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DEPORTATION OF MELANOIDIN IN DISTILLERY SPENT WASH USING BACTERIAL CONSORTIA - CONFIRMS THROUGH STRUCTURAL CHANGES AND AO-EB FLUORESCENCE

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ABSTRACT

Distillery spent wash maintains very dark brown color even after aerobic and anaerobic treatment due to the available of various organic and inorganic residues, obstinate and coloring compounds mainly melanoidin. In this study, melanoidin decolorizing bacterial strains were isolated and enhanced the growth rate of selected bacterial colonies at normal incubation temperature at laboratory conditions. The optimal decolourization of melanoidin was achieved at pH 7 and temperature is 37ºC on 3rd day of refinement. The phytotoxicity evaluation with mung bean (*Vigna radiata*) using two concentrations 1% and 5% revealed that the raw distillery effluent was environmentally highly toxic as compared to biologically treated distillery effluent, which indicated that the effluent after bacterial treatment is environmentally safe. This denotes the isolated bacterial strains competed to degrade and detoxification of melanoidin from the distillery effluent. Initially screening of 3 different bacterial strains were done in liquid cultures and were used as consortium for biosorption experiments and they have showed different decolourization ability. The minimum percentage in the consortium was observed on the first day by 14% and it increased up to 95% on the eighth day. The HPLC analysis of dye before and after degradation has five peaks during the retention time 3.00 and 6.50 min, whereas degradation products showed peaks at lower retention time, probably due to degradation of dye into small intermediate products. The melanoidin degrading products and growth population of bacterial strains were analysed by using UV- Visible Spectrometric, High Performance Liquid Chromatography (HPLC) and structural characterization through Scanning Electron Microscopy (SEM), Confocal Laser Scanning Microscopy (CLSM).

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INTRODUCTION

In India during the year 2006-07 the annual production of ethanol is about 2300 million liters (Subramanian *et al*., 2005). In 2009, 319 distilleries produced 3.25 × 109 L alcohols and have generated 40.40×10^{10} l of spent wash per year (Bharaghava *et al*., 2009). The primary product namely pure ethyl alcohol is processed in distilleries. Approximately 2.7 billion litres of alcohol were produced annually. Such production has resulted in the generation of spent wash, an unwanted residual liquid waste. As this waste contains organic compounds such as polysaccharides, reduced sugars, lignin, proteins, melanoidin and waxes it has increased the BOD in spent wash (Wedzicha *et al*., 1992). Conventional batch type and continuous type distilleries produce spent wash of about 10 – 15kL per 1kL of alcohol.

Distilleries generate two types of waste water such as non-process and process waste water. Derived products and the value added chemicals like ethyl acetate, acetic acid, and acetaldehyde are also produced as by products (Sankaran *et al*., 2014). As the highly acidic untreated distillery spent wash contain excess amounts of organic and inorganic residues, it affects the productivity of natural aquatic streams and thereby the water become unusable (Chandra, 2004). Release of distillery effluent into aquatic environment depletes the dissolved oxygen and interfering with respiratory metabolism cause heavy mortality of many living organisms (Quesim and Siddique, 1960; Venkataraman, 1966; Hingoroni *et al*., 1979). Further, the Ministry of Environment and Forests (MoEF), Government of India, announced the distilleries are top of the "Red Category" industries (Tewari *et al*. 2007).

Distillery industries discharge their waste waters into the water systems thereby it has reduced the water availability and has resulted in the outbreak of water related diseases (WPNWAC, 2003). Distillery spent wash are perilous and posing threat to the human life, water bodies and soil. Dye is one of the major pollutants present in the effluents of textile, plastic, distillery, food and cosmetic industries (Rafatullah *et al*., 2010). Raw industrial dyes are more toxic, carcinogenic, mutagenic and teratogenic in nature (Oxspring *et al*., 1996). Waste water released from fermentation and distillery industries results in aquatic pollution due to the melanoidin pigment present in the effluent too. This colouring compound is reported to be highly resistant to the microbial occurrence and to the conventional biological processes too (Evershed *et al*., 1997).

Anaerobically digested effluent is dark brown in colour with high chemical oxygen demand of about 90,000 mg/l (Fitzgibbon *et al*., 1995) and this offers the effluent bioremediation resistance (Sirianuntapiboon *et al*., 1988). Organic pollutants concentration is normally higher in anaerobically treated distillery effluent (Nandey *et al*. 2002).The effluent containing toxic chemicals such as heavy metals, acids, pesticides, persistent organic compounds and toxic salts when discharged into the soil they lead to environmental corruption (Kaushik *et al*., 2005; Garg & Kaushik, 2006&2008). Irrigation with industrial effluents is reported to damage the germination of seeds in various crops depending upon the types of effluent (Kaushik *et al*., 2005; Garg & Kaushik, 2006 & 2008).

Table 01. Physicochemical parameters of distillery spent wash. All values expressed in mg/L.

