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Biodegradation and decolourization of biomethanated distillery spent wash

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Abstract: A bioremediation method was optimized to degrade and discolour the biomethanated distillery effluent. This phytoremediaion involved a dual stage microbial treatment. During primary treatment, fungal consortium was employed using fluidized film aerobic system (FFAS) and during secondary treatment, algal biomass either in free state (powder form) or in immobilized condition (alginate beads) was employed. The analyzed effluent at the end of FFAS treatment showed a reduction of ~70% in BOD and ~63% in COD without causing any color change. However, at the end of the secondary treatment with algal biomass resulted in a reduction of ~80% in COD and effected 75% decolourization. The optimized conditions for discolouration in the packed bed column were 1.5mm size of immobilized beads, 3.5cm height of packing, 300ml/l spent wash flow rate, 20 H/D ratio of column for immobilized algae and 4cm packing height, 400ml/l spent wash flow rate and 20 H/D ratio of column for algae without immobilization. The efficiency of discolourization by algal biomass remained unaffected by immobilization. An approach of this study could be used to develop a cost effective, ecofriendly biotechnology tool for the bioremediation of spent wash.

Key word: Biodegradation, biomethanated distillery spent wash, industrial effluent treatment, fluidized film aerobic system, phytoremediation.

Introduction

In a developing country like India, distilleries have become a major source of pollution as 88% of its raw materials are converted into waste and discharged into the water bodies, causing water pollution. In the distillery, for every litre of alcohol produced, about 15 liters of spentwash is released as wastewater.

At present there are about 315 distilleries in India producing 50 to 60 billion liters of effluent annually. Due to high biochemical oxygen demand of raw spent wash, application of anaerobic treatment technology with biogas recovery has been reported to be highly effective (Nandy *et al.,* 2002). Anaerobic treatment is an accepted practice and various high rate anaerobic reactor designs have been tried at pilot and full-scale operation (Lata *et al.,* 2002). However, anaerobically treated effluent still contains high concentrations of organic pollutants and as such cannot be discharged directly (Nandy *et al.,* 2002). The recalcitrant nature of effluent is due to presence of brown polymers melanoidin, caramel and alkaline degradation products. Melanoidin pigments are formed by the non-enzymatic amino carbonyl reaction i.e. Millard reaction (Raghukumar & Rivonker 2001). It possesses antioxidant property causing toxicity to many microorganisms involved in conventional wastewater treatment processes. Reverse phase thin layer chromatography identified gallic and vanillic acid present in spent wash (Fitzgibbon *et al*. 1997). Microbial decolourization and degradation is an environment friendly and cost competitive alternative to chemical decomposition processes (Moosvi *et al*., 2005, Kumar *et al*., 1998). In order to reduce the colour and COD, it is considered highly desirable to exploit the biodegradation potential of soil microorganisms from polluted sites exposed to recalcitrant compounds of distillery spent wash for prolonged periods (Kumar *et al*., 1998). As such polluted soils can facilitate selection of biodegradative capability in microorganisms and may act as reservoir of fungal communities capable of degrading pollutants. In Erode region of Tamil Nadu, Sugarcane is grown as a cash crop in a total area of one lakh acres of land with varying types of soils mainly supplying two sugar factories situated in that region. Large volumes of spent wash, characterized by high Biochemical Oxygen Demand (BOD), low pH, obnoxious smell, high Chemical Oxygen Demand (COD), and melanoidin polymers and extremely dark brown color, are generated from these distilleries. Since the quantity of spent wash released/day/distillery is about one lakh liter, it cannot be consumed in total for biocompost production. So far, there has been limited success in search for dual system of fungi for

biodegradation and alga for decolourization. This study is centered on the concept that Biomethanated Distillery Spent Wash (BMSW) could be treated using cost effective FFAS with fungal consortium aiding the degradation followed by packed bed column with suitable algal biomass as bioadsorbents for effluent decolourization. Hence, investigations was focused on screening and isolation of distillery spent wash biodegrading organisms from contaminated soil and various sites of distillery unit, optimizing operational parameters for maximal biodegradation and decolourization of anaerobically treated distillery effluent.

