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BIOREMEDIATION OF DISTILLERY SPENT WASH (MELANOIDIN)-A NOBLE APPROACH

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Abstract

Melanoidin from distillery spent wash are natural condensation products of sugar and amino acids produced by non-enzymatic Maillard amino-carbonyl reaction taking place between the amino and carbonyl groups in organic substances. From environmental aspects melanoidins are very important due to their structural complexity, dark colour and offensive odor, which pose serious threat to soil and aquatic ecosystem This causes the problem, like reduction of sunlight penetration, decreased photosynthetic activity and dissolved oxygen concentration whereas on land, it causes reduction in soil alkalinity and inhibition of seed germination. Bioremediation is an ecofriendly technology for treating chemical spills and hazardous waste. It is considered highly desirable to exploit the biodegradation potential of soil microorganisms from polluted sites. Application of microorganisms like, Aspergillus niger , Leuconostocs sps , Bacillus sps, Staphyloccous aurius and Pseudomonas aeroginosa will be the cost effective biotechnology for treatment of water polluted by spent wash containing melanoidin.Experimental studies revealed that the individual organisms and their mixed consortia degraded the 75 to 80% concentrated spent wash, after the optimization of various physicochemical parameters the mixed consortia exhibited enhanced activity as compared to the individual cultures alone. The treated effluents were characterized by COD reduction, HPLC analysis.

Keywords: Melanoidin, Aspergillus niger, Leuconostocs sps , Consortia, Distillery Spent Wash, HPLC.

Introduction

Distillery spent wash is the residual liquid generated during alcohol production. It has been observed that a typical cane molasses based distillery generates 15 L of spent wash effluent per liter of ethanol produced. Around 212 distillery units in India generate more than 30 billion liters of spent wash annually (Chavan, M. N.; Kulkarani, M. V.; Zope, V. P.; Mahulikar,2006)) The most important characteristic of spent wash is it is strongly acidic, dark brown colored hydrophilic viscous liquid waste with strong objectionable odour. Dark brown color of spent wash is mainly because of the presence of polymeric melanoidin pigments formed by the non enzymatic amino carbonyl reaction means Millard reaction [31]Melanoidins are recalcitrant due to presence of caramel. Antioxidant natures of the pigments make them toxic to many microorganisms, including those present in waste water treatment processes. Agricultural land looses their fertility due to disposal of the spent wash directly into river. It also harms the aquatic system as its colored pigments reduce photosynthetic activity and depletes the dissolved oxygen in the water bodies. Spent wash polluted water has high biological oxygen demands, chemical oxygen demands , low pH, obnoxious smell.

Research of Objective

To study reduce the dark color, acidic PH, High BOD, high COD it is considered highly desirable to exploit the biodegradation potential of soil microorganisms from polluted sites microorganisms from the contaminated site. As such polluted soils can facilitates selection of biodegradative capability in microorganisms and may act as reservoir

Research Methodology

Materials: Sample Collection: Distillery effluent was collected from different Distillery division of Odisha. The contaminated soil was collected from the site nearby the distillery unit. The soil was collected by scrapping the top layer of soil and subsurface soil and packed in an air tight sterile PP bags.

Isolation of Organism by Enrichment Technique: As described by Kumar et.al, 1998 and some research Microorganism screening was done by enrichment the tubes showing decolorisation were subsequently sub cultured four times and isolation of microbial culture was carried out on minimal salt glucose medium by spread plate technique. The pure culture of different microbial isolates S1-S5 were maintained on minimal salt glucose agar medium containing 5% spent wash

Standard Melanodin Preparation: Standard melanoidin which is dark brown colored was prepared in laboratory by heating 1M glucose with 0.5M of glycine at 900C and pH-5.5 for 6 hr using hot air oven (Wedzicha, B.L.and M.T.Kaputo, 1992).The absorption maxima for standard melanoidin were measured at 450nm by double beam Spectrophotometer (Shimadzu) and a standard dose curve was prepared. For heating at 900C Hot air oven was used. Striking feature was that the absorption maxima of spent wash measured was also at 450nm.Hence the further degradation studies were performed at 450nm.

