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Caffeine removal using pretreated dead biomass of yeast *Trichosporon* sp. VITLN01 : Equilibrium, kinetics and thermodynamic studies

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Abstract : Dead biomass of yeast *Trichosporon* sp. VITLN01 pretreated with Tween 80, sodium dodecylsulfate (SDS) and NaOH were used as adsorbents for caffeine removal from aqueous solution. In batch system, maximum caffeine uptake was noted using Tween 80 treated adsorbent at pH 7.5 and adsorption equilibrium time was 3 h. The Langmuir, Freundlich and Temkin adsorption models were applied for mathematical description of adsorption equilibrium. Langmuir model was able to describe the adsorption equilibrium of caffeine on native and pretreated adsorbents. Thermodynamics studies indicated that caffeine adsorption process was spontaneous and exothermic in nature. SEM analysis revealed interesting changes in the surface texture of pretreated adsorbents before and after caffeine adsorption.Pretreated adsorbents immobilized in PVA-alginate matrix was used to remove caffeine from coffee processing industrial wastewater. The present study confirmed that immobilized Tween 80 treated dead biomass of yeast *Trichosporon* sp. VITLN01 may serve as efficient adsorbent for removal of caffeine from industrial wastewater.
Keywords: Adsorption;Caffeine; *Trichosporon* sp. VITLN01; Tween 80; Industrial wastewater

Introduction

Caffeine ($C_8H_{10}O_2$) is a xanthine alkaloid, naturally present in more than sixty plant species. Caffeine containing by-products and wastewaters generated from coffee and tea processing plants comprise a major part of the agricultural and industrial wastes in coffee growing area[1].Caffeine has been detected in surface water, ground water and wastewaters at a high concentration (~10g caffeine/L) due to the discharge of caffeine containing wastewaters in the surrounding water bodies[2,3]. Kolpinet al.[4] evaluated caffeine as fourth most frequently detected pollutant out of 95 different Organic Wastewater Contaminants (OWCs). In recent years caffeine has been considered as an emerging contaminant[5,6] which may pose a potential hazard to humans and aquatic animals [7]. Presence of caffeine in the agricultural soil, affects soil fertility as it inhibits seed germination and growth of seedlings. The ingestion of caffeine has severe effects on the physiological system.

The conventional methods used for caffeine removal viz. charcoal, carbon[8], membrane filtration[9,10], solid-liquid extraction[11] and supercritical carbon dioxide extraction [12]have major disadvantages. Therefore, there is a need for alternate method to conventional processes involved in the removal of caffeine from industrial wastewater.

Adsorption technique has attracted attention in this context and is being widely used for the removal of several pollutants. Limited number of studies is available on caffeine adsorption[13,14]. Biologically based adsorption process uses low cost biological materials viz. living or dead microorganisms[15]. So far, there is no report available on the usage of dead yeast biomass as adsorbent for caffeine removal.

Many adsorbents do not exhibit their full potential in the raw (untreated) form and their uptake capacity has been found to improve significantly upon chemical pre-treatment. So, in the present study, the possible use of dead yeast biomass pretreated with NaOH and surfactants viz. Sodium dodecyl sulphate (SDS) and Tween 80 have been investigated to improve the caffeine adsorption potential of yeast biomass.

Materials and Methods

Collection of Industrial wastewater

Coffee processing industrial wastewater was collected from Coffee Board, Yercaud, India and physic-chemical analysis was following the standard methodologies[16]. The initial caffeine concentration in the wastewater was measured using HPLC.

Isolation and identification of yeast

The yeast species was isolated from caffeine contaminated soil under coffee cultivation area, Coffee Board, Yercaud, India. Genomic DNA of the yeast cells was isolated as described by Cheng and Jiang[17]and molecular identification was done.

Preparation of adsorbent

The yeast cells were grown in Yeast Extract Peptone Dextrose (YEPD) medium containing (g l^{-1}): yeast extract, 10; peptone, 20; and dextrose, 20. The pH and temperature was maintained at 6.5 and 28 °C. Growth was allowed to proceed for five days on a rotary shaker operating at 120 rpm. After the yeast growth, the biomass and the culture medium were separated by centrifuging at 8000 rpm for 10 min and the resulting biomass was washed several times thoroughly with distilled water. The biomass obtained was subjected to drying at 60 °C until a constant weight of biomass was obtained. The dried native biomass of 1 g was suspended in each 100 ml of NaOH (0.1 M), Tween 80 (4 mM) and SDS (3 mM) and stirred at room temperature. After 24 h, biomass was separated by centrifugation, washed, dried and ground to a fine powder, which was sieved through a mesh (150 µm) sieve. The undersized fraction was used for caffeine adsorption studies.