Materials and methods

The biomethanated distillery effluent sample was collected from Bannari Amman Sugars Ltd -Distillery Division, Periyapuliyur Erode (Dt), Tamilnadu. The samples were centrifuged at 10,000 rpm for 15 minutes to remove the solid particles and stored at 4^0C. The effluent was characterized for pH, Chemical oxygen demand (COD), Biological Oxygen Demand (BOD), Total Solids (TS), Total Suspended Solids (TSS), Total Organic Carbon (TOC), Total Nitrogen (TN), Phosphates and sulphates employing standards methods for examination of water and waste water (APHA 1995). Microbial strains were isolated from different sites of distillery units from hot spent wash (without dilution from fermenters), cool spent wash (from cooling towers), effluent after anaerobic treatment, activated anaerobic sludge, sample effluent from lagoons, wet soil, dry soil, soil from nearby farmer's field, and effluent from site undergoing phytoremediation and effluent of ETP. Altogether a total of 42 fungi were isolated, among which the following 11 fungi i.e., *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. japonicus, A. ustus, A. versicolor, Cladosporium cladosporioides, Curvularia lunata, Nigrospora sphaerica, Penicillium oxallicum* and *P. purpurogenum* were found to be active in biodegrading BMSW and tested under enrichment culture technique (Mohana *et al*., 2007). A loopful of pure culture of each strain were transferred into a 250ml conical flask containing 20% BMSW and incubated at room temperature under optimum conditions. After 6 hour interval, 5ml aliquot was withdrawn to assess the decolourisation and odour reduction. There was no change in the colour but the odour was less compared to spent wash.

After initial screening, the fungal consortium were allowed to grow in a rectangular glass tank (Ramakrishna Rao *et al.,* 2005) of dimensions: length-61.4cm, breadth- 27.8cm and height - 30cm. Oxygen was supplied to the tank through diffusers attached to the bottom of the tank. Fluidized Film Aerobic System (FFAS) was used for the primary treatment. Process parameters *viz*. nutrient/ microorganism ratio, oxygen requirement, flow rate of the effluent, and specific surface area of the plastic media were optimized.

The primary treated spent wash was withdrawn for testing its characteristics. The spent wash from the fluidized film aerobic tank was allowed to pass through the peristaltic pump to the packed bed column containing alga for the secondary treatment.

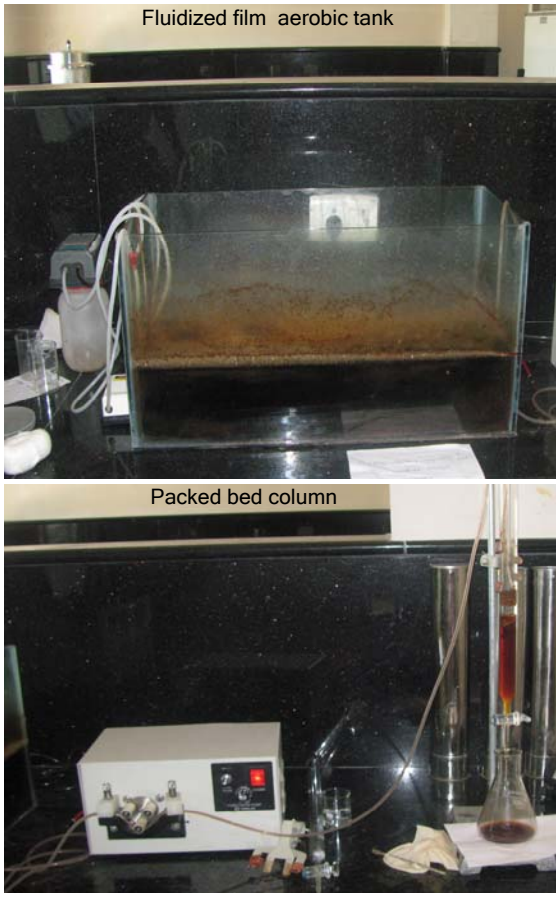
Initially ten different types of algae, collected from East coast of Tuticorin, were screened for their suitability in effluent treatment (results not shown) among which only three algae i.e. *Gracillaria edulis*, *Sargassum wightii,* and *Ulva lactuca* were subsequently chosen for the present study. In batch studies the dried algal biomass were used separately in the powdered form. Such experiment was carried out with 5g of algae each were mixed with primary treated spent wash in three 250ml standard conical flask under optimum conditions. The solution was agitated in a rotary shaker at 150rpm. The effluent characteristics were identified using standard method (Metgalf & Eddy,1998).

