CONJUGATIVE EFFECT OF LOW COST ADSORBENT WITH LACCASE ENZYME FOR REDUCTION OF PULP AND PAPER EFFLUENT LOAD, ITS KINETICS AND THERMODYNAMICS

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ABSTRACT: Pulp and paper industrial effluent has the foremost role in environmental pollution. The key factor of the current study is to determine the conjugative effect of charcoal (60%) and alluvial soil (40%) mixture with laccase enzyme to lessen the effluent load of the pulp and paper industry. The adsorbent mixture of charcoal (60%) and alluvial soil (40%) was used for the adsorption of biological oxygen demand (BOD) chemical oxygen demand (COD), color, and lignin of effluent. The impact of pH, temperature, adsorbent concentration, and adsorption time on the removal procedure were studied. Adsorption equilibrium was achieved after 50 minutes with an agitation rate of 500 rpm at pH 6.0 at 25°C. Results indicated that COD, BOD, color, and lignin were removed 86%, 80%, 60%, and 62% respectively. Moreover, the laccase enzyme also enhanced the reduction of these parameters as COD 95%, BOD 93% color 83%, and lignin 75%. The experimental batch equilibrium adsorption for COD and BOD was analyzed by Freundlich and Langmuir models and kinetics was also discussed by pseudo-first-order and pseudo-second-order. The BOD and COD data fitted to the pseudo-second-order kinetic model. Thermodynamics parameters ΔG°, ΔH°, and ΔS° results indicated that adsorption was nonspontaneous due to high TDS and lignin, endothermic in nature, and revealed an increase in randomness and degree of disorderliness at adsorbent mixture during the present study. Consequently, the use of laccase enzyme in combination with an adsorbent mixture presents promising results and is applicable.

Keywords: Adsorption; effluent; kinetics; isotherms; lignin.

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INTRODUCTION

The paper industry is among the prominent industry in Pakistan due to its product demand and it consumes a huge quantity of fresh water and generates a plenteous amount of wastewater in developing countries (Kumar and Chandra, 2021). The chemical composition of wastewater depends on the pulping process and use of various chemicals i.e. sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), and chlorine (Cl) compounds whilst washing, pulping, and bleaching process. It is comprised of hydrophobic, hydrophilic compounds, highly toxic and persistent chemicals that contribute towards high BOD, COD, dissolved solids, suspended solids, inorganic compounds, resins, acids, and dark brown color that have a severe impact on human health, animals, plants and food web (Ariaeenejad *et al.*, 2021).

The bio-magnification, bio-accumulation, and recalcitrance properties of effluent are challenging throughout the globe. So it requires an eco-friendly/economical method to reduce its pollution load. The traditional treatment methods i.e. membrane separation activated sludge, chemical oxidation, coagulation, aerated lagoons, and flocculation are ineffective and not feasible due to cost, in the reduction of pollution load BOD, COD, TDS, TSS, color, and lignin (Yang *et al.*, 2008). Subsequently, inexpensive, energy-effective, environment friendly, and cost-effective combinations of indigenous technologies are still required.

Adsorption is the main technique used to purify and drop off effluent load by removing dissolved components from effluent and by drawing the liquefied solutes to surface. Thus, it includes the interphase collecting of concentrate constituents at a surface and/or at the interphase (Mashhadi *et al.*, 2016; Adeyemo *et al.*, 2017). It has wide applications for the elimination of dye and heavy metals from pulp and paper, textile, leather, plastics, cosmetics, and food industries. Numerous researchers have reported on a range of adsorbents to improve the effluent quality and commonly used adsorbents are wood ash, rice husk, peat, slag, corn cob, groundnut hull, rice hull, banana stalk, spend tea leaves and so many others but the discovery of eco-friendly and cost-effective adsorbent is still required, the combination of alluvial soil and charcoal used for the treatment of effluent (Baek. *et al.*, 2010; Han *et al.*, 2015). The results indicated by Das *et al.*, (2014) that the sedimentary soil of River Bhagirathi was used as an economical and effective adsorbent to eradicate lead (Pb) ions from aqueous solutions. Moreover, literature depicted that

results after adsorption required further biological treatment to meet environmental standards.

In this respect isolated laccase enzyme from wood fungi have attracted growing attention for the deletion and detoxification of dyes, lignin, hazardous compounds, and other environmental pollutants (Du *et al.,* 2020; Mehandia *et al.,* 2020; Yang *et al.,* 2020; Iark *et al.,* 2019). Laccase relates to the multi-copper oxidase family and is generally available in plants, fungi, and bacteria. It can oxidize phenolic & non-phenolic compounds by decreasing the molecular oxygen in the water. Using laccase in a mixture with natural mediators, extensively accessible from pulping liquors and plant materials, exemplifies an auspicious substitute for the environment-friendly delignification of paper pulp (Camarero *et al.,* 2007; Motamedi *et al.,* 2021) applied laccase enzyme and decolorized 500 mg/L of Alizarin yellow, Carmine, Congo red and Bromothymol blue with 99.74–55.85% effectiveness afterwards fifteen minutes (at 50 ˚C). Kumar and Chandra (2021) determined that the isolated laccase has noteworthy effects on the detoxification of discharge pulp paper mill wastewater for the good environmental health.

