BIOLOGICAL AND PHYSICOCHEMICAL METHODS

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Abstract: -
Dyes which is a well-known synthetic organic product, is used by a wide number of industries. The colour is one such pollutant generally associated with water and wastewater in various forms. Bhilwara(Raj) is one such example where unabated and indiscriminate discharge of coloured effluents from dyeing and printing has posed a serious pollution problem of multi-facet dimension. The textile printing and dyeing industry of this town, the pride of our state, is adding to our state income by leaps and bounds at one hand and at the same time, it is shame to see the ugly sights of effluent disposal on the other hand.
In the present work physico-chemical method combined with biological process for removing the colour from the effluent (specifically target dye named CONGO RED) of dyeing and printing houses of Bhilwara district.

Introduction:-

Geographic location of Bhilwara: -

Bhilwara is located at 25212 N 74382 E / 25.35N 74.63E / 25.35; 74.63. It has an average elevation of 421-metres (1381-feet). It lies on delhi1-mumbai road, 135 Kms. South of Ajmer and 55 Kms north of Chittorgarh. It is 539 Kms away from the National capital of New Delhi and 267 Kms South west of the state capital of Jaipur. Physiographically the terrain of the city is nearly flat plain except some rolling topography along rocky outcrops towards Harni Hills (475 to 510m.) in south east and towards Pur Hills (500 to 520m.) in south-west and west which are remnants of the degraded Aravalli ranges.

Administratively Bhilwara city is the district head quarter with 12 Tehsils, 11 Panchayat samities and 381 gram panchayats spread over an area of 10455 sq. km with 20.14 lakhs population in 2001.

Problem in general: -

The use of the synthetic chemicals as raw materials or intermediates in textile industries has resulted in the generation of hazardous wastes as process effluents along with solids/hazardous residuals. The environmental fate of these organics depends on whether they undergo rapid or slow biodegradation or tend to the biological recalcitrant and persist in watercourses leading to bioaccumulation through food chain contamination [1].

The environmental impacts of the dyeing and printing industry are associated with its high-water consumption as well as by the colour, variety and amount of chemicals which are released in the wastewater. More than 8000 chemically different types of dyes are being manufactured and the biggest consumer of these dyes are textile industries and these are most serious polluters of our environment as far as colour pollution is concerned.

Solution of the Problem: -

The complete treatment of wastewater is the only acceptable solution looking to the seriousness of the pollution hazards. Although in the developing nation cost of treatment has always been one of the important factors while planning or introducing pollution control measures. It is therefore important that something that needs to be done should balance the imbalance. There are various method are available to remove the colour from dye wastewater some of which are Physical processes, Chemical and physico-chemical processes, and Biological processes etc.

The Present Study*:* -

The dye industry waste, which in high in term of quantity and concentration possess yet more serious problem for human ecologists. The origin of research problem is the same because Bhilwara is a small city with high density of textile industries. More than 20 well known process house running here as BSL, SUZUKI, SANGAM, etc. where the dying operations are highly water intensive.

In the present work physico-chemical method combined with biological process for removing the colour from the effluent of dyeing and printing houses of Bhilwara district. The specific objective of the present work is: -

(a) Choosing the representative dyes according to commercial use in industries
(b) Startup of the biological reaction
(c) Combined adsorption process
(d) Data analysis and interpretations

***Colour Removal By Physico-Chemical Processes*: -**

Various physico-chemical processes like coagulation, flocculation, adsorption, ion exchange, reverse-osmosis and electrochemical coagulation have been investigated for treatment of coloured effluents.