In another set of experiment, fifteen gms of *Sargassum weightii was* packed in the column and the primary treated spent wash was passed through it. Alternately the powdered biomass was immobilized and the results were compared. For immobilization, the powder of *Sargassum weightii* was sieved using 150µm dia pore size and powdered particles less than or equal to 150µm was used in immobilization. Fifteen gram of powdered biomass was mixed with 100 ml of 3% (wt.) sodium alginate solution. The concentration of sodium alginate varied between 6-12 % depending on the desired hardness. The beads were formed by dripping the polymer solution from a height of approximately 20 cm into an excess (100 ml) of stirred 0.2M $CaCl_2$ solution with a syringe and a needle at room temperature. The bead size was controlled by pump pressure and the needle gauge. A typical hypodermic needle produces beads of 0.5-2.5 mm in diameter. The beads were maintained in the calcium

Table 1. Process factors for optimization

Process factor	Conditions
Size of beads (mm) 0.5, 1, 1.5, 2 and 2. 5	Height of packing:3.5mm, Flow rate 200ml / lit, and Size of the column 400H-20D (cm)
Height of packing (cm) 2.5,3.0,3.5,4.0, and 4.5	Size of the beads 1.5mm, Flow rate 300 ml / lit, and Size of the column 400H-20D (cm)
Flow rate(ml/lit) 100,200,300,400 and 500	Size of the beads 1.5mm Size of the column 400H-20D (mm), Height of packing:3.0mm
Size of the column (cm) 400H-20D, 500H-15D, 600H – 15D.	Flow rate 300 ml / lit, Height of packing:3.0mm , Size of the beads 1.5mm

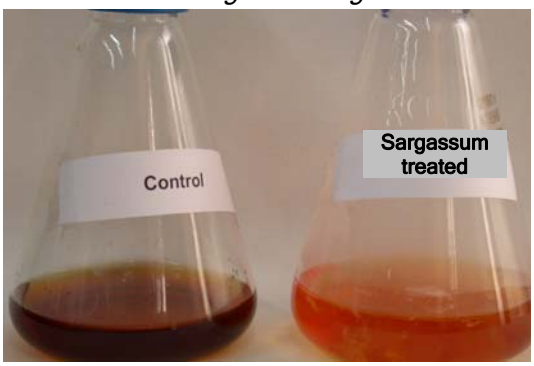
Fig. 1. Laboratory Experimental Setup for treatment of Biomethanated Distillery Spent Wash



Chloride solution to cure for 0.5-3 hours. The biomass was immobilized within the solidified sodium dried for 2days at room temperature and irregularly shaped beads were discarded. Sieve analysis was used to determine the size distribution of the beads. The sieve openings (mm) were 2.5, 2, 1.5, 1and 0.5. Beads greater than 2.5mm and smaller than 0.5 mm were discarded.

Different sizes of 0.5 to 2.5 mm immobilized biomass beads were packed in to glass column of diameter of 400 cm height and 20cm diameter size. At the top and bottom of the beads in the column, one layer of gravel with a height of 3 cm was used to distribute influent dye solution and support beads, respectively. To maintain a water head (~ 5 cm) above the top of the beads in the column, the outlet was set at a certain level, almost the same as the designed water level in the column (Fig. 1). The process parameters and other conditions varied for column studies were given in Table1.