In the present study, an endeavor was made to reckon the potential of a different combination of alluvial soil and charcoal as an adsorbent to treat pulp and paper effluent by integrating laccase enzyme to enhance further treatment efficiency. Furthermore, the effect of time, pH, temperature isotherms, adsorption kinetics, and thermodynamic factors were studied.

MATERIALS AND METHODS

Collection of sample and characterization: Wastewater samples were taken from M/s Century paper mill Lahore in pre-sterilized plastic jerry can (15L capacity) by using Van Dorn sampler, transported in ice containers, and measured characteristics on the same day. This paper mill used wheat straw, bagasse, bamboo, and waste paper recycling to make its product. In order to investigate the pollution load of wastewater grab sampling type was conducted and samples were investigated for COD) and BOD, and total Kjeldhal nitrogen (TKN), lignin, and total suspended solids (TSS), ammonia (NH3). The average and standard deviation (SD) of measured values of nine samples COD (1570 mg/L), BOD (865 mg/L), lignin (725%), TSS (545 mg/L), TKN (268 mg/L), NH_3 (123.5 mg/L) but reported three samples results from an average of nine samples. (Table-1).

Alluvial soil (AS) was collected from the bank of river Head Balloki, Punjab, and purified for adsorption of pulp/paper mill wastewater. It was dried under sunlight for three days afterward drying in a hot air oven at 38˚C for 24 hours. The dried soil was crushed and sieved from 100 mesh and stored in glass-bottles to be used as an adsorbent with charcoal (Table 2). Charcoal (AC) was collected from a biomass gasification plant. The charcoal was get washed with plenty of DDW (double distilled water) to eradicate any impurity and ash. It was dried under sun-light followed by drying in an oven for 3 hours at 100˚C and crushed to the size of 100 mesh. The iodine number was also calculated as per the guidelines of Olafadehan *et al.* (2012).

Table 1. Chemical profile of pulp and paper effluent using an adsorbent mixture (adsorbent concentration 10g/100ml; shaking time 50 min; pH.6.0; shaking speed 500rpm; temperature. 25˚C).

Parameters	Untreated	Adsorption Treated	Laccase Treated
pH	9.2	6.0	-
BOD (mg/l)	865	137	69
COD (mg/l)	1570	220	78
TKN (mg/l)	268	-	-
NH_3 (mg/l)	123.5	-	-
TDS (mg/l)	2879	893	-
TSS (mg/l)	545	153	-
Color Pt/Hz	4521	1800	768
Lignin (%)	725	275	181

*average values of nine samples

Table 2. Physicochemical characteristics of charcoal and alluvial soil

Parameters		Charcoal	Alluvial Soil
pH		7.58 ± 0.04	6.87 ± 0.060
Bulk Density	(kg/m3)	532.14 ± 3.3	139.58 ± 1.5
Porosity	(%)	35.21 ±2.01	42.69 ± 1.10
Carbon	(%)	34.69 ±1.08	1.54 ± 0.050
Hydrogen	(%)	2.01 ± 0.03	0.08 ± 0.010
Nitrogen	(%)	3.98 ± 1.01	0.12 ± 0.010
Ash	(%)	40.87 ± 1.50	55.74 ± 3.25
Silica	(%)	6.24 ± 1.03	32.54 ± 1.21
CEC	meq/g	62.5 ± 3.80	43.2 ± 2.450
Na	mg/l	3.5 ± 0.10	13.21 ± 1.02
K	mg/l	2.14 ± 0.20	10.7 ± 0.900
Ca	mg/l	23.5 ± 1.20	2.4 ± 0.5500
Mg	mg/l	20.5 ± 2.54	8.5 ± 1.1000
Pb	mg/l	0.02 ± 0.001	0.01 ± 0.000

± S.D (n = 3)

The chemical and physical key characteristics of produced charcoal were as pH (7.58 ±0.04), Bulk Density (532.14 ±3.3 kg/m^3), Porosity (35.21±2.01%), Carbon (34.69±1.08%), Hydrogen (2.01±0.03%), Nitrogen (3.98±1.01%), Ash (40.87±1.5%), Silica (6.24±1.03), CEC (62.5±3.8 meq/g), Sodium (3.5±0.1mg/l), Potassium (2.14±0.2 mg/l), Calcium (23.5±1.2mg/l), Magnesium (20.5±2.54mg/l)and Lead (0.02±0.001 mg/l) (Table-2).

