Biosorption of Reactive Red Dye from Textile Wastewater by Mango and Papaya Seeds

Tariku Ayala, Nedumaran Balasubramanian

Abstract— Presence of dyes in the aquatic systems has become a serious environmental problem. Focusing the environmental issue and economic point of view, the use of low cost and eco-friendly biosorbents has been investigated as an alternative to the current expensive method of removing the textile dyes from wastewater. This study ensures the feasibility of using Mango seeds (MS), Papaya seeds (PS), for removal of synthetic dye from aqueous solution. The biosorption characteristics of Reactive Red (RR) dye onto MS and PS have been studied. Full factorial design of experiment using Design Expert is used. Batch biosorption experiments were carried out for the biosorption of the dye from aqueous solution onto the PS and MS at constant room temperature 25°C and agitation speed 150rpm. The effects of different parameters solution pH, biosorbent dose and initial concentration of dye were studied and its significance tested with ANOVA. UV spectrophotometric technique was used for measuring of dye concentration before and after biosorption. Experimental results revealed that high biosorption took place at acidic pH. The effective solution pH, biosorbent dose, and contact time on the dye removal efficiency of the biosorbents were found to be 4.0, 15gL^{-1}, and 180min, respectively for the biosorption studies. At these experimental conditions the dye removal efficiency of 69% and 99.94% were achieved by mango seeds and papaya seeds respectively. The experimental results have been fitted well by the Langmuir isotherm model with the higher correlation coefficients of R^2 = 0.975 and 0.9985 for PS and MS respectively. Thus, indicating to the applicability of monolayer coverage of the dye on the surface of the biosorbents and they were found to be 2.028 and 3.754 mg/g of biosorbent and it was found that the biosorption kinetics follows pseudo second order model for PS and pseudo first order for MS.

Index Terms— Reactive Red dye, Mango seed, Papaya seed, Biosorption, isotherm, Kinetics models

———————————— ◆ ————————————

1 INTRODUCTION

WATER is vital to almost all life forms in existence and it is believed that, even, the first life started in water. Although more than 70% of earth surface is covered with water, majority of it is not suitable to sustain human life and only limited potable water resources are available. The extensive use of chemicals for various purposes in day-to-day life and the growing industrialization led to contamination of our existing natural resources by the release of diverse organic and inorganic pollutants into water system. Nowadays, the public has become more sensitive towards the protection of the environment and general awareness has now increased about the potential adverse effects of industrial effluents contaminate with various pollutants, including dyes on the environment [1], [2]. Dyes are mainly engaged in the textile, food, pharmaceutical, cosmetic, plastic, photographic and paper industries. Unfortunately, most of dyes escape into conventional wastewater treatment process. Although dye effluent escape into water bodies from various sources, Textile plant is classified as the most polluting industrial sector. It is noteworthy that some dyes are highly toxic, mutagenic and carcinogenic also decrease light penetration and photosynthetic activity, causing oxygen deficiency and limiting down-stream beneficial uses such as recreation, drinking water and irrigation [3].

Environmental legislation obliges industries to eliminate colour from their dye containing effluents before disposal into the water.

There are more than 100,000 commercially available dyes exist with over 7×10^5 MT of dyestuff produced annually. Among the chemical classes of dyes, azo dyes are more versatile and account to more than half of the annual dye production. It is estimated that 2% of dyes produced annually are discharged in effluent from different manufacturing operations. While in the textile industry, it is estimated that 10% to15% of the dye used during the manufacturing of textile products is released into the environment worldwide annually [4]. Reactive azo dyes extensively used in textile dying process in Ethiopia, have good water solubility and easily hydrolyzed into insoluble forms. Textile dyes wastewater, in particular characterized by intense color, high level of biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and highly fluctuating pH. Among all these color is the first wastewater contaminant to be recognized, since a very small amount of dye concentration in water even (<1ppm) are highly visible that affects aesthetic merit, transparency and water- gas solubility. Therefore, it is desirable to remove dyes from colored effluents for safe discharge in receiving water bodies to keep the environment sustainable [5].

The chemical structure classification describes dyes as being azo, nitro, nitroso, polymetine, annuleneds, aryl carbonium, sulphur, carbonyl, or one of many other types of dyes in this classification [6]. Reactive (Fig. 1), direct, disperse, acid and basic class of dyes are classified based on ther application and chemical type [7].

————————————————

- **Tariku Ayala,** Department of Chemical Engineering, School of Mechanical, Chemical and Materials Engineering, Adama Science and Technology University, P.O.Box 1888, Adama, Ethiopia. tarikuayala08@gmail.com +251910170090
- **Nedumaran Balasubramanian,** Associate Professor, Department of Chemical Engineering, School of Mechanical, Chemical and Materials Engineering, Adama Science and Technology University, Adama, Ethiopia. nedumaran_b@yahoo.co.in
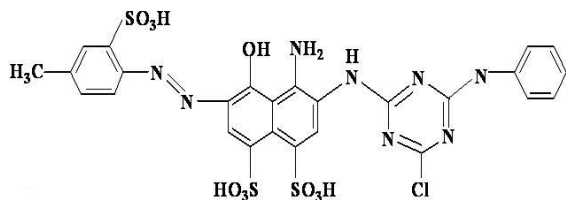
Fig.1. Structure of Reactive Red Dye

The principal chemical classes of reactive dyes are azo (-N=N-) (including metallized azo), triphendixazine, phthalocyanine, formazan, and anthraquinone, vinyl sulphone etc. High purity reactive dyes are used in the ink-jet printing of textiles, especially cotton [8]. These dyes have a variety of complex organic compounds and toxic substances with unknown environmental behaviour such as aromatic amines (C_6H_5-NH_2), which are suspected to have carcinogenic effect, phenyl (C_6H_5-CH_2) and naphthyl (NO_2-OH). The resistance of these organic compounds to decomposition due to the complex chemical structure of synthetic pigments in dyes results in a difficult to treat wastewater which is also resistant to degradation by biological methods [8], [9].

Biological treatment requires a large land area and is constrained by sensitivity toward diurnal variation as well as toxicity of some chemicals, and less flexibility in design and operation [10], [11]. In particular, due to their xenobiotic nature, azo dyes are not totally degraded.

There is also the possibility that a secondary pollution problem because of excessive chemical use [12]. Recently, other emerging techniques, known as advanced oxidation processes, which are based on the generation of very powerful oxidizing agents such as hydroxyl radicals, have been applied with success for pollutant degradation. Different physical methods are also widely used, such as membrane-filtration processes (nanofiltration, reverse osmosis, electrodialysis).

Various conventional methods of removing these dyes include coagulation [13] and flocculation, oxidation or ozonation and membrane filtration. However, these methods are not widely used due to their high cost, non-feasibility and economic disadvantage [14].

Treatment methods for removal dyes from textile wastewater can be divided into physical, chemical, and biological methods. Among physicochemical methods mainly adsorption process is one of the most effective and economically feasible methods for dye removal from textile wastewater due to simple, flexible design and easy operation [15]. Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals and other contaminant ions including dyes from even very dilute aqueous solutions.