Characterization of adsorbents

Physical characterization

The surface area and pore volume of the adsorbents were measured by BET (Brunauer Emmett-Teller nitrogen adsorption technique). Leachability of the adsorbents was characterized by agitating 1 g of adsorbent with 50 mL of deionized water in an Erlenmeyer flask on a rotary shaker for 24 h. The adsorbent was separated by settling and the Chemical Oxygen Demand (COD) of the supernatant was determined as per standard analytical method .

pH of point of zero charge

The pH of the point of zero charge (pHPZC) of the adsorbent was analysed using 0.01M of KCl solutions (each of 50 mL). The pH values of the solution were adjusted between 2 and 12 with and without adsorbent (10 g/L). These solutions were periodically agitated and allowed to equilibriate for 48 h. The pH values of the supernatant liquids were determined. The ΔpH was calculated by measuring the difference between the pH of the solutions with and without the adsorbent. The pH_{KCl} is the pH of the solutions without the adsorbent. The point of zero charge is the pH at which $pH_{KCl}$and ΔpH are zero. The point of zero charge was calculated by plotting ΔpH against pH_{KCl}.

Thermal analysis

Thermogravimetric analyses of native and pretreated adsorbents were carried out under high purity helium supplied at a purge gas flow rate of 0-1000 mL min^{-1} (Diamond TG/DTA, Perkin Elmer, USA). All samples were subjected to a 10 °C min^{-1} heating rate and were characterized between 50 and 1000 °C.

Scanning electron microscopic (SEM) analysis

SEM analysis of native and pretreated yeast biomass before and after caffeine adsorption was performed using scanning electron microscopy,Hitachi (Model: S-3400N).

Batch biosorption studies

Experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of synthetic caffeine solution. The flasks were agitated on a rotary shaker at 120 rpm for 12 h at room temperature (30 ±2 °C) to ensure equilibrium was reached. Adsorbent free blanks were used as control. For determining the effect of pH on caffeine adsorption by native and treated adsorbents, initial pH of each caffeine solution (10 g L^{-1}) was adjusted to desired value (4-8) using 0.1 M HCl or 0.1 M NaOH. The effect of temperature on the adsorption capacity was investigated in the temperature range of 20-40 °C. The adsorbent concentration was varied between 10 and 40 g L^{-1} caffeine solution for determining the effect of adsorbent dosage on caffeine sorption capacity. The effect of initial caffeine concentration on the adsorption capacity was studied at optimum pH, temperature and adsorbent dosage. The initial concentration in the adsorption medium was varied between 2-14 g L^{-1}. To optimize the contact time, caffeine sorption at 1 h interval was determined by analyzing residual caffeine in the supernatant. The samples collected at different time intervals were centrifuged at 6000 rpm for 5 min and the liquid supernatant was analyzed for the residual caffeine concentration

Equilibrium, Kinetic and Thermodynamic studies

The equilibrium data were analyzed using two parameter isotherms- Langmuir, Freundlich and Dubinin- Radushkevich (D-R). Kinetic and thermodynamic studies were conducted following the standard method.

Use of immobilized adsorbent for removal of caffeine from wastewater

PVA (10 g L^{-1}) and sodium alginate (10 g L^{-1}) were mixed thoroughly in distilled water at 80 °C to obtain a homogeneous suspension. After cooling PVA- alginate mixture to 40 °C, 20 g L^{-1} of the adsorbent (native or pretreated adsorbents) was added and mixed. The PVA- alginate mixture was extruded gently in 0.2 M $CaCl_2$ to form PVA-alginate beads of diameter ~2 mm.The resulting beads were washed with saline and used for adsorption experiments. PVA-alginate beads without adsorbent were also prepared as control. The beads were added to 100 mL of coffee industrial wastewater in 250 mL of Erlenmeyer flasks, adjusted to pH 7.5 and maintained at temperature 30°C. Liquid samples were collected at desired time intervals to estimate the caffeine adsorption capacity.

