INVESTIGATIONS ON BIOSORPTION OF COLORANTS IN DISTILLERY SPENT WASH BY NONVIABLE *ASPERGILLUS ORYZAE* MTCC 7691: KINETICS AND ISOTHERM STUDIES

Patil M. S. and Kulkarni M. V.

Abstract--This study presents the ability of dried and nonviable biomass of fungal strain Aspergillus oryzae MTCC7691 to absorb coloring components of distillery spent wash at different spent wash and biosorbent concentration. Increasing spent wash concentration was directly proportional to biosorption profile up to certain time. Maximum color removal was found in 10 % spent wash with 0.5 gm sorbent of Aspergillus oryzae MTCC7691. pH range 2-4 was observed as most suitable for biosorption. Decolorisation up to 58 % and 61% of 50% diluted spent wash was possible at pH 2 with 0.5 gm and 1 gm biosorbent concentration of Aspergillus Oryzae MTCC 7691. Desorption profile of the absorbed spent wash components was maximum by using 75 % alcohol in the period of 80 - 100 minutes. Statistical clarification of the biosorption equilibrium was presented with Langmuir and Freundlich adsorption model and the obtained information was the proof for an best possible fitness to these isotherms. Kinetic parameters point out the supremacy of pseudo second -order kinetic model by Ho for adsorption.

Key words: *Aspergillus oryzae* MTCC7691, Biosorption, Decolorisation, Distillery, Isotherm, Spent wash.

----------***----------

1. INTRODUCTION

Disposal of industrial effluent to soil and water bodies can have a profound impact on the environment, as it contains many potentially hazardous chemicals. Most of the industries release a heavily colored wastewater; which causes color contamination problems in natural water bodies. Most of the colored components in such wastewater are recalcitrant and remains unaffected through various stages of effluent treatment plants. Their recalcitrant nature protects it from biodegradation by various microbes, hence it remains persistent in soil and water bodies for long time. Contamination of soil, ground water and surface water with such untreated and partially treated effluents generate serious human health problems.

- Mayavati Patil[1]
  Dept. of Biotechnology, Institute of Sciences, Aurangabad., patilmsgis@gmail.com
- Mohan Kulkarni
  Department of Chemistry, Division of Biochemistry
  University of Pune, Pune.
  kulmv@yahoo.com

One such effluent is the distillery industry effluent- spent wash. Distilleries, alcohol fermenting industries are listed among the most environment polluting *industries, with 28 to 36 billion liters of spent wash generation for 2.4 billion liters of alcohol production per year in* India [1]. Mainly in India and brazil, where sugarcane is a major cash crop, distilleries uses molasses – a byproduct of sugar industries, as a raw material for the alcohol production. After fermentation by yeast, alcohol is recovered by distillation and spent wash- a dark brown colored liquid, with acidic pH (2.5- 4), remains behind. [2]. Due to presence of high organic and inorganic contents in concentrated form, it is a complex mixture with high biological (30,000-90,000mg/l) and chemical oxygen demand demands (45,000 to 75,000 mg/l) resulting in heavy pollution potential [3]. A recalcitrant brown polymeric pigments, 'melanoidins'is reported to impart intense brown color to it [4]. Such coloration of this colored effluent is responsible to cause color contamination problem in natural water bodies. An antioxidant property of melanoidins makes them toxic to many microorganisms [5, 6,7].

Thus, spent wash disposal even after conventional treatments (dilution, biomethanation, amendments with soil, etc.) is hazardous and has high polluting potential because of the accumulation of nonbiodegradable recalcitrant compounds [8,9;]. On the other hand Increasing awareness of harmful effects of such pollutants on ecology as well as on human health, is leading to significant increase in research to develop

those technologies, which may be applied to remove the contaminants from industrial waste effluent.[10].

Various methods like, oxidation process is used for treatment of waste water, such as ozone, single hydrogen peroxide, Fenton's reagent [11]. In most of the Indian distilleries biological treatment which includes anaerobic digestion generating methane as biogas is followed with decrease of BOD and COD. However, the success rate of most of these methods increases if applied after physico - chemical treatments [12]. The major problem encountered with distillery effluent treatment is the color which remains persistent even after biological treatment due to the presence of recalcitrant compounds like caramelized sugars, melanoidins, sugar decomposition products, anthocyanins and tannins, and other xenobiotic compounds Conventional processes hardly remove these constituents from the disti1lery effluent. [13]. Biodegradation of melanoidins by various microbes is also found to difficult at large scale due to its recalcitrant nature. Also high temperature of effluent, high concentration of polluting components, acidic pH, these conditions are inhibitory to the microbial growth[14].

In most of the earlier reports, the role of white rot fungi has been discussed for the decolorisation of spent wash. White rot fungus *Phenarichateae chrysosporium* decolorized 6.25 % spent wash GPY medium by 85 %, [15] and 74 % [16] after 10 days incubation. A facultative anaerobic pure bacterial culture achieved 31 % decolorisation & 57 % COD reduction after 7 days incubation [17]. Attempts for continuous decolorization of spent wash with immobilized *P. chrysosporium* on polyurethane foam disks was successful with easily available carbon source as glucose. [18] In above cases 2-3 % sugar as carbon source with other essential nutrients is reported to use and decolorisation was attempted after 7-10 days.

Few efforts were attempted to decolorize spent wash by using reticulated polyurethane foam which holded on a support nylon cloth core [19] alginate immobilized two-stage bioreactor system [20], synthetic resin polyurethane foam [21], pollutant degradation using immobilized fluidized bioreactor [22]. Anaerobic digestion through fixed-film, granular bed anaerobic baffled, upflow anaerobic sludge blanket, diphasic, and contact bioreactors [23,24,25]. Among white rot fungi, lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase activities were attributed for detoxification of recalcitrants from distillery spent wash [26]. However, the above studies employed (1) costly matrix for immobilization and (2) dilute spent wash demanding more water for biotreatment.

