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Deportation of Distillery Melanoidins Using Plant Based Adsorbents, Biosorbents and Microbial Inoculants-A Comparative Assessment

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ABSTRACT

The comparison of Adsorption and Bioremediation techniques were observed in the present study for the removal of Melanoidins from the distillery effluent. The bacterial cultures isolated from the sludge were tested for their decolourization potential. The identification of culturable bacteria by 16S rDNA based approach showed that the consortium composed of Micrococcus sp., Enterobacter sp., Shigella sp., Alkaligenessp., Klebsiella sp.,and Bacillus sp. The degradation studies affirmed up to a maximum of 85 % of decolourization of melanoidins with the bacterial consortium. The enzymatic activities of catalase determined the reduction by the microbes were may be purely due to the extracellular enzyme (catalase) that was producedduring the metabolization of melanoidins by the microbes. The equilibrium adsorption studies using both the type of adsorbents and biosorbents revealed that the degradation of melanoidins increased with increasing time intervals. SEM analysis confirms the adsorption of the melanoidins by the differences in the pores of the adsorbents and biosorbents used.

Keywords: *Distillery effluent, Melanoidins, Adsorption, Bioremediation, SEM analysis*

1. INTRODUCTION

Enrage medicinal, pharmaceutical and flavoring, alcohol constitutes the feedstock for large number of organic chemicals. India produces about 2.75 billion liters of alcohol annually. The demand for the alcohol for various purposes has been increasing year by year with the rise in the population in our Indian society. Though it is practiced all across the world, the use of alcohol as an amalgam in motor fuel were not permitted in India in olden days, which resulted in the neither capacity utilization of distillation facility. Due to the government's aggrandizement to mix ethanol in petrol there will be a drastic demand for ethanol, which could overcome the existing unutilized capacity and thus creating an excess demand [1].

The alcohol producing industries, called Distilleries are one of the major polluting Agrochemical Industry. About 88% of the raw material used in the industry ends up as waste. For a liter of ethanol production about 15 liters of spent wash is produced which in turn pollutes the environment. The major issue is treating of this spent wash as it contains high organic content and the color of the effluent. The effluent contains 2% (W/W) of recalcitrant brown colured polymer called Melanoidins and is the main source of the color of the effluent [2]. Melanoidins are the Nitrogenous polymer and the byproducts of the Maillards reaction. They affect the photosynthetic activity of the aquatic plants by preventing the penetration of sunlight into the aquatic ecosystems [3]. When the farm lands are exposed to these recalcitrant compounds containing spent wash for a prolonged period they are prominently converted into polluted sites.

Melanoidins not only affect photosynthesis but also exhibit antioxidant properties that pose toxicity to aquatic and terrestrial organisms. There are many conventional treatment processes for the treatment of the distillery effluent such as Activated sludge treatment process, Coagulation, Membrane treatment [4] and Chemical methods [5]. But only 6-7% of melanoidin degradation is reported to be exhibited by the aerobic-anaerobic effluent treatment processes [6]. Hence among the physico-chemical treatment methods adsorption is widely used due to its low cost and feasibility.

Hence in the present study the effect of operational parameters on decolourization of distillery effluent by prepared low cost adsorbents and the performance of the constructed bacterial consortium for treating the wastewater were investigated.

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2. EXPERIMENTAL

2.1 Sample collection:

Distillery effluent sample was collected from one of the distillery industries located at Trichy, Tamil Nadu, India.

2.2 Physicochemical analysis of the Distillery Effluent

Distillery wastewater is characterized primarily by measurement of BOD, COD, color, total dissolved and suspended solids. In addition to these parameters pH, E.C, alkalinity, turbidity, total hardness, calcium, magnesium, phosphate, sulphate and nitrate were also determined [7].

2.3 Isolation and Screening of micro-organisms from the sludge

The screening of microbes, having the ability to degrade the melanoidin containing distillery effluent was done by the enrichment technique as prescribed earlier [8]. The tubes that showed the maximum decolourization was chosen and sub cultured and the subsequent pure colony isolation was done by spread plate technique on a glucose containing melanoidin amended nutrient agar medium. The pure cultures of the isolates S1 to S6 were maintained in the same medium for further decolourization studies. Microbial consortium was prepared by inoculating all the obtained individual bacterial colonies into single nutrient broth. This consortium medium was used as an inoculum for the treatment of the distillery effluent.

2.4. Preparation of Adsorbents

2.4.1. Natural material

The dried plant of *P. juliflora* was collected from the nearby areas and they were powdered, sieved and preserved for the further studies. It is referred to as Prosopsis procured natural material.