S:No	Parameters	Values (mg/l)
1.	Color	Dark Brown
2.	pH	3.2
3.	Electrical Conductivity	6.6114mmhos
4.	Temperature	30. 70 C
5.	Total solids	626
6.	Total dissolved solids	360
7.	Total suspended solids	266
8.	Dissolved Oxygen	10.14
9.	Biological Oxygen Demand	58.33
10.	Chemical Oxygen Demand	1440

Different Tertiary treatment techniques were employed for the decolourization of distillery spent wash. It includes activated carbon (Bernardo *et al*., 1997), charcoal (Chandra and Pandey, 2000), pyorchar (Ramteke *et al*., 1989) an inorganic flocculent (Migo *et al*., 1993), ammonium sulfate (Chandra and Singh, 1999), oxidation process (Beltran *et al*., 1997) and wet air oxidation (Dhale and Mahajani, 2000.

Recently, waste water containing melanoidins were treated by the chemical precipitation and carbon adsorption. Though these processes are effective, there are certain disadvantages such as, high operation cost and consumption of chemical agents, fluctuation in color removal and high volume of sludge/solid waste production (SuntudSirianuntaiboon, 2003). Therefore, alternative treatment for industrial wastes and effluents minimization could be therefore, microbial mediated degradation and decolourization. Utilization of microorganisms like bacteria, fungi, yeast and algae for the biodegradation of complex, toxic and recalcitrant in the distillery spent wash is preferable as the process is eco-friendly and cost competitive (Mohana *et al*., 2007; Chavan, 2006, Pant and Adholeya, 2007). Molasses spent wash with a higher concentration of melanoidin was treated with *Coriolus versicolor* Ps4a (Aoshima *et al*., 1985), *Aspergillus fumigates* G-2-6 (Ohmomo *et al*.,1987), *Rhizoctonia sp*. D-90 (Sirianuntapiboon *et al*.,1991), *Lactobacillus hilgardii* (Ohmomo *et al*., 1988) and *Bacillus sp.* (Nakajima *et al*., 1999). Sharma *et al.* (2002) reported that the toxicity and higher concentration of distillery effluent may cause the defects on seed germination, seedling growth and inhibit the growth hormones. Consequently, parameters like pH, temperature, aeration and nutrients are involved in the microbial degradation and decolourization of industrial effluents (Kumar *et al*., 2006), Tewari *et al* (2007). Additionally, nano filtration (NF) and reverse osmosis (RO) were used by (Nataraj *et al*., 2006). Different studies have reported that various bacterial strains *Lactobacillus hilgardii* (Ohmomo *et al*., 1988a) *Bacillus* sp. (Kambe *et al*., 1999), *Pseudomonas putida* (Ghosh *et al*., 2002), *Bacillus thuringiensis* (Kumar and Chandra, 2006) and *Pseudomonas aeruginosa* (Mohana *et al*., 2007) decolourize the distillery spent wash. Since longer time biodegradation of complex, toxic and recalcitrant compounds present in distillery spent wash was decolourized by fungi, bacteria, yeast and algae (Moriya *et al*., 1990; Sirianuntapiboon *et al*., 2003).

Melanoidin

Melanoidin is a water soluble recalcitrant compound which gives colour to the distilleries and fermentation industries waste water (Evershed *et al*., 1997). The brown coloured pigment called melanoidin is reported to be produced by non-enzymatic amino carbonyl reactions, which is known as maillard reaction. Higher amounts of antioxidants present in melanoidin were reported to be the reason for the toxicity to the microorganisms (Chavan, 2006). However, Melanoidin are naturally present in foods and drinks too (Painter, 1998). But, Melanoidin do not have any definite structure still its elemental composition are not fully understood (Ikan *et al*., 1990). Melanoidin is reported to hinder the sunlight penetration in water bodies, reduce the photosynthetic activity and dissolved oxygen. It is reported to be pernicious to the aquatic biota. Brands *et al*. (2000) demonstrated that the ketose sugars showed a remarkably higher mutagenicity compared with their aldose isomers and generated active oxygen species resulting in DNA strand breaking and mutagenesis. The toxicity effect of undiluted distillery effluent spent wash in fresh water fish *Cyprinuscarpio*var *Communis* has been estimated (Mahimairaja and Bolan, 2004).Mutagenicity and DNA strand breaking activity of melanoidins from a glucose–glycine model was demonstrated by Hiramoto *et al*. (1997). Melanoidins are renowned as charged acidic compounds.

Different composition of melanoidin is based on the reaction conditions, temperature, heating time, pH, water and the nature of reactants (Coca *et al*., 2004). In vitro studies confirmed that the maillard reaction products (MRPs) act as reducing agents (Hayase *et al*.; 1996), metal chelators (Wijewickreme and Kitts, 1997) and radical scavengers (Monti *et al*., 1999). Physicochemical treatments may remove the melanoidin but it requires high amount of reagents and are found to produce large amount of sludge (Pena *et al*., 2003). Hence, microbial decolourization and degradation is an environment friendly and cost competitive alternative to chemical decomposition processes (Moosvi *et al*., 2005). Hence in the present study, the bacterial strains isolated from the physico chemically characterized distillery effluent were employed for the decolourization of the effluent. These strains were subjected for molecular characterization too.