Biosorption is a metabolically passive process, means it does not require energy, and the amount of contaminants a sorbent can remove is dependent on kinetic equilibrium and the composition of the sorbents cellular surface [16]. With the increasing demand of textile industries in Ethiopia discharge of colored effluents is also increased. Ayka Addis textile manufacturing industry is one of the largest economic sectors in Ethiopia, which discharge large quantity of effluent wastewater into the nearby water bodies. These colored effluents give undesirable perspective to the water streams where as some dyes may be threat to the environment due to their metabolites pose toxic, carcinogenic and mutagenic effects. Azo dye can be degraded to more dangerous substances under anaerobic conditions. The contamination of wastewater by dyes poses two types of problems. The first is due to its visibility and colour whereas the second problem is due to chemical effects of the dissolved toxic organics and dye molecules [17]. It is a well-known equilibrium separation process and an effective method for water de-contamination application. The process is influenced by many physicochemical factors such as dye/sorbent interaction, sorbent surface area, particle size, temperature, pH, and contact time [12]. Most of the solid adsorbents of great industrial applications possess a complex porous structure that consists of pores of different sizes and shapes. In terms of the adsorption science, total porosity is usually classified into three groups. Virtually every solid surface has the capacity to adsorb sorbate but the effectiveness of these solids in the wastewater treatment process is a function of its structure, degree of polarity, porosity and specific area. Some of the adsorbents include activated carbon, [15], [18], [19], [20], organic polymers and silica-based compounds [7], Palygorskite clay [21], Chitosan [22], fly ash [23], activated corchorus olitorius-L leaves [24], powdered marble [25], calcined bones [26] have been used for removal of dyes.

A sorbent can be considered low-cost if it requires little processing, is abundant in nature or is a by-product or waste material from another industry. Certain waste products from industrial and agricultural operations, natural materials and biosorbents represent potentially economical alternative sorbents [27], like Yellow Passion Fruit Waste [28], tamarid fruit shell [29], orange peel waste [30], date seed and palm tree waste [31]. Many of them have been tested and proposed for dye removal. Agricultural wastes usually have high molecular weight due to the presence of lignin, cellulose and hemicelluloses components [32].

About 3.7 Mha land area is used for production of mango trees world wide. Mango occupies fifth place after citrus, banana, grape and apple. 10% of mango is produced from African countries which includ 12,799 ha land producing 69,748.39 MT of mango in Ethiopia [33]. 11.22 MMT of papaya being produced world wide during 2010 and it stands at 3rd place in Ethiopia. About 40,435.06 MT of papaya produced across 3109.52 ha land during 2014-15 [34]. These two fruits have high nutritional value which is containing vitamin and that can prevents disease. Papaya and Mango seeds are used for removal of heavy metals [35], [36], [37], [38] and dyes like victazol orange [39], reactive red M-2BE [40], malachite green [41], and large pollutants [42]. Mango (*Mangifera indica*) is a perennial crop of the family Anacardiacea. It is grown practically all over tropical and sub-tropical regions of the world. The fruits are oval or kidney shaped with smooth, leathery skin and the colour ranges from light or dark green to clear yellow when ripe. Mango fruit contain amino acids, carbohydrates, fatty acids, minerals, organic acids, proteins, vitamins

(A and C) and dietary fiber [43]. The papaya, (*Carica papaya Linn*) is commonly known for its food and nutritional values throughout the world. The genus Carica papaya Linn is the most widely cultivated and best known species of the four genera that belongs to a small family Caricaceae [17]. The seeds are numerous, small, black, round and covered with gelatinous aril. Mango and Papaya seeds can be low cost biosorbents based on economic reasons and availability of agricultural waste.

The objective of this study is to remove Reactive Read dye from the textile industry effluent. Biosorption capacity of mango and papaya seeds for RR dye removal and kinetics and biosorption isotherm behaviors of the prepared biosorbent with the effects of parameters pH, concentration of reactive red dyes and biosorbent dosage on the adsorption efficiency were investigated in batch adsorption techniques and the optimum conditions for these parameters were evaluated [4].

2 MATERIALS AND METHODS

2.1 Materials

Dried and crushed mango seeds and crushed papaya seeds (collected from Local juice shop) were used for the study. Reactive Red dye (RR) powder was received from Ayka Addis Textile Industry, Ethiopia. Laboratory grade HCl, and NaOH, H3PO4, distilled water, grinder, 250ml plastic bottles, Orbital shaker (GFL 3074 Model), Sieves (250-500 μm), pH meter (JENWAY-3505), magnetic stirrer, UV spectrophotometer (Jenway 6300 England), electronic weighing balance, oven (Memmer), vacuum desiccators, glove and whatman filter paper available at laboratory were utilized.

2.2 Preparation of Biosorbents

Mango seeds (MS) and Papaya seeds (PS) were roughly washed with distilled water to remove the surface adhered particles and water soluble material. Then they were sliced, spread on trays and oven dried at 105-110°C for 24 hours. The dried slice was grounded and sieved to particle size of 1-2 mm. Then the powdered MS and PS were soaked in 3M phosphoric acid (H₃PO₄) at 45°C for 5h then washed with distilled water until the pH almost neutral, dried and stored in vacuum desiccators until required. The dried MS and PS were sieved at average geometrical mean size of 250μm-500μm so that all powdered adsorbent were at uniform size for the batch adsorption study. In order to find optimum operating conditions, the experiment is done for different times by changing parameters.

2.3 Characterization of Biosorbents

Ash content was determined using the ASTM D 2017 (1998). The moisture contents were determined in accordance with the ASTM D 1037 (1991). The volatile matter was determined according to ISO 562/1974. The carbon content was calculated sum up the moisture content; volatile matter content and ash content reduced from 100.

2.4 FTIR Analysis

Fourier Transmission Infrared Spectroscopy (FTIR) technique has been used for the prediction of functional group present in the given sample of organic compounds by the absorption of low energy light i.e. ultraviolet light of each wavelength. Absorption spectrum of the compounds which is a unique reflection of the molecular arrangement of the compound in the sample has been obtained. FTIR spectrum is a signature graph which contains percent transmittance along y-axis and frequency or wavelength along x-axis. The peak between a particular frequencies i.e. gap or band, type of the functional group present can be predicted with the help of available standard tables [44]. For the case of MS and PS, FTIR spectrum shows the change in properties of the surface of biosorbent on addition of RR dye.

2.5 Preparation of Stock Solutions

A stock solution of the RR dye, 1000 ppm was prepared by mixing 1g of the dye powder in 1L container and filling with distilled water up to 1L. The aqueous solutions of varies concentrations of RR dye was prepared by dilution from its stock solution. Azo type anionic RR dye has molecular weight 795.96 and has wavelength of 540 nm for maximum absorbance.

3 EXPERIMENTAL METHODS

3.1 Full Factorial Experimental Design

As the number of variables and levels are significantly less, full factorial design of experiment as given in Table 1 has been selected for its advantages over the other methods such as central composite design, Box-Behnken design [41], [45]. All possible combination of three factor levels was tested for its non-linear behavior [46] and the effects of individual factors assessed the effect of change of variables for dye removal efficiency E(%) as defined in (1) where C_o and C_e refers to initial and equilibrium concentration (mgL^{-1})

$$E(\%) = \frac{(Co-Ce)}{Co} \times 100 \tag{1}$$

TABLE 1
FULL FACTORIAL DESIGN EXPERIMENTAL METHODS

Independent factor	Factor levels
Dye concentration	3 (20, 50, 80mgL^{-1})
Dose of MS and PS	3 (5, 15, 25gL^{-1})
pH	3 (4, 6, 8)
Number of Run	27
Replicate: two times	2*27=54
Dependent/response variable	E

3.2 Batch Adsorption experiments

Equilibrium time is the time at which maximum amount of dye adsorbed by the biosorbent, denoted by tₑ. Two measuring flasks of 400 ml volume were taken. The 200 ml of 20 ppm concentration dye solution was placed in each flask and was put on the orbital shaker. The 3 g of MS and PS was added to one of the two flasks. This was the sample flask and the other served as the blank flask. These were shaken at constant speed. Samples were taken out from both flasks at regular intervals of time, centrifuged and absorbance of the supernatant was recorded for each sample. The concentration of dye in solution decreased at high rate at the start of the experiment

and then got steady. The time at which concentration became constant was taken as equilibrium time t_e(min).

The batch adsorption experiments were conducted in a series of conical flask of 250 mL capacity. In all the experiments, solution volume was 200 mL and the mixture of solution and the adsorbent was agitated by using rotary incubator shaker at 150 rpm, for desired time at room temperature. Initial pH of the solution was measured by adding 0.1M HCl or 0.1M NaOH before adding the biosorbent.