2.4.2. Preparation of Activated carbon:

Activated carbon was prepared by treating 50 g of dried plant material with 50 ml of concentrated sulphuric acid. It was kept in a hot air oven maintained at 105 ± 5 ºC for 24 h. The char resulted was washed with water followed by the addition of 2% solution of sodium bicarbonate solution until effervescence ceased and then it was kept in 2% solution of sodium bicarbonate overnight. The char was then separated and washed with water until it was free from bicarbonate and was dried. The acid washed material was repeatedly washed with water to remove the free acid and then dried it was at 110 ºC. Activated carbon with particle sizes in the range of 0.2-0.5 mm (400-500 ml) was used for further studies [9].

2.4.3. Preparation of microbial coated adsorbents (Biosorbents)

The above mentioned two adsorbents were mixed with the isolated bacterial consortium to be biosorbed and were maintained in boiling water bath for 2 hours. Then the dead cells are coated on the adsorbents to form biosorbent material. The Biosorbent material were then used for adsorption.

2.4.4. Characterization of Adsorbents and Bioadsorbents

The prepared adsorbents were characterized by different parameters like Apparent density, Moisture content, Ash content, Water soluble matter, Acid soluble matter, Decolourizing power, pH and Surface area. [10].

2.5. Biodegradation Assay

The degradation studies using the isolates were carried out in 250ml Erlenmeyer flasks containing 200 ml of nutrient broth containing 1 gm of glucose and 5% distillery effluent. The conical flasks were inoculated with 5 ml of the microbial consortium. The conical flasks were kept on a magnetic shaker and were agitated for a pre-determined time interval at a constant speed. The decolourization was recorded spectrophotometrically at 475 nm every day.

2.6. Characterization of microbes by Biochemical Analysis and Molecular Characterization

The isolated bacterial isolates were identified morphologically and biochemically using the standard procedures [11].The total DNA of the isolates was prepared from overnight grown cultures [12]. Further for the amplification 5 µl of the DNA sample was used along with the Universal 16s rDNA eubacterial primers 27 F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492 R (5'-TACGGYTACCTTGTTACGACTT-3') were used for the PCR amplification (Applied Biosystems Vertis Thermocycler). The amplicons amplified were gel purified using Quiagen Gel purification kit and partially sequenced. The sequences were compared using BLAST analysis to identify the identity of the isolates.[13].

2.7.Catalase Assay

Catalase assay was performed by Dichromate acetic acid method [14]. About 5 mL of the 0.1 M 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 mL of this reaction mixture was withdrawn and was injected into 2 mL of dichromate / acetic acid reagent. Each test tube was heated for 10 minutes in a boiling water bath to decompose the blue precipitate and produce a green solution. The absorbance was measured at 570 nm in the UV-Vis spectrophotometer (Systronics Double beam Spectrophotometer 2202). Using the "standard curve", the H_2O_2 left in the solution was estimated.

2.8. Evaluation of decolourization using Adsorbents

The batch type adsorption experiments were carried out in 250ml Erlenmeyer flasks by mixing a pre-weighed amount of the adsorbent with 100ml of diluted distillery effluent of a particular concentration (1-5%). The conical flasks were kept on a magnetic shaker and were agitated for a pre-determined time interval at a constant speed. After adsorption was over, the mixture was allowed to settle for 10min. The color remaining unabsorbed was determined spectrophotometrically at 475 nm. The adsorption experiments were carried out under the following conditions:

Initial concentration of distillery effluent : 1-5%
Amount of adsorbent: Prosopsis Procured Activated Carbon (PPAC): 0.2 gms; Prosopsis Procured Natural Material (PPNM): 0.5 gm; Commercially Activated carbon (CAC); 0.1 gm
Amount of Biosorbent: Microbial Coated Procured Activated carbon: 0.2 gms; Microbial Coated Natural Material: 0.5 gm; Microbial Coated Commercially Activated Carbon; 0.1 gm
Agitation time (min): 15 to 120 min

2.9. SEM analysis

To observe the porous structural difference of the used adsorbents and the biosorbents SEM analysis have been carried out using VEGA3 TESCAN Scanning Electron Microscope. The SEM measures the surface morphology of conducting and non-conducting materials by analyzing Back Scattered Electrons (BSE) and Secondary Electrons (SE). In the present study the SEM analysis was performed to observe the physical morphology of the initial and final adsorbents and biosorbents.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analysis of the Distillery Effluent

The physicochemical properties of the effluent such as colour, odour, Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), pH, Electrical Conductivity (EC), Total Hardness, Calcium (Ca), Magnesium (Mg), Alkalinity, Chloride (Cl), Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Phosphate were analyzed and tabulated (Table.1).The physicochemical status of the effluent clearly shows that it has to be subjected for pre-treatment before releasing into the water bodies. The effluent showed a considerably high load of pH, TSS, TDS, BOD, COD and total hardness compared to the prescribed National Environmental Quality Standards (NEQS).

3.2. Isolation and Screening of micro-organisms from the sludge

Six morphologically distinct species were isolated and screened (S1 to S6) based on their decolourising ability of the distillery effluent. The observed results revealed that out of the six isolates S1, S3 and S5 were found to be fastidious in both growth and in degrading the effluent. Whereas the isolate S2 showed moderate growth and decolourization and in contrast the isolates S4 and S6 showed moderate growth and were found to be very slow in degrading the colour of the effluent. This may be due to the recalcitrant compound called melanoidins, which are the major colouring compounds in the distillery effluent which may attribute to the delay in decolourization of the effluent [15].