In the present study the method which is used is **ADSORPTION** on different adsorption medium. Adsorption process is a sludge free-operation and produce high quality of treated effluent. Initially, activated carbon, prepared from various materials like coal, charcoal, wood, coke etc. has been used as sorbent and found to be highly efficient. Activated carbon has demonstrated its potential in removing colour from textile dye effluents [2,3,4]. Activated carbon is effective for removal of the organic substances of relative low solubility because it provides a large interfacial area (surface) at which such substances may accumulate. A number of biological adsorbents have also been investigated for the removal of reactive dyes, these include apple pomace, wheat straw comcob and barley husk, maizecob, wood and rice hull etc (robinson, etal., 2002 and low and lee, 1997).

Though activated carbon demonstrates its potentiality in removing the textile effluent colour, but few disadvantages are also associated with this as high initial cost of carbon problems associated with regeneration/reuse facilities etc.[5].

Colour Removal By Chemical Process:-

Various chemical processes like oxidation-reduction, precipitation, ozone oxidation etc. have been investigated for treating coloured textile industrial effluents. Chemical oxidation apart from destroying the colour causing chromophores and thereby causing a shift in the dominant wavelength of the waste from visible to UV or IR region, also renders the effluent more biodegradable. Chloride oxidation is widely used as terminal treatment to achieve the dual objective of colour destruction and disinfection of biologically treated wastes. Ozonation is the technique has been in practice from nearly a century [6,7]

Colour Removal By Biological Processes: -

Biological oxidation is most widely used in treatment for coloured (dye) effluents. However, the ability of biological treatment processes for decolourisation of industrial effluents is ambiguous, different and divergent[8]. The biological methods aim at degradation of dyes in to inorganic constituents, thereby they have an eco-friendly approach.

Several fungal species; ***Aspergillus fumigants***, A. oryzae [9]. ***Coriolopsis ployzona*** [10], **T*rametes Virsicolour*** [11] etc. have been found to degrade dyes in the experimental studies. Among them ***Phanerochaeta*** has received considerable attention, as it is known to degrade a wide range of recalcitrant xenobiotic compounds, including azo dyes [12] usually at a faster rate in presence of cellulose. Its extra cellular enzymes such as peroxidase and laccase degrade lignin as well as various recalcitrant compounds [13].

A large no. of bacterial species capable of degrading azo compounds are; ***Proteus sp., Enterococcus sp. Psuedomonas sp.*** [14]. Most of these microbial studies are still at the experimental stage.

It is advantageous over other methods, particularly chemical treatment , to employ biological processes for the treatment of effluent because active bacteria are continuously produced for long time, they have been used as standard methods of waste treatment, and efficiency of biological process can be measured by simple tests. Because of these advantages, biological treatment has been widely employed for industrial and domestic wastes.

Venkata Mohan and Karthikeyan (1997) investigated colour removal of direct and acid dyes by algae ***Spirogyra*** sp. and found it to be effective (99% ≈ colour removal) [15]

The present work attempts to evaluate the efficiency of physico-chemical and biological oxidation techniques for the removal of COD as well as parent compounds from aqueous solutions of dyes. The physico-chemical step is also as a potential pre-treatment to obtain biodegradable biological intermediates.

Principle Of Adsorption And Bio-Degradation:-

The use of low cost adsorbent has been investigated as a replacement for the current expensive methods of removing dyes from waste water. The adsorbent use in present study are ***Treated Sugar Cane Bagasse and Azadirachta indica (NEEM) leaf power***. The produced adsorbent was utilised to remove colours from wastewater and was given various characteristics. The main cause of the effluent's colour is unfixed dye. The colour in the effluent is mainly due to unfixed dye. The concentration of dye found in the effluent of textile industries depends upon the nature of dyes and dying process underway at the time (McMullan, *et al*., 2001). Lack in efficiency of dying process results in 15-30 % of all dye stuffs being lost directly to the wastewater (Perineau, *et al*., 1982). Even though they only make up a small part of the total amount of wastewater released following the dyeing process, textile dyes give it a highly coloured appearance (McKay, et al., 1985). The removal of dye in an economic fashion remains an important problem. The removal of dye from wastewater has been the subject of extensive research (Perineau, et al., 1982; McKay, et al., 1985; Gupta, 1985; Khattri, 2000; Low, et al., 2000; Liversidge, et al., 1997; Choy, et al., 1999, Asilian, et al., 2006).