At the end of each adsorption experiment, the samples were withdrawn from the shaker at predetermined time, and the solution was filtered using whatman filter paper to remove biosorbent and concentration of the residual dye was measured using UV-visible spectrophotometer at 540 nm. All experiments were performed in duplicate and the average value was used in the data analysis. Textile dye effluents have variety of chemical composition and their binding interactions with adsorbent depend on the chemical structure of a particular dye, the specific chemistry and morphology of the biosorbent surface and properties of the dye solution or wastewater [47]. Therefore, the effect of different experimental parameters upon adsorption efficiency of MS and PS was studied as follows.

The effect of pH on percentage removal of RR dye by the adsorbent was studied at initial concentration of dye solution 20mgL^{-1} for pH values at 4, 6, and 8 using 3 g of PS in 200mL of the solution at room temperature. The test solutions were shaken at agitation speed 150 rpm for 180 min in order to determine the effective pH for the dye removal. The effect of adsorbent dose was studied by adding different amount (1, 3 and 5) g of the biosorbent at 20mgL^{-1} in 200 mL of the dye solutions at constant pH for 180 min contact time. To determine the biosorbent dose at which maximum dye removal efficiency achieved.

The effect of initial dye concentrations was carried out by shaking 200 mL the dye solutions of desired concentrations (20, 50 and 80 mgL^{-1}) with constant amount 3 g of the adsorbent. All the samples were adjusted to constant pH 4.0 prior to adding the adsorbent. The test solution samples were then, withdrawn from the shaker at pre-determined time intervals of (60, 120, 180 and 240 min).

The amount of RR dye adsorbed onto the unit weight of PS and MS at equilibrium and at any time t were calculated based on mass balance equations given by the following equations (2) and (3), respectively.

$$q_e = \frac{V(C_o - C_e)}{m} \qquad (2)$$

$$q_t = \frac{V(C_o - C_t)}{m} \qquad (3)$$

Where, q_e and q_t are the amounts of dye adsorbed (mgg^{-1}) at the equilibrium and at any time t (min), respectively. C_o, C_e and C_t are the concentration of the dye in the solution (mgL^{-1}) at the initial, equilibrium and at time t, respectively; V is the volume of the solution (L); and m is the dry weight of PS and MS (g).

4 RESULTS AND DISCUSSION

4.1 Characterization of MS and PS

The proximate analysis of powdered MS and PS were summarized in Table 2.

TABLE 2
PROXIMATE ANALYSIS RESULTS OF MS AND PS

Contents	Value (%)	
	MS	PS
Ash content	3.51	6.27
Moisture content	6.1	2.73
Volatile matter	6.12	3.33
Carbon content	84.27	87.67
Bulk density	545kgm^{-3}	364kgm^{-3}

4.2 FTIR analysis of biosorbent

The FTIR spectrum of unloaded biosorbent is shown in figure 2 and 3. The obtained spectrum is similar with the spectra of other lignocellulosic materials obtained between wave lengths (4000 - 400 cm^{-1}) [48], [49]. The spectrum show the peaks typical for hydroxyl groups at the range 3420-3450cm^{-1}, confirming the presence of the free hydroxyl groups of carboxylic acids, alcohols and phenols on the biosorbent surface. Also, this band corresponds to the O–H stretching vibrations of cellulose, pectin, absorbed water and lignin. The band that appeared at 2923cm^{-1} could be attributed to –CH stretch. The carboxyl ions (COO-) give rise to two bands: a stretching band at 1628cm^{-1} and a weaker asymmetrical band at 1452cm^{-1} for the MS and PS. The peaks 1263cm^{-1} can be assigned to correspond to bending vibrations of O - C - H, C - C - H and C - O - H groups. The band at 1061cm^{-1} of the C-O stretching vibration is evident. The absorption peaks at 1165cm^{-1} and 1053cm^{-1} are indicative of P-O stretching vibrations.

As seen from the figure 2, the biosorbent (PS) surface before biosorption using FTIR analysis the bending shape is greater than the surface of biosorbent after biosorption, this shows the active site of biosorbent surface is exactly occupied by dye ions the bending shape of biosorbent surface decrease because the functional groups were removed from the biosorbent surface and also it can be observed there is a functional group in the structure of biosorbent.

Analysis of FTIR before and after biosorption by MS (Fig 3.) show a number of adsorption peaks, suggesting complex properties of the biosorbents. It could be seen that there is a much weaker characteristic stretching vibration absorption band at 1730.78cm^{-1} is assigned to carbonyl group C=O, this may be due to acetyl, uronic ester groups of hemicelluloses or the ester linkage of carboxylic groups [31]. The dominant peak at 3708.54 cm^{-1} attributed to O-H stretching vibrations in hydroxyl groups. A shift in hydroxide group from 3936cm^{-1} to 3708.54cm^{-1}, alcohol group from 1825cm^{-1} to 1682.80cm^{-1} while a shift in carbonate group from 1472cm^{-1} to 1304cm^{-1} was observed shifting in peaks was observed after adsorption and this shows that all the functional groups are completely involved in biosorption process.
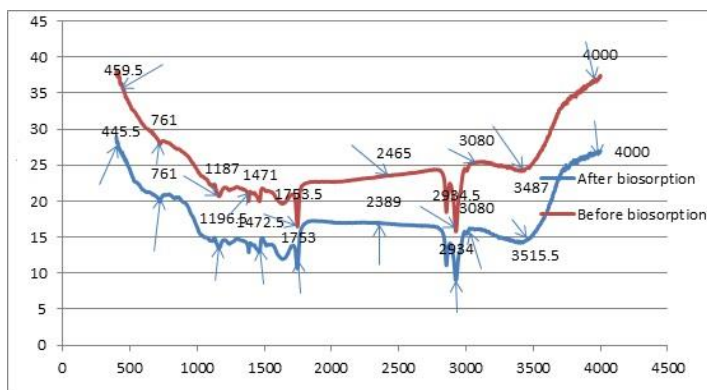
Fig. 2. FTIR Spectrum % transmittance vs. Wave length (nm) of biosorbent surface PS before and after biosorption
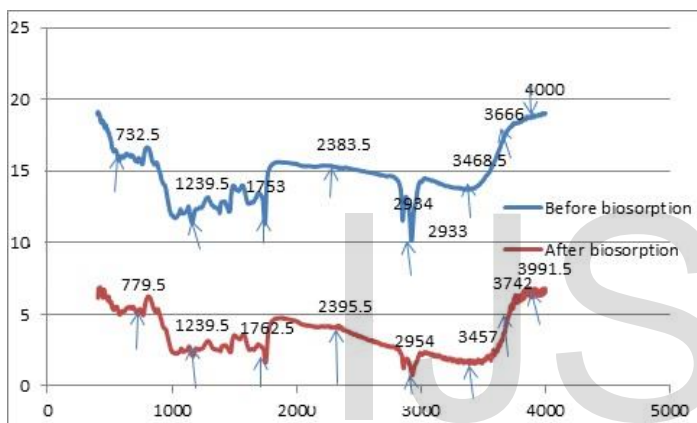

Fig. 3. FTIR Spectrum of %Transmitance vs. Wave length (nm) of biosorbent MS comparison before and after biosorption.

Also, this band corresponds to the O–H stretching vibrations of cellulose, pectin, absorbed water and lignin. The band that appeared at 2923cm^{-1} could be attributed to –CH stretch. The carboxyl ions (COO-) give rise to two bands: at stretching band at 1628cm^{-1} and a weaker asymmetrical band at 1452cm^{-1} for the MS & PS. The peaks at 1263cm^{-1} can be assigned to correspond to bending vibrations of O - C - H, C - C - H and C - O - H groups [28]. The band at 1061 cm^{-1} of the C-O stretching vibration is evident. The absorption peaks at 1165 and 1053 cm^{-1} are indicative of P-O stretching vibrations.