Physico-chemical treatment methods, are also widely reported to remove color and specific organic pollutants in the spent wash. Among these, activated carbon is a well-known adsorbent due to its extensive exterior area and high adsorption capacity [27]. Even low cost adsorbents such as pyorchar (activated carbon in granular and powdered form, manufactured from paper mill sludge) and bagasse flyash have also been reported for same application [28]. Bagasse fly ash contains high carbon content and adsorbed organic material further enhances its heating value, so it is not abetter alternative. Another one adsorbent derived from exoskeleton of crustaceans is a carbohydrate polymer chitosan also reported to use in treatment of distillery wastewater as an anion exchanger [29].

Biomass of some microorganisms, viz bacteria, fungi and algae, is capable of reducing color of different textile dyes either by biosorption, biodegradation or mineralization [30] *Geotrichum candidum* and *Rhizoctonia* species are reported to partially eliminate the maillaird colour compounds [31]. Miranda et al reported color reduction from molasses spent wash using *Aspergillus niger* through adsorption, Presence of melanoidin like pigment in the cytoplasm of mycelia cells was detected by electron microscopy [32].

In the focus of above studies, present investigations were undertaken to study the spent wash colorants adsorption pattern by nonviable biomasses of *Aspergillus oryzar* MTCC 7691 in batch cultures. The special effects of experimental circumstances such as effluent concentration, Biosorbent concentration, temperature and pH were also examined to obtain data on adsorption behavior of the fungal biomass.

Microbial cell surface carries various functional groups like hydroxyl, carboxylate, amino, and phosphate which are accountable for the confiscation of harmful materials from industrial sewage. [34]. The ability of dead biomass was found to be higher due to upper adsorption strength, change in surface property and increase in surface area due to cell rupture after death which was found in autoclaved biomass. Heat exposure can alter surface binding sites via denaturation of proteins present on the cell wall. Additionally, these pores decrease the mass transfer resistance and ease the diffusion of color particles because of high internal surface area with low diffusional conflict which implies high adsorption efficiency and rate .

This work stems from the work done on biosorption of dyes and their mixture using dry and dead fungal biomass [33,34,35] and removal of heavy metals in biological system [36,37]. Among the various sustainable techniques suggested, biosorption technology is gaining

attention of scientists, as easy and quick recovery of environmentally toxic pollutant can be achieved from the waste effluent. Particularly, it can be applied for effluents, containing organic pollutants and has been studied since 1980. It is a process that uses sorption ability of biological material for the removal of pollutants

2. MATERIALS AND METHODS

Distillery spent wash was collected from a locally situated distillery industry and stored at 4^0 C The λ_{max} values and optical density of different concentrations of spent wash was determined on UV- visible spectrophotometer (Shimadzu UV- 1601).

2.1 Isolation and taxonomic identification of fungus

The strain of *Aspergillus oryzae* MTCC 7691, isolated in our laboratory and identified at MACS Agharkar Research Institute, Pune and then deposited at MTCC Chandigarh, was used as a biosorbent. The culture was maintained on PDA slants (HI MEDIA) at 4^0C. The composition of PDA was (Gms/Lit.) Potato infusion from -200, Dextrose -20. Agar-15, final pH- 5.6+ or -0.2. and distilled water 1000 ml.

2.2 Preparation of fungal biomass

Five day old spore suspension (10^6 spore/ml) of *Aspergillus oryzae* MTCC 7691 was inoculated in 5000 ml capacity conical flask, containing 2000 ml of potato dextrose broth medium and incubated for 72 hrs. on a rotatory shaker having speed of 130-140 rpm. After good growth fungal mycelia pellets were strained through 150µm sieve, washed twice, with tap water and then with de-ionized water until free from media components and to neutralize the pH.

2.3 Nonviable biomass

To enhance the biosorption capacity of fungal biomass heat treatment is reported [35]. Hence biomass was autoclaved at 15 psi and at 121^0C, for 20 minutes. Nonviable, autoclaved biomass was dehydrated in an oven at 40 to 50^0C and then was crushed thoroughly in a mortar and pestle to get fine powdered form. This pulverized biomass was sieved through a 150 µm opening size sieve. Fine powdered particle size has been shown to give maximum biosorption, as it provides more surface area for adsorption and adsorption.

2.4 Batch mode experiments and Biosorption studies

Batch mode experimentation were conducted to investigate effect of parameters, like initial concentration of spent wash, agitation time, dosage of the biosorbent and pH influencing the rate and extent of uptake of colorants in the spent wash by dead fungal biosorbent. 1gm of dried powdered biomass as the absorbent was added in 100 ml of 30 % spent wash (30 ml spent wash +70 ml water) in a 500 ml capacity conical flask. Contents were mixed on the rotator shaker at 130-140 rpm, for 30 minutes and then filtered through what man filter paper No.1. Biosorption was confirmed with decreasing optical density of filtrate, which was monitored on UV- visible spectrophotometer (UV- visible, Shimadzu, 1601) at 475nm, as the λmax of melanoidin in the spent wash is 475nm. The initial and final absorbance values obtained were then used to calculate percentage decolorization of spen twash:

% Color removal = (Co- C1)/C0 x 100

C0=lnitial absorbance value

C1 =final absorbance value

2.4.1 Effect of spent wash concentration and agitation time

Bioorption profile of spent wash (adsorbate) of varying concentration at different time interval was studied by taking spent wash concentration in the range of 10 % - 60 % and time interval of 05 minutes from 10 to 30 minutes. 10 % -60 % concentrations of 100 ml spent wash was prepared in 250 ml Erlenmeyer flask and to these 1gm of dried powdered biomass was added as the absorbent. The mixture was agitated at 125 rpm on shaker at constant 30°C temperature. After the equilibrium period, absorbent was separated by centrifugation at 10000 rpm for 15 mins and the residual concentration of adsorbate left in flasks was determined. From this, the amount absorbed colorants was calculated. A plot was drawn to determine the optimum concentration and agitation time. Control experiments were carried out without absorbent to estimate the adsorbate removal due to adsorption on to the walls of the flasks. Residual part was dried at room temperature for 3 days and preserved for further desorption studies

2.4.2 Effect of Adsorbent dosage

Biosorption profile of spent wash (adsorbate) of varying concentration at different biosorbent concentration 0.5 - 2.5 gms in each 100 ml diluted spent wash having concentration in the range of 10 % - 60 %. The mixture was agitated at 150 rpm, at 30°C for the equilibrium period of 20 minutes. After the equilibrium period, the adsorbents and the adsorbate were separated and the amount adsorbed colorants was determined. Thus the optimum adsorbent dosage for maximum color reduction in each concentration of spent wash was determined. A

graph was plotted with adsorbent dosage vs percent adsorbate removal for 10-60% concentration of spent wash.

2.4.3 Effect of pH

The adsorbate solutions (50 %) spent wash were prepared at various levels of pH (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0) using either 0.l M HCl or 0.l M NaOH as per need. These are agitated with optimum dosages of absorbents (0.5 gm and 1 gm of dead biomass). The amount of color adsorbed was calculated. The optimum pH was determined from the plot drawn with pH against percent color removal.

2.5 Desorption profile

After determining the sorption profile of spent wash of different concentration by dead fungal biomass, the possibility of recycling the biosorbent was investigated. Desorption of the spent wash components was tried initially using 50 % ethanol. Sedimented and dried biosorbent in each flask were collected separately and unabsorbed components on the pellets were removed by smoothly washing the pellet with distilled water. Then one set of spent wash adsorbed samples was resuspended in flask having 50 ml of 50 % ethanol keeping it on magnetic stirrer and monitored at different time intervals of 0, 30, 60, 90, 120 and 150 mins using 1.0 N NaOH as a desorption agent and agitated for the equilibrium time of respective adsorbate. 5 ml aliquots were removed at time interval of 30 minutes and whatever color desorbed was estimated spectrophotometrically at 475nm.

2.6 Adsorption isotherms and kinetics:

To design any sorption system for removing the sorbets, it is important to set up the most suitable correlationship for the equilibrium curves [39]. These equilibrium sorption capacity curves can be studied by plotting the sorption isotherm. The Freundlich and Langmuir adsorption isotherm models are extensively reported to analyse data for water and effluent remediation treatment [34]. Hence, here also obtained data were fitted in the Freundlich and Langmuir models. To determine the appropriate model for the sorption system, the obtained data showing an amount of sorbate adsorbed on the sorbent surface are substitutded into an equilibrium isotherm model. By using this correlation, any deviation in the volume of sorbent with the amount of sorbate molecules present in solution is calculated

The Langmuir plot was plotted using the equilibrium time curves data (i.e. the adsorbate concentration was varied, while the adsorbent dose was fixed). While equilibrium data with adsorbent dose effect (i.e. the amount of adsorbate was fixed, while the adsorbent dose was varied) predicted the Freundlich plot.

3. RESULTS AND DISCUSSIONS

The main purpose of the this research investigation was to access the feasibility of biological removal and recovery of toxic colored components of spent wash by recycling the dead fungal biomass. Distillery spent wash discharged from distillery, needs pretreatment due to its hazardous impact on natural water bodies and on dumped soil. Due to very high pollution load and recalcitrant nature of melanoidins, spent wash is difficult to treat by conventional processes. Hence alternative, economic technology need to be investigated. Biosorption of heavy metals and other organic pollutants have been studied extensively by various scientists [33-38]. An adsorption potential of heavy metals by marine algae is well reported for economic feasibility of the method than removal by ion exchange chromatography [40]. Fungal biomass can be obtained economically and also as a waste from a variety of fermentation industries [41]. Use of nonviable fungal biomass has various advantages like no need of nutrients and easy regeneration [42]. Dried non living treated biomass of fungi may be a better alternative as biosorbent for removal of colored components present in waste effluent. Few studies are available removal of dye with dead fungal biomass [33,34]. Application of nonviable biomass of yeast is also reported for biosorption of some anionic textile dyes [35]. Reports are available onuse of native, heat-, acid- and alkali-treated biomasses of *Neurospora intennedia* for the adsorption of colored components of distillery effluent inbatch cultures [39]. However decolorisation of spent wash through biosorption by dead fungal biomass is a novel approach for spent wash treatment.

3.1 Effect of concentration of spent wash and agitation time

Initially at 10 % spent wash concentration, maximum sorption was possible within 10 minutes. Increasing spent wash concentration was directly proportional to biosorption profile up to equllibrium time. After 15 minutes no increase was found in 10-40 % spent wash concentration. While in case 50 % and 60 % spent wash decolorization was found up to the 20 minutes. Maximum color removal of 50 % and 60 % spent wash by dead fungal biomass was 47 % and 55% respectively after 20 minutes (Figure 1).