Absorption studies were carried out for different temperature, pH's and adsorbent doses.

Principle Of Biological Degradation: -

The term "biological degradation" or "biodegradation" refers to the phenomena of living organisms, typically microorganisms, biologically transforming organic substances. The role of micro-organisms in the decomposition of sewage and other organic wastes is long known.

It has been considered as a natural process in the microbial world as they use carbon as energy source for their growth and takes a pivotal role in the recycling of materials in the natural ecosystem. It brings about changes in the molecular structure of a compound ultimately yielding simpler and comparatively harmless (non-toxic) products like carbon dioxide, water, methane etc.

Organic debris in water will naturally decompose due to the presence of microorganisms in receiving bodies of water, making biological treatment a "natural process." High organic loads in a wastewater will upset the businesses of receiving bodies of water and cause other undesirable effects. Biological treatment is engineered to accelerate natural decay processes and neutralize the waste before it is finally discharged to receiving water. This has helped microbes to act as scavengers and reduce the pollution load natural ecosystem.

Methodologies: -

This chapter deals with the various experimental methodologies adopted in this study together with a description of the materials: -

Adsorption Techanique:-

Dye solution preparation: - Dye solution prepared by weighing accurate quantity and dissolved in double distilled water to prepared stock solution (500 mg/L). Experimental solution of the desired concentration was obtained by successively dilutions. Dye concentration was determined by using absorbance values measured before and after the treatment by shimadzu UV spectrophotometer. Experiment were carried out at initial pH values ranging from 2 to 9, initial pH was controlled by the addition of NaOH (Sodium Hydroxide) or HCL (Hydrochloric Acid).

Adsorption experiment:

Preparation of adsorbent :-

1. Treated sugar cane bagasse: - For this purpose the bagasse obtained from the site was dried under the open sky and sunlight until all the humidity evaporated and was then ground to fine powder. This ground bagasse was then sieved between -80 to +230 sizes. After this these ground bagasse then was treated with 2% formaldehyde for 5 hours. Then this bagasse filtered, washed with distilled water to removing formaldehyde and activated at 800C in the oven for 24 hours and kept in airtight container for further experiment.

2. Azadirachta Indica (Neem Leaves) -: Mature leaves of the Neem tree (Azadirachta Indica) was used after fine drying and grinding. It can also be used for adsorption of heavy metal ions from water [16].

Method employed :-

The method employed was very simple and effective. All adsorption experiments were carried out in batch processes. In each experiment 25 ml of the dye solution was mixed with 1gm of adsorbent in a 100 ml round bottom flask at room temperature (25±10C) and (mixture was stirred on a rotator shaker) left to stand for prefixed time (. After predetermined time interval the mixture was filtered and the quantity of dye not adsorbed that is the dye remain in solution, was measured using a shimandzu UV-Vis, spectrophotometer.

Biological Treatment: -

Generally mixed cultures are more useful in degradation due to the large variety of micro-organisms which are capable of utilizing a multitude of metabolic intermediates (end product of biodegradation by one species may act as the substrate for another species and so on.)

Sludge collected from Bhilwara dairy was used separately as seed to develop the culture for the biodegradation experiments. The diluted sludge was plated on to nutrient agar and the growth obtained after 24 hours, scraped, washed (4-5 times) with distilled water and used as the inoculums for the batch reactors.

Determination of COD (Chemical Oxidation Demand), Colorimetric Method: -

The chemical oxidation demand is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. The dichromate reflux method is preferred over procedures using other oxidants because of superior oxidizing ability, applicability to wide variety of samples. A sample is refluxed in an exceedingly strong acid solution with potassium dichromate ($K_2Cr_2O_7$). Colorimetric reaction vessels are sealed glass ampules. Oxygen consumed is measured against standards at 600 nm with spectrophotometer.