Degradation Studies: For mixed consortia loopful of pure culture of each isolate Pseudomonas aeroginosa, Aspergillus niger , Streptoccous sps , Bacillus sps and Staphyloccous, aurius from minimal salt spent wash agar media plate was transferred in 100 ml minimal medium with 60 % and spent wash in 500 ml flask and incubated at room temperature to study the degradation ability. After 10 hr interval, 5ml aliquot was withdrawn for assaying degradation. Non inoculated minimal salt medium and minimal medium with 60% and spent wash were used as a blank and control respectively (Chavan, M. N.; Kulkarani, M. V.; Zope, V. P.; Mahulikar, 2006). Isolates showing excellent results were selected for further study. Same procedure was followed for Aspergillus niger and Pseudomonas aeroginosa. COD and BOD Measurement: Chemical and Biological oxygen demand of the samples before and after the treatment were determined using potassium dichromate and Winkler's method respectively (Yamuna, B.G, Prasanth Kumar, M.K, Ranjini, T.N,)

Biochemical and Morphological Studies of the Isolates: Biochemical and microbial characterization of the isolates was done according to standard protocols (Harley J H &Prescot L M (eds),1996),.Species identification was supported by VITEK 2 System at Bac-test laboratory Nasik. Optimization of Physicochemical Parameters: Various parameters were optimized to achieve better degradation and COD removal activity by mixed consortia. In previous studies the same was performed for Staphyloccous,aurius ,Aspergillus niger , Pseudomonas aeroginosa . To study the effect of externally added carbon source on degradation activity and COD reduction activity 1g% each of glucose, fructose, maltose, sucrose and starch was added separately in minimal salt medium containing 60% spent wash (Chavan, M. N.; Kulkarani, M. V.; Zope, V. P.; Mahulikar, 2006).

Optimum glucose concentration required for color removal and COD reduction by the isolate was determined by taking glucose concentrations in the range of 0.1-1.0 %. To study

the effect of various nitrogen sources, ammonium chloride, ammonium sulphate, peptone, yeast extract and casein hydrolysate were added in the minimal medium at 0.4 g% concentration. Effect of pH on the degradation activity was determined by using the spent wash medium adjusted to pH values within the range of 4 to 9. Optimum temperature required for decolorisation was determined by incubating the isolate in the culture medium at different temperatures within the range of 25 to 45 0C. Different concentrations of spent wash such as 20, 40, 60, and 80% supplemented in the mineral medium as optimized above, were inoculated with the isolate and maximum spent wash concentration utilized by mixed consortia for decolorisation Different concentrations of spent wash such as 20, 40, 60, and 80% supplemented in the mineral medium as optimized above, were inoculated with the isolate and maximum spent wash concentration utilized by mixed consortia for decolorisation and degradation was studied.

HPLC Analysis: HPLC Analysis was carried out and about 25 µl of sample was injected to the HPLC unit, mobile phase was acetonitrile: water in the proportion of 44:55ml (v/v) +1.0 ml glacial acitic acid +0.5g of sodium acetate 3H2O, pH of the system was 5.2. Flow rate of the system was 0.8ml/min changes in the peak length were the clue for the degradation of melanoidin in spent wash.

Biodegradation Studies: Decolorisation was followed by spectrophotometric measurements at 450nm, which is λmax of melanoidin and spent wash. The effect of various sugars as externally added carbon source in the spent wash medium on decolorisation and COD reduction activity was studied.

Declorisation was found to be more in presence of all carbon sources used with respect to control, and was found to be maximum in the presence of glucose.