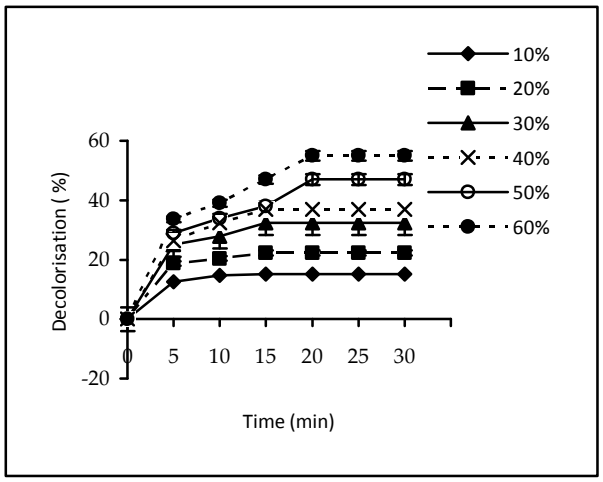

Fig.1: Sorption profile of spent wash of varied concentration at different time interval by 1gm biosorbent of *Aspergillus oryzae* MTCC 7691.

3.2 Effect of Adsorbent dosage

Increasing biosorbent concentration did not show any significant increase in biosorption (Figure 2). This effect can be explained as 'relatively high concentration of the biosorbent in the solution might be blocking the binding sites of biosorbent due to reduced distance between biosorbent [44,45]. Two way interactions of increasing spent wash and biosorbent concentration were not found effective because maximum color removal was found in only 10 % spent wash with 0.5 gm sorbent of *A. oryzae* MTCC7691.

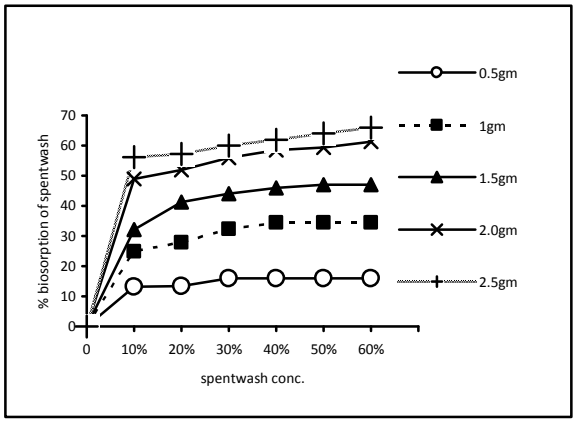

Fig.2: Sorption profile of spentwash at varied biosorbent concentration of *Aspergillus oryzae* MTCC 7691 after 20 minutes.

3.3 Effect of pH

Process of biosorption is governed by solution pH [46]. pH range 2-4 (acidic) was observed as most suitable. As shown in figure 3, Increasing pH found to reversibly affect on biosorption mechanism. It was a significant observation because original pH of the spent wash was also 2- 4 , which offers the advantage that no needs to adjust pH before biosorption treatment. We could achieve 61 % and 58 % decolorisation of 50 % diluted spent wash at pH 2 with 0.5 gm and 1 gm biosorbent concentration of *Aspergillus. oryzae* MTCC 7691 .

3.4 Desorption profile

Optimum time required for desorption of spent wash colorants of various concentrations from dead biomass was found to be 100 minutes. Desorption profile of the absorbed spent wash components by using 50 % alcohol in the period of 80 - 100 minutes. (figure 4). Maximum desorption of 69.9 % at 50 % spent wash concentration and 66 % at 60 % was observed at 100 mins and a similar pattern of desorption was observed after 120 minutes.

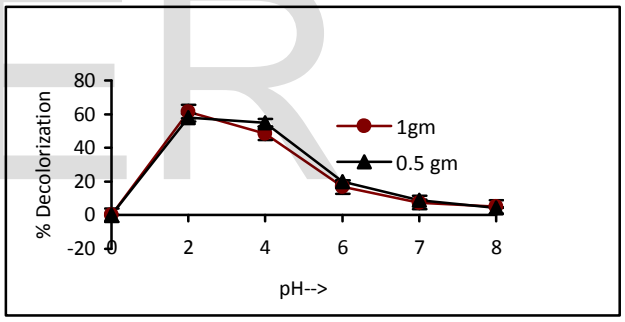

Fig. 3: Effect of pH on biosorption of 50% diluted spent wash by dead biomass of fungus *Aspergillus oryzae* MTCC 7691

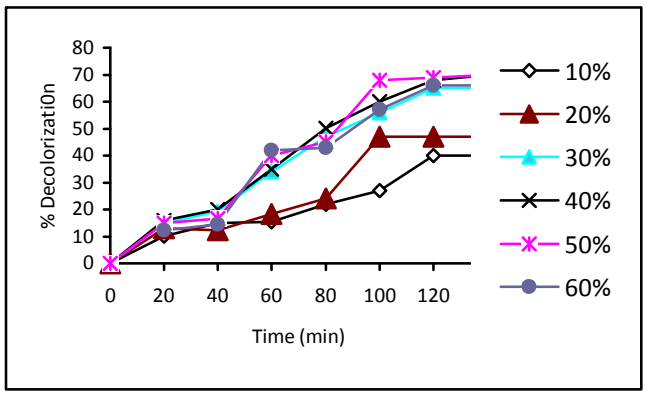

Fig. 4: Desorption profile of spent wash by dead biomass of fungus *Aspergillus oryzae* MTCC 7691.

