Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(11):4682-4694

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Removal of methylene blue dye from waste waters using new bio-sorbents derived from *Annona squamosa and Azadiracta indica* plants

B. Srinivasa Reddy, V. Krishna Veni and K. Ravindhranath*

Department of Engg. Chemistry and Post Graduate Chemistry, Bapatla Engineering College, Bapatla-422101, Guntur Dt., Andhra Pradesh

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ABSTRACT

Leaves, stems and their ashes of Annona Squamosa, and Azdirachta Indica have been probed for their sorption abilities towards Methylene Blue using simulated waste waters. The physicochemical parameters such as pH, time of equilibration and sorbent concentrations are optimized for the maximum removal of Methylene Blue. These sorbents are found to be effective at pH 8. Sorbent dosage and time needed for the maximum removal of Methylene Blue are found to be less for the ashes than with the raw powders of the plant materials Methodologies have been developed for the extraction of good quantities of Methylene Blue dye from waste water samples. Fivefold excess of common anions ions, have not interfered the extraction while Cation like Ca^{2+}, Mg^{2} and Cu^{2+} have shown some interference but Fe^{2+} and Zn^{2+} have synergistically maintained the maximum extraction of the dye. The procedures developed are successfully applied for the samples collected from dyeing industries.

Key Words: Methylene Blue; pollution control; bio-adsorbents; applications.

INTRODUCTION

Synthetic dyes are being extensively used in various industries such as textile, paper and plastics to import ascetic color to the products. The effluents of these industries contain notable amounts of the dyes [1] and the effluents are to be treated for the removal of these synthetic dyes before discharging the effluents into the environment.

If proper treatment methods are not followed, the synthetic dyes ultimately reach to the nearby water bodies and pose threat to the aquatic life as they are non-degradable in nature. The dyes blocks sunlight and curtails some photo-sensitive chemical reactions necessary to aquatic life [2,3]. Further, some of the dyes are toxic even carcinogenic in nature [4].

Due to the harmful effects of dyes, EPA is instructing industries to eliminate dyes from effluents before discharging into the mainstreams.

Effluents containing dyes are difficult to treat because most of these chemicals are not prone to aerobic digestion [5]. There are methods reported in literature for the removal of dyes from effluents or waste waters but these methods suffers from one or the other disadvanges and an universally acceptable method is still eluding the researchers. S.M.Ghoreishi and R. Haghighi [6] studied the chemical catalytic reactions and biological oxidation for the treatment of non-biodegradable dyes in textile effluents. S.Al-Aseh et al [7] studied the removal of methylene blue dye from aqueous solutions using activated and non-activated bentonites. M.A.Khraisheh and M.S. Alg-Houti [8] enhanced dye adsorption characteristics by microemulsion of modified calcined diatomite (E-CD). A.R.Cestari et al [9] probed chitosan beads in the removal of an anionic red dye from aqueous solutions.

Increasing research interest is being envisaged during the recent past, in evolving procedures using bioprocess of microorganisms and bio-adsorbents derived from flora and fauna materials in controlling the polluting ions. These bio-processes along with other chemical processes are proving to be potential alternative to the existing methods of detoxification and for the recovery of toxic and valuable ions from industrial discharges/ polluted waters . These biological approaches have stimulated continuous and expanding research in this field. Fly ash [10], peat [11], unburned carbon [12], oil palm fiber activated carbon [13] and activated charcoal [14] have been explored for their efficiency in removing dyes from wastewater. S.B.Bakaullah et al [15] studied the removal of Methylene Blue from aqueous solution by adsorption on sand. M.Lehocky and A. Mracek, [16] studied the improvement of dye adsorption on synthetic polyester fibers by low temperature plasma pre-treatment. G.Cirini, [17] reviewed the non conventional low cost adsorbents for dyes.

Our research labs are also making efforts in this aspect of pollution control methods and some successful procedures have been developed for some polluting ions and they have been reported to the Literature [18-24].

Methylene blue is an important cationic dye and is used in many textile manufacturers and it releases aromatic amines (e.g., benzidine, methylene) and is a potential carcinogen [4]. The attempt to remove Methylene Blue from waste water are few and far between [7, 15, 25-28]. Bamboo-based activated carbon [25], gypsum [26], treated olive pomace and charcoal [27], water hyacinth [28], have been explored for their sorption abilities towards Methylene Blue dye from waste waters.

In the present work, sorption abilities of thermally activated bio-adsorbents derived from leaves and barks/ stems of some plants have been explored with an object of controlling the concentration of Methylene Blue dye in the waste waters by optimizing various physicochemical parameters such as pH, time of equilibration and sorbet concentrations.

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EXPERIMENTAL SECTION

(A)CHEMICALS: All chemicals used were of analytical grade.

1. **Stock solution of Methylene Blue:** 50 ppm of Methylene blue solution was prepared by dissolving a requisite amount of A.R. grade Methylene Blue dye in double distilled water. It was suitably dilute as per the need.