Fig. 2. Flasks showing decolourisation of secondary treated Spent Wash by Sargassum wightii



Results and discussion

Decolourization and degradation of distillery spent wash has been a serious environmental concern, which is evident from the magnitude of research done in this field in the last decade (Dahiya *et al.*, 2001 a, b; Malik & Malik Amrita, 2000; Mohan *et al* ., 2007; Pant & Adholeya 2007; Pena *et al.*,1996; Sirianuntapiboon *et al.*, 2004a, b). The recalcitrance of melanoidins to biodegradation is apparent from the fact that these compounds escape from various stages of waste water treatment plant and finally, enter the environment, stringent regulations on the discharge of coloured effluent help to check the direct discharge of such effluent into the environment. In the present investigation bioremediation of the biomethanated distillery effluent was studied by employing dual stage microbial treatment. During primary treatment,

fungal consortium was employed as FFAS and immobilized algal biomass during secondary treatment.

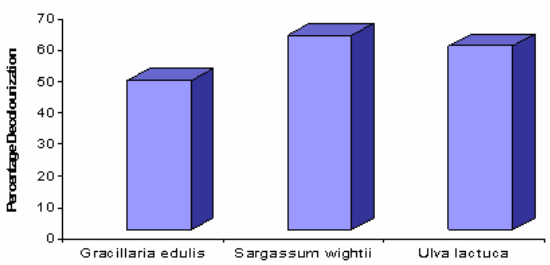
percentage decolourization is high for hight to diameter ratio of 20 (400mm/H- 20mm/D).

Table 2. Physico chemical analysis of the effluent before and after treatment

Parameters	Before treatment	After FFAS Treatment (Primary)	After Algal treatment (Secondary)	
			With immobilization	Without immobilization
Colour	Dark brown	Dark brown	Light brown	Light brown
Odour	Odour	Less odour	Less odour	Less odour
pH	3	6.8	7.2	7.2
BOD (mg/ L)	70800	20500	15000	12500
COD (mg./L)	156380	58150	32250	30150
Total sugar (mg/ L)	135000 - 95,000	500- 1000	500- 1000	450 - 880
Total dissolved solids (mg/ L)	9000	1580	500- 1000	450 - 880
Sulphate	1250	485	485	385
Phosphopus	5650	460	460	325
Free chlorine	6250	860	860	728

The characteristics of biomethanated distillery spent wash before and after FFAS treatment is presented in the table 2. Physical chemical analysis of the effluent showed 71.05%BOD and 62.8% COD reduction. There was no change in the colour of the effluent.

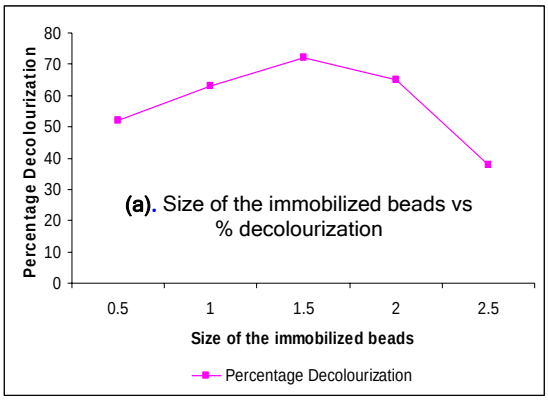
Fig. 3. Effect of different algae on percentage decolourization

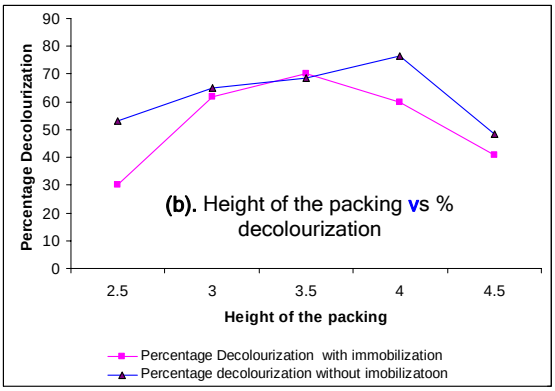


Batch studies were conducted using three algae and the % decolourization was shown in the Fig. 3. Among the three organisms *Sargassum wightii* showed maximum (62.5%) decolourization (Fig.2) which has been used further for column studies in comparison with immobilized beads.