3.5 Adsorption isotherms and kinetics

3.5.1 Langmuir isotherm

This isotherm is supported on theory of monolayer adsorption on a surface holding restricted capacity of adsorption sites of homogeneous energies of adsorption with no transmigration of adsorbate on the plane of the surface. Langmuir isotherm is given by [47],

$$q_e = Qbc_e / 1+ bc_e$$

Here, C_e is the equilibrium concentration of spent wash solution (%), q_e is the quantity of spent wash colorants sorbed on to fungal biomass (%), Q is the Langmuir constant associated with sorption ability (%), b is the Langmuir constant related to sorption energy (%). Data obtained for the sorption of spent wash colorants in the concentration range of 10 to 60 % of spent wash was fitted to the Langmuir isotherm (Figure 5). A plot of C_e/q_e versus C_e presented a straight line, the slope and intercept of it correspond to Q and b respectively. The computed correlation coefficients and the Langmuir constants for different concentrations of spent wash are presented in Table 1. The R^2 value was 0.9716.

One more dimensionless equilibrium parameter (R_L) can be estimated using the following relation,

$$RL = 1/1+bc_o$$

Where b is the Langmuir constant and C_o is the initial Spent wash concentration (%).

.It is used to predict if an adsorption system is 'favorable' or 'unfavorable' . RL is > 1.0 then (system is Unfavourable), L= 1.0 (Linear), 0< RL< 1.0 (Favorable) and RL = 0 (Irreversible) [34].

Numerical calculations demonstrate that this parameter gives an hint of type of isotherm. [48] The values of R_L (Table 2) were between 0 and 1 for selected spent wash concentrations which indicate the applicability of the Langmuir isotherm.

3.5.2 Freundlich isotherm

Another form of Langmuir approach for adsorption on amorphous surface is Freundlich isotherm [49], which was tested in the linear form

$$\text{Log } q_e = \log K_f + 1/n \log C_e$$

Where, C_e is the equilibrium concentration of spent wash colorants (%), q_e is colorants (%) in the spent wash adsorbed/min on to biomass of *A. oryzae* MTCC7691 and When log q_e was plotted against log C_e, a linear plot was obtained for each of the concentration tested. Freundlich plot obtained for Figure 6. The Freundlich constants 1/n (Intensity of adsorption) and K_f (Adsorption capacity) were computed from the slope and intercept of the plot. The computed correlation coefficients and Freundlich constants for the different spent wash concentrations were studied are presented in the Table 1. The R^2 values for Langmuir equation (0.9716) were higher than those obtained for Freundlich equation (R^2= 0.9639). The constant 1/n showed the sorption intensity and its fractional value (0<1/n<1) showed the heterogeneous nature of sorbent surface. The calculated sorption capacity of dead biomass for spent wash was 5.28% / min.

3.6.1 Lagergrens first order model

The study of adsorption kinetics explains the solute uptake rate, which is most important for batch experiment study [34]. For a batch, where the rate of adsorption of color on the surface is proportional to the amount of colorants adsorbed from the liquid phase, for this pseudo first order kinetic equation may be expressed as,

$$dq_t/qt=K_{ad} (q_e-q)$$

Where q and q_e are the amount of color of adsorbed (%) at time t and at equilibrium time, respectively and K_{ad} is the rate constant of adsorption.

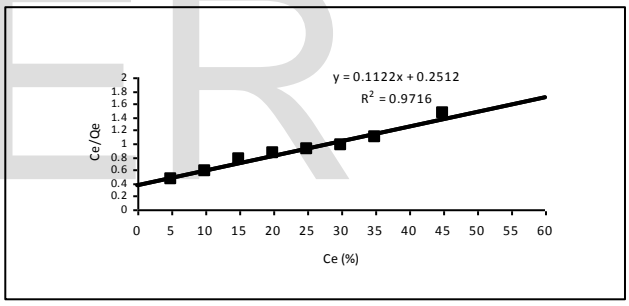

Fig. 5: Langmuir plot for spent wash biosorption by dead biomass.

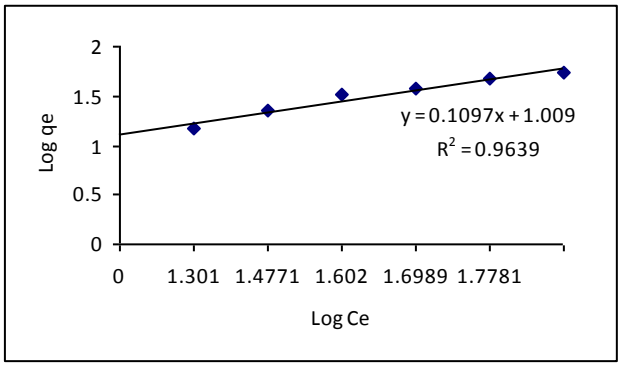

Fig. 6: Freundlich plot for spent wash biosorption by dead biomass.

Integrating above Eq. for boundary conditions $t = 0$ and $t = t$ and $q = 0$ and $q = q$, gives [34],

$$\text{Log } (q_e\text{-}q) = \log q_e - K_{ad}t/2.303$$

Figure 7 showed a linear plot of pseudo first order kinetic model at all studied spent wash concentrations. The values of pseudo first order rate constants K_{ad} and equilibrium adsorption capacity q_e is calculated from slope and intercept of plot of log $(q_e\text{-}q)$ verses t. The calculated K_{ad}, experimental and predicted q_e, with corresponding correlation coefficient values (R^2) are presented in Table 2. Correlation coefficient for pseudo first order kinetic model obtained for all concentration of spent wash were low and the predicted q_e values deviated reasonably from the calculated values. The rate constant (calculated K_{ad},) of pseudo first order increased with increasing initials pent wash concentrations.

3.6.2 Ho's pseudo second order model

This type of kinetic model is expressed as,

$dq_1/qt = K_2 (qe\text{-}q_1)^2$

Here, K_2 is the pseudo second order rate constant, q_e and q_1 represents the percentage of color adsorbed at equilibrium and time t.