4.3 Effect of Initial Solution pH on Biosorption

The pH value of the solution will determine the surface charge of the adsorbent which will affect the interaction between the adsorbate and biosorbent. Experiments were carried out with the pH values of up to 8 due to the fact that dye molecules and biosorbent deterioration appeared at higher pH values. The pH of the system exerts profound influence on the uptake of adsorbate molecules most probably due to its influence on the surface properties of the biosorbent and ionization or dissociation of the adsorbate molecule [19]. Figure 4 and 5, shows the

variations in the percentage removal of the RR dye from aqueous solution at different initial solution pH by PS and MS respectively. It is evident that, percentage removal of the dye significantly decreased from 99.94 % to 56.45 % as the solution initial pH increases from 4 to 8. This indicates that acidic pH is favourable for the dye adsorption by the biosorbent.

This is due to the fact that low pH value leads to an increase in H^{+} ion concentration in the system and the surface of PS may acquires positive charge by absorbing H^{+} ions. As the PS is positively charged at low pH value, a strong electrostatic attraction appears between the negatively charged anionic dye and the PS leads to maximum adsorption of the dye. On the other hand, increase in pH value led to increase in the number of negatively charged sites on the adsorbent [28]. The negatively charged surface on PS doesn't favor the sorption of anionic dyes due to electrostatic repulsion appears between the negatively charged anionic dye molecules and the adsorbent surface leads to minimum adsorption of the dye. Based on the result high dye removal efficiency of 99.94% was achieved at pH 4.0. From the figure 4 and 5, when compare the removal efficiency of PS and MS, in both cases as the dye concentration and pH increases percentage removal efficiency decreases but PS is more effective than MS as it is observed from the graph.

4.4 Effect of Initial Dye Concentration on Biosorption

The effect of initial dye concentration (20 - 80 mgL[-1]) on the adsorption of dye by PS and MS was studied as shown in figure 6 and 7 respectively. The removal of dye decreases with an increase in dye concentration. This may be due to the saturation of the available active sites on the powdered PS, beyond a certain concentration of dye. For a fixed amount of powdered PS at a low dye concentration, biosorption of the dye proceeds faster due to less number of dye ions in the solution. As initial concentration increased, the number of dye ions in the solution increases against a fixed amount of available active sites, thus leading to a decrease in dye removal with an increase in initial dye concentration [9], [50]. It is evident that using fixed amount of 15gL[-1] of the PS and MS bio sorbent, the percent removal of the dye was decreased from 99.94% to 58.72% and 69% to 47% respectively when the initial concentration of the dye increased from 20mgL[-1] to 80mgL[-1] at equilibrium contact time 180 min. This can be explained by the fact that the initial concentration of dye had a restricted effect on dye removal capacity; simultaneously the biosorbent media had a limited number of active sites, which would have become saturated at a certain concentration. This had lead to the increase in the number of dye molecules competing for the available functions groups on the surface of adsorbent material (PS) [17]. In addition, it was found that the biosorption capacity of the biosorbent increased from 1.78mgg[-1] to 8.96mgg[-1] as the dye concentration increased from 20mgL[-1] to 80mgL[-1]. This effect might be attributed to an increase in the driving force of the ionic gradient with the increase in the initial dye concentration. This explained the increase in sorption capacity due to increase in the amount of interactions between dye anions and the biosorbent.
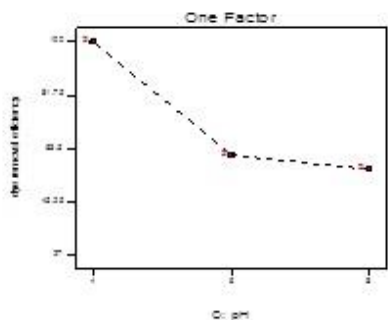
Fig 4: Effect of initial solution pH at dye concentration of 20mgL^{-1} and dosage of 15 gL^{-1} on percentage removal of dye onto PS (as generated by Design-Expert Software)
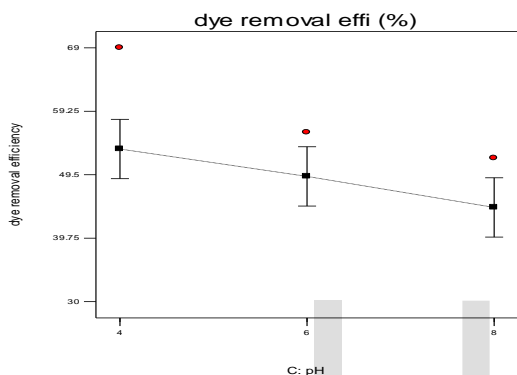

Fig. 5. Effect of initial solution pH at 20mgL^{-1} on percentage removal of dye onto MS
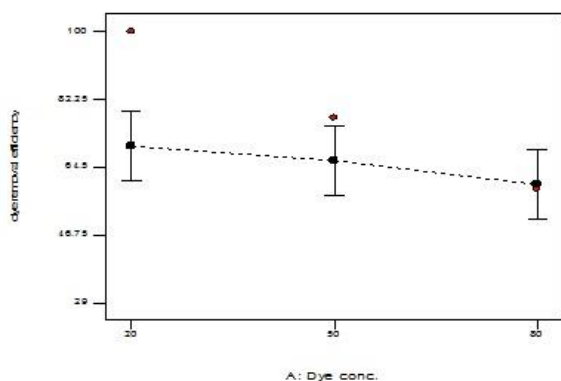

Fig. 6. Effect of initial dye concentration at pH 4.0 on percentage removal onto PS

4.5 Effect of Biosorbent Dose on Biosorption

Study concerning the biosorbent dosage gives information about the effectiveness of a biosorbent and the ability of a dye to be sorbed with minimum dosage. The effect of the PS used for RR dye biosorption was tested in the range of 1 gL^{-1} to 25gL^{-1}. The sorbent dose influence was studied for 20mgL^{-1} RR dye concentration, at 180min and 4.0 respectively. The results

shown in figure 8 revealed that the removal of dye increased with an increase in PS dosage. Figure 9 for MS shows similar trend.
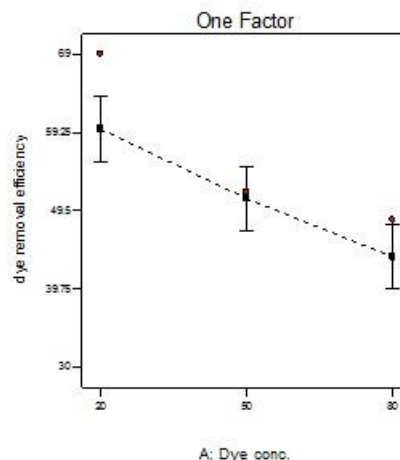

Fig. 7. Effect of initial dye concentration on percentage removal onto MS

This may have been as a result of the increase in number of active sites on the adsorbent surface with increasing dosage amount, resulting in an increase in dye removal. Beyond a certain amount, 20mgL^{-1}, the percentage removal of dye leveled off at 99.94%. This may be due to a reduction in the concentration gradient of the dye molecules [50]. Usually the removal capacity, increases with increasing of the biosorbent doses, since the amount of pollutant adsorbed per mass unit decreases with increasing of adsorbent dose in the liquid-solid system [51].

When the PS dose is increased from 5gL^{-1} to 25gL^{-1}, the biosorption capacity decreased from 1.174mgg^{-1} to 0.640mgg^{-1}. The increase in the extent of removal of RR dye is found to be relatively low after a dose of 15gL^{-1}, which has been considered as optimum dose of PS for further experiments. The decrease in amount of RR dye adsorbed with increasing PS mass may be considered the effect of overlapping or partial aggregation of biosorption on the biosorbent surface, resulting in a decrease in total surface area available for dye molecules. From figures 8 and 9 (PS and MS) it is concluded that as the pH increases dye removal decreases and as biosorbents increases dye removal efficiency increases because the type of dye is basic dye with higher pH due to the presence of –OH ions in the solutions easily removed in a lower pH so their interactions exactly affects biosorption.