The aqueous slurry (5%) of alluvial soil and charcoal after filtration were used to check the pH (HANNA-1122). The concentration of carbon, hydrogen, nitrogen of alluvial soil, and charcoal were determined by a CHNS analyzer (Elementar-Germany). The physio-chemical properties (ash, silica, porosity, bulk density) of both adsorbents were measured by using AOAC-2017 methods. The CEC (cation exchange capacity) of adsorbents was detected by the ammonium acetate method (Iqbal *et al.,* 2010). The concentration of magnesium (Mg), calcium (Ca) and lead (Pb) were determined by ABS (atomic absorption spectroscopy) while Na^+ and K^+ were detected by a flame photometer (Jenway). Furthermore, wastewater parameters like pH, BOD, COD, TDS, TSS, Kjeldhal nitrogen, ammonia, lignin, and color were analyzed from time to time following standard methods (APHA, 2017).

Method for Treatment of Pulp and Paper wastewater: The pure wastewater samples of industry were filtered through a mixture of gravel and sand to remove the heavy particles and analyzed the characteristics of effluent before and after the filtration from the adsorbent mixture AC (60%) and AS (40%). The concentration of COD and BOD adsorbed by AC & AS mixture at equilibrium qt (mg/g), qe (mg/g), and time t were calculated by following relationships.

$$R(\%) = \frac{Co-Ce}{C0} \; X100 \qquad (1)$$

$$qt = \frac{Co-Ct}{m} \; X\,V \quad (2)$$

$$qe = \frac{Co-Ce}{m} \; X\,V \quad (3)$$

Where q_t depicts the concentration of COD and BOD adsorbed by adsorbent mixture at time t. C_o, C_t and C_e represent the concentration of BOD and COD in an original sample at time t and equilibrium correspondingly whereas V is the volume of wastewater sample (l) and m is the mass/weight of the adsorbent mixture in grams.

The grave sand filtered wastewater samples were adsorbed at lab temperature (25°C ± 1) using the optimized concentration of adsorbent mixture (10g), pH (6) and time (50 min). The wastewater sample 100 ml mixed with adsorbent 10 g in 250 ml glass stoppered Erlenmeyer flask and shake at constant speed @ 500 rpm continued for 50 minutes. The subsequent solution was then filtered and analyzed for the concentration of BOD, COD, lignin and color.

Impact of Laccase enzyme on Wastewater: Further wastewater samples were treated with laccase enzyme which was produced from the white-rot fungus *Ganoderma lucidium* in optimized conditions. The fungus was collected from rotted wood plants. The charcoal and alluvial soil mixture treated wastewater 50 ml was mixed in 250ml Erlenmeyer's flasks containing phosphate buffer (25mM) laccase enzyme (15U/ml) at pH (6) and temperature (25°C). The mixture was further stirred for four hours on a shaking bath and finally calculate the BOD, COD, lignin and color of control and treated samples.

RESULTS AND DISCUSSION

Adsorption of Effluent: Adsorption is the physicochemical procedure developed an effective wastewater treatment due to its economical property. In the present study effluent TDS, lignin, and color parameters depicted the decline in concentration due to adsorption. The utmost decline was found in TDS, lignin and color 69%, 65% and 60% respectively after 14 days of adsorption. These parameters exhibited the pollution load of effluent and industrial processes.

Effect of Adsorbent: The increase in solute adsorption with the rise in the quantity (concentration) of adsorbent since increasing in adsorbent level resulted in augmented dynamic altercation adsorption sites. Yet, the whole solute adsorption (as per the unit weight of adsorbent) can drop subsequent rise in adsorbent concentration because of interference produced by the interaction of the active sites of an adsorbent or at equilibrium (Iftekhar *et al.,* 2018). In the present study charcoal (60%) and alluvial soil (40%) mixture was consumed for the adsorption of pulp and paper wastewater at pH 6.0 for 50 min at 25°C with the shaking a speed @ 500 rpm. The adsorbent mixture concentration was increased from 2 to 16g/l with a persistent volume of 100ml. The removal percentage increased as the concentration of adsorbent mixture quantity improved but from 12 to 16 g/L no significant change had been observed (Fig.1a). It means that the surface area available for effluent BOD, COD was decreased due to the overlapping of adsorption sites. Therefore maximum removal (85%) was achieved by 10g/L of the adsorbent mixture. Das *et al.,* (2014) used the alluvial soil for the reduction of lead results are in line with the present study. Furthermore, based on a broad literature study it looks that the adsorbent g/L was too high in the present study but Gasser and Aly (2013) also exercised 10g/L optimum dose for La adsorption due to a maximum concentration of 5000-10000 mg/L. Similarly, Silva *et al.,* (2020) appraised the efficacy of orange albedo as an adsorbent for the elimination of cationic dyes in an aqueous solution and concluded the optimized conditions when 1% biomass,100rpm and 30°C.

Influence of pH: The pH is amongst the utmost significant parameters that can straight impact the uptake of solute by adsorbents as it can affect the surface characteristics of an adsorbent and the degree of ionization. Furthermore, it also impacts the rising or reducing the adsorption capacity since it alters the state of binding groups of both (adsorbate and adsorbent). Litefti *et al.,* (2019) presented in their study the impact of pH on the elimination proficiency of BOD, COD was

investigated at varying pH values ranging from 2 to 14 (Fig.1b). It was discerned that maximum COD and BOD was removed at pH 6 whereas it presented an increasing trend from pH 2 to 6. The pH 6 was optimum, which was selected to optimize further the adsorbent concentration and adsorption time for COD and BOD removal. The removal percentage exhibited a decreasing trend at a pH higher than 6 due to the increasing hindrance of organic compounds in effluent with OH- ions. Moreover, the maximum adsorption percentage at pH 6 may be credited at a low pH value, positive charge sites are created by the adsorbent mixture. A similar observation has been reported by Abeyemo *et al.* (2017) discussed the adsorption of sewage sludge COD, BOD on charcoal.