MATERIALS AND METHODS

Sample Collection

Distillery spent wash was collected from a distillery unit located at Perundurai, Erode district, Tamil Nadu, India. The effluent was characterized in terms of pH, Electrical conductivity, color, temperature, total dissolved solids (TDS), total suspended solids (TSS), Dissolved oxygen (DO) ,biological oxygen demand (BOD), chemical oxygen demand (COD) and conductivity as per standard methods(APHA,2005).

Isolation of microorganisms from the distillery effluent

Accurately 1ml of the distillery effluent was added to 9ml of sterile distilled water. Then, 1ml from each dilution (10^{-4}–10^{-7}) was transferred aseptically into sterile nutrient agar containing Petri plates. The plates were further subjected for incubation at 37°C ± 2°C for 24hrs for the isolation of bacterial strains. The morphologically divergent colonies were picked, streaked and stored for further use.

Decolourization studies

A loopful of pure culture of each isolate from nutrient broth spent wash media plate was transferred into the nutrient medium with 1% and 5% spent wash in 250 mL flask and were incubated at room temperature to study the decolourisation ability. Dilution of distillery effluent was found to be necessary to reduce the level of toxic ingredients in the spent wash. After 24 h interval, 5-mL of the aliquot was withdrawn for assaying decolourisation. The extent of decolourization was calculated by measuring the optical density of supernatant of treated medium at 475 nm using an UV visible spectrophotometer (systronics). Uninoculated nutrient agar medium and nutrient broth medium with 1% and 5% effluent were used as blank and control. Consortium was found to show better results when compared with individual isolates.

Bacterial resistance

The isolated bacterial cultures were inoculated with glass spreader and 100µl of the synthetic melanoidin of each concentration (10 ppm, 50 ppm, 100 ppm) was added in the corresponding well of agar plates. Then the plates were incubated for 24Hrs and zones of resistance were observed.

Phytotoxicity test

Mercuric chloride

The seeds of mung bean *(Vigna radiata)* were taken and were washed with 0.1% of mercuric chloride. The germination tests were carried out in petri dishes at 26 ± 2^0C. Ten seeds were sown at equidistant in sterilized 10 cm petri dishes lined with Whatman filter paper. Ten replicas were used for each treatment. Then 5ml of untreated, treated, consortium of 1% and 5% concentration was added to the petridish.
A control experiment was carried out using raw effluent. Seed germination was observed at an interval of 24 h for 8 days.

Catalase assay

Qualitative test

The enzyme catalase splits hydrogen peroxide into water and oxygen. When small portion of the colony is introduced into H_2O_2, rapid evolution of bubbles indicates the positive result. To the 24 hrs old culture plate, 1ml of 3% H_2O_2 was added. Release of bubbles was observed and it was compared with the control.

Quantitative test

About 5 ml of 0.1M phosphate buffer was added to test tubes. 1 ml of the enzyme preparation was added to the buffer. The flask was swirled gently. About 1.0 ml of this reaction mixture was withdrawn and was injected into 2.0 ml of dichromate/ acetic acid reagent. Each test tube was heated for ten minutes in a boiling water bath to decompose the blue precipitate and produce a green solution. The absorbance was measured at 570nm in the spectrophotometer. Using the "standard curve", the H_2O_2 left in the solution was estimated (Sinha, 1972).

Scanning Electron Microscopy (SEM)

VEGA3 TESCAN Scanning Electron Microscope is used to identify the porous structure difference of the treated and untreated effluent. This SEM set up was at a resolution 486x,712x, 1.03kx, 1.12kx and the magnification at 50µm and 20µm to the before and after treatment of effluents.

High performance liquid chromatography (HPLC) analysis

The HPLC analysis was performed for estimation of degradation efficiency and degradation products. The degraded samples (100 ml) were centrifuged at 10,000 rpm for 15 min at 25 ± 1 _C, and mixed with equal volumes of ethyl acetate and kept in the desiccators for water removal using anhydrous sodium sulphate. The solution was further dried using rotary evaporator. The control was also exposed to similar step. The residue thus obtained was dissolved in 5 ml of methanol and subjected to HPLC analysis (Water HPLC, Model no. 2690) on a reverse phase C18 column (5 mm, 4.6, 250 mm) at 35 _C. Acetonitrile at 10–90 % gradient was used as the mobile phase and the flow rate was adjusted to 1.0 ml/min. Analysis was carried out for 20 min and the peaks were identified using a photodiode array detector at 294 nm.

Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy is one of the highest resolution light microscopy methods available. Using 63X objectives (Zeiss LSM710, with 1009 oil objectives) with very

high optical aperture and oil immersion, one can reach a maximum resolution of approximately 0,2µm in XY axis and 0.7 µm in XZ axis. In our particular setting, the smallest and most important object to observe are the individual GFP expressing bacteria, which are around 2 µm long and 0.2 µm of diameter. Therefore, it is possible to detect individual bacteria with a reasonable resolution, using confocal microscopy (Bettencourt, 2010). Acridine orange (AO) was first reported as a fluorescent microscopy stain in 1940. It can enter both live and dead cells and has unique properties in differentiating between DNA and RNA as it gives green fluorescence (at 502 nm) when bound to DNA and orange fluorescence (at 650 nm) when bound to RNA(wing-tak wong,2015).

