Adsorptive Characteristics of Coag-flocculation Studies of *Carica papaya* Leaf Protein Isolate in Paint Industry Effluent Medium

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***Abstract:-* The aim of this study is to explore the potential of *Carica papaya* leaf proteinisolate as an adsorbate applicable in wastewater treatment. The protein content of *Carica papaya* leaf was fully isolated by precipitation adopting the method of salting out and re-solubilisation in distilled water to obtain pawpaw leaf protein concentrate also known as pawpaw leaf coagulant (PLC). The phytochemical analysis of the PLC indicated its potency as an adsorbate applicable in industrial effluent coag-flocculation where the colloidal particles within the effluent medium are the adsorbent.The effluent used in this study was paint industry effluent (PIE). The adsorption of PLC onto the colloidal particles, triggering particles aggregation and subsequent agglomeration, were studied varying the adsorbate dose, solution pH and contact time. The highest turbidity percentage removal of 99.5% were obtained at contact time of45 minutes, solution pH of 6.0 and dosage of 300 mg/l at room temperature. It was also discovered that the colour of the paint effluent, as a result of the treatment, reducedfrom 2015 PCU to 200 PCU while total solids dropped from 1600 mg/l to 122 mg/l. The trend indicates that at $4 < \text{pH} > 9$, the sorption of PLC is more favourable. Adsorption isotherms – Langmuir, Freindlich, Tempkin and Henry – were plotted and the data were found to be best represented by the Henry isotherm, having coefficient of determination, R^2 of 1. This is followed by Langmuir isotherm with R^2 of 0.9978. Adsorption kinetics studies favoured pseudo-second order kinetic model. It is concluded that *Carica papaya* leaf protein isolate is a useful adsorbate capable of initiating particles agglomeration in an effluent medium prior to gravity settling and detoxification.**

***Keywords:* pawpaw leaf coagulant, coag-flocculation, adsorbate, adsorption isotherms.**

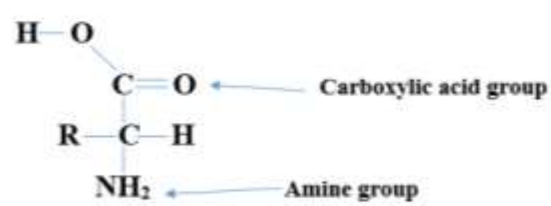
I. INTRODUCTION

Paint industries are one of the major industries engaged in production activities in Nigeria due to the fast developing nature of the nation's real estate subsector. In the production of paint, water is a major component used. As a result, there is discharged into the environment paint effluents contaminated with colour and odour. The effluent is also very turbid with both suspended and dissolved solid particles in it.Alam*et al* (2013) reported that effluent from a paint factory contained TSS of 2040 mg/l. This value is high but smaller than the average TSS of 9532 mg/l and turbidity of 26.8NTU reported by Aboulhassan*et al* (2014). Olaoye and Oladeji (2015) reported that the paint industry wastewater obtained from a paint manufacturing location at Ibadan, Nigeria contained TDS and TSS values of 987 mg/l and 1665 mg/l respectively. These high values of TDS and TSS are smaller than the following higher values reported by Menkiti*et al* (2015) for paint effluent sourced from a factory at Onitsha, Anambra State: TDS (1600 mg/l), TSS (2679 mg/l) and TS (4279 mg/l).

It is a common practice to detoxify industrial effluents prior to disposal to avoid endangering the lives of economically important species within the ecosystem. However, paint manufacturing factories in Nigeria are yet to key into this practice. Majority of them discharge their effluents directly into the environment, thus constituting a threat to the flora and fauna of the receiving environment. In effluents predisposal treatment, as well as in general water treatment practice, coagulation/flocculation is widely applied as a precursor to the disinfection stage. One of four mechanisms – double-layer compression, adsorption and charge neutralisation, enmeshment by a precipitate (sweep-floc coagulation) and interparticle bridging (polymer bridging) – is the mechanism by which coagulation occurs (Jin, 2005). This research focuses on the adsorption as a critical aspect of coagulation.

The coag-flocculation characteristics of natural substances are linked to their protein content (Ramanan*et al*, 2014). Proteins are very high molecular mass polymers. They are made up of basic building blocks or units (monomers) called amino acids. An amino acid has two functional groups in its molecule – an amine group, NH_2 and a carboxylic acid group, COOH ((Matthews,

2002). Thegeneral structure of an amino acid is shown below.