Effect of Time of Adsorption: The adsorption time considerably ($P<0.05$) affects the adsorption process and affects the economy of the process efficiently in addition to the adsorption kinetics. Hence, adsorption time is an alternative factor governing the performance in the adsorption process [28]. The influence of adsorption time for the removal of BOD and COD from pulp and paper effluent by 10g/L adsorption mixture, pH 6 at temperature 25^{o}C and agitation rate 500 rpm was evaluated for the period of 10-70 min (Fig.1c). The COD percentage removal at 10, 30, 50 and 70 min was 35%, 67%, 86% and 70% respectively while for BOD at 10, 30, 50 and 70 min was 28%, 59%, 78% and 62% respectively. The reduction of BOD, COD by adsorption mixture exhibited fast as the adsorption time increased. The first 50 min reduction was maximum but after 50 min there was no significant change in results. It means that active sites of the adsorption mixture are unoccupied for the adsorption of BOD and COD. The current research results are in line with Abeyemo *et al* (2017). Das *et al.* (2014) studied the impact of time on the uptake of lead (II) ions by alluvial soil, and concluded that the exclusion rate was fast firstly and then progressively decreased with time until it reached equilibrium yonder when no noteworthy increase in elimination was noted.

Adsorption Isotherms: Adsorption isotherms present a relationship between adsorbate concentrations with the adsorbent surface at optimized conditions. There are a number of isotherm equations, however, the most common isotherm equations for investigating experimental sorption equilibrium parameters is the Langmuir and Freundlich isotherm models (Langmuir,1918) expressed as follows.

$$Ce/qe = Ce/qm + 1/qm\,Kl \quad (4)$$

Where q_o and k are Langmuir constants, q_o represents the adsorption capacity (mg/g); k represents energy of adsorption (L/mg) respectively.

The Freundlich isotherm model is applied to adsorption on heterogeneous surfaces and is not restricted to the formation of a monolayer. The model accepts that as the adsorbate level rises, the concentration of adsorbate on the adsorbent surface also increases and, consistently, the sorption energy declines exponentially on completion of the sorption centers of the adsorbent.

$$log\ qe = log\ kf + 1/n\ log\ Ce \quad (5)$$

Where 1/n is a measure of the adsorption intensity and capacity.

In the present study BOD and COD adsorption capacities (q_e) of the adsorbent mixture and residual amount of COD and BOD at equilibrium (Ce) were analyzed with these two isotherms at optimized temperature 25˚C.

Liner isotherms were plotted between (q_e) and Ce to evaluate the best isotherm suitable for the adsorption of pulp and paper BOD and COD by adsorbent mixture (Fig 3). In Langmuir model a graph was plotted between Ce/qe against Ce for COD and BOD and obtained a straight line. The R^2 for BOD (0.9992) for COD (0.9996) approves that the Langmuir model is appropriate for the adsorption of both parameters by adsorption mixture. In Freundlich isotherm, the log (q_e) against log Ce was also plotted and calculated the constant 1/n and k from the slope (See figure 2) and all parameters for both models are represented in table 3. The value of n between 1 and 10 explained the best and most favorable adsorption process by Slejko (1986), whereas in the present experiment the value of n is 6.607 and 3.902 for BOD and COD respectively.

The data presented in table (3) indicated that both isotherms are suitable for the adsorption of COD and BOD by adsorption mixture. Moreover, R^2 values of both parameters COD and BOD by adsorption mixture also concluded that the Langmuir & Freundlich isotherms have a well-defined model of linear. The same observation has been found by Yang *et al.* (2020).

The maximum monolayer coverage capacity (q_o) for Langmuir isotherm was calculated as COD (15.85mg/g) and BOD (16.13 mg/g) while k is 0.012 l/mg, 0.003 l/mg for COD and BOD respectively presented that Langmuir model is fit for homogeneous surfaces.

Table 3. Freundlich and Langmuir Isotherms constant for COD and BOD by adsorbent mixture (adsorbent concentration 10g/100ml; shaking time 50 min; pH.6.0; shaking speed 500rpm; temperature. 25˚C).

	Langmuir Isotherms constant			Freundlich Isotherms constant			
	qm (mg/g)	KL (l/mg)	R^2	Kf (mg/g)	n	R^2	1/n
COD	16.13	0.003	0.9992	1.428	6.607	0.9992	0.15
BOD	15.85	0.012	0.9996	2.88	3.902	1	0.25

Adsorption Kinetic Modeling: To analyze the adsorption process mechanism 2 kinetic models Lagergren 1st order (Lagergren 1898) and Pseudo second order were applied to the present study and determine the best-fitted model.