Data analysis

Graph pad prism 6 was used to analyses the data. Due to the higher toxicity concentration in environmental samples, samples data may not be able to produce a full sigmoidal curve. Therefore linear regression was applied on the linear portion of the data points and the obtained values were used to calculate the sample values. Melanoidin and hydrogen peroxide (H_2O_2) standard curve also plotted by the same batch of experiment. The data points showing decolorizing values were fitted with the linear regression.

RESULTS AND DISCUSSION

Characteristics of the aerobically treated distillery spent wash

After sample collection physicochemical characteristics were analysed. The raw distillery spent wash contain higher concentration of pH, BOD and COD. The results were shown in (Table no: 1). Distillery spent wash is reported to be a highly complicated waste with higher BOD, COD, TDS and colour possessing high quantities of melanoidin posing threat to the environment and remaining as a challenge for proper and effective treatment. The conventional distillery spent wash treatment process includes need high operation cost and consumption of chemical agents, colour pertaining even after treatment, high amount of sludge production, hazardous by products and higher energy requirements (suhuttaya jiranuntipon 2009).

Isolation of bacteria from the distillery effluent

About 3 different morphologically distinct colonies were screened and were isolated from the distillery effluent in the melanoidin containing nutrient agar medium. The cultures were purified and stored for further use. The organisms identified from the distillery waste water include the bacterial genus such as *Micrococcus sp., Enterobacter sp., Shigella sp.*, (Table no: 3). Hence, decolourization and detoxification of distillery spent wash is a serious environmental concern which is evident from the research done in the last decade (sirianuntipiboon 2004). However, microbes have been employed for the treatment of distillery spent wash *P.putida* produces hydrogen peroxide which is a strong decolourizing agent (Jain *et al* 2000). Sirianuntipiboon 2004 first reported the decolourization activity of the acetogenic bacteria. Three different bacterial strains *B.megaterium, B.cereus and B.fragairae* isolated from the activated sludge of distillery industry to remove the 68% colour of the spent wash (Jain *et al* 2000).

Further, Valderrna *et al*., 2002 reported that micro algal treatment reduces the nutrients and organic sources in the waste water. Melanoidin pigment was decolourized by the extracellular hydrogen peroxide and peroxidase production by *coriolus hirusulus* (miyata *et al* 1998).

Table 03. Biochemical assay confirms the bacterial genus such as *Micrococcus sp., Enterobacter sp., Shigella sp*.

Test name	S1	S2	S3
Gram staining	+	-	-
Shape	Coccus	Rod	Rod
Motility	-	+	-
Oxidase	-	-	-
Methyl red	-	+	-
Voges proskauer	-	-	-
Citrate	-	+	-
Catalase	+	+	+
TSI	-	-	-
Indole	-	-	-
Urease	+	-	-
Name of the genus	*Micrococcus sp.,*	*Enterobacter sp.,*	*Shigella sp.,*

Decolourization of distillery spent wash using bacterial strains

Decolourization and degradation of distillery spent wash has been a severe environmental concern (Pena *et al*., 1996; Dahiya *et al*., 2001a,b; Sirianuntapiboon *et al*., 2004a,b). The recalcitrance of melanoidin compounds escape various stages of waste water treatment plant and finally, enter the environment. Inspection of literature specified that there is only less studies regarding microorganisms as pollution alleviators and there is none on degradation of melanoidin. In the present investigation, four strains, belonging to four different genera were screened for their degrading/decolorizing ability of 1:1 (v/v) diluted aerobically treated spent wash without any nutrient amendment. The capacity of the organisms to reduce colour ranged from 10–80% and the order of their degrading efficiency is higher in consortium than individual strains (Fig: 4).

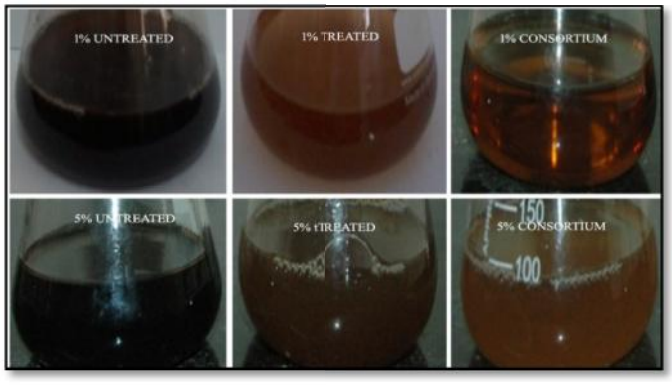

Fig 4: The sample containing 1% and 5% nutrient broth were prepared and inoculated with the isolated three individual colonies and consortium cultures which were isolated previously from the distillery effluent were subjected for incubation over shaker at 32°C for 8 days

Hence the study reveals that the indigenous microbes have great potential and are likely to be used as bioremediation agents for distillery effluent. Initially screening of 3 different bacterial strains were done in liquid cultures and were used as consortium for biosorption experiments and they have showed different decolourisation ability.

