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STUDIES ON DECOLOURISATION OF MOLASSES WASTE BY VARIOUS MICROORGANISMS

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ABSTRACT

Although decolorization of industrial effluents has been achieved by degradation using bacterial [6]and fungal[7] isolates, increasing demands for effective and economical technologies for colour removal have led to research into a biosorption-based process that utilizes the sorption capacity of biological material for the removal of pollutants [7].The biosorption techniques have been used effectively in the effluent treatment processes mainly for the heavy metals and dyes [8].By keeping these above facts in mind the present work is aimed at to collect the sample from the sugar industry,to isolate and identify the microbes such as bacteria and fungi,to study the degradation efficiency of selected microbes on molasses,to know the physico-chemical characteristics of molasses.

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INTRODUCTION

Across the world 125 to 130 million tons of sugar are produced every year. About 2/3rd of sugar is produced from sugarcane and 1/3 rd from sugar beet. Process of sugar from cane or beet releases molasses, which is typically 4% by weight on the quantity of cane or beet processed are 40% on the sugar produced. Melanoidin, the molasses pigment in distillery wastes is one of the sources of water pollution[1] Molasses wastewater is disposed into nature after treatment by biological method and diluted with water. But almost all molasses pigment remains in wastewater and so the removal of molasses pigment by physical method such as by adsorption to chemicals was also studied [2].The production of dextran is dependent on the amount of glucose in the fermentation broth *Leuconostoc mesenteroides* acted up on glucose and converting them into glucose-saccharides or glucans[3]. The influence of uranium, thorium, Zirconium and thallium on fermentation production of lactic acid from molasses by *Lactobacillus bulgarius* as was studied [4].MDA has already been found in filamentous fungi-mould such as Basidiomycetes, Ascomycetes, Deuteromycetes and soil microorganisms, nevertheless there have been hardly any reports of microbial decolorization of melanoidin by bacteria.

MATERIALS AND METHODS

Sources of Molasses

For the present study the samples were collected from Sugar industry from Aranthangi, Tamilnadu, South India. Samples were collected in large sterilized bottles and brought to the laboratory. Physico-chemical characteristics were done on the same day when the samples were brought to the laboratory. The molasses samples were filtered through cotton to remove suspended solids.

Isolation of bacteria

10 ml of the molasses sample was taken in a 250ml conical flask containing 90ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and transferring serially 10ml of the molasses suspension to 90ml of sterile distilled water to make different dilutions viz., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. One ml of 10^{-5} dilution was plated in petridishes containing nutrient agar medium.

Composition of nutrient agar medium

The inoculated plates were incubated at 25 ± 2°C for one or two days and bacteria appearing over the medium were picked up and mounted on a clean slide, stained with crystal violet, Grams iodine and safranine and observed under the microscope.The bacteria were identified based on colony characteristics. Gram staining methods and by various biochemical tests as given by Bergey's Manual of Determinative Bacteriology.

Physiological and Biochemical tests

The physiological and biochemical tests were conducted following the methods of Somasegaran and Hoben (1985) and Josey *et al.,* (1979) respectively[9], as described by Cappuccino and Sherman (1999) [10] to identify the bacteria.

Gram staining: The bacterial cultures were subjected to gram staining procedures and observed.

Growth on media: The pure culture from the slants was spread on peptone glucose agar and on nutrient agar media and the growth was observed.

Mac Conkey agar test: Mac Conkey agar medium was prepared by mixing bacto peptone, 17.0g; proteose peptone, 3.0g; lactose, 10.0g; bile salts mixture, 1.5g; sodium chloride, 5.0g; agar, 13.5g; neutral red, 0.03g and crystal violet, 0.001g in 1000ml of distilled water. The pH of the medium was adjusted to 6.5 before adding agar and sterilized. The sterile medium was poured into the sterile Petri plates and allowed to solidify. The plates were inoculated with of the isolates**.** The inoculated plates were incubated at 28 ± 2ºC for 3 to 5 days and observed.