4.6 Effect of Contact Time on Biosorption

The relation between removal of the RR dye and reaction time were studied to see the rate of dye removal by PS and MS using fixed amount of 15gL^{-1} at different initial concentration. The results indicate that the rate of dye removal increased with increasing contact time and get saturated at 180min. For the first 120 minutes, the percentage removal of the dye by the biosorbent is rapid and thereafter it proceeds at a slower rate and finally attains saturation at different contact time for different initial concentration. The higher concentration solution of dyes employed, the longer equilibrium time was needed.

The rate of removal of the dye is higher in the beginning due to the large surface area of the bio sorbent available for the biosorption of dye ions.
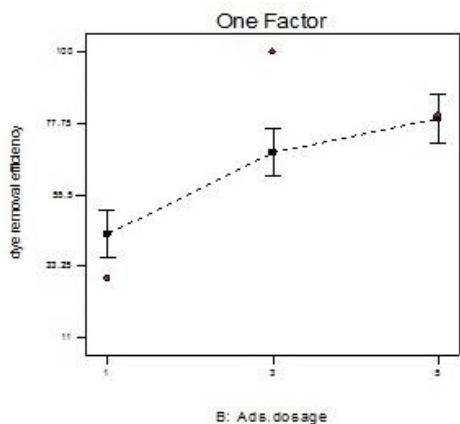

Fig. 8. Effect of PS dose on biosorption at 20mgL^{-1}, 180min and pH 4.0
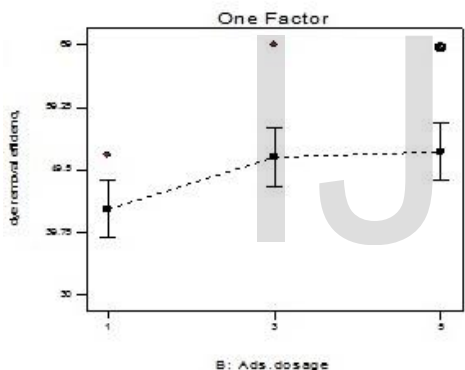

Fig. 9. Effect of MS dose on percentage removal of dye at 20mgL^{-1}, 180min and 4.0

After a certain period, only a very low increase in the dye uptake was observed because there are few active sites on the surface of the bio sorbent. From the contact time studied, it was revealed that 180 min of agitation time is sufficient to reach equilibrium when 80 mgL^{-1} of dyes concentration was employed. Therefore, equilibrium time of 180 min was selected for the adsorption of RR dye for further studies. The percentage removal efficiency of PS is higher than MS.

ANOVA Analysis

Analysis of variance (ANOVA) of factorial model indicating classical sum of squares of type II is given in Table 3 for PS. The Model F-value of 4.82 implies the model is significant. There is only a 0.34% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B and C are significant model terms. Values greater than 0.1000 indi-

cate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The ANOVA analyses of the selected parameters are significant. So the model is applicable for biosorption of reactive red dye onto PS.

From ANOVA Table 4 for MS, the Model F-value of 12.46 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C is significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy). Model reduction may improve your model. The ANOVA analyses of the selected parameters are significant. So the model is applicable for biosorption of reactive red dye onto MS.

TABLE 3
ANOVA ANALYSIS OF PS

Source	Sum of Squares	df	Mean square	F-value	p-value Prob>F
Model	5343.48	6	890.58	4.82	0.0034
A-Dye conc.	462.36	2	231.18	1.25	0.003074
B-Ads.dosage	4447.98	2	2223.99	12.04	0.0004
C-pH	433.15	2	216.57	1.17	0.003299
Residual	3693.01	20			
Cor Total	9036.49	26			

TABLE 4
ANOVA ANALYSIS OF MS

Source	Sum of Squares	df	Mean square	F-value	p-value Prob>F
Model	1948.26	6	324.71	12.6	<0.0001
A-Dye conc.	1145.50	2	572.75	21.97	< 0.0001
B-Ads.dosage	439.68	2	219.84	8.43	0.0022
C-pH	363.08	2	181.54	6.96	0.0051
Residual	521.33	20			
Cor Total	2469.59	26			

4.8 Biosorption Isotherms

The relationship between the amount of a substance adsorbed at constant temperature and its concentration in the equilibrium solution is called the biosorption isotherm. Biosorption isotherm is important from both a theoretical and a practical point of view. In order to optimize the design of the biosorption system to remove the dye, it is important to establish the most appropriate correlations of the equilibrium data of each system [47]. Equilibrium isotherm equations are used to describe the experimental biosorption data.

These data provide information about the capacity of the biosorbent or the amount required for removing a unit mass of

pollutant under the system concentrations [52]. The most widely accepted surface biosorption models for single-solute systems are the Langmuir [53] and Freundlich isotherm [54]. These two most common isotherm models have been tested to analyze equilibrium data of solute between biosorbent and solution. The parameters obtained from these different models provide important information on the biosorption mechanisms and the surface properties and affinities of the biosorbent. Linear regression is frequently used to determine the best-fitting isotherm.

The Langmuir isotherm is valid for adsorption of a solute from a liquid solution as monolayer biosorption on a surface containing a finite number of identical sites. Therefore, the Langmuir isotherm model was chosen for estimation of the maximum biosorption capacity corresponding to complete mono-layer coverage on PS and MS surface. The experimental data are analyzed according to the linear form of the Langmuir isotherm equation. The linear Langmuir isotherm as in (5), plots of C_e/q_e vs C_e suggest the applicability of the Langmuir isotherms for the removal of RR dye onto PS and MS.

$$\frac{C_e}{q_e} = \frac{1}{q_m k_L} + \frac{C_e}{q_m} \qquad (5)$$

The values of q_m (maximum uptake) and k_L (Langmuir parameter) of linear expression of Langmuir biosorption isotherm was calculated from the slopes and intercept of the linear plot are shown in figure 10. The predicted results of these models for the removal of RR dye by PS and MS are represented in Table 5. Results show strong positive evidence on the biosorption of the dye onto the biosorbent follows the Langmuir isotherm represents the best fit of experimental data than the Fruendlich isotherm of (6).

$$\log C_e = \log k_f + \frac{1}{n} \log C_e \qquad (6)$$

The fact that Langmuir isotherm fits the experimental data very well confirms the monolayer coverage of dye onto PS and MS (q_m= 2.028mgg-1 and 3.754mgg-1) and also the homogeneous distribution of active sites on the biosorbents, since the Langmuir equation assumes that the surface is homogeneous [55].

The essential features of the Langmuir isotherm can be expressed in terms of a dimensionless constant called separation factor R_L, also called equilibrium parameter which is defined by (7). The value of R_L indicates the shape of the isotherms to be either unfavorable ($R_L > 1$), linear ($R_L = 1$), favourable ($0 < R_L < 1$) or irreversible ($R_L = 0$) [56].