There was an increase in the decolourization percentage of the medium containing effluent day by day. The minimum percentage in the consortium was observed on the first day as 14% and it increased up to 95% on the eighth day (fig:5).

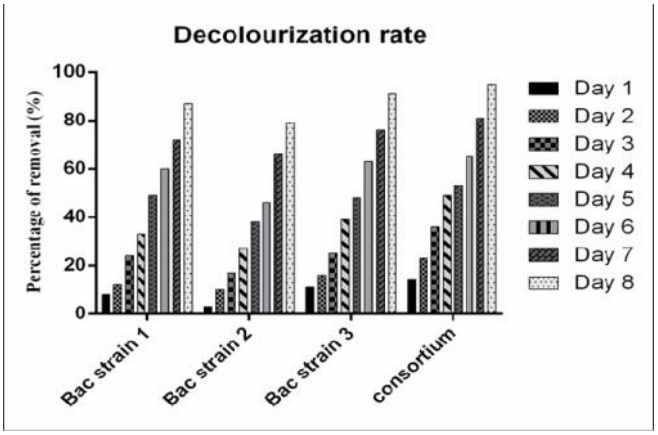

Fig 5. The minimum percentage in the consortium was observed at the first day by 14% and it increased up to 95% on the eighth day. Similarly among the individual cultures the S3 culture was better for decolourization as it went up to 91% of decolourization followed by the S1 with 87% and S2 with 79%. There was a steady growth of the organism hence removal was found to be in steady state too.

Bacterial resistance against melanoidin

Bacterial cultures isolated from the distillery effluent were tested against melanoidin in volume of 100µl of 10 ppm, 50 ppm and 100 ppm concentrations. All the isolated bacterial strains showed resistance towards the distillery effluent. Since the strains were isolated from the distillery effluent they had shown resistance towards the effluent Zones of resistance ascertaining significant activity were tabulated (Table No: 02). Similar result was reported by Geetha, (2002).

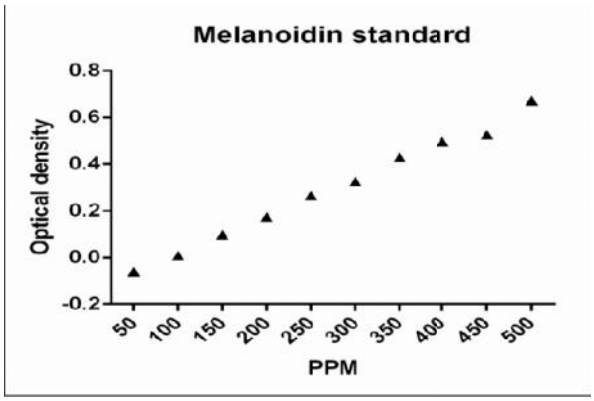

Fig 1: The 50 ppm to 500 ppm concentrations was prepared and the melanoidin standard values were observed at 475 nm in UV spectrophotometry

Phytotoxicity test in *Vigna radiata:*

Petri dish having *Vigna radiata* seeds with 1% and 5% treated distillery spent wash showed initiation of germination on first day and attained well growth in final day, whereas no germination was observed with non-treated raw distillery effluent. It was observed that the effluent before treatment was very toxic which inhibited the growth of seeds but the toxicity of effluent decreases after treatment.

Table 2. Bacterial cultures were tested against melanoidin in volume of 100µl of 10 ppm, 50 ppm and 100 ppm concentrations. Zones of resistance ascertaining significant activity were tabulated

S. No	Isolated cultures	Resistance against Melanoidin (mm)		
		10 µm	50 µm	100 µm
		1.1	0.4	0.0
1.	S1	0.7	0.0	0.0
2.	S2	1.6	0.8	0.3
3.	S3			

Various parameters were showed % germination of No of leaves, Stem length, Root length, Total length, and Sub root (Fig: 9 & 10).

Fig 9: First day germination and fifth day germination. It shows 100 % germination of *Vigna radiata* was observed with treated wastewater. Only 50 and 10 % germinations were observed for wastewater contains high dye concentrations. Final day 5% untreated effluent plate seeds growth were inhibited by fungal growth.

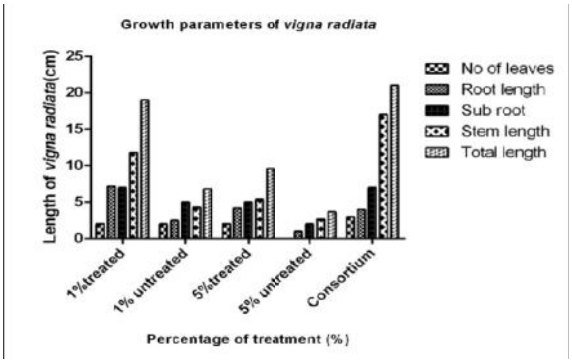

Fig 10. Growth parameter shows the total number of leaves, root length, sub root, stem length and total length of the *Vigna radiata* germinated in treated, untreated and consortium treated distillery spent wash