Adjacent amino acids in a given protein are linked by a peptide bond which are formed as a result of two amino acids jointly releasing a water molecule, the – OH of which comes from the carboxylic acid group of one of the amino acids while the – H comes from the amine group of the other amino acid. These groups, having lost – OH and – H, then bond together to form the peptide bond or peptide link. The mechanism is represented by equation 1.

$$\underset{\text{Amino acid}}{NH_2CHR_1COOH} + \underset{\text{Amino acid}}{NH_2CHR_2COOH}$$

$$\rightarrow \underset{\text{Dipeptide}}{NH_2CHR_1CO - NHCHR_2COOH} + \underset{\text{Water}}{H_2O} \qquad (1)$$

When amino acids dissolve in water, they ionize. At a particular pH, they exist in solution as dipolar ions called zwitterions. This occurs as a result of intra-molecular proton transfer from the carboxyl group to the amine group (of the same molecule). The pH at which this occurs is known as the isoelectric point for the amino acid.

When a protein-based coagulant is dosed into water sample, the mechanism of the hydrolysis of the protein may be written thus:

a. If there are n monomers (amino acids) in a polypeptide (protein), then the general equation for the hydrolysis of the protein may be written as:

$$\underset{\text{Protein}}{\text{Polypeptide}} + \underset{\text{water}}{(n-1)(H_2O)} \rightarrow \underset{\text{monomers}}{n(\text{Monomer})} \qquad (2)$$

b. Each monomer is ionized by 'carboxylic acid group-amine group' proton transfer to form a zwitterion, thus:

$$\underset{\text{Amino acid}}{NH_2CHRCOOH} \rightleftharpoons \underset{\text{Zwitterion}}{NH_3^+CHRCOO^-} \qquad (3)$$

Since water also ionizes to form hydroxonium ion, the carboxyl group in the zwitterion is protonated by the hydroxonium ion to form protonated amino acid, thus:

$$\underset{\text{Zwitterion}}{NH_3^+CHRCOO^-} + \underset{\text{Hydroxonium ion}}{H_3O^+}$$

$$\rightleftharpoons \underset{\text{Protonated amino acid}}{NH_3^+CHRCOOH} + \underset{\text{Water}}{H_2O} \qquad (4)$$

Colloidal particles in water possess net negative surface charges which make them stable due to very strong electrostatic repulsion between adjacent particles. Since the protonated amino acids are positively charged, they are readily adsorbed onto the surfaces of the negative colloidal particles and neutralise surface charge (Bagwell et al, 2001). This leads to a reduction of the zeta potential to a level where the colloids are destabilised and thus enhances particle aggregation. In any case, the coagulant dosage should be proportional to the quantity of colloids present. If overdose is applied, charge reversal on the colloids occurs and the colloids are not destabilised (Jin, 2005). The goal of this research is to present pawpaw leaf protein concentrate as an adsorbate that can be adsorbed by colloidal particles in paint industry effluent leading to particle agglomeration and floc settling.

II. MATERIALS AND METHODS

2.1 Pawpaw Leaf Coagulant (PLC)

Green leaves of pawpaw were collected from Ihembosi town in Anambra State of Nigeria, washed and sun-dried for seven days. The leaves were then crushed and reduced to fine powder with the help of a high speed laboratory electric blender and packed in an air-tight -container. 150 grams of the powder was added in 500 ml of distilled water and the mixture was blended in high speed mixer for 30 seconds and allowed to stand for 24hours to allow proteins go into solution. The suspension so obtained was shaken and filtered through muslin cloth to give a stock solution (Patil and Sadgir, 2013). The protein-rich filtrate was treated with saturated solution of sodium chloride to salt out the protein. Again, this enabled the proteins to precipitate out of solution (Martinez-Maqueda*et al*, 2013). The suspension was then fed into a centrifuge machine and rotated at a speed of 415rpm for 20 minutes. After centrifugation, the suspension was filtered by N0.1 whatman filter paper to recover the protein isolate from the salt-water solution. The protein isolate was then resolubilized in distilled water (Martinez-Maqueda, *et al*, 2013) to form colloidal solution, protein concentrate, and stored at room temperature. This was later used as the coagulants.

2.2 Effluent

The effluent used in the experiments were collected from a paint factory at NkporAgu, Anambra State and taken to the laboratory for characterisation.