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

Where, C_o(mgL-1) is initial dye concentration and k_L(L.mg-1) is the Langmuir constant related to the energy of adsorption [55]. The influence of isotherm shape on whether adsorption is favourable or unfavourable has been considered.
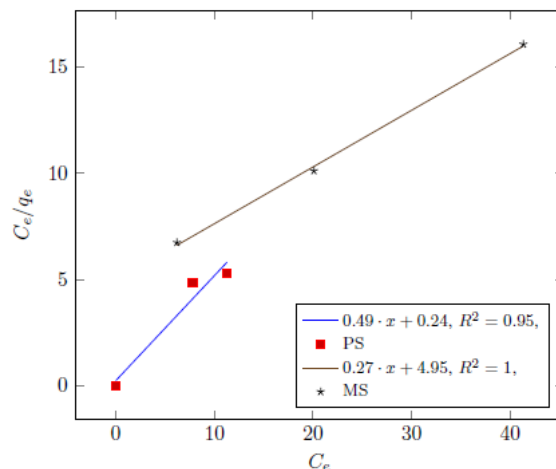

Fig.10. Langmuir isotherm for dye removal biosorption onto PS (C_l/q_e=0.49.C_e+0.24, R^2 = 0.95) and MS (C_l/q_e=0.27.C_e+4.95, R^2 = 1)
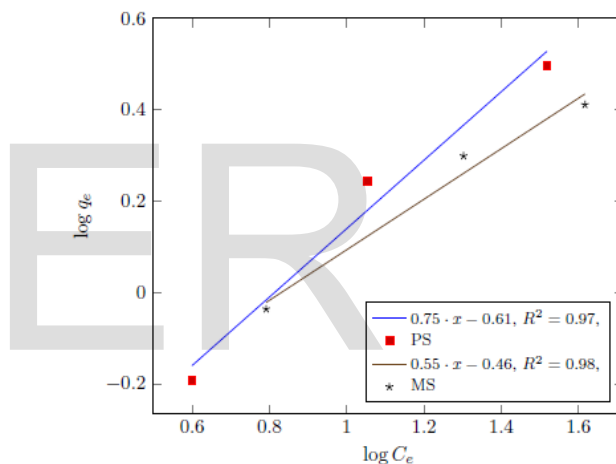

Fig.11. Freundlich isotherm for dye removal biosorption onto PS (log q_t=0.75.log C_e-0.61, R^2=0.97) and MS (log q_t=0.55. log C_e-0.46, R^2=0.98)

TABLE 5
LANGMUIR AND FREUNDLICH ISOTHERM MODEL FOR BIOSORPTION OF DYE ONTO PS AND MS

Bio-sorbent	Langmuir isotherm			Freundlich isotherm		
	q_m (mgg-1)	K_L (L.mg-1)	R^2	K_f	n	R^2
PS	2.028	2.034	0.975	0.280	1.427	0.969
MS	3.754	0.0538	0.999	0.347	1.81	0.980

The calculated value of R_L for PS and MS were (0.024 and 0.482) respectively. So the value of R_L is between 0 and 1 for PS and MS suggesting the isotherm to be favorable at the concentrations studied. While Langmuir isotherm assumes that en-

thalpy of biosorption is independent of the amount adsorbed, the empirical Freundlich equation, based on biosorption on heterogeneous surface, can be derived assuming a logarithmic decrease in the enthalpy of adsorption with the increase in the fraction of occupied sites. Freundlich isotherm plots between logqe versus logCe for the biosorption of RR dye were drawn in figure 11 and the estimated parameters given in Table 5 for PS and MS. The applicability of the Freundlich biosorption isotherm was also analyzed, using the same set of experimental data. It was found that the correlation coefficient values were less than 0.99 at the dye concentrations studied indicating that Freundlich model was not applicable to the present study. The favourable adsorption of this model can be characterized; the magnitude of the exponent n gives an indication on the favourability of biosorption. It is generally stated that values of sorption intensity, n in the range 2 to 10 represent good, 1 to 2 moderately difficult, and less than 1 poor adsorption characteristics [54]. In the present study the value of n (n= 1.43 and 1.81 for PS and MS) is greater than 1, indicating that the biosorption process is favourable.

Freundlich and Langmuir parameters have been presented in the Table 5. It is noticeable that Langmuir model is better fit in the biosorption isotherm model since it has the higher value of correlation coefficient, in comparison with Freundlich model for both PS and MS onto RR dye.

4.9 Kinetics Studies

Kinetic models have been used to investigate the mechanism of sorption and potential rate controlling steps, which is helpful for selecting optimum operating conditions for the full-scale batch process. The kinetic parameters, which are helpful for the prediction of adsorption rate, give important information for designing and modeling the adsorption processes [28]. Thus, the kinetics of RR dye adsorption onto PS and MS were analyzed using pseudo-first-order and pseudo-second-order kinetic models. The pseudo-first-order rate expression based on solid capacity is generally expressed as in (8) [5]:

$$\frac{dq_t}{d_t} = k_1(q_e - q_t) \tag{8}$$

After integration and applying boundary conditions, t = 0 to t and q_t= 0 to q_e; the integrated form of equation becomes (9):

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \tag{9}$$

Where, q_e (mgg^{-1}) is the amount of dye adsorbed at equilibrium, q_t(mgg^{-1}) is the amount of dye adsorbed at time t and k_1(min^{-1}) is the rate constant of pseudo-first-order adsorption. The values of log $(q_e - q_t)$ were linearly correlated with t from which k1 and predicted q_e can be calculated from the slope and intercept of the plot, respectively and the results are presented as in figure 12. Pseudo-second-order kinetic rate equation is expressed as in (10) [57]:
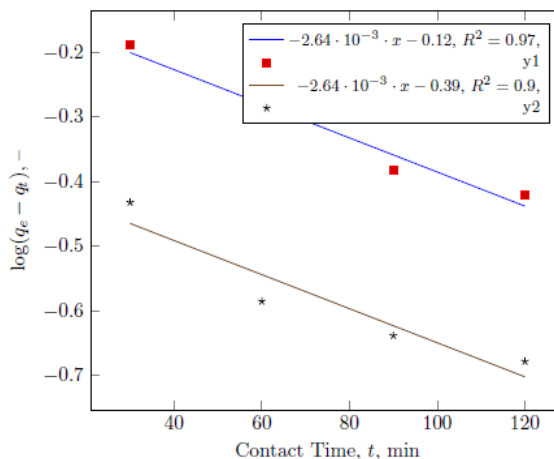

Fig.12. Pseudo First order for biosorption of dye onto PS (log (qe-qt) =-2.64.10-3t-0.12, R²=0.97) and MS (log (qe-qt) =-2.64.10-3t-0.39, R²=0.94)
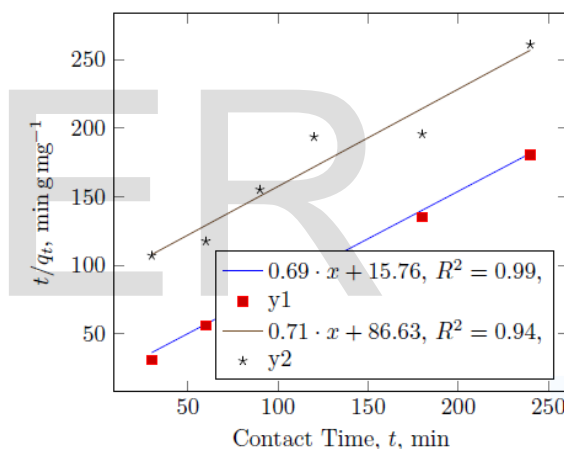

Fig.13. Pseudo second order for biosorption of dye onto PS (t/qt=0.69t+15.76, R²=0.99) and MS (t/qt=0.71t+86.63, R²=0.94).

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{10}$$

Where, k_2 (g.mg^{-1}min^{-1}) is the equilibrium rate constant of pseudo second order adsorption. t/q_t vs t is ploted as shown in figure 13 for MS and PS. From figure 12 and figure 13 it is evident that when compare pseudo first order and pseudo second order by using correlation coefficient (R^2) the pseudo second order is the best model and also q_e estimated is near to q_e experimental from analysis it observed that favourable model is the pseudo second order kinetics. For MS the value of q_e found as 0.88mgg^{-1} for pseudo first order and 1.4mgg^{-1} for pseudo second order and k_1 as 9.99E-03 min^{-1} and k2 as 5.81E-03. As seen the result that are obtained from the experiment the pseudo first order is favourable for biosorption of dye onto MS because the constants and correlation coefficients more applicable in the pseudo first order.