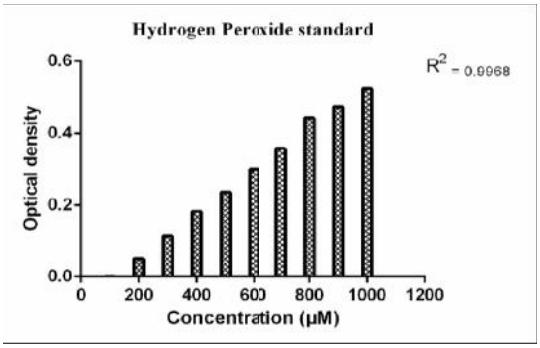

Fig 6: The standard graph of hydrogen peroxide was constructed using the 0.2 M hydrogen peroxide and the graph corresponds to 100 µM to 1000 µM concentration of the hydrogen peroxide

Catalase Assay

The qualitative analysis for catalase suggested that all the three cultures produced catalase positively. In quantitative assay, the standard graph of hydrogen peroxide was constructed using the 0.2 M hydrogen peroxide and the graph corresponds to 100 µM to 1000 µM concentration of the hydrogen peroxide(Fig 6). The production of catalase peaked on the fifth day of the cultivation and gradually decreased thereafter. The S3 culture produced high unit of the enzyme that it catalysed 900 µM of hydrogen peroxide. Followed by the S1 isolate produced the enzyme which decomposed 600 µM of hydrogen peroxide (Fig: 7).

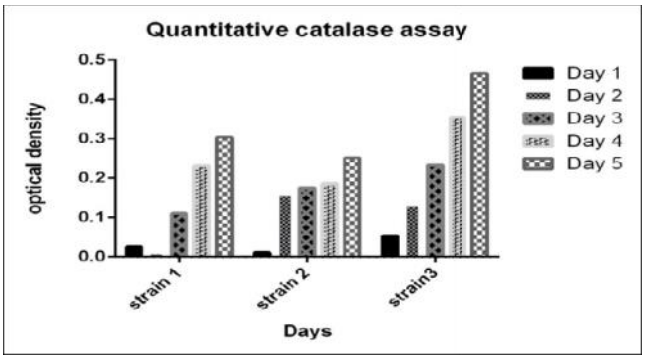

Fig 7. The production of catalase peaked on the fifth day of the cultivation and gradually decreased thereafter. The S3 culture produced high unit of the enzyme that it catalysed 900 µM of hydrogen peroxide. Followed by the S1 isolate produced the enzyme which decomposed 600 µM of hydrogen peroxide

Degradation analysis using HPLC

Melanoidin, which is the main pigment, instigated the color in the distillery spent wash. The amount of degradation was confirmed by the analytical HPLC analysis. The dye before and after degradation has resulted in the low retention time of 3.00 and 6.50 min probably due to the degradation of dye into small intermediate products.

A reduction in the peak areas of degraded samples compared with the control sample was observed by Bharagava *et al.* (2009). There were differences in the rate of color disappearance between control and treated 1% and 5% distillery spent wash and consortium while no differences were shown in two treated groups. Major decolourization process proves that *Micrococcus sp., Enterobacter sp.,* and *Shigella sp.,* could degrade the dye from the spent wash as confirmed by HPLC and UV spectrometry. The major achievement of this method is that consortium could degrade the recalcitrant melanoidin from the spent wash, as evidenced by HPLC and spectrophotometry.

Marine fungus *Flavoden flavus* was employed for the decolourization and detoxification of the 10% distillery spent wash. *Penicillium decumbens* was found to reduce 74% of the phenolics content and 40% of the color in beet spent wash (jimnez *et al* 2003). Mohana *et al* (2007) reported that bacterial consortium has decolorized both organic and in organic nitrogen sources from melanoidin containing distillery spent wash. The bacterial strains have showed highest decolourization of 82% and 80% when they were added along with yeast extract within short span of 5 days (sirianuntipiboon *et al* 1999).

Scanning electron microscopy (SEM)

Scanning electron micrograph images of the adsorbents were obtained in order to understand its surface morphological characteristics. The SEM images of bacterial consortia culture samples before and after usage as biosorbents. The samples were exposed for 192 hours of biosorption in the aqueous solutions. Over the sorption period it can be seen that the morphology of the particles had undergone remarkable physical disintegration. SEM images clearly showed the morphological disintegrated structure of the sorbents.

Confocal laser scanning Microscopy (CLSM)

The CLSM micrograph confirms the mortality and immortality rate of bacterial cells in different effluent samples.