Indole test: Peptone agar broth was prepared by mixing peptone, 30.0g; beef extract, 3.0g ferrous ammonium sulphate, 0.2g; sodium thiosulfate, 0.025g and agar, 3.0g in 1000 ml of distilled water. The pH of the medium was adjusted to 7.3 before adding agar and sterilized. The sterile sim's agar broth in test tubes was inoculated with isolates of various industrial molasses. Inoculated and uninoculated tubes were maintained at 35 ± 2ºC for 48 hrs. 1.0 ml of Kovac's reagent was added to each tube, including control, after 48 hrs. The tubes were gently shaken at an interval of 10 to 15 minutes and allowed to stand until the reagent reaches the top. Production of a red reagent layer is the indicative of indole positive.

Methyl red test: Methyl Red Voges Proskauer broth was prepared by mixing peptone, 7.0g; dextrose, 5.0g and potassium phosphate, 5.0g in 1000ml of distilled water. The pH of the medium was adjusted to 6.9. Five ml of the broth was poured into each tube and sterilized. MR-VP tubes were inoculated with the isolates and the uninoculated tube was maintained as control. All the tubes were incubated at 35 ± 2ºC for 48 hours. After 48 hrs, five drops of methyl red indicator was added to the tube of each set. The methyl red indicator at pH 4 will remain red (throughout the

tube), which indicates the positive test, while turning of methyl red to yellow is a negative test.

Voges Proskauer test: Five ml of Methyl red - Voges Proskauer broth was poured into different tubes and sterilized. These tubes were inoculated with isolates and the uninoculated tube was maintained as control. All the tubes were incubated at 35 ± 2ºC for 48 hrs. A few drops of Barritt's reagent was added to each tube and observed for the appearance of deep rose colour.

Citrate utilization test: Simmon's citrate agar slants were prepared by mixing ammonium dihydrogen phosphate, 1.0g; dipotassium phosphate, 1.0g; sodium chloride, 5.0g; sodium citrate, 2.0g; magnesium sulfate, 0.2g; bromothymol blue, 0.08g and agar, 15.0g in 1000ml of distilled water. The pH of the medium was adjusted to 6.9 before adding agar and sterilized. These slants were inoculated with the isolates and control was maintained without inoculation. They were then incubated at 27 ± 2ºC for 48 hrs and observed for colour change from green to blue.

Starch hydrolysis test: Starch agar medium was prepared by mixing soluble starch, 10.0g; beef extract, 3.0g and 12.0g agar in 1000ml of distilled water. The pH of the medium was adjusted to 7.5 before adding agar at 25 ± 2ºC. the sterilized medium was poured into the sterile Petri plates, inoculated with the isolates and observed for colour change. A clear zone of hydrolysis surrounding the growth of the organism is a positive result.

Urea hydrolysis test: Urea agar medium was prepared by mixing peptone, 1.0g; sodium chloride, 5.0g; potassium monohydrogen phosphate, 2.0g and agar 20.0g in 1000 ml of distilled water. The pH of the medium was adjusted to 6.8 before adding agar and sterilized. The medium was poured into the sterile Petri plates and allowed to solitify. The Petri plates were then inoculated with the isolates and incubated at 37± 2ºC for 48 hrs. Appearance of deep pink colour is the positive result.

Nitrate reduction test: Nitrate broth was prepared by mixing peptone, 5.0g; beef extract, 3.0g and potassium nitrate, 5.0g in 1000ml of distilled water. The pH of the medium was adjusted to 7.2. The medium was poured into test tubes and sterilized. After cooling, the tubes were inoculated with bacterial cultures. The uninoculated tubes were maintained as control. They incubation period, 3 drops of sulfanilic acid reagent and 3 drops of alphanaphthylamine reagent were added to each tube and observed for cherry red colour.

Hydrogen sulfide production test: Sim's agar medium was prepared by mixing peptone, 30.0g; beef extract, 3.0g; ferrous ammonium sulfate, 0.2g; sodium thiosulphate, 0.025g and agar 3.0g in 1000ml of distilled water. The pH of the medium was adjusted to 7.3 before adding agar. The medium was poured into test tubes and sterilized. The sterilized tubes were inoculated with the isolates. They were then incubated at 35 ± 2ºC for 48 hrs and observed for hydrogen sulfide production.

Cytochrome oxidase test: A piece of filter paper was moistened with a few drops of freshly prepared 1% solution of tetramethyl-p-phenylene diamine dihydrochloride. The isolates were picked up aseptically from the slant with a sterile wooden applicator for stick smear. Then it was smeared on the moistened filter paper and observed immediately for the development of a violet colour as a positive result.