III. RESULTS AND DISCUSSION

3.1 Physiochemical Characterisation of the effluent

Physiochemical characterisation of the effluent was carried out before and after treatment and the results are as shown in table 3.1. The results indicate that turbidity, colour and BOD are the factors of interest. They were reduced by adsorptive coag-flocculation at room temperature to permissible levels as shown in the table.

Table 3.1: Physiochemical characteristics of the paint industry effluent

S/N	Parameter	WHO (2006, 2011)	FEPA (Ekanem *et al*, 2016)	Before Treatment	After Treatment
1	Temperature (^oC)	25	<40	30.6	31
2	pH	6.5 – 8.5	6 – 9	8.2	7.9
3	Conductivity (µs/cm)	8-10,000	1000	140	148
4	TDS (mg/l)	–	2000	91	20
5	TSS (mg/l)	30	30	1509	102
6	TS (mg/l)	500	–	1600	122
7	Turbidity (NTU)	< 5.0	–	205	4
8	Colour (PCU)	≤ 15	–	2015	200
9	BOD (mg/l)	5	50	1006.67	9.0
10	COD (mg/l)	10	100	12583.38	14.4
11	Lead (mg/l)	0.01 (A, T)	<1	0.056	0.049
12	Iron (mg/l)	0.3	20	0.615	0.10
13	Copper (mg/l)	2	1.5	0.704	0.09
14	Zinc (mg/l)	≤ 3.0	<1.0	0.09	0.07
15	Chromium (mg/l)	0.05 (P)	0.05	0.2	0.002
16	Arsenic (mg/l)	0.01 (A, T)	–	$3x10^{-6}$	$3x10^{-8}$

A:provisional guideline value because calculated guideline value is below the achievable quantification level; **P:** provisional guideline value because of uncertainties in the health database; **T:**provisional guideline value because calculated guideline value is below the level that can be achieved through practical treatment methods, source protection, etc.

3.2 Proximate composition of pawpaw leaf powder

Table 3.2 shows the proximate composition of pawpaw leaf powder. The result shows that there is significant amount of crude protein in the extract as shown.

Table 3.2: Proximate composition of pawpaw leaf powder

Trial No.	Moisture	Crude protein	Fat	Ash	Crude fibre	Carbohydrate	Dry matter
			Percentage by mass (%)				
1	6.09	13.6	0.01	10	31.3	39	93.91
2	4.43	13.4	0.02	10.9	31.4	39.3	95.57
3	5.09	13	0.01	10.8	32	39.1	94.91
Total	15.61	40	0.04	31.7	94.7	117.4	84.39
Mean±STDev.	5.20±0.84	13.33±0.31	0.01±0.01	10.57±0.49	31.57±0.38	39.13±0.15	94.80±0.84

These values are the means of triplicate determinations as shown.
Note: moisture content + dry matter = 100%
Crude protein + fat + ash + crude fibre + carbohydrate = dry matter

3.3 Phytochemical analysis of pawpaw leaf extract

The results of the phytochemical analysis (table 3.3) indicates high presence of protein in the pawpaw leaf extract thus showing that it can be effectively applied as a bio-coagulant (adsorbate) in water and wastewater treatment.

Table 3.3: Qualitative phytochemical composition of the protein concentrates

Phytochemical	Pawpaw Leaf
Alkaloids	+
Flavonoids	+
Steroids	+
Saponins	+
Glycosides	+
Terponoids	+
Resins	-
Reducing sugars	-
Carbohydrates	-
Proteins	+++
Tannins	+
Fats and oils	-
Phenols	+
Oxalates	-
Gallic acid	-
Anthroquinone	+
Coumarins	+
Naphthoquinone	-
Anthocyanin	+
Leucoanthocyanin	+

Key: + Slightly Present; ++ Moderately Present; +++ Highly Present; - Absent

3.4 Coagulation/flocculation analysis

3.4.1 Coag-flocculation efficiency studies

The efficiency of the process was calculated using the equation 5 and the results plotted as figure 3.1 through 3.5. The figures indicate high efficiency of the system showing that PLC is a good adsorbate (coagulant) for wastewater treatment

$$\text{Contaminat removal } (\%) = \frac{C_r - C_t}{C_r} \times 100 \qquad (5)$$

Where: C_r stands for contaminant in raw water

C_t stands for contaminants in treated water

The contaminants identified to be at higher concentration than the World Health Organisation's minimum acceptable level are turbidity, colour and phosphates.