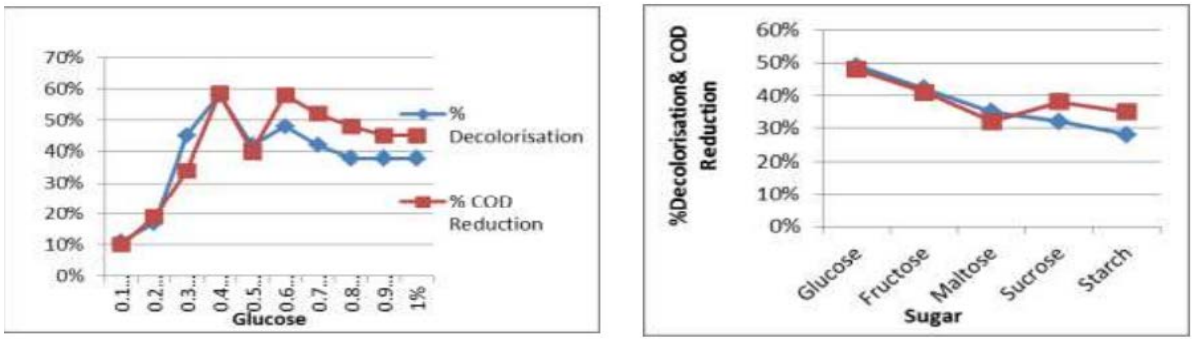

Figure 1 Effect of Different Concentration of Glucose on % Degradation and COD Removal and Effect of Different Types of Sugars on % Degradation and COD Removal

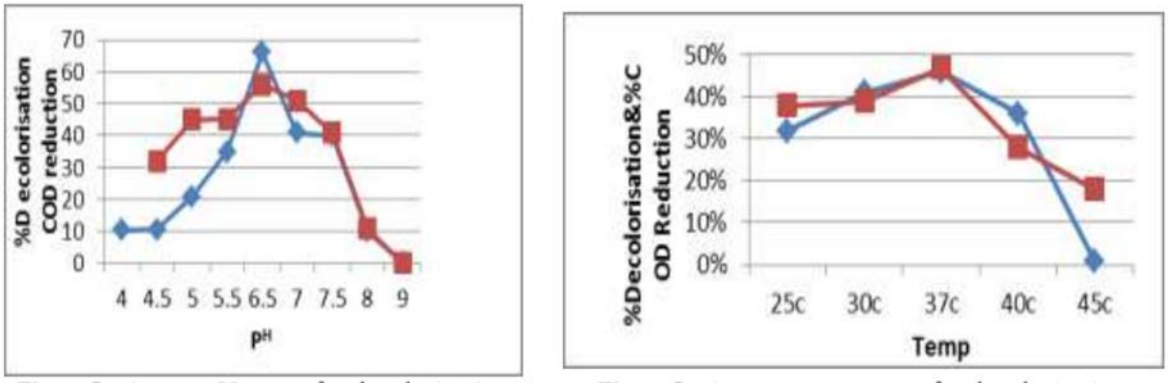

Figure 2 Optimum pH range for decolorization COD and COD removal and Optimum temperature for decolorization and removal

Table 1 Percent Reduction in Color and COD of Treated Spent Wash under Optimum Performance.

Time (hr)	% Decolorisation	%COD Reduction
24	25	28
48	38	41
72	58	59

Whether or not melanoidin is the main component responsible for color in the spent wash was confirmed by HPLC analysis.

Table 2 HPLC Profile of Melanoidin Degradation Study

Sample	Retention time	Area	Height	Concentration
A(Before treatment)	2.423	1180608	50575	100.0000
B(48Hr treatment)	2.435	1084859	46092	100.0000
C(72 Hr)	2.699	895421	45650	87.3203

After spectrophotometric observations further analysis of degraded spent wash was performed by HPLC. Various peaks of degraded spent wash using mixed consortia were compared to the peaks of standard melanoidin as good results were obtained by mixed consortia. The observed changes in peaks of IR spectra analysis with respect to control indicate the conversion of complex compound in to simple forms .An important structural feature of melanoidin is the presence of conjugated C=C and C=N bonds which imparts color these polymers .The above four bacterial and one fungal strain employed during the present research work might have brought about the cleavage of ethylinic C=C and Azomethine C=Nlinkages. This conjugation may be broken down due to enzymatic oxidation. This is very significant from the toxicological implications of discharging spent wash effluent in the water bodies.