Results and discussion

Identification of the yeast isolate

A yeast isolate was obtained from the caffeine contaminated soil through the standard serial dilution technique. In this study, Partial ITS1, 5.8S rRNA, ITS2 and partial 28S rRNA gene sequences of the yeast isolate, comprising of 988 nucleotides was determined. Similarity search using BLASTn tool for conserved sequences of the isolate showed 100 % query coverage and 97 % homology with *Trichosporon asahii* strain (accession no. AM900369.1). Therefore, the isolate was identified and designated as *Trichosporon* sp. VITLN01.

Characterization of native and pretreated adsorbent

Surface area and leachability test

The external surface area and total pore volume of the adsorbents were measured from N_2 adsorption isotherms with a sorptiometer. These properties were listed in the Table 1. Among all the adsorbents, Tween 80 treated adsorbent was found to have the highest surface area and pore volume. The adsorbents exhibiting a large surface area are generally assumed to adsorb large amount of adsorbate than the adsorbents with lower surface area. The micropore volume of adsorbent has major contribution on the adsorption capacity of adsorbate molecules to pass through the pores. The leaching characteristics of the adsorbents were measured in terms of

COD. Tween 80 treated adsorbent showed the least COD value and NaOH treated showed the highest values of about 10 mg L^{-1} and 29 mg L^{-1} respectively (Table 1).

Table 1.Physical properties of native and pretreated adsorbents.

S.No.	Adsorbents	BET surface area ($m^2 g^{-1}$)	Total pore volume ($cm^3 g^{-1}$)	Leachability (mg L^{-1})
1	Native	15.16	0.012	13
2	Tween 80 treated	29.14	0.023	10
3	SDS treated	23.21	0.017	14
4	NaOH treated	19.36	0.009	29

pH of point of zero charge (pH_{PZC})

The pH_{PZC} values of native and treated adsorbents were shown in the Table 2. It was observed that pretreatment of the native adsorbent affected the pH_{PZC}value. When the pH of solution was higher than $pH_{PZC,}$ the adsorbent surface exhibited net negative charge and could interact with positive charge containing species while at pH lower than $pH_{PZC}$the surface was positively charged and could interact with negative species[18].

Table 2. The pH values of the point of zero charge (pH_{PZC}) attained by adsorbents.

S.No.	Adsorbents	pH_{PZC}
1	Native	5.0
2	Tween 80 treated	7.2
3	SDS treated	6.9
4	NaOH treated	6.3

Thermal analysis

Thermogravimetric and differential thermal (TG/DTA) analysis of the native and pretreated adsorbents were carried out for better understanding of the influence of temperature on the properties of adsorbents. The adsorbents underwent two or three decomposition process when heated from 50 to 1000 °C. The initial weight loss (7-9 %) in the small temperature range of 100 to 200 °C could be due to the loss of adsorbed water molecules. The drastic weight loss due to decomposition was observed in the temperature range of 200 to 700 °C. The maximum weight loss (76.155 %) was observed in case of NaOH treated adsorbents followed by SDS treated adsorbent (70.355 %), native adsorbent (68.21 %) and Tween 80 treated adsorbent (65.352 %). This might be due to the degradation of the organic fractions of the studied adsorbents.

Batch adsorption studies

The effect of pH on caffeine adsorption was studied by varying the pH from 4 to 8. For each pH value, temperature (30 °C), adsorbent dosage (10 g L^{-1}), initial caffeine concentration (2 g L^{-1}) were kept constant. In all the cases, caffeine uptake increased with increasing pH and reached maximum at pH 7.5. These results suggest that caffeine adsorption is controlled by ionic attraction between caffeine and functional groups at the adsorbent surface.

The effect of temperature on caffeine adsorption onto the native and pretreated adsorbents at the range of temperatures between 20 and 40 °C and at constant pH (pH 7.5) was tested. The results showed that the adsorption capacity increased with increasing the temperature up to 30 °C and above this temperature, the adsorption capacity of caffeine was decreased. This decrease at high temperature may be due to the denaturation of active binding sites in the adsorbents [19].