An integral form of above equation is,

$qt = t/ 1/ K_2 q_e^2 + t/q_e$

$t/q1 = 1/ K_2 q_e^2 + t/q_e$

The kinetic data was analysed using the equation . The values of the K_2 and q_e were calculated from intercept of the slope of the plots t/q_t verses t . Corresponding R^2 values are given in Table No 3

The constant K_2 was used to calculate the initial sorption rate h as follows [34] ,

$$h = K_2 q_e^2$$

Initial sorption rate decreased with increasing spent wash concentration. Linearised appearance of pseudo second order kinetic model at all concentrations of spent wash is shown in figure 8 The correlation coefficient (R^2) for the pseudo second order kinetic model obtained for all concentrations of spent wash were high and prediacted qe values deviated reasonably from the experimental values. Further, the pseudo first order model did not fit well for the given rate of contact time suggesting that the adsorption system for this experiments follows second order kinetic model.

4. CONCLUSION

Despite of having variety of physico-chemical and biological process, an efficient environment friendly approach for distillery spent wash treatment has stimulated the interest in exploring novel means. In this method under optimized conditions developed in our laboratory, a biological matrix is used to specifically adsorb ecotoxic color components including melanoidin from spent wash. This surface binding involves specific chemical sites or functional groups on the cell wall, performance of which is affected by pH and other ions.

Application of this technique for the treatment of distillery effluent would provide an answer to its economic feasibility. The experiment carried out on this subject prove the ability of nonviable microbial biomass to treat distillery waste water and to remove selectively toxic, coloured components (melanoidins) of spent wash. Further recovery of remaining components from used biomass focus the steps of depollution. Such advantages makes it an easy, cost-effective, ecofriendly and a rapid technology to reduce the pollution potential of the distillery spent wash. Thus microbial biosorption of spent wash can become viable, future technology for spent wash treatment. *The* feasibility of the method was cross checked by subjecting the results obtained in Langmuir isotherm and Freundlich isotherm models. Among the kinetic models studied, Ho's pseudo second order modelwas found to relevant to best explain the above type of biosorption mechanism carried out with fungal biomass.

TABLE 1
Langmuir and Freundlich Isotherm Constants

Langmuir constants			Freundlich constants		
Q(mg/g)	b (L/mg)	R^2	K_f	1/n	R^2
	0.3257	0.9716	3.4584	0.4382	0.9639

TABLE 2

R_L, R_2 and Rate Constants of Lageren I Order Kinetic Model

Initial DSW conc. (%)	**R_L**	**q_e (expected)**	**q_e (calculated) (%/Min)**	**R^2**	K_{ad}
10	0.29210	5.567	5.2845	0.90339	0.0010453
20	0.17103	4.2656	3.243	0.87879	0.0028326
30	0.12091	3.2867	2.507	0.78556	0.016534
40	0.09351	2.9674	1.891	0.78328	0.019835
50	0.07623	2.24756	1.536	0.97045	0.020176
60	0.06435	1.5677	1.298	0.92253	0.020846

TABLE 3:

Values of K_2, h and R^2 in Ho's pseudo second order model:

Initial DSW conc. (%)	K_2	q_e (calculated)	h	R^2
10	0.01684	3.486	0.2046	0.9802
20	0.01063	3.9871	0.1689	0.9472
30	0.00657	4.5871	0.1382	0.9902
40	0.00578	4.9648	0.1424	0.9829
50	0.00295	5.5633	0.0913	0.9801
60	0.00084	5.8859	0.0291	0.9489

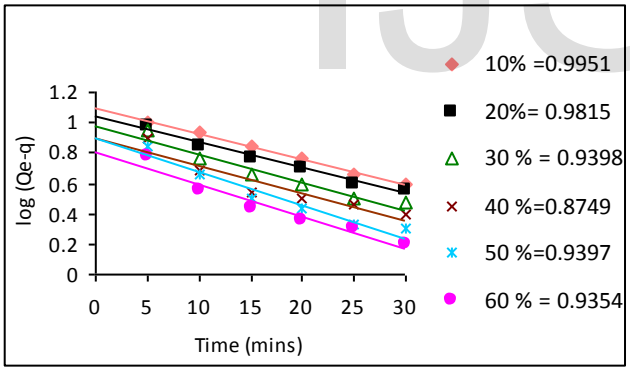

Fig. 7: Pseudo-first order plot for spent wash biosorption by dead biomass.

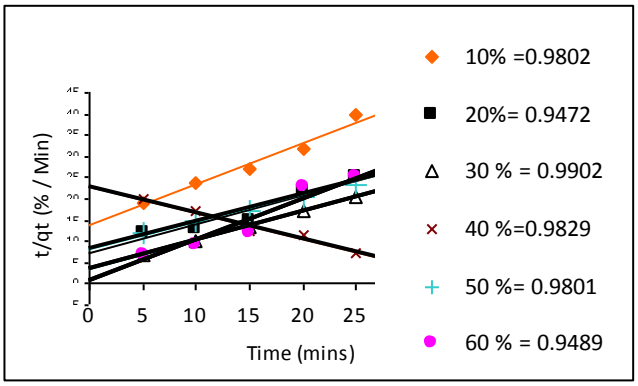

Fig. 8: Pseudo-second order plot for spent was biosorption by dead biomass.

REFERENCES

[1].Singh P, Robinson N & Singh D, Treatment of industrial effluents; Distillery effluent. In Pandey A (ed) Coincise encyclopedia of BioresourceTechnology. Food products press, The Hawarth press, 135-142, 2004.

[2]. Tiwari, S., Rai, P., Yadav, S. K., & Gaur, R. (2012). A novel thermotolerant Pediococcus acidilactici B- 25 strain for color, COD, and BOD reduction of distillery effluent for end use applications. Environmental Science and Pollution Research 20(6), 4046–4058.