A. Reagents :

(a) Digestion solution : Add to about 500 ml distilled water, 10.216 gm ($K_2Cr_2O_7$), primary standard grade, previously dried at 1000 C for 2 hours, 167 ml. Concentrate H_2SO_4 and 33.69 gm. $HgSO_4$ dissolve, cool to room temperature , and dilute to 1000ml.

(b) Sulphuric acid reagent: Add Ag_2SO_4 reagent to con. H_2SO_4 at the rate of 5.5 gm. Ag_2SO_4 /Kg H_2SO_4. Let stand for 1-2 day to dissolve Ag_2SO_4.

(c) Potassium hydrogen phthalate standard: Lightly crush and then dry potassium hydrogen phthalate to constant weight to 1200C. Dissolve 425 mg in distilled water and dilute to 1000ml. Potassium hydrogen phthalate (KHP) has a theoretical COD of 1.176 mg O_2/mg and this solution has a theoretical COD of 500 mg O_2 /l this solution is stable when refrigerated for up to 3 months in the absence of visible biological growth.

B. Procedure:
Table No. 1
Measure suitable volume of sample and reagents into tube or ampoule as given in table below: -

Table number :- 1 Digestion vessel	Volume (ml) Sample	Volume (ml) Digestion solution	Volume (ml) Sulphuric acid reagent	Total final volume (ml)
Culture tubes 16 x 100 mm	2.5	1.5	3.5	7.5
20x150 mm	5.0	3.0	7.0	15.0
25x150 mm	10.0	6.0	14.0	30.0
Standard 10 ml ampules	2.5	1.5	3.5	7.5

Wash culture tubes and caps with 20% H2SO4 before first use to prevent contamination. Place sample in culture tubes or ampules and add digestion solution. Carefully run sulphuric acid reagent down inside of vessel so an acid later is formed under the sample digestion solution layer. Tightly cap tubes or seals ampules and invert each several times to mix completely and reflux for 2 hours at 1500C and then cool to room temperature.

Invert cooled samples, blank and standards several times and allow solids to settle before measuring absorbance. Dislodge solids that adhere to container wall by gentle tapping and settling. Then pore these samples into quartz cuvette and place it into light path of UV-VIS spectrophotometer set at 600 nm. Read absorbance and compare to calibration curve and find out the COD of unknown samples.

Estimation of Residual Dye Concentration:-

Absorptionmetric measurements have been commonly employed for the investigation of concentration of coloured substances in the solution. Such studies have gained popularity due to simplicity and versatility of light absorption measurements. Such measurements are termed as spectrophotometric or absorptiometric.

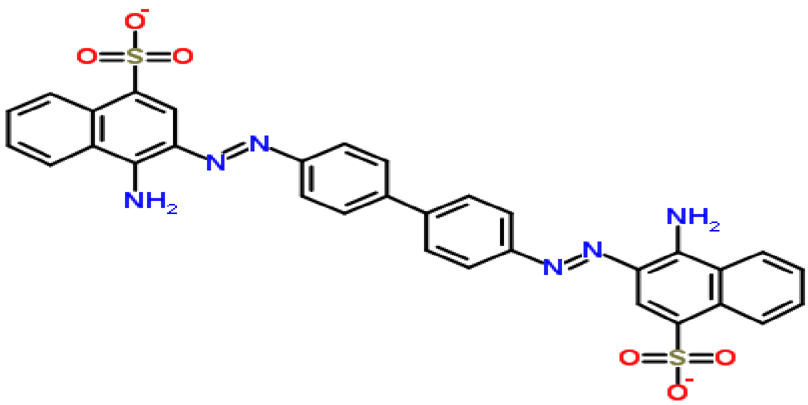
n the spectrophotometric measurements, it is possible to employ almost all types of monochromatic light of a very narrow bandwidth by the dispersion of light through a prism or a grating. This fact is of great importance for precise measurement of absorbance, since the Beer- Lambert's law, holds good only with monochromatic radiations.