Pseudo 1st order kinetic model: The Lagergren first-order model is presented as

$$Log(qe - qt) = logqe - \frac{Kf\,t}{2.303} \qquad (6)$$

In the above equation qe and qt are the amounts of BOD and COD adsorbed (mg/g) at equilibrium and at time t correspondingly whereas k_l is the rate constant of Lagergren first-order adsorption (min^{-1}).The graph was plotted between log (qe-qt) versus t to calculate the values of q_e and rate constant K_l.

Pseudo second-order kinetic model

$$\frac{t}{qt} = \frac{1}{ksqe2} + \frac{t}{qe} \qquad (7)$$

Where k_s is the rate constant of the second order (g/mg/min). The values of R^2, Ks and q_e were calculated by plotting a graph between t/qt and t at different temperatures but only 25˚C results have been presented here (fig.5). The data presented in table (4) indicated that the kinetic model pseudo-second order exhibited seamless linearity with correlation coefficient R^2 (1) for COD and R^2 (0.9997) for BOD. In the same way for COD the calculated qe (10.87) values were coherent with experimental results (10.836) in this kinetic model. Furthermore, BOD values were also showing the same trend. The values of Ks for both parameters decreased with increasing concentration due to less struggle for active sites at lesser levels but at high concentrations, the competition for active sites will be higher (Das *et al.*, 2014). In addition rate, constant Ks decreased with rising the temperature, showing endothermic behaviors of the adsorption process. The present study results are supporting the literature. The data presented in table (4) also depicted that R^2 values are upper for the second order model than for first-order model. So pseudo-second-order is fit to obtain the experimental data for COD and BOD adsorption from the adsorbent mixture.

Table 4. Calculated parameters of Pseudo- first order and pseudo-second-order kinetics models for the adsorption of COD and BOD by adsorbent mixture (adsorbent concentration 10g/100ml; shaking time 50 min; pH.6.0; shaking speed 500rpm; temperature. 25˚C.

		Pseudo- first order					Pseudo- second order			
	T (˚C)	C_0 (mg/L)	K_f (min^{-1})	qe (mg/g) exp	qe (mg/g) cal	R^2	Ks (mg/min)	qe (mg/g) exp	qe (mg/g) cal	R^2
COD	25	1260	0.118	10.836	1.691	0.9192	0.582	10.836	10.87	1
BOD	25	415	0.065	3.320	1.796	0.8958	0.25	3.320	3.381	0.999

The kinetic process explained the adsorption rate of effluent under different temperatures. The increase in temperature augmented the rate of adsorption because the structures of the adsorbent mixture showed vide surface area. The chemical adsorption is caused by the reaction force and the coordination process between the effluent functional groups on the surface of the adsorbent mixture. Furthermore, it revealed that the presence of various sheet particles of the adsorbent mixture was dispersed well during the present study process. The physical appearance of the treated adsorbent mixture was highly different from natural mixture constituents. The present study results were eloquent with Lu *et al.,* (2016).

Thermodynamic Parameters: The thermodynamic parameters accompanied by temperature were used to know the behavior of the adsorption method; endothermic or exothermic, randomness and spontaneity. Moreover also determined either the temperature is promising for the adsorption procedure or not. The significant thermodynamic parameters are the ΔG˚, ΔH˚, and ΔS˚ representing the changes in Gibbs free energy, enthalpy and entropy respectively. T is the absolute temperature in Kelvin and R is the general gas constant (R=8.134 J/mol K).

$$\Delta G^{\circ} = -RT\,In\,(Kc) \qquad (8)$$

$$Log\,Kc = \Delta S^{\circ}/2.303\,R - \Delta H^{\circ}/2.303\,RT \qquad (9)$$

The ΔG˚ for the adsorption of BOD and COD on the adsorbent mixture at 5, 15 & 25˚C were determined from the equation. The values of ΔS˚ and ΔH˚ were also determined from the intercept and slope of the graph between log Kc and 1/T (K). The values of ΔG˚, ΔH˚, and ΔS˚ are presented in table 5 and it is determined that the positive value of ΔH˚ indicated the endothermic behavior of BOD and COD adsorption process by adsorbent mixture. The present result of the study also supports the increasing uptake value capacity of adsorbents with an increase in temperature as reported by Han *et al.* (2006) who stated that the increased sorption capacity of the sorbent with temperature is attributed to the enlargement of pores and/or the activation of the sorbent surface. The positive values of ΔG˚ approve the non-spontaneous behavior of the process. It was observed that the presence of a higher concentration of TDS, TSS and lignin are the main factors for nonspontaneous behavior of adsorption reaction. Wirnkor (2019) removed

the COD and BOD from vegetable oil industry effluent by adsorbent and concluded that the process was also nonspontaneous. Therefore the present results are highly coherent with the latest research. Moreover, the positive value of ΔS˚ specified the randomness and disorderliness in the present adsorption process. Furthermore, similar observations have been found by Nayl *et al.,* (2017) during the adsorption of COD and BOD by charcoal.