Optimisation of Physicochemical Parameters: Glucose in 0.4% concentration as carbon source was found to be optimum for decolorizing activity and above 0.58% glucose there was decreasing in decolorizing activity. This effect might be due to the acidic conditions produced in the medium after incubation by all organisms, inhibiting to the microbial growth. There was no effect of externally added organic and inorganic nitrogen on decolorisation efficiency. Nitrogen from the spent wash might be sufficient for the growth of the mixed consortia from

the data it is clear that the mixed consortia were utilizing nitrogen from spent wash contents. Maximum decolorisation and COD reduction was found within the pH range of 6-6.5 the preferred range for the growth of the mixed consortia .Temperature range of 370C was found to be suitable for activity of the isolate (Fig 4).The medium composition was optimized as (g/l, glucose-4; KH2PO4-0.2; MgSO4- 0.009; pH-6.5, and temperature of 370C. Under the optimum conditions mixed consortia were able to decolorize the spent wash by 59% and COD reduction by 57% after 72hr of incubation for 80% spent wash.

Percent reduction in color and COD of treated spent wash under optimum performance conditions are shown in Fig.1 to Fig.2.

Discussion of Research Results

As the melanoidin content molasses spent wash is highly recalcitrant waste product. Treatments of Distillery spent wash (Gaurang Trivedi, I.R. Gadhv,2015) by physical and chemical methods are found mainly unsuitable on industrial scale (Shah, B. A.; Shah, A. V.; Singh, R. R.,2009).It is now realized that microbial treatment provides safer, more efficient and less expensive alternative to physico-chemical methods for decolorisation, degradation as well as mineralization of spent wash.

Degradation of melanoidins was also confirmed by spectrophotometric analysis as decrease in optical density of melanoidin at its λmax; appearance of new peak in the spectra with respect to control was the clue for suggesting effective degradation. Physicochemical analysis of the spent wash effluent before and after treatment with the mixed consortia is presented in the table.

Table 3 Physicochemical Analysis of the Spent Wash Effluent Before and After Microbial Treatment

Sr No	Parameters	Before treatment	After treatment
1	Color	Dark Brown	Light Brown
2	Odor	Strong pungent	Mild
3	PH	4-4.3	6.5
4	BOD(mg/L)	60,540	34,350
5	COD(mg/L)	95680	43500
6	Total sugar(mg/L)	12,3000-90,000	700-1300
7	Total dissolved solids	7800	1600
8	Iron(mg/L)	124	80
9	Magnesium(mg/L)	2550	240
10	Sulphates (mg/L)	980	770
11	Free chlorides(mg/L)	7000	650
12	Phosphorus(mg/L)	4850	400
13	Oil and Grease(mg/L)	174	170

Appreciable reduction in case of most of the parameters were observed, especially color, BOD, COD, etc. This is very significant from the toxicological implications for discharging spent wash effluent in the water bodies.

Several researchers have investigated the role of microbial community in the degradation of melanoidins in the spent wash. Bacillus and Xanthomonas in immobilized form are reported to degrade the color causing material in the spent wash (Ghosh M,Verma SC,Mengoni A& Tripath A K,2004) Most of the bacterial strains like Pseudomonas, Acetobactor, Aeromonas sp. are reported to be capable of degrading some of the recalcitrant

compounds in the an aerobically digested distillery spent wash. In a two stage bioreactor using Pseudomonas putida and Aeromonas sp. has achieved color and COD reduction by 60 and 44.4% respectively (Ghosh M,Verma SC,Mengoni A& Tripath A K, 2004).

The fungus Corriolus hirstus . The fungus Corriolus hirstus, exhibited ability to decolorize melanoidin by 74% in GPY medium Malakootian, M.; Nouri, J.; Hossaini, H., ,2009), White rot fungi Phanerochaete chrysosporium, decolorised MSW (6.25% v/v) supplemented with glucose 9.25g/L) by 85% after 10 days of incubation. . Lactobacillus hilgardi is reported to decolorize melanoidin solution 28 %. In all of these cases 0.4-3% sugar either glucose or sucrose with essential nutrients were added and decolorisation required 7-10 days also the spent wash concentration was less. Pseudomonas aeroginosa used in this work was capable of giving 55% & 57% reduction in color and COD respectively with 80% spent wash within 72hr with externally added glucose 0.5% .

The indigenous isolate Aspergillus niger used in this work was capable of giving 58% & 57% reduction in color and COD, respectively with 60% and 80% spent wash. Within 72hr with externally added dextrose 0.4%.