The number of sites available for the caffeine adsorption depends on the concentration of adsorbents. The effect of adsorbent concentration (10-40 g L^{-1}) on caffeine uptake was tested. Caffeine uptake was found to increase with increase in adsorbent concentration from 10 to 20 g L^{-1}. Beyond this dosage the uptake was reduced. From these results, it may be concluded that at lower adsorbent dosage (i.e., below 20 g L^{-1}), caffeine

molecules were competing for adsorption at limited amount of adsorption sites. However, as the adsorbent concentration was increased, the adsorption sites were easily available resulting in higher uptake of caffeine. The significant decrease in caffeine uptake at adsorbent dosage greater than 20 g L^{-1} may be attributed to the presence of surplus binding sites on the adsorbent surface than the available caffeine molecules in the solution at constant concentration of 10 g L^{-}

The initial caffeine concentration provides an important driving force to overcome mass transfer resistance of caffeine between solid and aqueous phases[20].Caffeine uptake capacity of native and pretreated adsorbents as a function of initial caffeine concentration was examined. The initial caffeine concentration in the solution was varied between 2-14 g L^{-1}. The uptake of caffeine increased linearly with increase in initial caffeine concentration and then reached a plateau at 12 g L^{-1} demonstrating the saturation of binding sites.

The contact time for an adsorption process has quite practical application significance[21,22]. Typical adsorption process shows a speedy initial uptake, followed by a slower process. At the initial 180 min, caffeine molecules in the solutions were rapidly adsorbed by the four adsorbents. Afterwards the uptake decreased or attained equilibrium. The adsorption equilibrium time of native adsorbent was 10 h, while the equilibrium time of Tween 80 treated, SDS treated and NaOH treated adsorbents were attained in about 3 h, 6 h and 9 h respectively. Among all adsorbents, Tween 80 treated adsorbents showed the maximum caffeine uptake (0.5 g g^{-1}) and shorter equilibrium time.

Adsorption isotherms

The experimental data were analyzed using Langmuir, Freundlich and Temkin isotherm models. The R^2 values shown in Table 3 suggested that the Langmuir isotherm provides a good fit to the isotherm data.The R^2 values of Freundlich and Temkin isotherm models were lower than Langmuir isotherm. It can be seen from the isotherm model parameters and correlation coefficient (R^2) values that Langmuir isotherm model showed better fit than Freundlich and Temkin isotherm models. Thus in this study, the results indicated that caffeine adsorption by native and pretreated adsorbents was apparently with monolayer coverage on adsorbent surface that is homogenous in adsorption affinity.

Table 3.Isotherm model parameters for caffeine removal by native and pretreated adsorbents.

S.No.	Isotherm model	Parameters	Adsorbents			
			Native	**Tween 80 treated**	**SDS treated**	**NaOH treated**
1	Langmuir	q_m(g/g)	0.379	0.766	0.471	0.448
		B	0.245	0.613	0.482	0.407
		r^2	0.922	0.964	0.999	0.996
2	Freundlich	K_f(g/g) $(l/g)^{1/n}$	10.6	3.5	6.44	9.5
		N	1.745	2.217	1.561	1.527
		r^2	0.866	0.884	0.837	0.876
3	Temkin	A (l/g)	0.562	1.105	0.587	0.726
		B	0.065	0.106	0.103	0.076
		r^2	0.848	0.778	0.872	0.885

Adsorption kinetics

The value of pseudo-first-order rate constants (k_1) and calculated sorption capacity ($q_{eq,\ cal}$) were determined from the equations of linear plots of log (q_{eq}-q_t) against time. Pseudo-second-order rate constants (k_2)and calculated sorption capacity ($q_{eq,\ cal}$) were determined from the equations of linear plots of t/q_t against time. Table 4 shows the comparison of the pseudo-first-order, pseudo-second-order adsorption rate constants, calculated and experimental q_{eq}values obtained for different adsorbents. In case of Tween 80 treated adsorbent, the data showed well fit to pseudo-first order kinetics, since the variations between the calculated ($q_{eq,cal}$) and experimental ($q_{eq,exp}$) sorption capacity were minimal and correlation coefficient (R^2) value was also found to be 0.999. Therefore, caffeine sorption may be explained as the passive uptake through physical adsorption or the

adsorbent surface ion exchange. The data for other adsorbents showed better fit to pseudo-second order kinetics, thus in agreement with chemisorptions being the rate limiting step in these cases.