Table 5. Thermodynamic parameters/factors for adsorption of COD and BOD by an adsorbent mixture.

Parameters	Temperature (˚C/K)	Δ G˚ (kj/ mole)	Δ H˚ (kj/ mole)	Δ˚S (J/ mole/K)
COD	5/278	9.037		
	15/288	7.707	38.22	105.38
	25/298	6.887		
BOD	5/278	8.240	11.87	13.06
	15/288	8.120		
	25/298	7.970		

Effect Laccase Enzyme on Effluent Properties: The physicochemical characteristics of wastewater were dark brown and alkaline in nature and is the best enzyme to oxidize phenolic and non-phonic compounds. In the current study, the wastewater treated with charcoal (60%) and alluvial soil (40%) mixture was further oxidized by laccase enzyme to reduce the effluent load BOD, COD, color and lignin. It was observed that a significant reduction in BOD 92%, COD 95%, color 83% and lignin 75% was achieved by using the laccase enzyme under standardized conditions. (Table.1). Sonica *et al.,* (2017) also treated the pulp and paper wastewater with laccase enzyme and found extremely promising results. The laccase enzyme also reduces the treatment time for other microorganisms. Camarero *et al.* (2007) used the laccase enzyme with some natural mediators for delignification and found encouraging results. Pramanik and Chaudhuri (2018) decolorized five high-molecular azo dyes with laccase enzyme and recorded the highest 70.41% reduction in color. Moreover, Cifci *et al.,* (2019) also clarified various dye chromophores proficiently using laccase enzyme as they investigated the chromophore structure of the dye is pivotal in enzymatic color elimination proficiency.

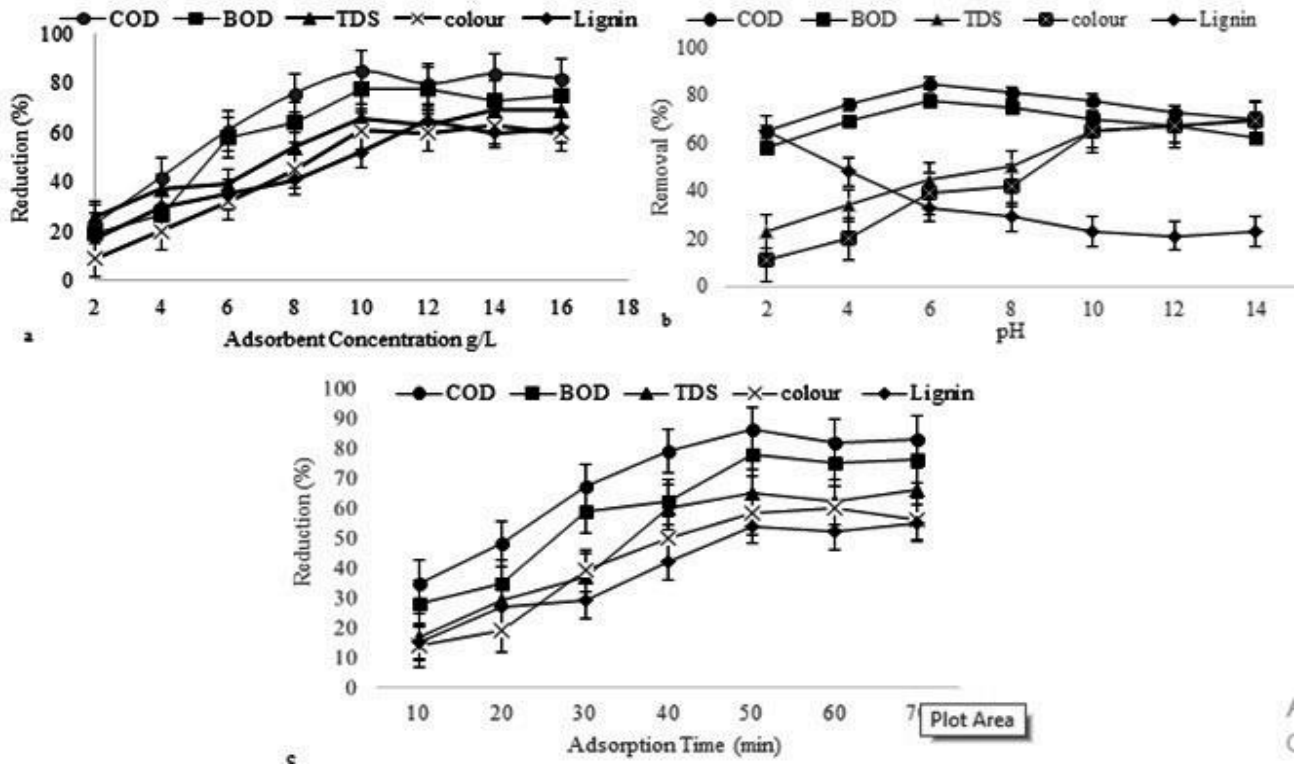

Fig.1. (a) Effect of adsorbent mixture (charcoal 60% & alluvial soil 40%) on removal percentage of COD,BOD,TDS, color and lignin from 100 ml of effluent (time 50min; pH.6.0; shaking speed 500rpm; temp. 25˚C) (b) Effect of pH (adsorbent concentration 10g/100ml; time 50min; shaking speed 500rpm; temp. 25˚C) (c) Effect of adsorption time (adsorbent concentration 10g/100ml; pH.6.0; shaking speed 500rpm; temp. 25◦C