While mixed consortia was capable to give 59% decolorisation and 57%COD removal for 80% spent wash with externally added glucose 0.4%. Use of mixed consortia for decolorisation of 90% and 100% spent wash in progress. Better decolorisation was observed with 20%, 40% and spent wash using the above strains like Pseudomonas aeroginosa, Aspergillus niger, Streptococcus sps, Bacilli sps and by mixed consortia.

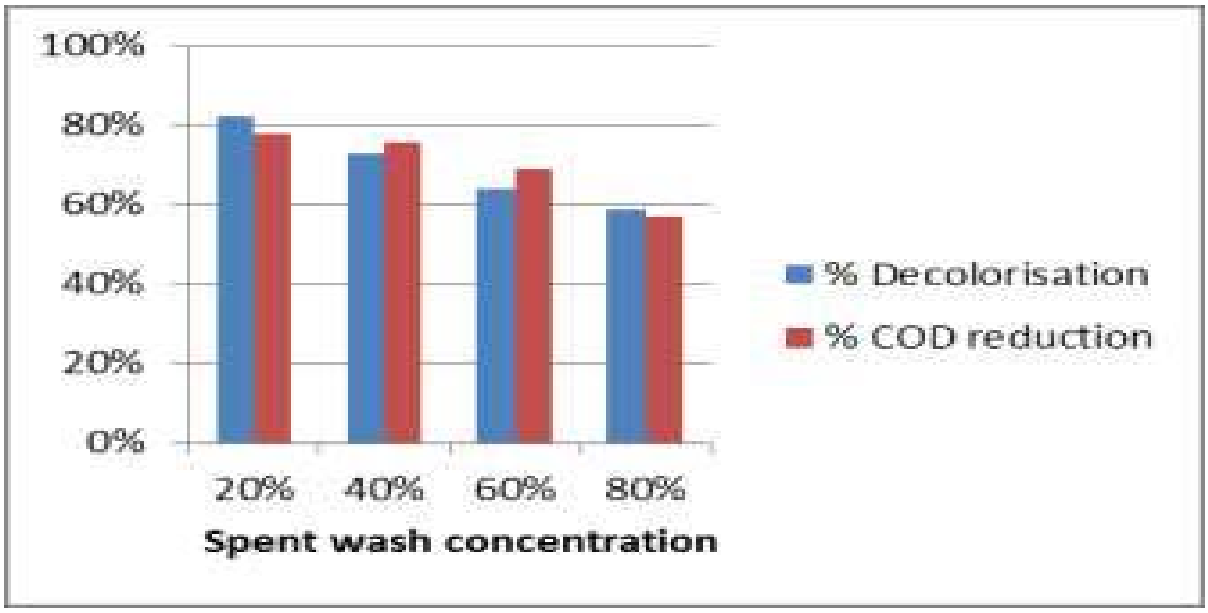

Figure. 5 Optimization of Spent Wash Concentration for Degradation by Mixed Consortia

Addition of readily available external carbon source was found to be necessary for metabolism of microbes in the spent wash medium. Although spent wash contains huge amounts of sugar but its easily metabolisable carbon content is almost negligible Gaurang Trivedi, I.R. Gadhvi,2015) Growth pattern of the isolate with respect to color removal indicated that within first 24 hr growth was initiated but without any decolorisation, but after 24 hr gradual increase in growth with decolorisation was observed up to 72 hr.

This effect can be explained as during initial phase organism utilizes easily metabolisable carbon source added to the medium and later on begins to degrade spent wash

components for carbon source (Gaurang Trivedi, I.R. Gadhvi, 2015). When both decolorisation and COD reduction were monitored as a function of time, the results showed that with the increasing decolorisation activity there was notable COD reduction and it was profound between 24-72 hr.

The probable mechanism of decolorisation and COD reduction might be through enzymatic degradation as sugar oxidase, and manganese dependant peroxidase have been reported for microbial degradation of melanoidins (Deppendra Singh, Reddy, P.B,2015)

Suggestions

Mixed consortia were found to be more efficient in decolorization of 60% spent wash along with melanoidin degradation in comparison to earlier reports. As the highly concentrated spent wash is decolorized by the above strains and mixed consortia this approach can be further exploited to develop a cost effective, eco-friendly biotechnology package for the treatment of concentrated distillery spent wash.

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