3.3. Characterization of Adsorbents and Bioadsorbents

The characteristics of the adsorbents were understood by performing the required analysis. The decolourizing power of MCCAC and CAC is higher when compared with that of all the other adsorbents which may be due to higher surface area of MCCAC and CAC.

The higher surface area of MCCAC and CAC may be due to the carbonization process involved. The surface area of MCCAC and CAC is 318 and 297 m^2/gm, which is a higher value when compared with that of all the other used adsorbents (Table. 2). Even the surface area of carbon prepared from *Calymperes delessertii Besch*, moss is reported to be 190 m^2/gm [16].

3.4. Biodegradation Assay

The cultures in the exponential phase were used for the experiments. The sample containing nutrient broth at 5 % concentration was prepared and inoculated with consortium cultures which were isolated previously from the distillery effluent were subjected for incubation over shaker at 32^{o}C for 8 days. In order to maintain the growth of the bacteria, 0.5 % of glucose was added as carbon source in the medium. An aliquot of 5 ml was withdrawn after decolourization and was centrifuged at 5000 rpm for ten minutes and the residual melanoidin contents were monitored spectrophotometrically at 475 nm. 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 (Table. 3 and Figure No.1) [17].

There was an increase in the decolourization percentage of the medium containing effluent day by day. The minimum percentage was observed on the first day and it was 52% and it increased up to

Table 1: Physicochemical analysis of the Raw Distillery Effluent.

S. No	Parameters	Values (mg/l)
1.	Color	Dark Brown
2	Odour	Objectionable
3.	pH	5.71
4.	Electrical Conductivity	39.74 mmhos
5.	Temperature	30.2°C
6.	Total Solids	11,40,000
7.	Total Dissolved Solids	7,00,000
8.	Total Suspended Solids	4,40,000
9.	Sulphate	2217.46
10.	Silicate	763.35
11.	Phosphate	255.8
12.	Nitrate	2324.28
13.	Chloride	23,742.60
14.	Dissolved Oxygen	-
15.	Chemical Oxygen Demand	1,56,000
16.	Biological Oxygen Demand	72,000

All values are exposed in mg/L except pH and EC

Table 2: Characterization of Adsorbents and Bioadsorbents

Parameters	PPAC	CAC	PPNM	MCPPAC	MCCAC	MCPPNM
Apparent density gm/ml	0.47	0.89	0.35	0.54	0.92	0.41
Moisture content %	13.84	6.62	18.62	12.32	5.35	15.7
Ash content %	0.94	2.56	0.46	1.23	3.07	0.77
Water soluble matter %	0.76	1.03	0.21	0.89	1.21	0.57
Acid soluble matter %	3.41	1.95	4.61	1.52	1.76	4.96
Decolorizing power mg/g	33.45	57.67	26.32	37.24	64.32	29.76
pH	6.9	7.1	7.2	6.9	7.1	7.3
Surface area (m^2/gm)	194	297	128	213	318	153

Table 3: Biodegradation Assay of distillery effluent by microbial consortium.

Days	% removal
1st day	52%
2nd day	59%
3rd day	63%
4th day	67%
5th day	71%
6th day	76%
7th day	80%
8th day	85%

be 85% on the eight day. There was a steady growth of the organism hence the removal was found to in steady state too. After eighth day the organisms entered death phase and hence no further decolourization was observed.

3.5. Characterization of microbes by Biochemical Analysis and Molecular Characterization:

Based on the biochemical analysis, the bacterial isolates S1 and S6 were found to be Gram positive rod and cocci respectively. The other isolates from S2 to S5 were identified as Gram negative rods. All the isolates showed negative results to Indole production test, Methyl Red test and Triple Sugar Iron test and were all positive for catalase test. The isolates S1, S3 and S5 where non-motile whereas in contrast S2, S4 and S6 were found to be motile. The strains S1, S2, S3 and S5 were observed as oxidase negative, while S4 and S6 showed positive results. Strains S1, S3, S4 and S6 showed negative results for V-P test and Citrate Utilization assay while S2 and S5 showed positive for the tests. S1, S5 and S6 showed positive reaction for urease activity while the other strains showed only negative reaction.