[3] Lekshmi S.R. 2013. Treatment and Reuse of Distillery Wastewater, International Journal of Environmental Engineering and Management 4 (4), 339-344.[

[4] Kitts D D, Wu C H, Stitch H F & Poerie W D, Effect of glucose-glycine mailared reaction products on bacterial and mammalian cells mutagenesis. *J Agric food chem.*, **41**(1993) 2353 – 2358.

[5] Kitts D D, Wu C H, Stitch H F & Poerie W D, Effect of glucose-glycine mailared reaction products on bacterial and mammalian cells mutagenesis. *J Agric food chem.*, **41**(1993) 2353 – 2358.

[6] Dandi, B. N., Dandi, N. D., Shelar, R. D., Chaudhari, A. B., & Chincholkar, S. B. (2010). Biological decolorisation of high concentration distillery spent wash using newly isolated microbial strains. Journal of Advances in Science and Technology, 13(2), 73–78.

[7] Dandi, N. D., Dandi, B. N., & Chaudhari, A. B. (2013a). Bioprospecting of thermo- and osmo-tolerant fungi from mango pulp–peel compost for bioethanol production. Antonie van Leeuwenhoek, 103(4), 723–736.

[8] Ghosh, M., Varma, S. C., Mengoni, A., & Tripathi, A. K. (2004). Enrichment and identification of bacteria capable of reducing chemical oxygen demand of anaerobically treated molasses spent wash. Journal of Applied Microbiology, 96(6), 1278–1286.

[9] Dandi, B. N., Dandi, N. D., Chaudhari, A. B.& Chincholkar, S. B. (2013). Biodegradation of high gravity distillery effluent using microbes from different ecological habitats. Environmental Engineering and Management Journal (in press).

[10] Mohana, S., Acharya, B. K., & Madamwar, D. (2009). Distillery spent wash: treatment technologies and potential applications. Journal of Hazardous Materials, 163(1), 12–25.

[11] Ravikumar R.Vasanthi S, and Saravanan 2011. Single factorial experimental design for decolorizing anaerobically treated distillery spent wash using cladosporium cladosporioides Int. J. Environ. Sci. Tech., 8 (1), 97-106.

[12] Naik N,.. Jagadeesh S and.. Noolvi M. N.2010 Enhanced Degradation of Melanoidin and Caramel in Biomethanated Distillery Spentwash by Microorganisms Isolated from Mangroves Iranica Journal of Energy & Environment 1 (4): 347-351

[13] Chavan M. N (Patil M. S.) Dandi N. D. & Kulkarni M. V. &. Chaudhari A. B. 2013. Biotreatment of melanoidin containing distillery spent wash effluent by free and immobilized *Aspergillus oryzae* MTCC 7691.

[14] P. Rath, G. Pradhan, M.K. Mishra. 2010. Effect of sugar factory spent wash on growth pattern of sugar cane crop.J ournal of Phytology 2(5): 33–39.

[15]Fahy V, Fitzgibbon F J, & mcMullan G, Decolorisation of molasses spent wash by *Phanerochaete chrysosporium. Biotechnol Lett,* **19** (1997) 97-99.

[16]Kumar V., Wati L., Fitzgibbon F., Nigam P.,Banat I.M., Singh D. and Marchant R (1997 **A**). Bioremediation and decolourisation of anaerobically digested distillery spentwash. *Biotechno. Lett.* 19, 311-313.

[17] Miyata M., Mori T., Iwahori K. & Fujita M. Microbial Decolorisation of melanoidins-containing wastewater: Combined use of Activated Sludge and the Fungus *Coriolus hiristus . J Biosci. and Bioengi,* **89** (2000) 145-150.

[18] Guimarães, C., Porto, P., Oliveira, R., & Mota, M. (2005). Continuous decolourization of a sugar refinery wastewater in a modified rotating biological contactor with Phanerochaete chrysosporium immobilized on polyurethane foam disks. Process Biochemistry, 40(2), 535-540.

[19] Henry, D. P., & Thomson, R. H. (1993). A new process to treatstrong biological waste. Water Science and Technology, 27(1), 213–218.

[20] Ghosh, M., Ganguli, A., & Tripathi, A. K. (2002). Treatment of anaerobically digested distillery spent wash in a two-stage bioreactor using Pseudomonas putida and Aeromonas sp. Process Biochemistry, 37(8), 857–862.

[21] Raghukumar, C., Mohandass, C., Kamat, S., & Shailaja, M. S. (2004). Simultaneous detoxification and decolorization of molasses spent wash by the immobilized white-rot fungus Flavodon flavus isolated from a marine habitat. Enzyme and Microbial Technology, 35(2–3), 197–202.

[22] Andleeb, S., Atiq, N., Robson, G. D., & Ahmed, S. (2012). An investigation of anthraquinone dye biodegradation by immobilized Aspergillus flavus in fluidized bed bioreactor. Environmental Science and Pollution Research, 19(5), 1728–1737.

[23] Pant, D., & Adholeya, A. (2007a). Biological approaches for treatment of distillery wastewater: a review. Bioresource Technology, 98(12), 2321–2334.

[24] Mise1 S.R, Saranadgoudar R., Lamkhade R .2013 Treatment of distillery spent wash by anaerobic digestion process. International Journal of Research in Engineering and Technology. IC-RICE Conference Issue, 310-313.

[25] Prakash N. B., Sockan V., Sitarama V. R. 2014. Anaerobic Digestion of Distillery Spent Wash . ARPN Journal of Science and Technology,. 4,(3). 134-140.