Table 4.Kinetic model parameters for caffeine removal by native and pretreated adsorbents.

Adsorbent	$q_{eq,exp}$ (g/g)	Pseudo-first order model			Pseudo-second order model		
		K_1	$q_{eq,cal}$ (g g^{-1})	R^2	K_2 (g g^{-1} min^{-1})	$q_{eq,cal}$ (g/g)	R^2
Native	0.268	0.002	3.597	0.916	13.922	0.268	0.967
Tween 80 treated	0.500	0.007	0.51	0.999	0.012	0.401	0.971
SDS treated	0.345	0.006	0.373	0.953	9.21	0.349	0.971
NaOH treated	0.312	0.002	0.303	0.935	10.272	0.313	0.971

Intraparticle diffusion

The above studied kinetic models cannot identify the diffusion mechanism. The kinetics results can be analyzed by intraparticle diffusion model in order to elucidate the diffusion mechanism[23,24]. The linear plots revealed the occurrence of intraparticle diffusion in the sorption process of caffeine by the native and pretreated adsorbents. The rate constants for the intraparticle diffusion k_{id} ,were listed in Table 5. The results showed that the rate constant was higher for Tween 80 treated adsorbents compared to other adsorbents.

Table 5.Intraparticle diffusion model constants and correlation coefficients for adsorption of caffeine on native and pretreated adsorbents.

Adsorbents	K_{id} (g g^{-1} min^{-1})	C	r^2
Native	0.01	0.007	0.988
Tween 80 treated	0.048	0.188	0.977
SDS treated	0.020	0.047	0.960
NaOH treated	0.013	0.002	0.957

Thermodynamics of sorption

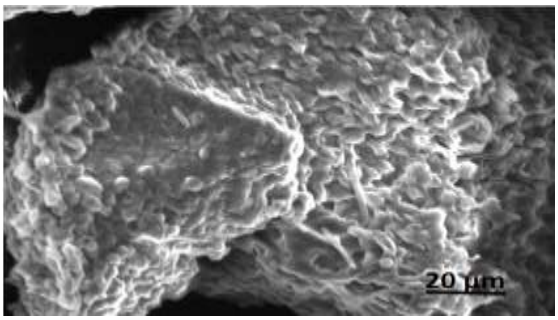
In order to describe thermodynamic behavior of caffeine adsorption on native and pretreated adsorbents, thermodynamic parameters viz. Enthalpy change (ΔH), Gibbs energy (ΔG) and entropy change (ΔS) were estimated using the standard equations and the computed values are listed in the Table 6. The negative values of ΔG indicated that caffeine removal by all adsorbents were thermodynamically feasible and spontaneous in nature. The values of ΔH and ΔS in the caffeine adsorption process were determined from slope and intercept of the plot of log q_e/C_e vs. 1/T. The negative value of ΔH indicated the exothermic nature of caffeine adsorption on native and pretreated adsorbents. The positive value of ΔS showed the increased randomness at the solid/liquid interface during adsorption process and suggested good affinity of the caffeine towards the adsorbents (native and pretreated). Thus the thermodynamic studies clearly demonstrated that adsorption of caffeine on native and pretreated adsorbents were favorable, spontaneous and exothermic in nature.

Table 6.Thermodynamic constants obtained for native and treated adsorbents during caffeine adsorption process.

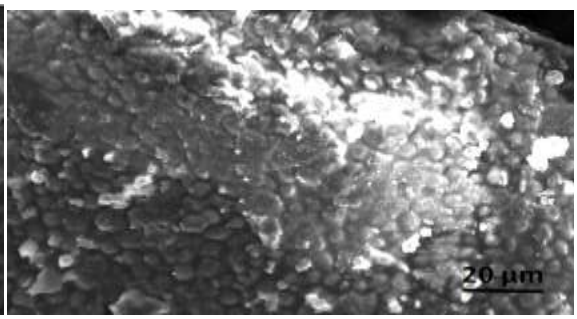
Adsorbents	Temperature (K)	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	R^2
Native adsorbent	283	-2.96	-2.76	0.0007	0.999
	293	-2.97			
	303	-2.97			
Tween 80 treated adsorbent	283	-9.4	-7.23	0.00769	1.000
	293	-9.48			
	303	-9.56			
SDS treated adsorbent	283	-7.55	-6.57	0.00347	0.990
	293	-7.58			
	303	-7.62			
NaOH treated adsorbent	283	-4.87	-4.39	0.0017	0.993
	293	-4.89			
	303	-4.91			