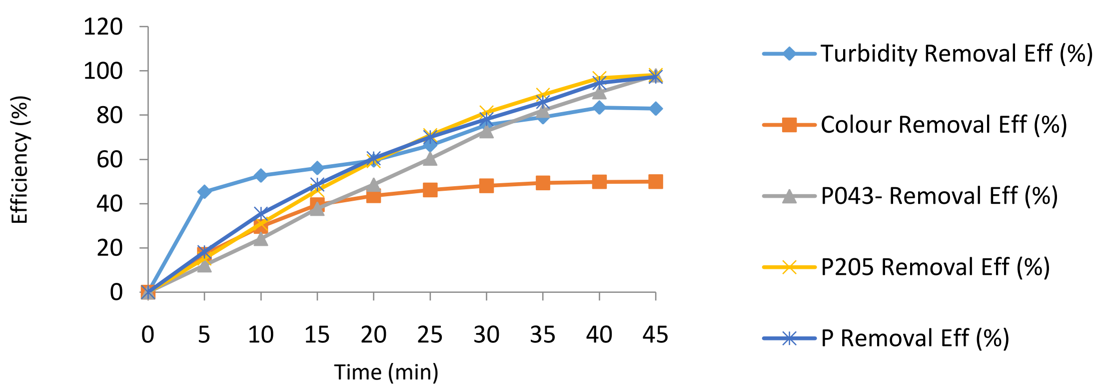


Fig. 3.1: Efficiency versus time at pH of 2.0 and PLC dose of 300mg/lit for PIE at room temperature

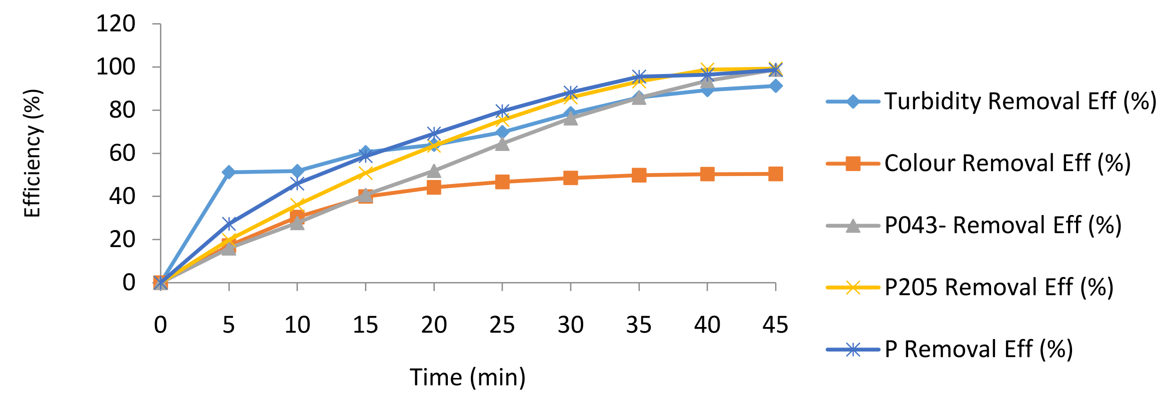


Fig. 3.2: Efficiency versus time at pH of 4.0 and PLC dose of 300mg/lit for PIE at room temperature