The amplicons that were amplified were 1500-bp long gene and were subjected to DNA sequencing with the universal internal primers (Plate No.1) [18].The sequences were then compared using NCBI BLASTN program. Pair wise alignments were done to find out a closest match. The

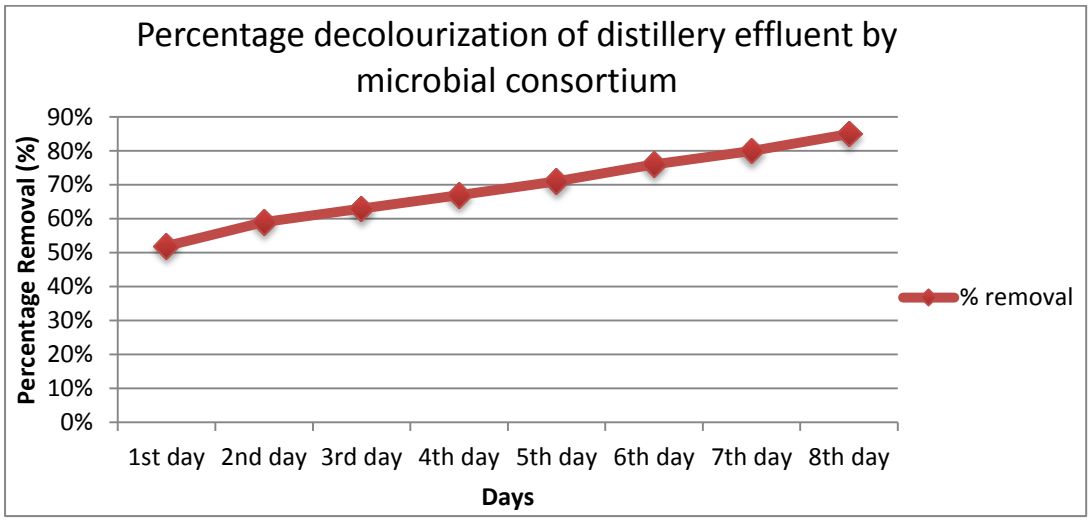

Figure 1: Biodegradation Assay of distillery effluent by microbial consortium.

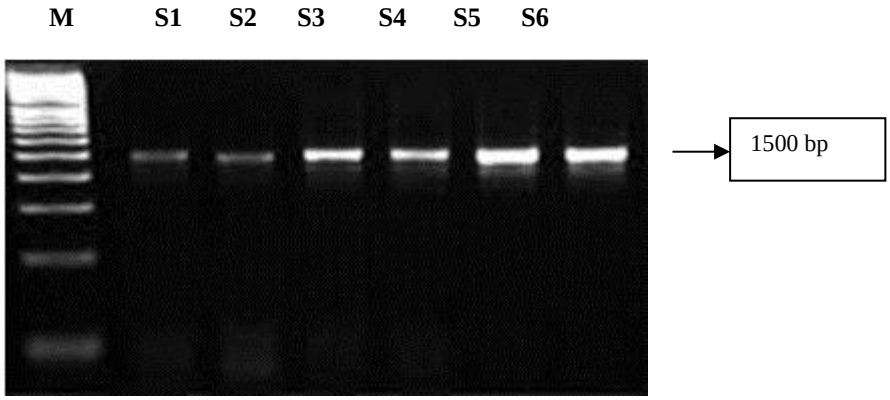

Plate 1: PCR Amplification Profile of the microbial isolates.

alignments of 99% or more were chosen for the analysis. Hence based on the biochemical characterization of the isolates, the genus of the isolates was identified as *Micrococcus*, *Enterobacter*, *Shigella*, *Alkaligenes*, *Klebsiella* and *Bacillus*.

3.6. Catalase Assay:

The enzymatic activities of catalase was determined by the oxidation of hydrogen peroxide to water and oxygen.H_2O_2 is a potent oxidizing agent that can break havoc in a cell; because of this, any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the peroxide. The production of catalase peaked on the fourth day of the cultivation and gradually decreased thereafter. As it is shown in Figure, qualitative assay of catalase production indicated that all strains had catalase activity. The maximum catalase activity was achieved in the consortial culture (0.519 U/ml) followed by S1 (0.409 U/ml) culture which can efficiently help it in melanoidin bioremediation (Table No. 4 and Figure No.2).

3.7. Evaluation of decolourization using Adsorbents:

The effect of initial effluent concentration and contact time on the removal of the color of the effluent from different dilutions from 1% to 5% was maintained at ambient temperature and different agitation time period was found out. The percent colour removal increased with increasing

Table 4. Catalase Assay of the isolates isolated from distillery effluent.

Isolate Name	Enzyme Activity (Unit/ ml)
S1	0.409
S2	0.295
S3	0.307
S4	0.154
S5	0.320
S6	0.125
Consortium	0.519

agitation time and reached a maximum value at a particular time after which there was a very less percent increase in colour removal. That particular time which is termed as equilibrium time varied depending on the dilution and the adsorbent.

The maximum colour reduction % and the equilibrium time for the removal of the 1%effluent solution in 150 min with PPNM was 66%,PPAC was 81%, CACwas84%, PPMCNM was 71%, PPMCAC was 85% and MCCAC was 89% irrespective of the initial concentration (Figure No.3, 4, 5, 6, 7, 8)respectively. With increase in initial dye concentration there was a reduction in the percentage dye removal in all the diluted distillery effluent samples.