5 CONCLUSIONS

The efficiency of powdered MS and PS biosorbents for the removal of RR dye from aqueous solution had been studied and its effects on different operational parameters namely solution pH, dose of MS and PS, dye concentration and contact time. It was found that maximum biosorption of the dye took place at acidic pH. The effective solution pH, dose of MS and PS, and contact time were found to be 4.0, 15gL^{-1} and 180min, respectively for the biosorption of dye at constant temperature (25°C). Dye removal efficiencies of 69% and 99.94% were achieved for MS and PS respectively. Langmuir isotherm is the best model for biosorption of dye of monolayer coverage of the dye onto the surface of the biosorbent. Biosorption kinetics study shows that the biosorption follows pseudo second order model for PS and pseudo first order is best model for MS. Thus, PS and MS can be used as a low-cost, effective alternative for the removal of textile reactive red dyes from aqueous solution.

6 ACKNOWLEDGMENT

REFERENCES

[1] Sarita Yadav, Tyagi D.K, Yadav O.P., "An Overview of Effluent Treatment for the Removal of Pollutant Dyes", *Asian Journal Research Chem*, vol. 5, no. 1, pp. 01-07, 2012.

[2] Malinauskiene, L., "Contact allergy to textile dyes: clinical and experimental studies on disperse azo dyes", MSc. Thesis, Lund University, Sewden, 2012.

[3] Mohammed Basim Alqaraguuly, "Removal of Textile Dyes (Mexilon Blue, and Methyl Orange) by Date Stones Activated Carbon", *International Journal of Advanced Research in Chemical Science*, vol. 1, no. 1, pp. 48-59, 2014.

[4] Tan L.S., Jain K, and Rozaini C.A., "Adsorption of Textile Dye from Aqueous Solute on Pretreated Mangrove Bark, An Agricultural Waste: Equilibrium and Kinetic Studies", *Journal of applied Sc. In Env. Sanitation*, vol. 5, pp. 283-294, 2010.

[5] N. Koprivanac and H. Kusic, "Hazardous Organic Pollutants in Colored Wastewaters", *New Science Publishers*, New York, 2008.

[6] Stevens, C.B., "Dye Classes: General Structure and Properties in Relation to use. In: The Dyeing of synthetic-polymer and Acetate Fibres", D.M Nunn, ed., The Dyers Company Publications Trust, Bradford, West Yorkshire, England, pp. 2-53, 1979.

[7] Koyuncu, M., "Removal of Maxilon Red GRL from Aqueous Solutions by Adsorption onto Silica", *Oriental Journal of Chemistry*, vol. 25, pp. 35-40, 2009.

[8] Velmurugan and Dhinakaran, "Dye Removal from Aqueous Solution Using Low Cost Adsorbent", *International Journal of Environmental Sciences*, vol. 1, pp. 1492-1503, 2011.

[9] Khataee, A.R., Ayazloo, M, and Pourhassan, M, "Biological Decolorization of C.I. Basic Green 4 Solution by *Chlorella sp.*: Effect of Operational Parameters", *Chinese J. Appl. Env. Biol.*, vol. 15, pp. 110-114, 2009.

[10] Kassa, R.M., "Biological Organic Matter and Nutrient Removal from Textile Wastewater using Anaerobic-Aerobic Bioprocess", MSc. thesis, Addis Ababa University, Ethiopia, 2007.

[11] Abbas F.S., "Dye Removal from Wastewater using Agricultural Waste", *Advances in Environmental Biology*, vol. 6, pp. 1019-1026, 2013.

[12] K.R. Ramakrishna and T. Viraraghavan, "Dye Removal using Low Cost Adsorbents", *Water Sci. Technol.*, vol. 36, pp. 189-196, 1997.

[13] J.B. Veeramalini, K. Saravanakumar and D. D Joshua Amarnathamarnath, "Removal of reactive yellow dye from aqueous solutions by using natural coagulant (Moringa oleifera), International Journal of Environmental Science and Technology, vol. 1, pp. 56-62, 2012.

[14] S. Babel and T.A. Kurniawan, "Low-cost Adsorbents for Heavy Metals Uptake from Contaminated Water: A Review", *J. Hazardous Mater.*, vol. B97, pp. 219–243, 2003

[15] Beyene, H.D., "The Potential of Dyes Removal from Textile Wastewater by using Different Treatment Technology", *International Journal of Environmental Monitoring and Analysis*, vol. 2, pp. 347-353, 2014.

[16] F. Kargi and S. Ozmihci, "Bio Sorption Performance of Powdered Activated Sludge for Removal of Different Dyestuffs", *Journal of Microbiology and Technology*, vol. 35, pp. 267–271, 2004.

[17] Krishna KL, Paridhavi M, Jagruti Patel A. "Review on Nutritional, Medicinal and Pharmacological Properties of Papaya (Carica papaya Linn)", *Nat prod Rad*, vol. 7, no. 4, pp. 364-373, 2008.

[18] Muhi Mohammed, F., "Modelling and Design of Water Treatment Processes Using Adsorption and Electrochemical Regeneration", Msc.thesis, University of Manchester, 2011.

[19] Santhi, M., and Smitha, "Removal of Methyl Red from Aqueous Solution by Activated Carbon Prepared from the Annona Squmosa Seed by Adsorption", *Chemical Engineering Research Bulletin*, vol. 14, pp. 11-18, 2010.

[20] K.A. Nevine, "Removal of Reactive Dye from Aqueous Solutions by Adsorption on to Activated Carbon Prepared from Sugarcane Bagasse Pith", *Desalination*, vol. 223, no. 1-3, pp. 152-161, 2008.

[21] Dakhil Nassir Taha, I.S., "Adsorptive Removal of Dye from Industrial Effluents Using Natural Iraqi Palygorskite Clay as Low-cost Adsorbent", *Journal of Asian Scientific Research*, vol. 3, pp. 945-955, 2013.

[22] Hasan, M.B., "Adsorption of Reactive Azo Dyes on Chitosan/Oil-palm Ash", MSc., Thesis, University of Sains, Malaysia, 2008.

[23] Maria, P.U., "Novel Materials Based on Fly Ash for Advanced Industrial Wastewaters Treatment", MSc. Thesis, University of Sains, Malaysia, 2014.

[24] Subasri, A.M., S. Arivoli, V. Marimuthu and Mani, N. "Equilibrium, Kinetic and Thermodynamic Study on Rhodamine-B Removal from Aqueous Solution Using Activated Corchorus Olitorius-L Leaves", *International Journal of Plant, Animal and Environmental Sciences*, vol. 5, no.1, pp. 101-110, 2015.

[25] Aseel M. and Kadim Aljebori, A.N., "Effect of Different Parameters on the Adsorption of Textile *Dye Maxilon Blue GRL* from Aqueous Solution by using White Marble", *Journal of Chemical Engineering*, vol. 8, pp. 142-151, 2010.

[26] Mohammadine El Haddad, R.S., "Removal of Two Textile Dyes from Aqueous Solutions onto Calcined Bones", *Journal of the Association of Arab Universities for Basic and Applied Sciences*, vol. 1, pp. 51–59, 2013.

[27] Eng-Cheong Khoo, S.-T.O., "Utilization of Agricultural Waste as a Bio-sorbent for the Removal of Dyes from Aqueous solution", 1st World Sustainability Forum, November 1-9, 2011.

[28] F.A. Pavan, E.C. Lima, S.L.P. Dias, and A.C. Mazzocato, "Methylene Blue Biosorption from Aqueous Solutions by Yellow Passion Fruit Waste", *J. Hazard. Mater.*, vol. 150, pp. 703-712, 2008.