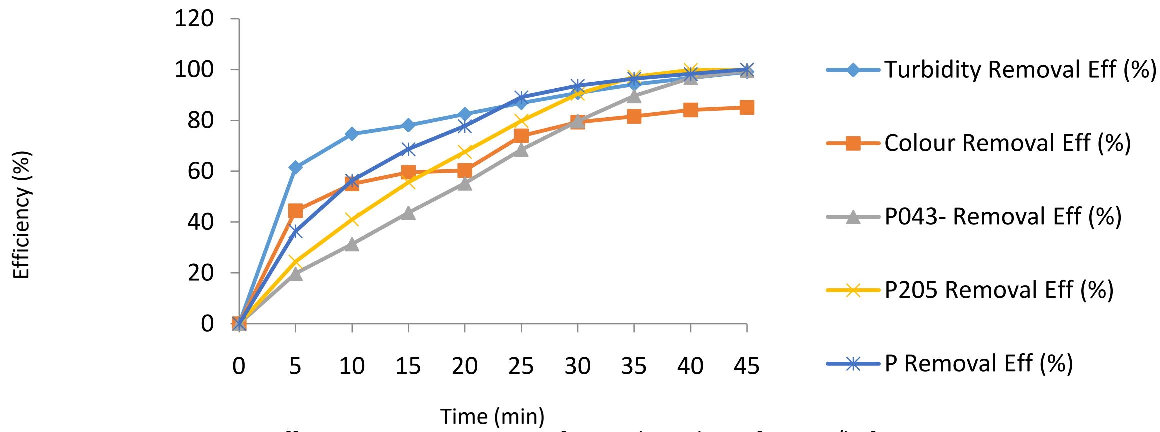


Fig. 3.3: Efficiency versus time at pH of 6.0 and PLC dose of 300mg/lit for PIE at room temperature

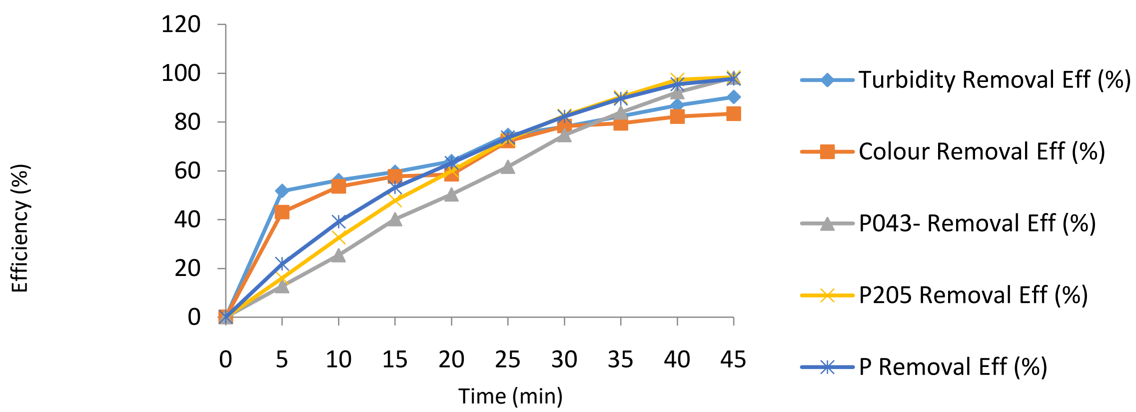


Fig. 3.4: Efficiency versus time at pH of 8.0 and PLC dose of 300mg/lit for PIE at room temperature

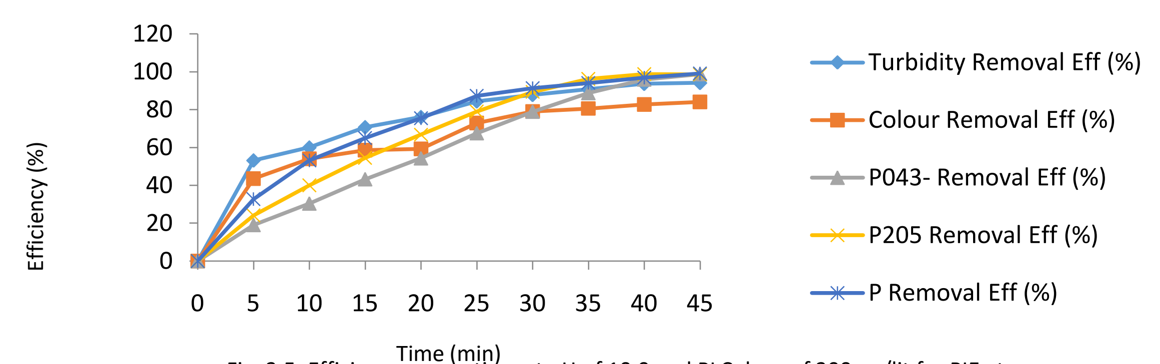


Fig. 3.5: Efficiency versus time at pH of 10.0 and PLC dose of 300mg/lit for PIE at room temperature