(B) ADSORBENTS: Powders of leaves, stems and their ashes of various plants were tried for the removal of Methylene Blue from synthetically prepared simulated waters by optimizing various physicochemical parameters viz., pH, concentration of sorbent and time of equilibration. It was observed that the sorbents derived from leaves and stems of Annona Squamosa and Azadiracta Indica have affinity towards the dye.

A: Annona Squamosa ***B: Azadiracta Indica***

Fig No. 1: Plants showing affinity towards Methylene Blue dye

Annona squamosa is a small well-branched shrub that bears edible fruits called sugar-apple; belongs to *Annonaceae family* and grows well in lower altitudes. *Azadirachta indica, or Neem tree,* is an evergreen tree native to Southeast Asia and it belongs to *Meliaceae family*.

The leaves and stems of *Annona Squamosa and Azdirachta Indica* were freshly cut from trees, washed with tap water, then with distilled water and then sun dried. The dried materials were powdered to a fine mesh of size: > 75 microns and activated at 105^{o} C in an oven and then employed in this study. Further these leaves and stems were burnt to ashes and these ashes were also used in this work.

(C)Adsorption experiment:
Batch system of extraction procedure was adopted **(**29-31). Carefully weighted quantities of adsorbents were taken into previously washed 1 lit/500 ml stopper bottles containing 500 ml/250 ml of Methelene Blue solution of predetermined concentrations. The various initial pH values of the suspensions were adjusted with dil HCl or dil NaOH solution using pH meter. The samples were shaken vigorously in mechanical shakers and were allowed to be in equilibrium for the desired time. After the equilibration period, an aliquot of the sample was taken for the determination of Methelene Blue using Spectrophotometric method. The dye has λmax at 661 nm and obeys Beers-Lambers law at low concentrations. The O.D. measurements were made at the said λmax using UV-Visible Spectrophotometer (Systronics make). (12). The obtained O.D value for un-known solution was referred to standard graphs (drawn between O.D and concentration) prepared with known concentrations of Methylene Blue by adopting method of Least Squares.

The sorption characteristics of the said adsorbents were studied with respect to the time of equilibration, pH and sorbent dosage. At a fixed sorbent concentration, the % removal of Methylene Blue from sample waters was studied with respect to time of equilibration at various pH values. The results obtained were presented in the Graph Nos. A: 1-8. To fix the minimum dosage needed for the maximum removal of the Methylene Blue ions for a particular sorbent at optimum pH and equilibration times, extraction studies were made by studying the % of extraction with respect to the sorbent dosage. The results obtained were presented in the Graph Nos. B: 1-2.

(D) **Effect of Other ions (Interfering Ions):**
The interfering ions chosen for study were the common ions present in natural waters viz. Sulphate, Fluoride, Chloride, Nitrate, Phosphate, Carbonate, Calcium (II), Magnesium (II), Copper(II), Zinc(II) and Nickel (II). The synthetic mixtures of Methylene Blue and of the foreign ions were so made that the concentration of the foreign ion was maintained at fivefold excess than the dye concentrations as cited in the Table: 1. 500ml of these solutions were taken in stopper bottles and then correctly weighted optimum quantities of the promising adsorbents (*as decided by the Graph Nos. A and B)* were added. Optimum pH was adjusted with dil. HCl or dil. NaOH using pH meter. The samples were shaken in shaking machines for desired optimum periods and then small portions of the samples were taken out, filtered and analyzed for Methylene Blue concentration. % of extraction was calculated from the data obtained .*The results were presented in the Table: 1.*

(E) Applications of the developed bio-sorbents:
The adoptability of the methodology developed with the new bio-sorbents derived from *Annona Squamosa and Azdirachta Indica plants* in this work for removing the dye, is tried with some real sewage/effluent samples collected from some dyeing industries at Hyderabad and Mangalore. For this purpose, samples were collected from effluents of industries and the samples were analyzed for actual amounts of Methelene blue and samples were fed with known amounts of Methelene blue.

Then these samples were *subjected to extraction for the dye using the bio-sorbents developed in this work at optimum conditions of extraction. The results obtained were presented in the Table 2.*

RESULTS AND DISCUSSION

Leaves, stems/barks and their ashes of Annona Squamosa and Azdirachta Indica have *been found to have affinity towards Methylene Blue. The extractability of Methylene Blue has been studied with* respect to various physicochemical parameters such as *pH, time of equilibration and sorption concentration* and the results obtained are presented in the Graph No.A: 1-12 and Graph No. B: 1-4. The following observations are significant:

1. ***Time of equilibration***: Percent of extractability increases with time for a fixed adsorbent at a fixed pH and after certain duration, the extractability remains constant, i.e. an equilibrium state has been reached. In other words, there will not be any further adsorption after certain time of equilibration time (vide Graph Nos. A: 1-8). As for example, in the case of powders of leaves of *Annona Squamosa, % of extraction of_Methylene Blue has been found to be 45.0% at 0.5 hrs, 65.0% at 1.0 hrs, 75.0% at 1.5 hrs or more at pH :2; 60.0 % at 0.5 hrs, 70.0% at 1.0 hr, 80.0% at 1.5 hrs or more. The same trend is found in other sorbents of our present study.*