[29] M.C. Somasekhara Reddy, "Removal of Direct Dye from Aqueous Solutions with an Adsorbent Made from Tamarind Fruit Shell, an Agricultural Solid Waste", *Journal of scientific & industrial research*, vol. 65, pp. 443-446, 2006.

[30] Serin, F.G., and Selen., "Adsorption Study on Orange Peel: Removal of Ni(II) ions from aqueous solution", *Afrian Journal of Biotechnology*, vol. 11, pp. 1250-1258, 2012.

[31] Z. Belala, M. Jeguirim, M. Belhachemi, F. Addoun and G. Trouve, "Biosorption of Basic Dye from Aqueous Solutions by Date Stones and Palm-Trees Waste: Kinetic Equilibrium and Thermodynamic Studies", *Desalination*, vol. 271, pp. 80-87, 2011.

[32] Salleh, 2011, Pal R.K., "Ripening and Rheological Properties of Mango as Influenced by Ethereal and Carbide", *J. Food Sci. Technol.*, vol. 35, pp. 358-360, 1988.

[33] Tewodros B. Neguse, Fredah K. R. Wangala, Wassu M. Ali, Willis O. Owino, Githiri S. Mwangi, "Mango (Mangigera indica L.) Production Practices and Constraints in Majour Production Regions of Ethiopia", *African Journal of Agricultural Research*, vol. 14, no. 4, pp 185-196, 2019.

[34] Ayele L, Etissa E, Dagnew A, Assefa W, Kebede G, Girma M, Firde K,

Ayalew M. "Development of Hermaphrodite Papaya (Carica papaya L.) Varieties for Production in Ethiopia", *Acad. Res. J. Agri. Sci. Res.*, vol. 5, no. 7, pp 561-569, 2017.

[35] Igwegbe W.E., Okoro, B.C., Osuagwu, J.C., "Use of Carica Papaya as a Biosorbent for Removal of Heavy Metals in Wastewater", *International Journal of Environmental and Biological Engineering*, vol. 9, no. 12, pp 1410-1414, 2015.

[36] Samiksha V. Ashtikar, Amruta D. Parkhi, "Adsorption of Copper from aqueous Solution Using Mango Seed Powder", *International Journal of Engineering Research and applications*, vol. 4, no. 4, pp 75-77, 2014.

[37] Dimple C Parekh, Jignesh B Patel, Padmaja Sudhakar and V.J. Koshy, "Removal of Trace Metals with Mango Sees Powder", *Indian Journal of Chemical Technology*, vol. 9, pp 540-542, 2002.

[38] Tiyasha Kanjilal, Swaminathan Babu, Kalpana Biswas, Chiranjib Bhattacharjee and Siddhartha Datta, "Application of Mango Seed Instrements as Biosorbent in Lead Removal from Industrial Effluent", *Desalination and Water treatment*, vol. 56, no. 4, pp 984-996, 2015.

[39] Wagner S. Alencar, Elie Acayanka, Eder C. Lima, Betina Royer, Felipe E. de Souza, Jeronimo L.A., "Mangifera Indica (mango) Seeds as a Biosorbent for Removal of Victazol Orange 3R Dye from Aqueous Solution and Study of Biosorption Mechanism", *Chemical Engineering Journal*, vol. 209, pp 577-588, 2012.

[40] F.M. Machado, C.P. Bergmann, T.H.M. Fernandes, E.C. Lima, B. Royer, T. Calvete, S.B. Fagan, "Adsorption of Reactive Red M-2BE Dye from Water Solutions by Multi-wallede Carbon Nanotubes and Activated Carbon", *Journal of Hazardous materials*, vol. 192, pp 1122-1131, 2011.

[41] Adriana S. Franca, Leandro S. Oliveira, Silvanio A. Saldanha, Pedro I.A. Santos and Samantha S. Salum, "Malachite Green Adsorption by Mango (mangifera india L.) Seed Husks: Kinetic, Equilibrium and Thermodynamic Studies", *Desalination and water treatment*, vol. 19, no. 1-3, pp. 241-248, 2010.

[42] Edson Luiz Feletto, Caroline Trevison Weber, Daniel Assumpcao Bertuol and Marico Antonio Mazutti, "Application of Papaya Seeds as a Macro/ Mesoporous Biosorbent for the Removal of Large Pollutant Molecule from Aqueous Solution: Equilibrium, Kinetic and Mechanism Studies", *Separation Science and Technology*, vol. 48, no. 12, pp. 1-8, 2013.

[43] Pal R. K, "Ripening and Rheological Properties of Mango as Influenced by Ethereal and Carbide", *J. of Food Science and Technology*, vol. 35, pp. 358-360, 1998.

[44] Silverstein R.M. and Webster F.X., "Spectrometric Identification of Organic Compounds", John Wiley and Sons, Inc, New York, 6th edition, 2004.

[45] Rakić Tijana, Kasagić-Vujanović Irena, Jovanović Marko, Jančić-Stojanović Biljana, Ivanović Darko, "Comparison of Full Factorial Design, Central Composite Design, and Box-Behnken Design in Chromatographic Method Development for the Determination of Fluconazole and Its Impurities", *Analytical Letters*, vol 47, no. 8, pp 1334-1347, 2014.

[46] Montgomery D.C., "Design and Analysis of Experiments", John Wiley and Sons, Inc, New York, 5th edition, 2005.

[47] Asgher, M., "Utilization of Citrus Waste Biomass for Sorption of Reactive Dyes from Aqueous Solutions", *International Journal of Environmental Science*, vol. 3, pp. 179-191, 2011.

[48] B. Nedumaran, "Removal of Heavy Metals from Aqueous Waste Using Fresh Water Macro Algae", PhD Thesis, Anna University Chennai, India, 2008.

[49] V. Jaikumar, K. Sathish Kumar, and D. Gnana Prakash, "Biosorption of Acid Dyes Using Spent Brewery Grains: Characterization and Modeling", *Int. J. Appl. Sci. Engi.*, vol. 7, pp. 115-125, 2009.

[50] Khataee, A.R., Dehghan, G., Ebadi, A., Zaraei, M., and Pouhassan, M., "Biological Treatment of a Dye Solution by Macroalgae Char asp.: Effect of operational parameters, Intermediates Identification and Artificial Neural Network Modelling", *Bioresource Technology*, vol. 101, pp 2252-2258, 2010.

[51] C. Smaranda, "Study Concerning the Behavior of Some Organic Pollutants in the Environment, PhD Thesis, Gh. Asachi Technical University of Iasi, Romania, 2011.

[52] Seeds and Sepehr, B., "Removal of Orange 7 Dye from Wastewater Used by Natural Adsorbent of Moringa Oleifera", *American Journal of Environmental Engineering*, vol. 1, pp. 1-9, 2011.

[53] Langmuir I., "The Constitution and Fundamental Properties of Solids and Liquids", *Journal of American Chemical Society*, vol. 38, pp. 2221–2295, 1916.

[54] Freundlich H.M.F., "Over the Adsorption in Solution", *Journal of. Phys. Chem.*, vol. 57, pp. 385–470, 1906.

[55] A. Mohammad Ismail, M. Loganathan, and P.A. Gastian Theodar, "Effect of

Bio Adsorbents in Removal of Colour and Toxicity of Textile and Leather Dyes", *Journal of Eco Biotechnology*, vol. 4, no. 1, pp. 1-10, 2012.

[56] Aksu Z., "Application of Biosorption for the Removal of Organic Pollutants: A Review", *Process Biochem., vol.* 40, no. 3-4, pp. 997- 1025, 2005.

[57] B. Nedumaran, "Adsorption Kinetics of Copper (II) ions from Aqueous Solutions: An Alternate Way of Finding Pseudo Second Order Rate Constant", India's Magazene on Environmental Science and Engineering, vol. 6, no. 4 , pp. 29-34, 2006.