Scanning electron microscopy (SEM)

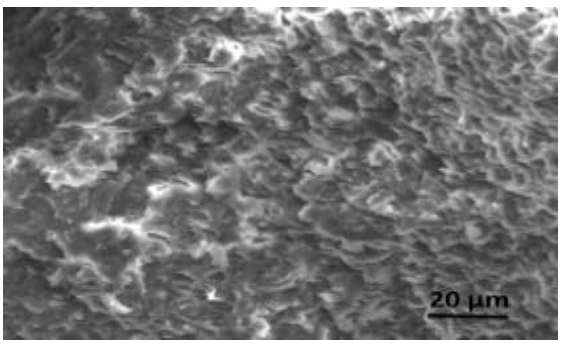
Surface texture and morphology of the adsorbents before and after caffeine adsorption were examined using SEM analysis. The native adsorbent surface appeared smooth and clear with oval shaped cell (Fig. 1a). No noticeable changes were seen in the native adsorbent after caffeine adsorption (Fig. 1b). Due to pretreatments, structural distortion and roughness of



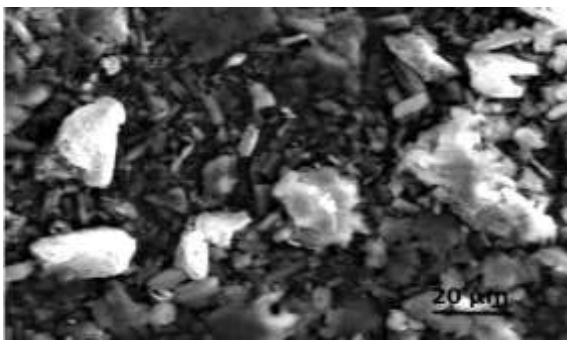
(a)



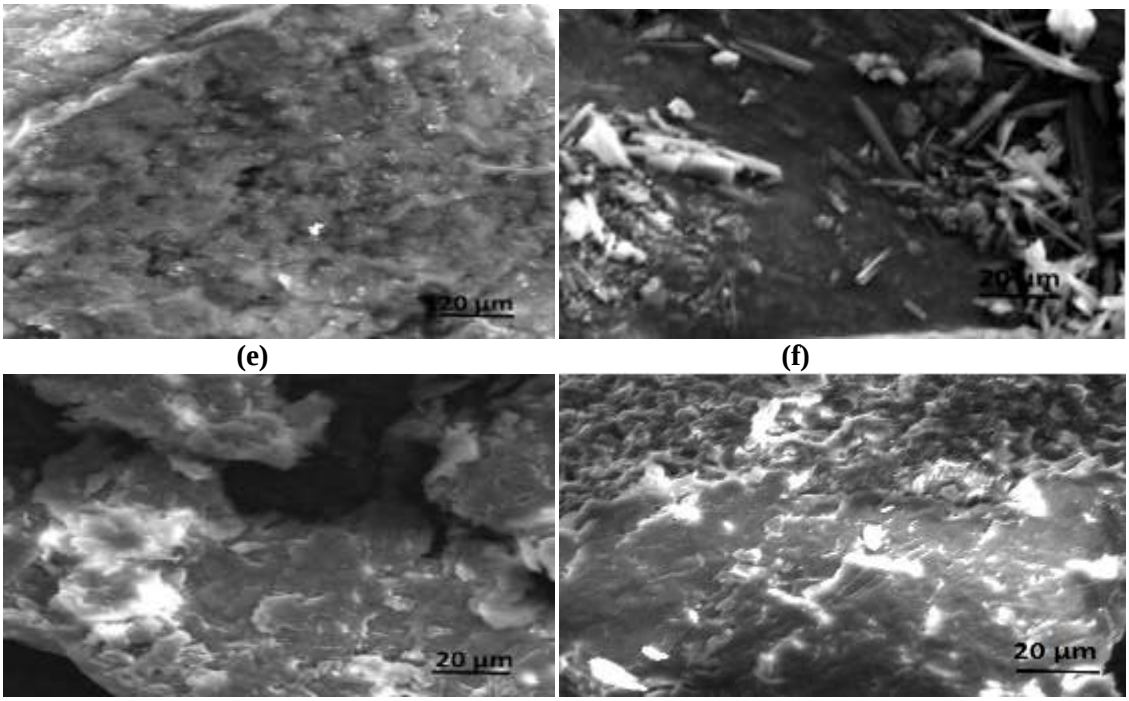
(b)