3.8. SEM Analysis

Scanning electron micrograph images of the adsorbents were obtained in order to understand its surface morphological characteristics (Plate No 2). The SEM images of activated carbon and natural material of *Prosopsis juliflora* and commercially activated carbon particles, before and after, both coated and uncoated with microbes were analysed. The samples were exposed for 150 minutes of adsorption in the effluents. In case of the activated carbon (PPAC), due to dehydration, decomposition of organic matter takes place that causes high porosity of activated carbon under the effect of chemical activation (Plate No 2a, 2b, 2c, 2d, 2e and 2f). Micrographs of activated carbon of Prosopsis show that activation plays a key role in porosity development which is largely responsible for the extent of surface area and adsorptive capacity of carbon than the natural material. Based on the micrographs there was no significant difference in the porous structure of coating of microbes on the adsorbents.

Over the adsorption period it can be seen that the morphology of the particles had undergone remarkable physical disintegration. SEM images clearly showed that the pores were completely filled after the adsorption of the used adsorbents. The literature suggests that the adsorption is observed when the micropores present in the adsorbent serves as a passage for the adsorbed adsorbates through the micro pores. These micropores are present in the outer surface of the carbon which further facilitates the adsorption [19].

4. CONCLUSION

The present investigation enables us to review about the comparison of the prepared low cost adsorbent, biosorbents and biological material (Microbes) for the treatment of the distillery effluent. Among all the microbially coated adsorbents (Biosorbents) were concluded as an effective technology for the treatment of distillery effluent. Microbiologically, the distillery effluent was flourishing with a variety of potential microbes (Bacteria). 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 6 different bacterial strains were done in liquid cultures and were used as consortium for bioremediation experiments and showed different decolourisation ability for the diluted distillery effluent. Hence the results of the study may be utilised for the treatment of the distillery effluent efficiently.

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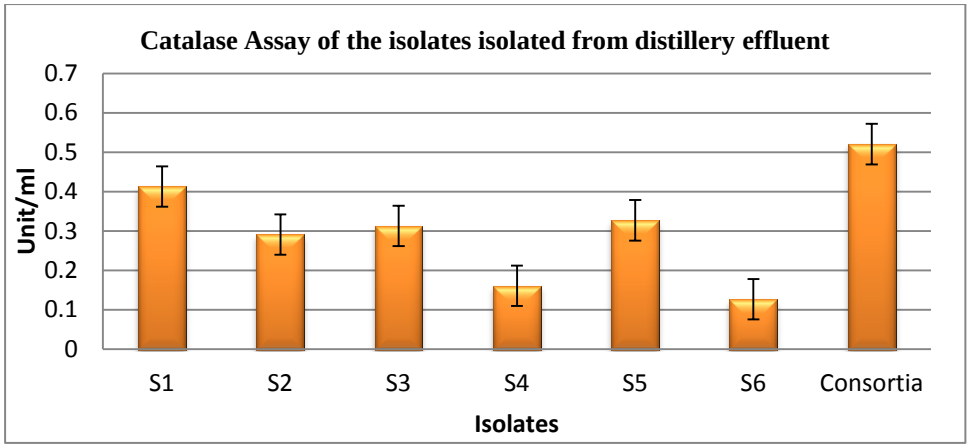


Figure 2: Catalase assay of the isolates isolated from the distillery effluent.

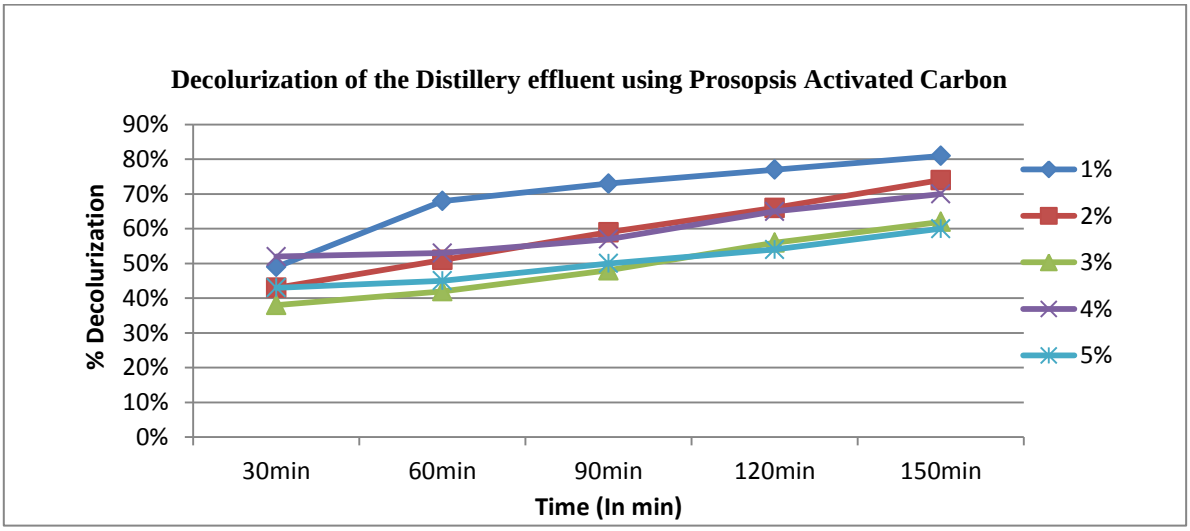


Figure 3: Percentage colour Removal using *Prosopsis* Activated Carbon.