3.4.2 Adsorption studies

Four adsorption isotherms – Langmuir isotherm, Freundlich isotherm, Temkin isotherm and Henry isotherm - were tested in this study to explore the fitness of experimental data to these isotherms. The Langmuir model is based on the assumption that there exists fixed individual sites on the surface of the adsorbent and that each of these sites is capable of adsorbing one molecule, culminating in a monolayer over the adsorbent surface, the thickness of which is the size of one molecule. Langmuir (1918) contendedthat maximum adsorption occurs when the surface is covered by a monolayer of adsorbate. The linearized Langmuir equation is given as equation 6:

$$\frac{C_1}{q} = \left(\frac{1}{q_\infty K_g}\right) + \frac{C_1}{q_\infty} \qquad (6)$$

Where C_1 (mg/l) is the equilibrium concentration of the adsorbate, q (mg/l) is the amount of adsorbate adsorbed per unit mass of adsorbent, q_∞ (mg/g) and K_g(L/mg) are Langmuir constants which are related to adsorption capacity and rate of adsorption, respectively.

The essential characteristics of Langmuir equation can be expressed in terms of dimensionless separation factor, R_L, defined as equation 7 (Obiora-Okafo*et al*, 2014):

$$R_L = \frac{1}{1 + K_g C_o} \qquad (7)$$

Where C_o (mg/l) is the initial concentration of the adsorbent.$R_L$values have the following interpretations: adsorption is unfavourable if $R_L > 1$, linear if $R_L = 1$, favourable if $0 < R_L < 1$ and irreversible if $R_L=0$. The values of R_L for the various systems studied are presented in table 1. The value of the R_L obtained shows that the adsorption is favourable; that is,$0 < R_L < 1$. This is in agreement with the result obtained by Obiora-Okafo, *et al*, (2014).

The Freundlich model is based on sorption on a heterogeneous surface of varied affinities (Obiora-Okafo*et al*, 2014). The linear form of this modelis given as equation 8:

$$\mathrm{Log}q_\infty = \mathrm{Log}K_f + \frac{1}{n}\mathrm{Log}C_1 \qquad (8)$$

Where $K_f(\mathrm{mg/g}(\frac{L}{mg})^{\frac{1}{n}})$ is a Freundlich equation constant that indicates the adsorption capacity of the adsorbent (Obiora-Okafo*et al*, 2014). n is also a Freundlich equation constant that represents the parameter characterizing Quasi-Gaussian energetic heterogeneity of the adsorption surface (Roop and Meenakshi, 2005b). In general, $n > 1$ suggests that adsorbate is favourably adsorbed on the adsorbent. The higher the n value the stronger the adsorption intensity (Obiora-Okafo*et al*, 2014).

On the condition that the heat of adsorption decreases linearly with surface coverage (Roop and Meenakshi, 2005c), the Langmuir isotherm reduces to the Temkin adsorption isotherm, This isotherm assumes that the fall in the heat of adsorption is linear rather than logarithmic, as implied in the Freundlich equation. The Temkin equation is expressed in linear form as equation 9:

$$\frac{2.303RT}{b_T}LogA + \frac{2.303RT}{b_T}LogC_1 \qquad (9)$$

Where$^{RT}/_{b_T}$ = B (J/mol), which is the Temkin constant related to the heat of sorption, whereas A(l/g)is the equilibrium binding constant corresponding to the maximum binding energy(Obiora-Okafo*et al*, 2014). R (8.314J/mol k) is the universal gas constant and T (K) is the absolute solution temperature.

The Henry model is expressed as equation 10:

$$q = K^{'}C_1 \qquad (10)$$

Where $K^{'}$ is the Henry adsorption constant.

Among these isotherms, Henry isotherm (fig. 4) gave the best fit with $R^2$of 1. This is followed by the Langmuir isotherm (fig. 1) which gave $R^2$of 0.9978. Fig. 2 depicts the Freundlich isotherm for this system. The non-linearity of this linear form of the Freundlich isotherm validates the fact that Freundlich isotherm does not work very well (Sánchez-Martin and Beltrán-Heredia, 2010). The Temkin isotherm plot of fig. 3

shows that Tempkin isotherm does not work very well for this system. Table 3.5 presents the summary of these isotherms.