The primary treated spent wash is allowed to flow in a packed bed coloumn packed with *Sargassum wightii.* Decolourization was found by Bio Spectro Photometer (BL198 Elico, UV visible) by measuring the effluent at 475 nm, which is the λmax of melanoidin. Fig.4. shows that decolourization percentage decreases with increase in size of the biomass above 1. 5mm, packed height of 3.5mm. The algal biomass effectively decolorized up to 300ml/l and above which the percentage remains constant. The

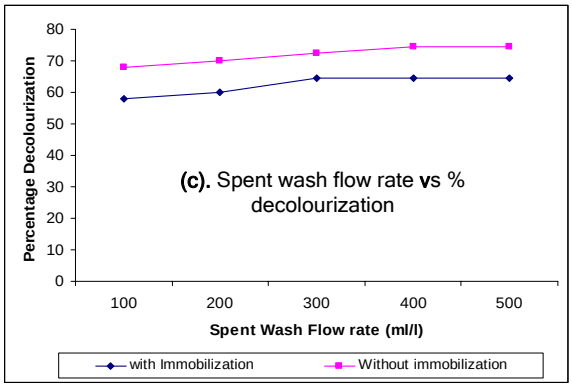
Fig 4. Percentage decolorization with various process parameters (Fig.a - d)



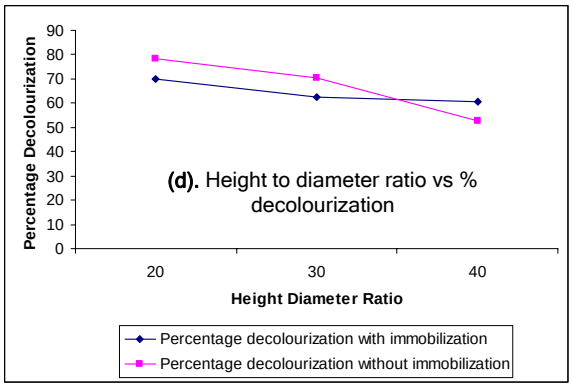


The optimized process parameters are 1.5mm size of immobilized beads ,3.5cm height of packing,300ml/l spent wash flow rate, 20 H/D ratio of column for immobilized algae and 4cm packing height,400ml/l spent wash flow rate and 20 H/D ratio of column were optimized process parameters for algae

without immobilization. With the optimized parameters the spent wash was allowed to



(c). Spent wash flow rate **vs** % decolourization



(d). Height to diameter ratio vs % decolourization

flow in the column packed with and without immobilized biomass. The treated effluent from the column was collected, analyzed for its characteristics and the results were shown in the table 2. Physico chemical analysis of the effluent before and after microbial treatment shows that 79.35% COD, 74% reduction in colour with immobilized alga and 80.72% COD and 76% reduction in colour without immobilization. While the hydrolytic property of the fungal degradative enzymes is well known, the discolouration of effluent by algal treatment might be due to various factors including the production of hydrogen peroxide, hydroxyl anions and molecular oxygen, released by the algae (Francisca *et al* ., 2001).

Conclusion

Application of fluidized film aerobic system with fungal strains isolated from the sites of distillery for biodegradation and the use of algal strains in the packed bed column could be a pragmatic approach for treating the biomethanated distillery spentwash. This study for the first time, has opened up the possibility of treating distillery spent wash using the dual system.

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