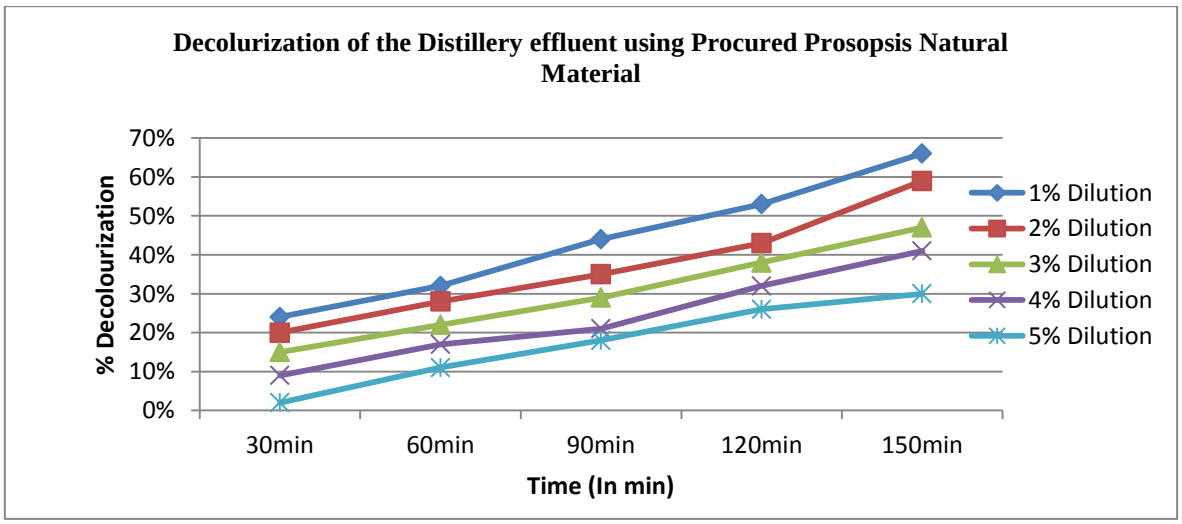


Figure 4: Percentage colour Removal using Procured *Prosopsis*Natural Material

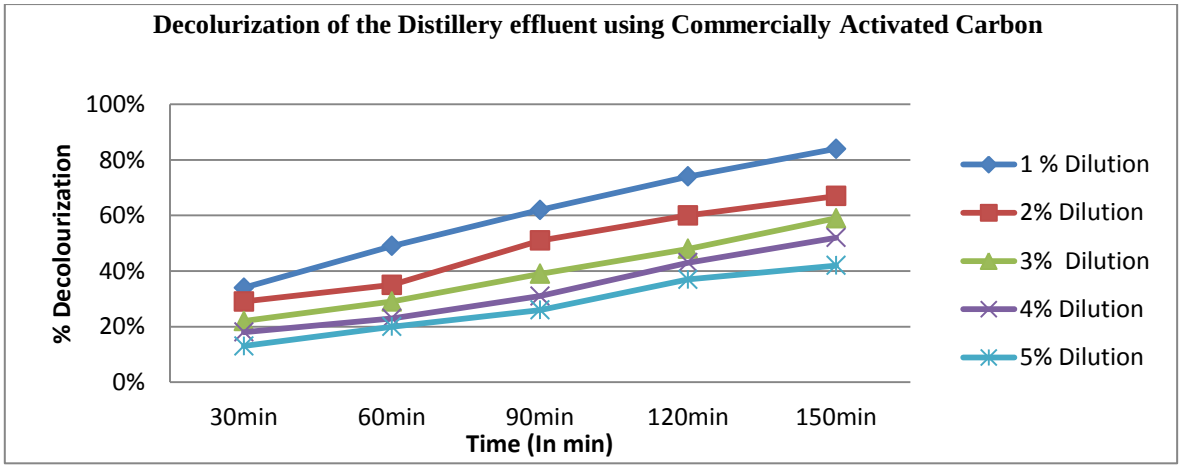


Figure 5: Percentage colour removal using Commercial Activated Carbon.