The Material (Dyes) :-
Three dyes used for the present study purchased in pure form. All are synthetic dyes and used frequently in the textile industries of bhilwara (Rajasthan). They can be categorised as:-

Congo-Red dye :
(a) Common name - Direct Red 28

(b) Chemical name - Sodium tetraazodiphenyl-naphthionate

(c) Formula –



(d) Compact Formula -: C32H22N6O6Na2S2

(e) Colour Index No. -22120

(f) Molecular weight – 696.7

(g) Solvent- Water

(h) Group – Direct dye

Result And Discussion :-

This chapter presents the detailed results of the several batch experiments using aqueous solution of selected dyes, which are generally regarded to be with limited water solubility and negligible volatility besides having wide industrial uses.

Adsorption Study:-

In each experiment(for pH effect) 25 ml of the dye solution was mixed with 0.5 gm of adsorbent in a 100 ml round bottom flask at room temperature (25±10C) and (mixture was stirred on a rotator shaker) left to stand for prefixed time(. After predetermined time interval the mixture was filtered and the quantity of dye not adsorbed that is the dye remain in solution, was measured using a shimandzu UV-Vis, spectrophotometer.

The experiment was done by varying the amount of absorbents 0.1 to 1.0 mg 100 ml-, concentration of the dye solution from 25 to 250 mg L- (by diluting the stock solution) and pH at different time intervals.

Congo-red dye:-

Adsorbent: A. Treated sugar cane bagasse B. Azadirachta Indica (Neem Leaves)

Contents :-

1. Effect of initial adsorbent dose 2. Effect of initial concentration of dye 3. Effect of pH

Table 2. Effect of the initial adsorbent dose (g/100ml-) on dye removal, initial pH

6.5, concentration of dye=100mg L- Initial

adsorbent dose (g/100mL-)			Observed percentage of removal of colour with time (min)			
10	30	50	70	90	110	
Treated sugar cane bagasse						
0.1	6.2	8.4	11.9	15.2	17.2	20.0
0.3	17.3	19.6	22.5	27.4	38.9	42.0
0.5	29.0	30.3	35.0	38.0	39.4	45.15
0.7	38.5	42.0	46.5	49.3	55.0	60.10
1.0	41.25	55.55	62.15	68.20	72.15	79.5
Azadirachta Indica (Neem Leaves)						
0.1	7.2	8.0	10.5	12.6	14.2	19.7
0.3	21.50	25.20	28.60	32.20	38.00	43.25
0.5	28.0	34.50	38.65	45.50	54.25	58.35
0.7	32.25	38.00	44.55	52.35	55.0	65.30
1.0	40.25	48.25	58.25	68.35	72.55	85.25

Results and discussions :-

The water demand of industrial sector in the country has increased at a fast pace during the recent years. More particularly after implementation of liberalized policies for industrial development. Among the leading textile printing centers in the country, Rajasthan states own many.

By the present study we can conclude that biodegradation process can be effectively used for the colour removal of the dyes used in textile industries. The studies were carried out in reference to the change in the COD. As we see the result are somehow less than the adsorption technique. So it is advisable to use both the technique in combination to maximize the removal percentage. Both techniques are cheap, eco-friendly, and not generate any other toxic byproducts. So the need of future is that to develop the onsite feasible combine study which requires little bit patience, money and the awareness regarding environment.

Using physico-chemical treatments [28], colour reduction was significant (80-90%) but the problem was sludge disposal. By having biological step in addition to physico-chemical treatment, both sludge disposal and the toxicity of the effluent can be reduced.

As we see from above experiments that by biological treatment alone, much colour reduction could not be achieved. Hence, the combinations of biological and physico-chemical treatment methods are ideal for the colour removal.

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