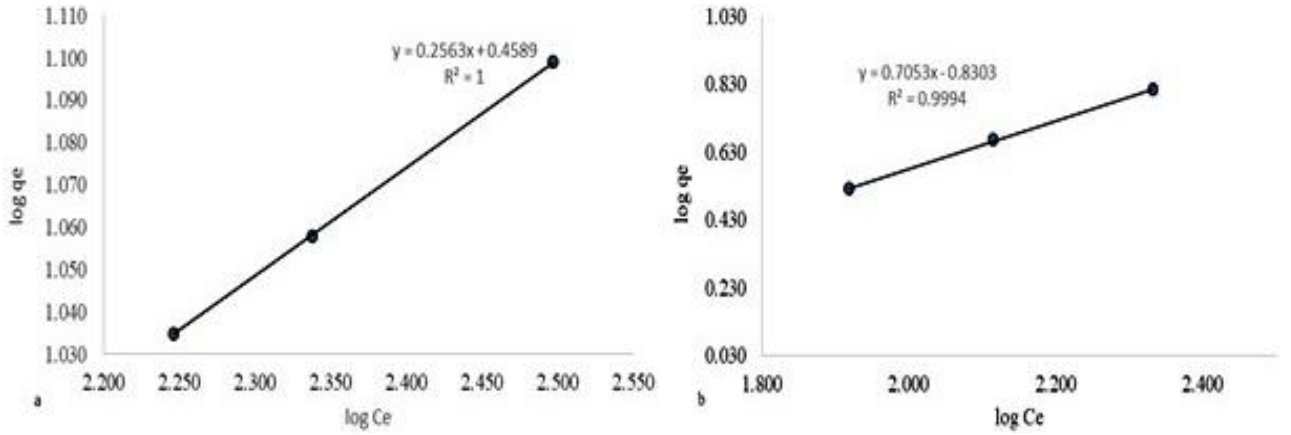

Fig 2. Freundlich adsorption isotherms of COD (a) and BOD (b) by adsorbent mixture (adsorbent concentration 10g/100ml; shaking time 50 min; pH.6.0; shaking speed 500rpm; temperature 25˚C)

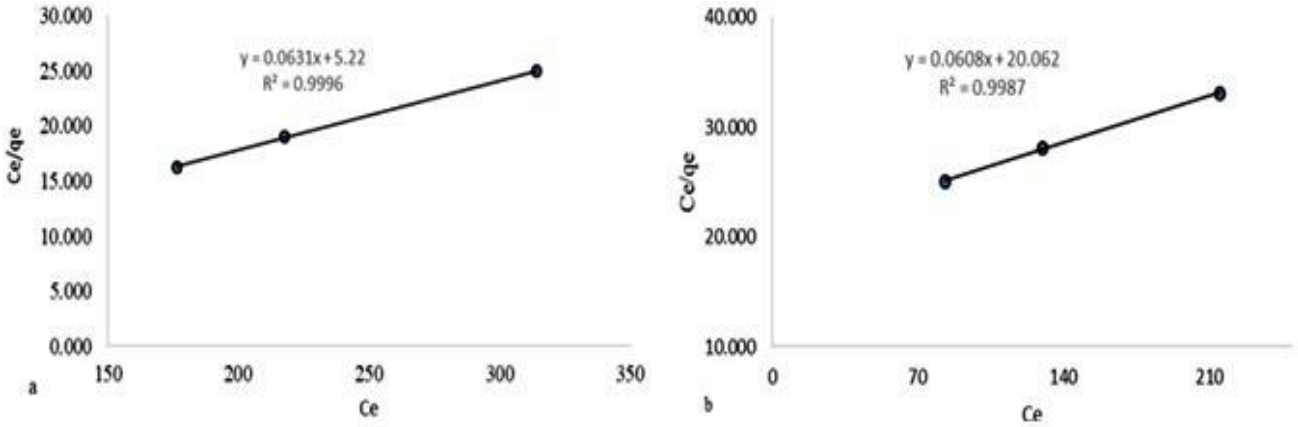

Fig 3. Langmuir adsorption isotherms of COD (a) and BOD (b) by adsorbent mixture (adsorbent concentration 10g/100ml; shaking time 50 min; pH.6.0; shaking speed 500rpm; temperature 25˚C)