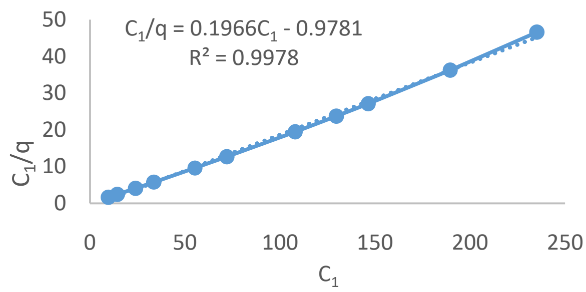


Fig 3.6: Langmuir Isotherm for PLC-treated PIE

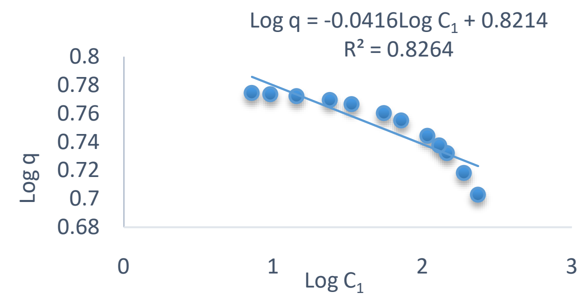


Fig. 3.7 : Freundlich Isotherm for PLC-treated PIE

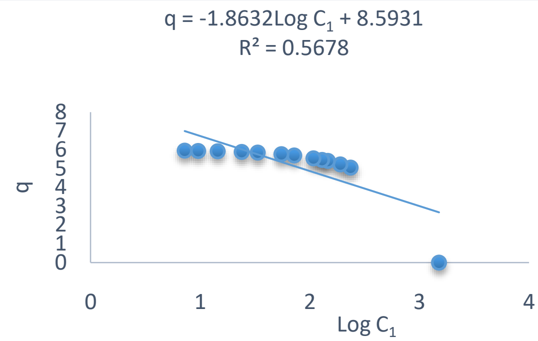


Fig 3.8: Temkin Isotherm for PLC-treated PIE

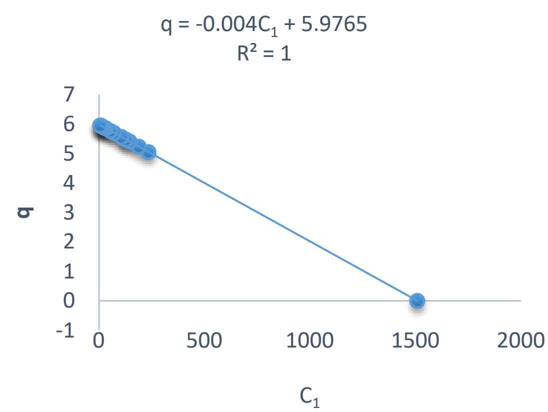


Fig 3.9: Henry Isotherm for PLC-treated PIE

The kinetic parameters obtained from the isotherms are presented in table 3.4.

Table3.4:Adsorption isotherms kinetic parameters for adsorption of PLC onto colloidal particles

Kinetic parameters												
Langmuir Isotherm				Freundlich Isotherm			Tempkin Isotherm				Henry Isotherm	
q_∞	k_g	R_L	R^2	k_f	n	R^2	A	B	b_T	R^2	$K^{'}$	R^2
5.086	0.201	0.019	0.998	-0.085	-24.039	0.826	2.44E-05	-0.809	-3113.77	0.568	-0.004	1

Table 3.5: Summary of the Mono-Layer Adsorption Isotherms

S/N	Name	Fundamental equation of isotherm	Linear form	Straight line plot for
1	Langmuir	$q = q_\infty \frac{K_g C_1}{1 + K_g C_1}$	$\frac{C_1}{q} = \left(\frac{1}{q_\infty K_g}\right) + \frac{C_1}{q_\infty}$	$\frac{C_1}{q}$ vs C_1
2	Freundlich	$q = k_f . C_1^{n_f}$	$\mathrm{Log}q = \mathrm{Log}K_f + \frac{1}{n}\mathrm{Log}C_1$	Logq vs $\mathrm{Log}C_1$
3	Temkin	$q = \frac{2.303RT}{b_T} Log(AC_1)$	$q = \frac{2.303RT}{b_T} LogA + \frac{2.303RT}{b_T} LogC_1$	q vs $\log C_1$
4	Henry	$q = K^{'} C_1$	$q = K^{'} C_1$	$q = C_1$

IV. CONCLUSIONS

On the strength of the findings of this work, it is affirmed that PLC is an effective adsorbate applicable in adsorptive treatment of paint industry effluent prior to disposal. The data obtained are well represented by Henry and Langmuir isotherms.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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