2. ***Effect of pH****: % of extraction of Methylene Blue is found to be pH sensitive. Extraction of Methylene Blue* has been found to *be increasing with the increase of pH of the equilibration system* for a fixed adsorbent at optimum time of agitation. As for example in case of powders of *Annona Squamosa leaves* the maximum extractability has been found to be 75% at pH: 2; 80% at pH: 4; 85.0% % at pH: 6; 100% at pHs: 8 after an equilibration period of 1.5 hrs (vide Graph No. A:1). With the *ash of leaves of Annona Squamosa, the* % of extraction has been found to be 80.0% at pH: 2; 85.0% at pH:4; 90.0% at pH:6 and 100% at pH: 8 after an equilibration time of 1.0 hrs (vide Graph No.A:2). Similarly at pHs 2, 4, 6 and 8, the % of extractions has been found to be respectively 60.0%, 65.0%, 75.0% and 100% after 2.0 hrs of agitation time for *stem powders of Annona Squamosa* and 55.0%, 60.0%, 70.0% and 100% after 1.5 hrs of agitation time *for their ashes* (vide Graph No.A:3 and 4).

In the case of Azdirachta Indica, the % of extraction has been found to be 60.0%, 71.0%, 83.0%, 91.0% at pH 2, 4, 6, and 8 respectively with *the leaves powders(vide Graph No.A:5)*; 65.0%, 70.0%, 85.1%, and 100% *with leaves ash (Vide Graph No.A:6)*; 80.1%, 85.2%, 88.3%, and 100% with *the stem powders (vide Graph No.A:7)*; and 85.0%, 90.5%, 95.4% and 100% with *the stem ashes(vide Graph No A:8)*.

3. *The maximum % of extractability* is found to be *100% with the sorbents derived from Annona Squamosa* at optimum pH value of 8 but with varying times of equilibrations: 1.5 hrs for leaves powders, 1.0 hr with the ashes of leaves, 2.0 hrs with stem powers and 1.5 hrs with stem ashes (vide Graph No. A: 1-4)

4. In case of sorbents pertaining to *Azdirachta Indica*, % of extraction has been found to be 91.0% with leaves powder and 100% with the rest of the sorbents at optimum pH of 8 but at different optimum equilibration times namely, 2 hrs for powders of leaves and their ashes, 1.5 hrs with stem powders and 1.0 hr for stem ash (Graph No. A: 5-8).

5. ***Time of equilibration****: In most of the bio- sorbents, the equilibration time* needed for maximum extractability of Methylene Blue has *found* to be less for ashes than with the raw powders of leaves or stems. The equilibration time needed for maximum extraction has been found to be 1.5 hrs for the leaves powders of *Annona Squamosa*, where as it is reduced to 1.0 hr with their ashes. In case of stem powders of *Annano squamosa*, the equilibration time required for the maximum extraction is found to be 2.0 hrs, with its ashes it is decreased to 1.5 hrs. Similar trend is observed in the case of sorbents derived from *Azdirachta Indica* (vide Graph No. A: 1-8).

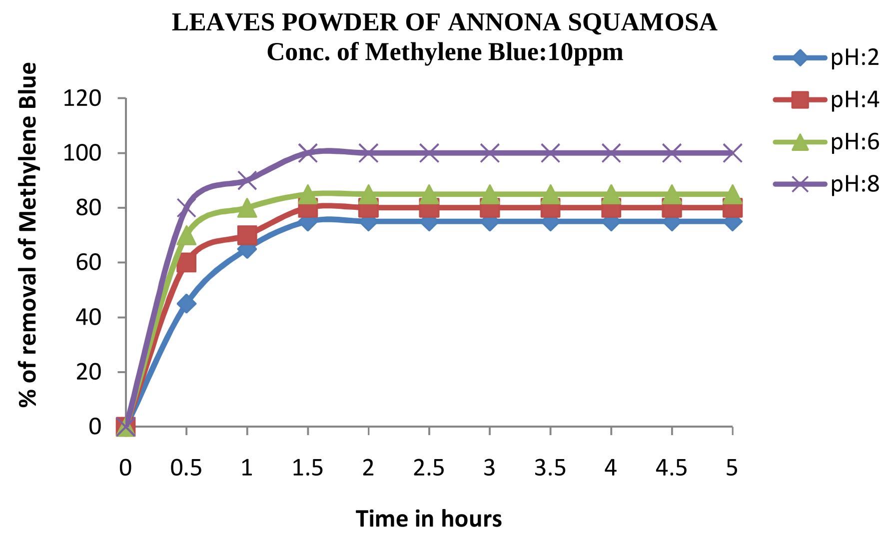
6. ***Sorbent Concentration***: The optimum bio-sorbent concentration required for maximum extractability of the Methylene Blue is found to be in the order : *Leaves powder (2.0 gm/lit) >leaves ash (1.5 g/lit) >stem(1.0 g/lit) > Stem ash (0.5 g/lit)* in the case of the plant *Annona Squamosa* at optimum conditions of pH and equilibration time (vide Graph No. B:1).