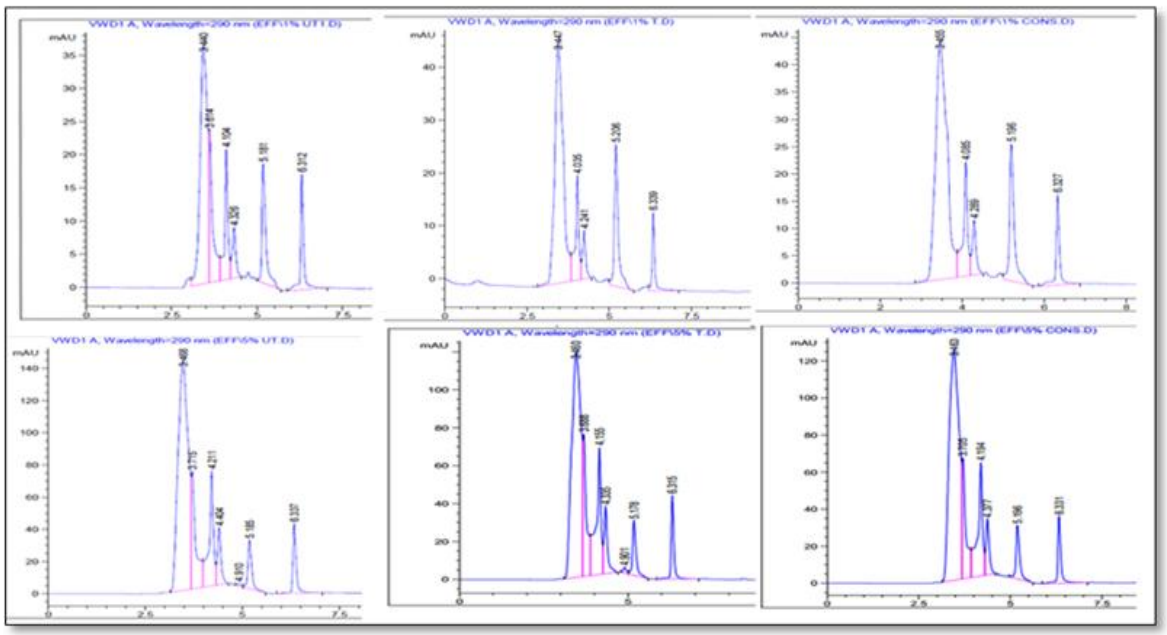

Fig 11: Before and after degradation shows five peaks during the retention time 3.00 and 6.50 min, whereas degradation products showed peaks at lower retention time. Differences in the rate of color disappearance between control groups (1% and 5%untreated) and treatment groups (1% and 5% treated effluent and consortium) while no differences were shown in two treatment groups

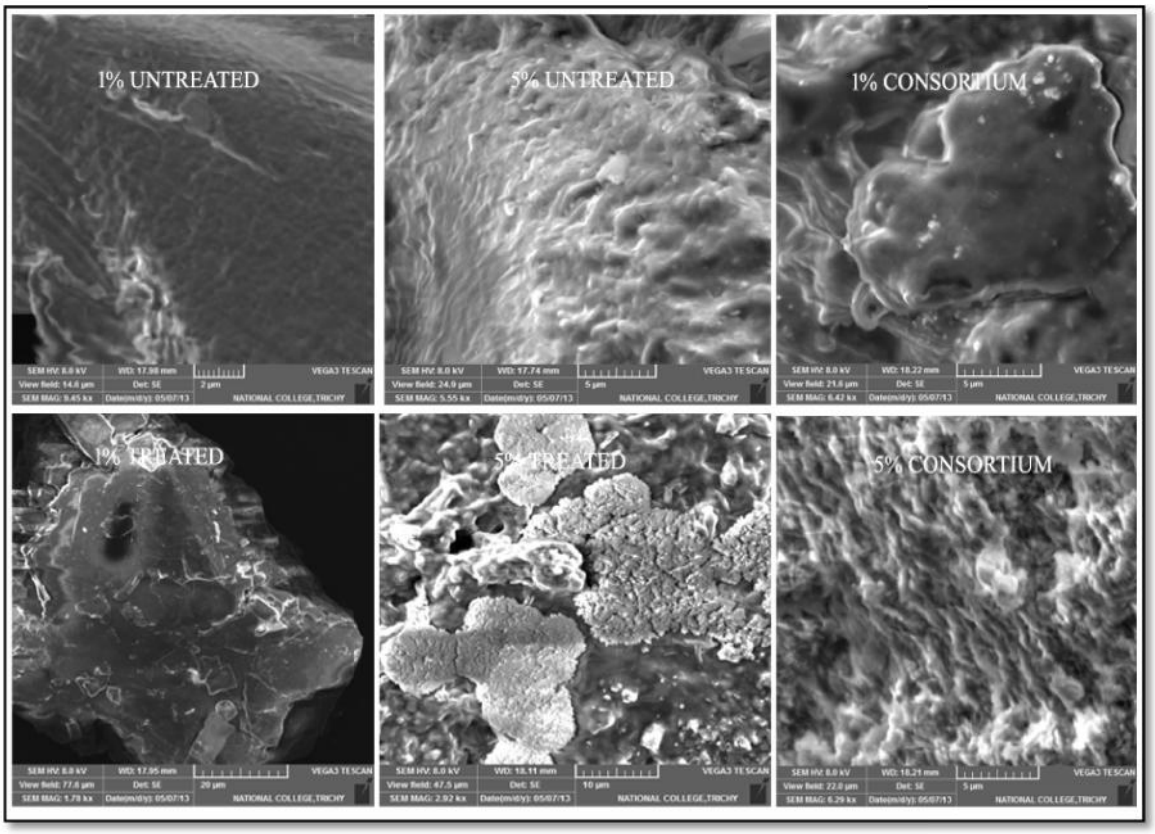

Fig 12: The SEM micrograph of bacterial consortia culture samples before and after usage as biosorbents. The samples were exposed for 192 hours of biosorption in the aqueous solutions. Over the sorption period it can be seen that the morphology of the particles had undergone remarkable physical disintegration. SEM micrograph clearly showed the morphological disintegrated structure of the sorbents