(c)



(d)



(e) **(f)**

(g) **(h**

Fig. 1 SEM analysis of native and pretreated adsorbents before and after caffeine adsorption

Adsorbent surface was observed in case of all pretreated adsorbents before caffeine adsorption (Fig. 1c, e, g). In case of Tween 80 treated adsorbent, formation of large amount of agglomerative mass of caffeine binding to the adsorbent surface was noted after caffeine adsorption (Fig. 1d).The surface of SDS treated adsorbent was changed after caffeine adsorption and caffeine was adsorbed as cylinder or fibers-like structure (Fig. 1f). The NaOH treated adsorbent showed non-uniformly distributed caffeine bodies on irregular adsorbent surface after caffeine adsorption (Fig. 1h).

Caffeine removal from industrial wastewater using immobilized adsorbents

The coffee processing industrial wastewater collected from Coffee Board, Yercaud, India, was found to contain 11.4 g L^{-1} caffeine. Wastewater pH was adjusted to 7.5 and experiments were conducted in batch mode at 30 °C. Removal of caffeine from industrial wastewater was investigated using pretreated adsorbents immobilized in PVA-alginate matrix. Caffeine uptake was found to be maximum (0.570 g g^{-1}) in case of immobilized Tween 80 treated adsorbents followed by SDS treated adsorbent (0.378 g g^{-1}), NaOH treated adsorbent (0.347 g g^{-1}) and native adsorbent (0.291 g g^{-1}). Tween 80 treated adsorbents immobilized in PVA-alginate matrix acquired the best caffeine uptake capacity compared to other adsorbents.

Conclusion

PVA-alginate immobilized pretreated dead biomass of *Trichosporon* sp. VITLN01has been successfully used as adsorbent for the removal of caffeine from aqueous medium. The batch experiments showed that all the parameters viz. pH, temperature, initial adsorbent dosage, initial caffeine concentration and contact time had effect on caffeine removal. Adsorption equilibrium data fitted well to the Langmuir model suggesting homogeneous monolayer adsorption. Kinetics of adsorption by Tween 80 treated adsorbent was better explained by pseudo-first order model which suggested physical adsorption. The thermodynamic calculation showed the feasibility, exothermic and spontaneous nature of caffeine adsorption. Based on the findings of present research, it may be concluded that PVA-alginate immobilized Tween 80 treated dead biomass of *Trichosporon*sp. VITLN01can serve as potential adsorbent for the removal of caffeine from coffee processing industrial wastewater.