In the case of Azdirachta Indica, the optimum concentration of sorbents has been found to be in order: *Leaves powder (1.5 gm/lit) >leaves ash (1.0 g/lit); Stem (1.5 g/lit) > Stem ash (1.0 g/lit)* (Vide Graph No. B: 2).

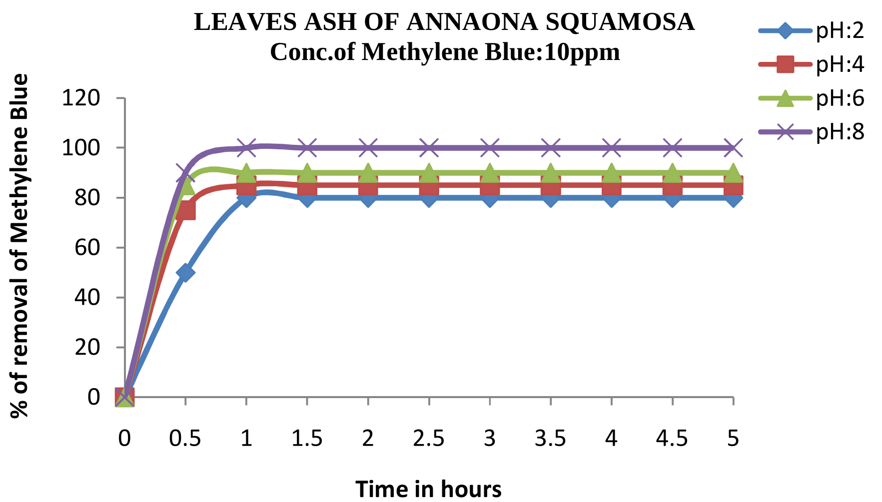
7. ***Interfering Ions***: The extractability of Methylene Blue in presence of *fivefold* excess of common ions found in natural waters, namely, Sulphate, Phosphate, Chloride, Carbonate Fluoride, Calcium, Copper, Iron, Zinc and Magnesium ions, has been studied. The results are presented *in* Table No.1.

- *Anions* envisaged *marginal effect* on the % extractability of Methylene Blue with the sorbents of the present work at the optimum conditions of time of equilibration, pH and sorbent concentration.

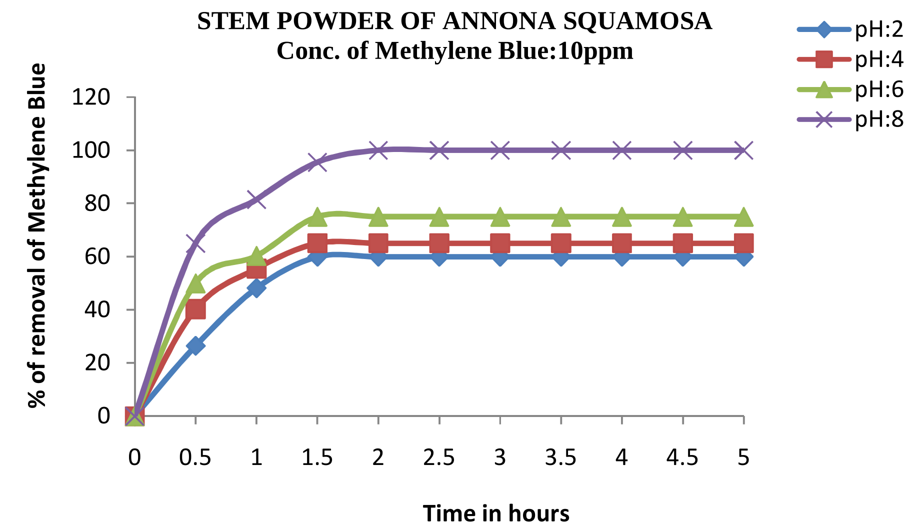
- *Cations like* Fe^{2+} and Zn^{2+} have not interfered and synergistically maintained the maximum% of extraction while cations like Ca^{2+}, Mg^{2+} *and* Cu^{2+} have less interfered the % of extraction of the dye.