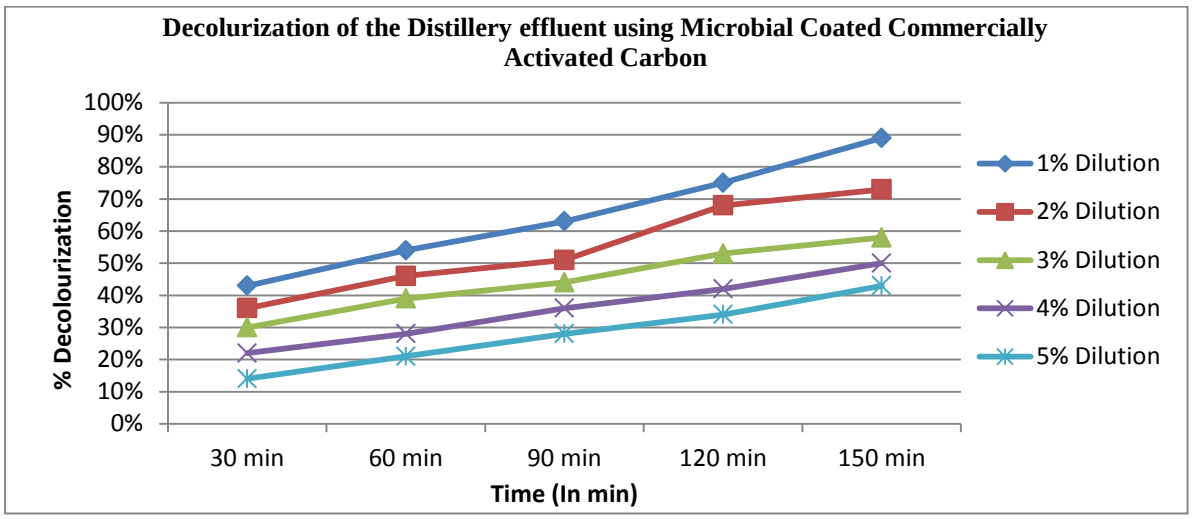


Figure 6: Percentage color removal using Microbial Coated Commercial Activated Carbon.

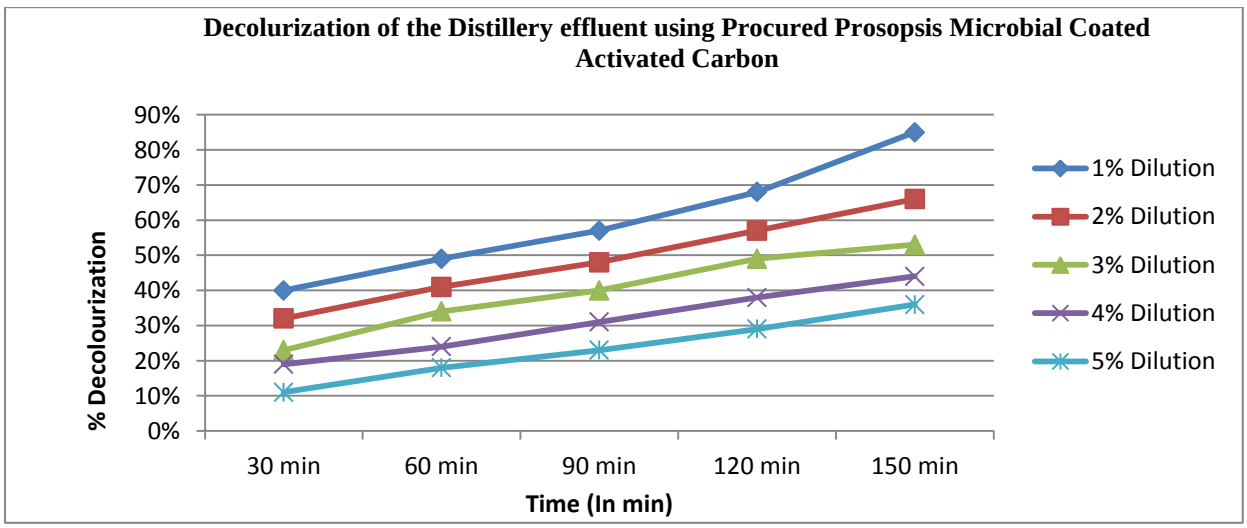


Figure 7: Percentage color removal using Procured Prosopsis Microbial Coated Activated Carbon