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References

1. Adams, M.R. and Dougan J., Biological management of coffee processing, Tropical Sci. 1981, 123, 178–196.
2. Buerge, I.J., Poiger.,Muller M.D. and Buser, H.R., Caffeine, an anthropogenic marker for wastewater contamination of surface waters, Environ. Sci. Technol. 2003, 37, 691-700.
3. Glassmeyer, S.T., Furlong E T., KolpinDW.,CahillJ.D., Zaugg S.D. et al., Transport of Chemical and Microbial Compounds from Known Wastewater Discharges: Potential for Use as Indicators of Human Fecal Contamination, Environ. Sci. Technol. 2005, 39 ,5157-5169.
4. Kolpin, D.W., Furlong E.T., Meyer M.T.,Thurman E. M.,Zaugg, S.D. et al., Pharmaceuticals, hormones, and other organic wastewater contaminants in United States streams, 1999–2000: A national reconnaissance, Environ. Sci. Technol. 2002, 36 ,1202–1211.
5. Bruton, T., Alboloushi, A., de la Garza B., Bi-O. Kim andHalden, R.U., Fate of Caffeine in the Environment and Ecotoxicological Considerations. In: Contaminants of Emerging Concern in the Environment: Ecological and Human Health Considerations (Ed.: R.U. Halden) American Chemical Society, 2010, 1048, 257-273.
6. Sodre, F.F., Locatelli M.A.F. and Jardim, W.F. 2010, Occurrence of emerging contaminants in Brazilian drinking waters: A sewage-to-tap issue, Water Air Soil Pollt.2010, 206 ,57-67.
7. Bolong, N., IsmailA.F., Salim, M.R. and Matsuura, T., A review of the effects of emerging contaminants in wastewater and options for their removal, Desalination 2009, 239, 229-246.
8. Feldman, J.R. and Katz S.N., Caffeine. In: Encyclopedia of chemical processing design Marcel Dekker Inc, 1977, pp. 424–440.
9. Snydera, S.A., Adhamb S., Reddingc A.M., Cannonc F.S. J., et al. Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals, Desalination 2007, 202, 156–181.
10. Yoona, Y.P. Westerhoffb, S.A. Snyderc, E.C. Wertc, and Yoon, J., Removal of endocrine disrupting compounds and pharmaceuticals by nanofiltration and ultrafiltration membranes, Desalination 2007, 202 , 16–23.
11. Senol, A and Aydin, A., Solid-liquid extraction of caffeine from tea waste using battery type extractor: Process optimization, J. Food Eng. 2007, 75,565–573.
12. Kim, W.J., Kim, J.D., Kim, J., Oh S.G., and Lee, Y.W., Selective caffeine removal from green tea using supercritical carbon dioxide extraction, J. Food Eng. 2008,89, 303-309.
13. Lin, A.Y., Lin , C., Tung , H., and Sridhara Chary, N., Potential for biodegradation and sorption of acetaminophen, caffeine, propranolol and acebutolol in lab-scale aqueous environments, J. Hazard. Mater. 2010, 183 ,242–250.
14. Sotelo, J.L., Rodríguez, A.,Álvarez, S., J and García G, Removal of caffeine and diclofenac on activated carbon in fixed bed column,Chem. Eng. Res. Des. 2012, 90, 967–974.
15. Šafaříková, M., Ptáčková, L., Kibriková, I. and Šafařík, I., Biosorption of water-soluble dyes on magnetically modified *Saccharomyces cerevisiae* subsp. *uvarum* cells, Chemosphere 2005, 59, 831-835.
16. APHA: Standard methods for the examination of water and wastewater, 19thEdn. American public health Association, Washington DC, USA, 1995.
17. Cheng, H.R., and Jiang, N., Rapid extraction of DNA from bacteria and yeast, Biotechnol. Lett. 2006, 28, 58-59.
18. Nurchi, V.M., Crisponi, G., Villaescusa, I., Chemical equilibria in wastewaters during toxic metal ion removal by agricultural biomass. Coordin. Chem. Rev. 2010, 254, 2181-2192
19. Özer, A., and D. Özer, D., Comparative study of the biosorption of Pb (II), Ni (II) and Cr(VI) ions onto *S. cerevisiae*: Determination of biosorption heats, J. Hazard. Mater. 100 , 2003, 219-229.
20. Chou, W., Wang, C., Huang, K., Chang, Y., and Shu, C., Investigation of indium ions removal from aqueous solutions using spent coffee grounds, Int. J. Phys. Sci. 2012, 7(16) , 2445-2454.

21. Marques, P.A., Pinheiro, H.M., Teixeira, J.A., Rosa, M.F., Removal efficiency of cu^{2+}, cd^{2+}, Pb^{2+} by waste brewery biomass: pH and cation association effect, Desalination 1999, 124(1), 137-144.
22. Goksungur, Y., Uren, S., and Guvenc, U., Biosorption of copper ions by caustic treated waste baker's yeast biomass, Turk. J. Biol, 2005, 27 , 23-29
23. Weber, W. J., and Morris, J.C., Kinetics of adsorption on carbon from solution. J. Sanitary Eng. Division: Am. Soc. Chem. Eng. 1963, 89, 31-59.
24. Wu, J., and Yu, H.Q., Biosorption of 2,4-dichlorophenol from aqueous solution by *Phanerochae techrysosporium* biomass: Isotherms, kinetics and thermodynamics. J. Hazard. Mater. B 2006, 137, 498-508.

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