Time Vs %removal of Methylene Blue
Graph No: A:1

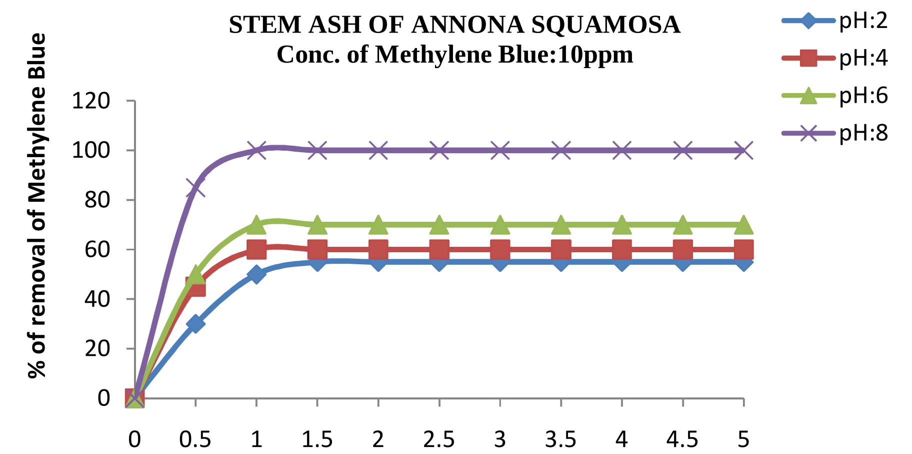


Time Vs % removal of Methylene Blue
Graph No: A:2



Time Vs %removal of Methylene Blue

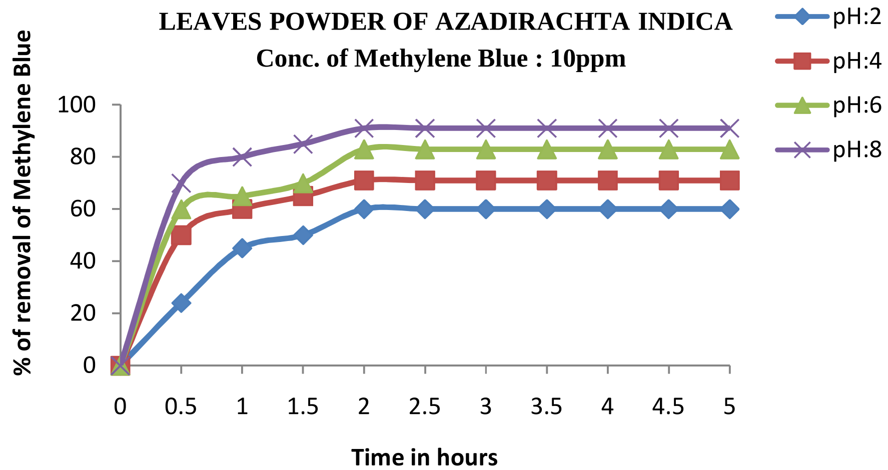
Graph No: A: 3



Time in hours

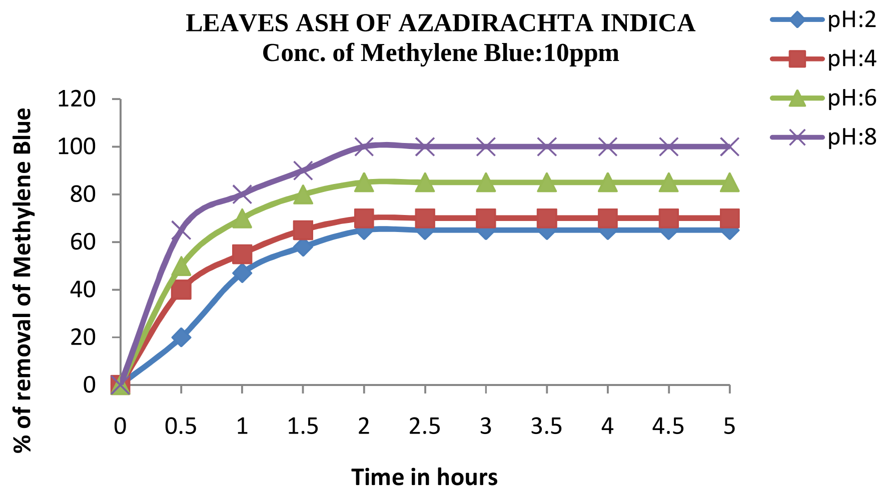
Time Vs % removal of Methylene Blue

Graph No: A:4