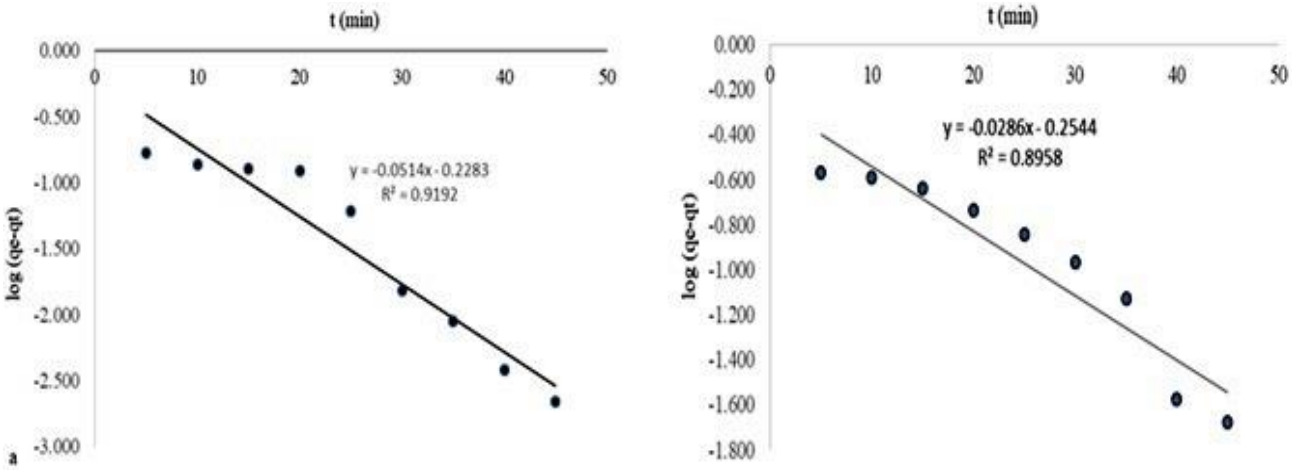

Fig.4 Pseudo-first order adsorption kinetics of COD and BOD from adsorbent mixture (a) COD (b) BOD by adsorbent mixture (adsorbent concentration 10g/100ml; shaking time 50 min; pH.6.0; shaking speed 500rpm; temperature 25˚C)

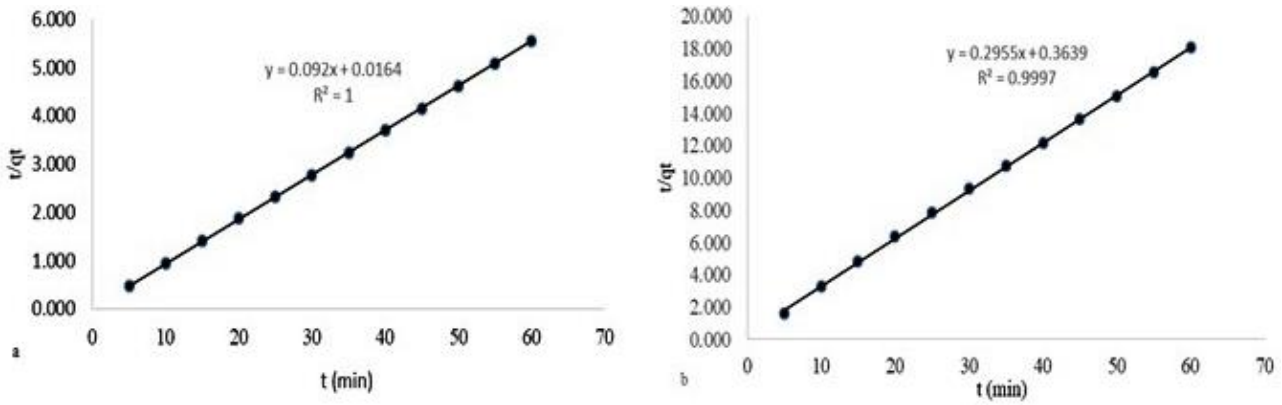

Fig.5.Pseudo-second order adsorption kinetics of COD and BOD from adsorbent mixture (a) COD (b) BOD by adsorbent mixture (adsorbent concentration 10g/100ml; shaking time 50 min; pH.6.0; shaking speed 500rpm; temperature 25˚C).

Conclusion: The use of alluvial soil in combination with charcoal collected from biomass gasification plants is best for adsorption. The operating parameters pH, temperature, adsorption time, adsorbent concentration, and stirring time were much more effective and conjugative with the laccase enzyme to reduce the COD 95%, BOD 93% color 83%, and lignin 75%. The adsorption isotherms results were fitted best to Langmuir and Freundlich models. Results of the present study concluded that adsorption kinetics followed more by pseudo-second order model than the pseudo-first order model. Thermodynamics results revealed that the positive value of ΔG° indicated the nonspontaneous process due to the high concentration of TDS, TSS and lignin concentration available in the effluent. The calculated ΔS° values also presented the disorderliness and randomness that occurred due to interfaces in this adsorption process. Moreover, the ΔH° positive values confirm the endothermic behavior of the process. The present finding advocate that the combination of laccase enzyme with the adsorbent mixture is the cost effective, eco-friendly and applicable to treat any time of effluent in the future.

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