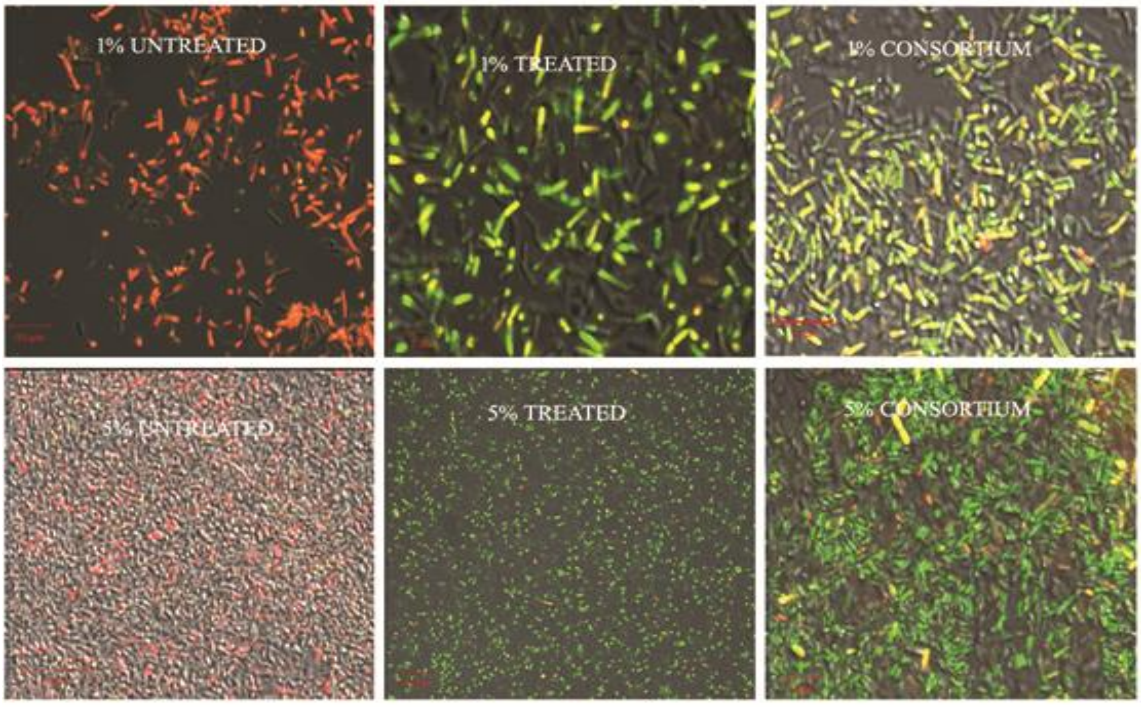

Fig 13: (AO/EB) staining image confirms the absence of live bacterial cells in the untreated effluent samples. The treated distillery effluent both 1% and 5% shows the presence of live a bacterial cell which has been proved by the confocal laser scanning microscopy (CLSM) images. Similarly the consortium has fluoresced well than the individual cells

Whereas the treated distillery effluent both 1% and 5% shows the presence of the live bacterial cells which has been proved by the fluorescence. However the performance was better with 1% than with 5%. Similarly the consortium has fluoresced well than the individual cells.

It proves that high bacterial population increases catalase production higher in consortium which may be the reason for decolourization of melanoidin in distillery spent wash.

Conclusion

The main interest of recycling water is to be related with the management of the effluents by reducing fresh water consumption and waste water treatment costs, small disposal volumes which will minimize the waste disposal costs and reduction. Microbiologically, the distillery effluent was flourishing with a variety of potential microbes (Bacteria). Similarly among the individual cultures the S3 culture has shown higher efficiency for decolourization, up to 91% followed by S1 with 87% and S2 with 79%. Further 100 % germination of *Vigna radiata* was observed with treated wastewater and control, whereas, only 50 and 10 % germinations were observed for wastewater having dye concentrations 100 and 1,000 mg/l, respectively .The consortium has fluoresced well than the individual cells in the 1% treated effluent which may be the possible reason for the higher efficiency of the consortium to decolourize the effluent Hence the study reveals that the indigenous microbes have great potential and are likely to be used as bioremediation agents for distillery effluent. Initially screening of 3 different bacterial strains were done in liquid cultures and were used as consortium for biosorption experiments and showed different decolourisation ability for the aqueous melanoidin solutions. Hence the results of the study may be used for the treatment of the distillery effluent.

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