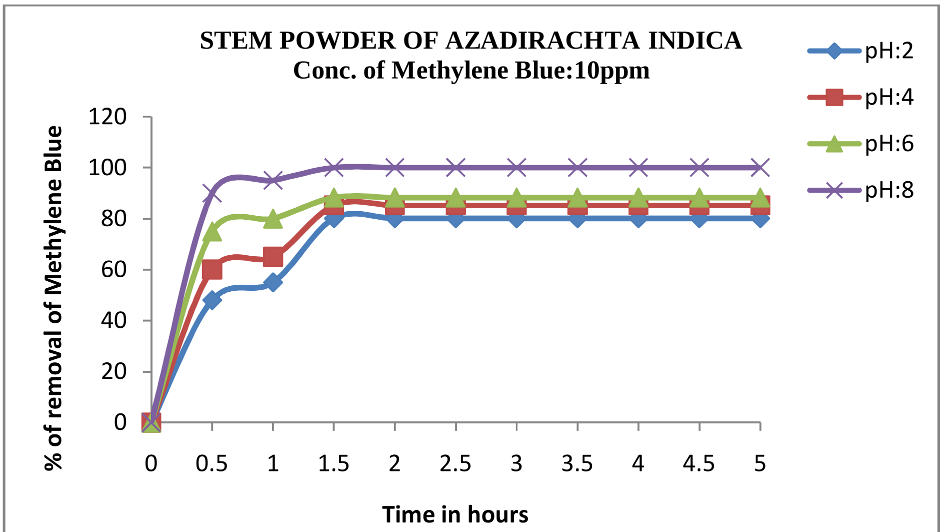
Time Vs % removal of Methylene Blue

Graph No : A-5

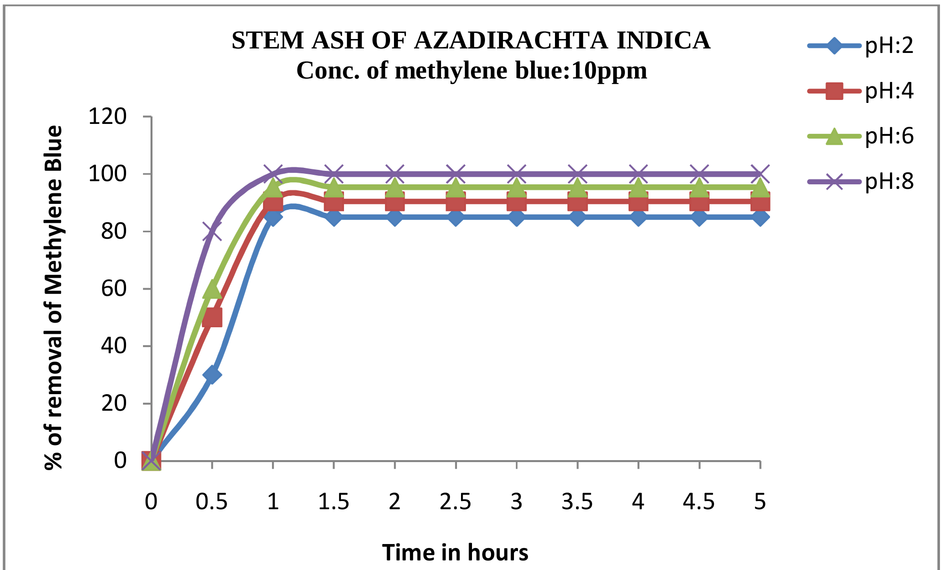


Time Vs % removal of Methylene Blue

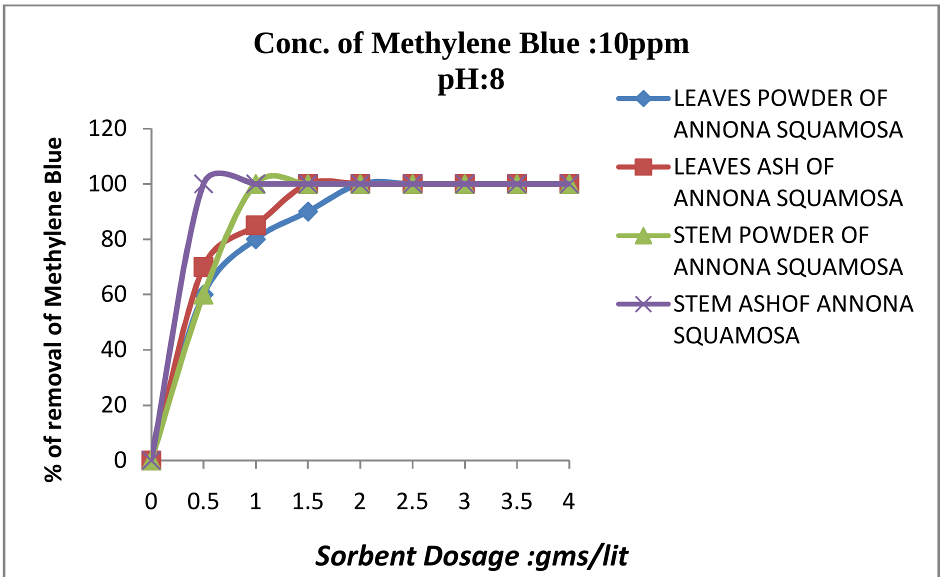
Graph No: A-6



Time Vs % removal of Methylene Blue
Graph No:A-7



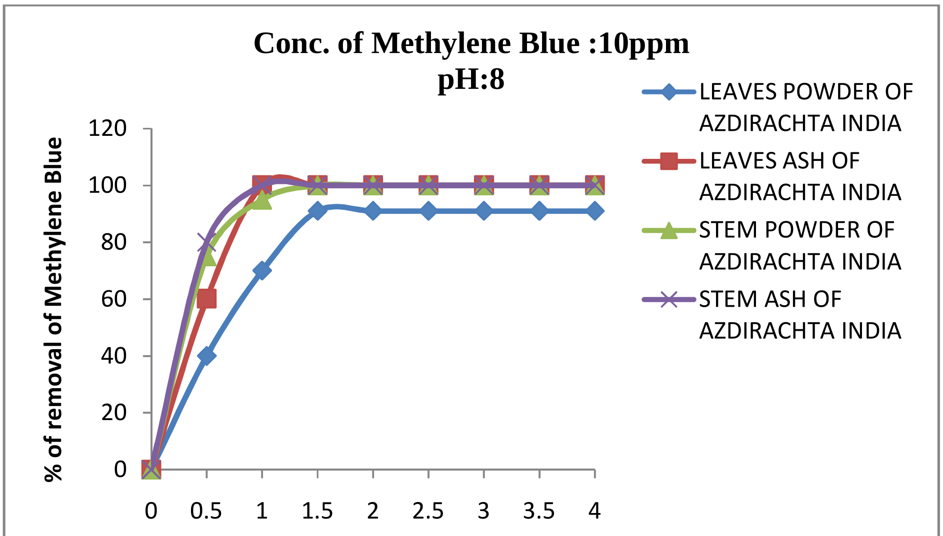
Time Vs % removal of Methylene Blue
Graph No:A-8



