		7.42	2.25			Amini et al. (2008)
PSAC	*A. niger*	65	72	936	809	This study
	B. subtilis	65	74	936	773	

significant extent of adsorption. Increase of *1/n* values along the decrease in solution' pH is in line with the effect of pH on lead removal observed for the Langmuir adsorption isotherms. The highest lead uptake was by PSAC-BS at pH 6 comprising 74 mg/g; slightly lower lead uptake was by PSAC-AN, 72 mg/g, and the lowest

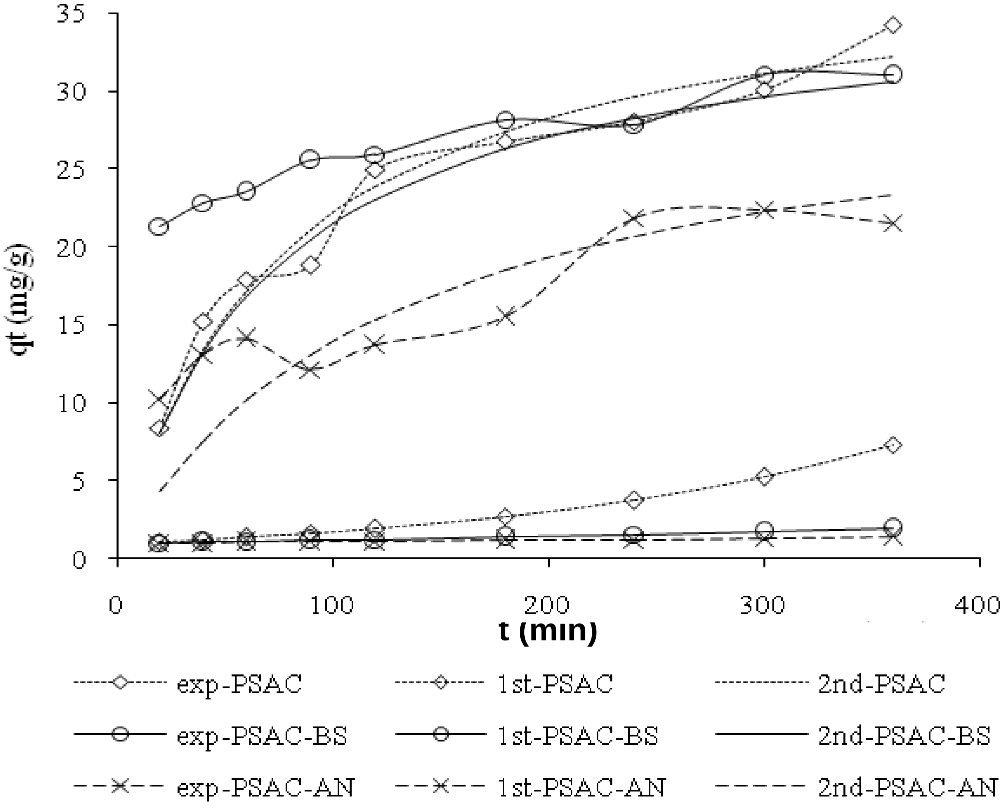


Figure 8. Experimental and modeled rates of lead removal by PSACs at pH 5.

uptake was by the original PSAC, 65 mg/g. Although, the surface area of the biomodified PSACs was smaller compared to the original PSAC, it did not have a strong effect on the adsorption capacities which were still higher for the biomodified PSACs. Lower uptake of lead on biomodified PSACs at pH 3 as compared to the original PSAC is associated with a higher sensitivity of bacterial biomass towards acidic pH in general; a change which is interrelated with more complex composition and structure of the bacterial cell wall as compared to the activated carbon surface. Table 4 shows data on lead removal by various carbons, most of which were modified with chemical agents. Such comparison signifies that activated carbon based biosorbents have higher adsorption capacity and that microbial bio-agents have a greater modifying potential to enhance adsorption systems performance.