[26] Raghukumar, C., D'Souza-Ticlo, D., & Verma, A. K. (2008). Treatment of colored effluents with lignin-degrading enzymes: an emerging role of marine-derived fungi. Critical Reviews in microbiology, 34(3–4), 189–206.

[27] Satyawali Y., Balakrishnan M. 2007. Removal of color from biomethanated distillery spent wash by treatment with activated carbon. Water research 43 (6), 1577-1588.

[28] Shivayogimath C. B., Inani S. 2014. Treatment of biomethanated distillery spent wash by adsorption process on bagasse activated carbon (6),1070-1075.

[29] Qiu-X, Nian Y, JIN X. Y., Chang Z. 2007. Effects of chitosan on growth of an aquatic plant (*Hydrilla verticillata*) in polluted waters with different chemical oxygen demands, Journal of Environmental Sciences, 19, 217-221.

[30] Carliell, C.M., Barclay S. J., Naidoo, N., Buckley, C.A., Mulholland, D.A and Senior, E., 1995. Microbial decolorization of a reactive azo dye under anaerobic conditions. Water SA., 21: 61-69.

[31] K. Rajasundari and R. Murugesan 2011. Decolourization of Distillery Waste Water – Role of Microbes and their Potential Oxidative Enzymes. *J. Appl. Environ. Biol. Sci.*, 1(4) 54-68.

[32] Miranda, P.M., Benito, G.G., Cristobal, N.S., Nieto, C.H.,1996. Colour elimination from molasses wastewater by *Aspergillus niger*. Bioresour Technol 57, 229–235.

[33] Bhole B D, Bharati G, Madhuram A, Deshpande D & Joshi J, Biosorption of methyl violet basic fuchsin & their mixture using dead fungal biomass, *Curre Sci*, **86 (**2004) 1641- 1645.

[34] K. Nanthakumar, K. Karthikeyan and P. Lakshmanaperumalsamy 2009 Investigation on Biosorption of Reactive Blue 140 by Dead Biomass of *Aspergillus niger* HM11: Kinetics and Isotherm Studies, Global Journal of Biotechnology, anf Biochemistry 4 (2): 169-178.

[35] Bireller E.S., Aytar P., Gedikli S. and Cabuk A. 2012. Removal of some reactive dyes by untreated and pretreated *Saccharomyces cerevisiae*, an alcohol fermentation waste.71, 632-639.

[36] Bishnoi N. R., Kumar R., Bisnoi.2007. Biosorption of Cr(VI) with *Trichoderma viride* immobilized fungal biomass and cell free Ca-alginate beads. Indian Journal of Experimental Biology. 45, 657-664.

[37] Taghi Ganji M., Khosravi M and Rakhshaee R. 2005. Biosorption of Pb, Cd, Cu and Zn from the wastewater by treated *Azolla filiculoides* with H2O2/MgCl2 International Journal of Environmental Science & Technology. 1, (4), 265-271,

[38] Fu Y & Viraraghanvan T, Column studies on biosorption of dyes from aqueous solutions on immobilized *Aspergillus niger* fungal biomass. *Water SA* **29** (2003) 465- 472.

[39] Kaushik G k and Thakur I S 2009 Adsorption of colored pollutants of distillery spent wash by native and treated fungus: Neurospora intermedia . Environment Sci and pollution research 20, (2), 1070-1078.

[40] Lee P C, Park C J & Yang J F Screening of hexavalent chromium biosorbent from marine algae. *Appl Microbiol Biotechnol,* **54** (2000) 597-600.

[41] Kapoor A & Viraraghavan T Fungal biosorption- An alternative treatment option for heavy metal bearing waste water; A review. *Bioresource technol,* 53 (1995) 195-206.

[42] Gadd G M Biosorption *Chem and Ind,* **13** (1990) 421-426.

[43] Shivyogimath C.B. and Inani S. 2014 Treatment of biomethanated distillery spent wash by adsorption process on bagasse activated carbon Int. Journal of Applied Sciences and Engineering Research, 3, 6, 1066-1075.

[44] Jaspers C J & Pennincks M J Adsorption effects in the decolorisation of a kraft bleach effluent by *P. chrysosporium, Biotechnol Lett* **18** (1996) 1257-1260.

[45] Volesky B, Advance in biosorption of metals: selection of biomass types. *FEMS Microbial Rev* **14** (1994) 291-302

[46] Kulkarni M.V (patil M.S.). Chavan M. N. A method for decolorization of distillery spent wash using dead fungal biomass. Indian Patent no. 229515. 18-February 2009.

[47] Langmuir, I., 1918. The adsorption of gases on plane surfaces of glass, mica and platinum. J. Am. Chem. Soc., 40: 1361-136.

[48] Hall, K.R., L.C. Eagleton, A. Acrivos and T. Ver , 1966. Pore and Solid diffusion kinetics in fixed bed adsorption under constant pattern conditions. Ind. Eng. Chem. Fund. 5(2): 212-223.

[49] Freundlich, H., 1906. Adsorption in solution. Phys. Chem. Soc., 40: 1361-1368.

BIOGRAPHIES

Dr. Mrs. M. S. Patil Working as the Assistant Professor in the Department of Biotechnology, Institute of Sciences, Aurangabad, Maharashtra, India since,2012. Teaching Post Graduate students. Actively involved research in areas viz, Bioremediation, Biodegradation, Phytochemistry, etc.

Dr. M. V. Kulkarni Working as the Professor in the Division of Biochemistry, Dept. of Chemistry Savitribai Phule University of Pune, Maharashtra, India since, Teaching Post Graduate students. Actively involved research in areas viz, Environmental Biotechnology, Abiotic stress, Medicinal Biochemistry, Bioremediation.