Sorbent Dosage Vs % removal of Methylene Blue
Graph No: B-1…

Table No. 1: Effect of interfering Ions on the Extractability of Methylene Blue with different Bio-sorbents

S. No	Adsorbent and its concentration	Maximum Extractability at optimum conditions	*Extractability of Methylene Blue in presence of fivefold excess of (50 ppm) interfering ions at optimum conditions: Conc of Methylene Blue: 10 ppm*									
			SO_4^{2-}	PO_4^{3-}	Cl^-	CO_3^{2-}	F^-	Ca^{2+}	Cu^{2+}	Fe^{2+}	Zn^{2+}	Mg^{2+}
1	Leaves powder of Annona Squamosa	100.0%; pH: 8; 1.5 hrs; sorbent conc.: 2.0 g/l	99.0%	98.5%	100.0%	100. 0%	99.5%	95.2%	93.5%	100.0%	100.0%	94.0%
2	Leaves Ash of Annona Squamosa	100.0%; pH:8;1.0 hr; sorbent conc.:1.5g/lit	100.0%	98.5%	100.0%	100. 0%	99.5%	93.2%	94.2%	100.0%	100.0%	93.5%
3	Stem powders of Annona Squamosa	100.0%; pH:8; 2.0 hrs; Sorbent conc.:1.0 g/lit	100.0%	98.5%	100.0%	100. 0%	99.5%	95.2%	91.5%	100.0%	100.0%	94.6%
4	Stems ash of Annona Squamosa	100.0%;pH:8, 1.5 hrs; Sorbent conc.: 0.5 g/lit	100.0%	98.5%	100.0%	100. 0%	99.5%	93.2%	92.5%	100.0%	98.5%	93.5%
5	Leaves powder of Azdirachta Indica	91.0%; pH:8; 2.0 hrs; Sorbent conc.:1.5 g/lit.	90.0%	89.5%	90.5%	89.8%	91.0%	85.8%	88.3%	90.2%	90.0%	89.0%
6	Leaves Ash of Azdirachta Indica	100.0%; pH:8; 1.5 hrs; Sorbent Conce.: 1.0 g/l	99.5%	98.0%	100.0%	100. 0%	99.5%	93.6%	92.5%	100.0%	99.1%	92.5%
7	Stem powders of Azdirachta Indica	100.0%; pH:8; 1.5 hrs; Sorbent Conce.: 1.5 g/l	98.5%	97.5%	100.0%	100. 0%	99.5%	94.5%	93.8%	100.0%	99.2%	92.8%
8	Stems ash of Azdirachta Indica	100.0%; pH:8; 1.0 hr; Sorbent Conc.: 1.0 g/l	97.8%	98.5%	100.0%	100. 0%	99.5%	93.6%	95.0%	100.0%	98.8%	93.5%



Sorbent Dosage : gms/lit
Sorbent Dosage Vs % removal of Methylene Blue
Graph No: B-2

TABLE NO.2: % Of extractability of methylene blue from different industrial effluents with bio-sorbents developed in this work

Bio-Sorbent	% of Extractability of Methylene Blue				
	Sample 1: Fed with 10.0 ppm of Methlene Blue	**Sample 2 Fed with 15.0 ppm of Methlene Blue**	**Sample 3 Fed with 20.0 ppm of Methlene Blue**	**Sample 4 Fed with 25.0 ppm of Methlene Blue**	**Sample 5 Fed with 30.0 ppm of Methlene Blue**
Leaves powders of Annona Squamosa :at pH:8; Equilibration time: 1.5 hrs and sorbent concentration: 2.0 g/l	96.5%	95.5%	94.0%	93.5%	97.5%
Leaves ashs of Annona Squamosa : at pH:8; Equilibration time: 1.0 hs and sorbent concentration: 1.5 g/l	97.5%	94.5%	93.5%	91.5%	92.5%
Stem powders of Annona Squamosa :at pH:8; Equilibration time: 2.0 hrs and sorbent concentration: 1.0 gms/l	93.5%	92.5%	91.5%	94.3%	92.5%
Stems ashs of Annona Squamosa :at pH:8; Equilibration time: 1.5 hrs and sorbent concentration: 0.5 gm/lit	98.5%	96.5%	97.5%	95.7%	94.5%
Leaves powders of Azdrirachta Indica :at pH:8; Equilibration time: 2.0 hrs and sorbent concentration: 1.5 g/l	87.5%	85.5%	84.5%	83.5%	86.5%
Leaves ash of Azdirachta Indica : at pH:8; Equilibration time:1.5 hrs and sorbent concentration: 1.0 g/l	96.0%	94.5%	92.0%	95.0%	94.6%
Stem powders of Azdirachta Indica :at pH:8; Equilibration time: 1.5 hrs and sorbent concentration: 1.5 gms/l	95.0%	93.5%	93.0%	94.0%	93.0%
Stems ash of Azdirachta Indica :at pH:8; Equilibration time: 1.0 hr and sorbent concentration: 1.0 g/l	94.0%	95.8%	91.0%	93.0%	96.2%