Kinetic parameters

Previously, Khambhaty et al. (2008) showed that the non-linear method is more preferable to express the kinetic rate of the sorption process as compared to various linear methods. Figure 8 shows a non-linear representation of the kinetics study results for all biosorbents at pH 5. Table 5 presents the estimated parameters for the pseudo-first and pseudo-second order reaction models at all tested pHs. The estimated constants' values and correlation coefficients for both pseudo-first and second order equations in linear forms were quite close and sufficiently high, with a slight gain towards the pseudo-first order model. However, graphical presentation of the experimental and modelled data in Figure 8 clearly shows that the adsorption process for lead followed the pseudo-second order model, which suggests a chemisorption mechanism.

Desorption

The results for desorption experiments was carried out in three cycles for the same samples of original PSAC in 0.1 M HCl. In the first 100 min, desorption of lead from biosorbent surface into the acidic solution was slow, then it increased reaching maximum of 97, 95 and 92% desorption efficiency within 5 h period in the three cycles, respectively. pH of the acidic solution was measured continuously and showed insignificant fluctuations within 1.67 to 1.74 range. The results show that high desorption efficiency of lead can be achieved and sustained in consecutive cycles. Insignificant decrease of the efficiency in the second and third cycles is associated with partially irreversible bounding of lead ions to the

Table 5. Kinetic parameters of lead adsorption.

pH	$q_{e,exp}$ (mg/g)	Pseudo-first-order rate constants			Pseudo-second-order rate constants		
		k (min^{-1})	$q_{e,cal}$ (mg/g)	R^2	k_2 (mg min^{-1})	$q_{e,cal}$ (mg/g)	R^2
Original	**PSAC**						
3	15.2	8.52×10^{-3}	13.3	0.97	1.63×10^{-3}	14.7	0.99
4	31.5	8.98×10^{-3}	30.4	0.97	4.1×10^{-4}	34.3	0.99
5	37.1	5.53×10^{-3}	29.0	0.94	3.3×10^{-4}	39.1	0.99
PSAC-*B. subtilis*							
3	20.4	4.15×10^{-3}	7.0	0.89	1.68×10^{-3}	20.7	0.99
4	33.9	3.69×10^{-3}	13.7	0.95	7.6×10^{-4}	34.6	0.99
5	35.5	3.46×10^{-3}	14.5	0.95	6.6×10^{-4}	36.2	0.99
PSAC-*A. niger*							
3	14.4	3.46×10^{-3}	9.02	0.60	7.07×10^{-4}	15.2	0.99
4	22.2	3.9×10^{-3}	16.8	0.80	1.1×10^{-3}	18.5	0.99
5	26.2	4.4×10^{-3}	17.7	0.85	1.2×10^{-2}	27.6	0.99

biosorbent surface; a trend which was also observed for desorption of lead ions from the modified spent grain (Li et al., 2009) and poly(*m*-phenylenediamine) (Huang et al., 2007) indicating that bio- and chemical adsorbents share similar tendency in desorption. The results are quite encouraging for PSAC based adsorbents applications in heavy metal removal systems in view of the overall process performance improvement through determination of optimal desorption conditions, utilization of efficient desorbing agents, and their concentrations to extend the biosorbent material life cycle.

Conclusion

The experimental data for the removal of lead in aqueous solutions onto original and biomodified PSACs showed promising results in the application of a hybrid adsorption system. The effect of pH was similar for all biosorbents; lead uptake increased when pH of the solution increased. The biomodified PSACs showed higher removal of lead at the tested pH range, except for pH 3. The desorption experiments showed high desorption efficiency of PSAC, indicating its potential for extended reusability. It is anticipated that optimization of the biomodification procedure and treatment process design should show remarkable metal removal efficiencies. The presented data are to promote development of adsorption configurations, merging low-cost biosorbents biomodified with various microbial live and/or dead biomasses to increase efficiency of metal ions adsorption systems for industrial wastewater treatment.

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