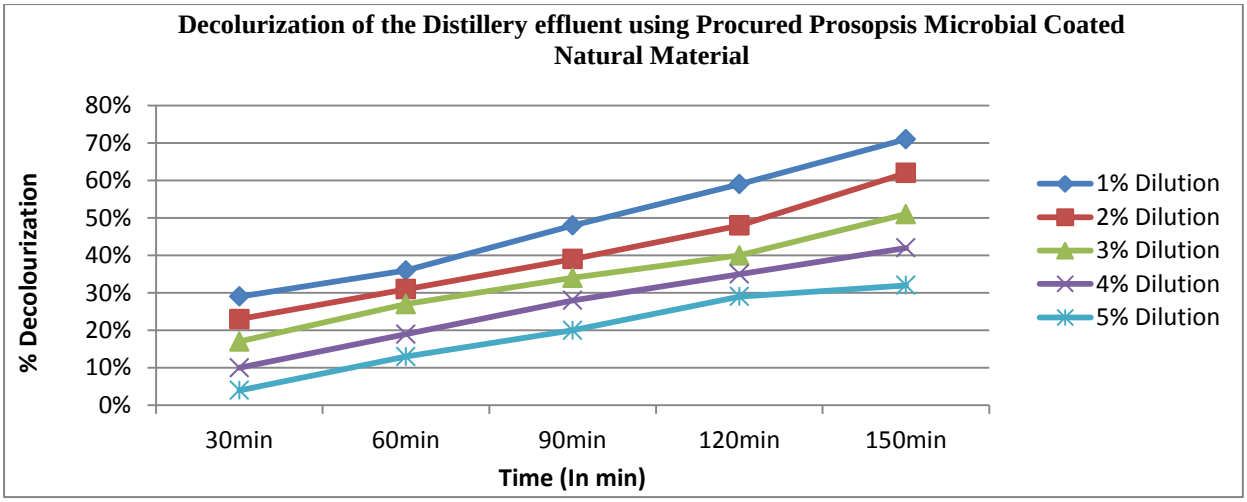
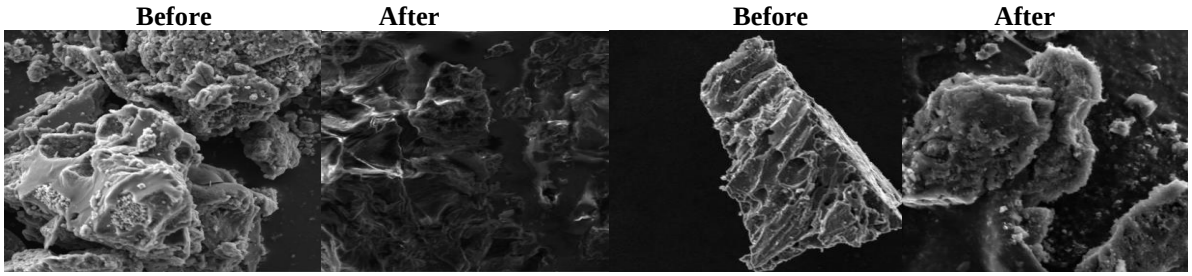
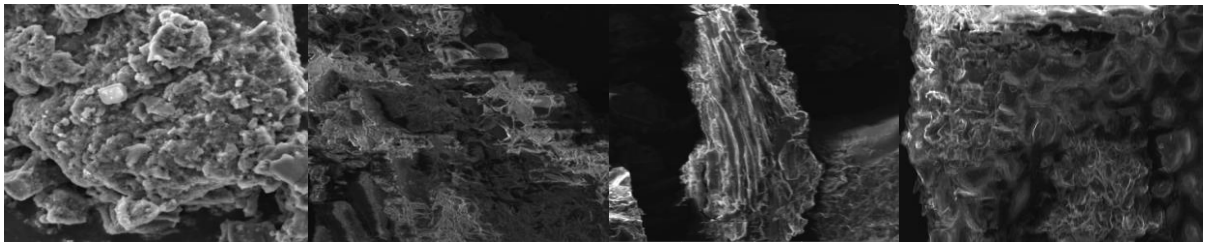


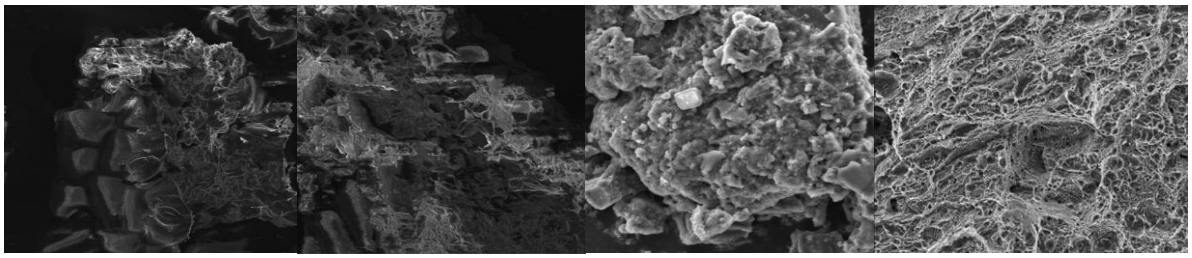
Figure 8: Percentage color removal using Procured Prosopsis Microbial Coated Natural Material.



2a. Prosopsis Procured Activated Carbon (PPAC). 2b. Commercial Activated Carbon (CAC);



2c. Prosopsis Procured Natural Material (PPNM); 2d. Microbial Coated Prosopsis Procured Activated Carbon



2e. Microbial Coated Commercially Activated Carbon (MCCAC) 2f. Microbial Coated Prosopsis Procured Natural Material (MCPPNM)

Plate 2: SEM analysis of the used Adorbents and Biosorbents

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