Applications

The Applicability of the methodologies developed in this work have been tested with respects to the real samples of diverse nature, collected from the sewages/effluents of dyeing industries which are fed with varying quantities of the dye Methylene Blue. *The results have been presented in the Table No: 2.*

It is found that the sorbents developed in this work are successful in removing Methylene Blue at optimum conditions of pH, equilibration time and sorbent dosage as cited in the Table No.2. Percentage of removal of Methylene Blue is found to be between: *93.5% to 97.5%* with leaves powder of *Annona squamosa* and *91.5% to 97.5%* with *their ashes*; *91.5% to 94.3 %* with stems powder of *Annano squamosa* and *94.5% to 98.5%* with *their ashes*; *83.5% to 87.5%* with the leaves powders of *Azdirachta Indica* and *92.0% to 96.0%* with *their ashes*; *93.0% to 95.0%* with the *stem powder of Azdirachta Indica* and *81.0% to 96.2%* with *their ashes*.

DISCUSSION

For a thorough understanding of the sorption mechanism of these bio-adsorbents, surface morphological studies using such modern instruments like X-ray Photo Electron Spectroscopy (XPS), Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM) and Energy Dispersive Spectrum (EDS) in addition to the classical elemental chemical analysis before and after the sorption of the Methylene Blue species on the bio-sorbent surface, are needed. It is beyond the aims of this work.

However, a rough nature of sorption characteristic may be accounted from the pH-dependent dissociation of surface functional groups namely –OH or –COOH present in these biomaterials. As pH increases, the dissociation of the functional groups increases imparting negative charge to the surface and so the surfaces thus charged negatively has electrostatic thrust for positively charged ions.

Methylene Blue being a cation in the pH range 2 to 8, is glued to the surface resulting in the increase in the % of extraction.

Ashes are the oxides of some heavy metals containing large amounts of silica. The ashes, contains '-OH' groups and '–O-'. The observed behaviors of extractability as pH varies may be understood in the same lines as described in the case of raw leaves or stem powders. In fact, in the literature it is reported that the silica possesses cation exchanging nature [32-34] and this supports the proposed logic for the observed behavior.

The decrease in the rate of adsorption with the progress in the equilibration time may be due to the more availability of adsorption sites initially and are progressively used up with time due to the formation of adsorbate film on the sites of adsorbent and hence, the decrease in sorption capability of the adsorbent with the increase in the time.

The observations made with respect to the interfering ions are interesting to note. Anions have not interfered with the extraction of the Methylene Blue at the optimum conditions of extraction as cited in the Table 1 but some catios have marginal interference. This is excepted because the negatively charged surface at the high pH values show less affinity towards anions. The cations like Ca^{2+}, Mg^{2} *and* Cu^{2+} *competes with methelene blue for sorption sites on the sorbents resulting inference. But this is not found in the case of Zn and Fe because the Zn ion forms negatively charged zincate at the experimental pH resulting no affinity towards the sorbent while* Fe^{2+} *gets precipitated as ferrous hydroxide at the pH:8 and thus resulting precipitate also adsorbs or traps the methylene dye resulting the complete removal of Methylene Blue dye.*

CONCLUSION

1. Leaves, stems/barks and their ashes of *Annona Squamosa, and Azdirachta Indica* been found to have *strong affinity towards Methylene Blue at pH : 8*.

2. % of removal of *Methylene Blue* is pH sensitive and also depends on sorption concentration and time of equilibration
3. The conditions for the maximum extraction Methylen Blue *at* minimum dosage and equilibration time have been optimized.

4. Sorbent dosage and time needed for the maximum removal of *Methylene Blue* is less for the ashes than with the raw powders of the plant materials.

5. *Fivefold excess* of common anions ions present in natural waters, have not interfered the extractability of Methylene Blue at optimum conditions of pH, equilibration time and sorbent concentration. *Cation like* Ca^{2+}, Mg^{2}

and Cu^{2+} have shown some interference but while Fe^{2+} *and* Zn^{2+} have synergistically maintained the maximum extraction of the dye.

6. We claim 100% of extraction of Methylene Blue dye with the sorbents derived from *Annona Squamosa* plant from the simulated waters at the optimum experimental conditions of extraction. With the sorbents pertaining to *Azdirachta Indica plant* also experimental conditions have been optimized to extract 100% the dye except in the case of leaves powders wherein the maximum % of extraction has been found to be 91.0% from the synthetically prepared simulated water.

7. The developed methodologies in this work are tested with respect to diverse waste water samples collected from dyeing industries. The methods have been found to be remarkably successful in removing the Methylene Blue from industrial effluents as detailed in Table No: 2.

Acknowledgement
The authors thank UGC for financial aid.

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