Catalase test: Some bacterial cells were picked up aseptically from the slant with a sterile wooden applicator. The mass of bacterial cells adhering to the stick was deposited on to a clean glass microscope slide. Then a drop of hydrogen peroxide was pipetted out and added to the mass of bacterial cells adhering to the slide. The production of gaseous bubbles indicates the presence of catalase.

Carbohydrate utilization test: Solutions of carbohydrates (0.1%) namely adonitol, arabinose, cellobiose, dextrose, dulcipol, fructose, galactose, inositol, inulin, lactose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, xylose and glucose were prepared separately and filtered. 1.0 ml aliquots of the specific carbohydrate were added to each of the phenol red broth tubes. These media were added with trypticase as a protein source to support the bacterial growth. The pH indicator, phenol red, turns from red to yellow at acid pH. The test bacterium was inoculated to each set of carbohydrate-supplemented tubes and incubated at 37 ± 2ºC for 48 hrs. After incubation the colour of the tubes was observed. Yellow colour indicates the acid production. Partial filling of the Durham tube indicates positive gas production. After the identification of the genus of the bacterium, the organism was used for further experiments.

Isolation of mould

10 ml of the molasses sample was taken in a 250ml conical flask containing 90ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and transferring serially 10ml of the molasses suspension to 90ml of sterile distilled water made different dilutions viz., 10^{-1}, 10^{-2} and 10^{-3}. One ml of 10^{-3} dilution was plated in petridishes containing Potato Dextrose Agar medium (PDA).

Composition PDA medium

Streptomycin sulphate (100 mgl^{-1}) was added to the media to prevent the bacterial growth. The plates were incubated at 25 ± 2°C for five days and fungi appearing on the medium were mounted over a clean slide, stained with lacto phenol cotton blue and observed under the microscope and photomicrographs were also made.

Isolation of Yeast

10 ml of the molasses sample was taken in a 250ml conical flask containing 90ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and transferring serially 10ml of the molasses suspension to 90ml of sterile distilled water made different dilutions viz., 10^{-1}, 10^{-2} and 10^{-3}. One ml of 10^{-3} dilution was plated in petridishes containing Sabouraud's dextrose agar medium

Composition SDA medium

The mould and yeast were identified by using standard manuals, such as Manual of soil fungi Dematiaceous Hyphomycetes, More Dematiaceous Hyphomycetes Hyphomycetes.

Experimental condition

The molasses was filtered through cotton before use and the initial physiochemical analysis of molasses was made following the standard (APHA, 1975). The following treatments were employed. Molasses uninoculated – control for physiochemical analysis. Molasses inoculated with Aspergillus niger .Molasses inoculated with *Pseudomonas* putida. Molasses inoculated with Saccharomyces cervisiae

Uniform suspensions of the above microorganisms were inoculated in one of the flask with 100 ml of molasses. The first was kept as control without inoculation.

The experiment was conducted in duplicates, under controlled conditions (temperature 27 + 2^{o}C with a light intensities of 1500 lux provided form over head cool white fluorescent tubes) for 20 days. After 20 days the cultures were filtered through ordinary filter paper. The filtered molasses (inoculated and control) were used for physicochemical and decolourization studies.

Physico-chemical analysis of molasses

Determination of pH

pH was recorded at the collection site with BDH indicator paper. At laboratory the pH was checked again with (Elico-India) pH meter.

Estimation of alkalinity (Carbonate and Bicarbonate)

To 10ml of filtered sample, 2 drops of phenolphthalein indicator was added and titrated against 0.1N HCl. This sample was used for both phenolphthalein and total alkalinity.

Estimation of Free Carbon dioxide

To 10ml of filtered sample a few drops phenolphthalein indicator was added and titrated against 0.05 N NaOH. The end point was the appearance of pink color.

Estimation of Dissolved oxygen

Standard flasks (15 ml) were filled with filtered samples without air bubbles. Then the samples were Winkler's by adding 0.2 ml of Manganese sulphate followed by 0.2 ml of alkaline iodide. The precipitate was dissolved by the addition of 0.2 ml of sulphuric acid. The clear yellow color solution was titrated against sodium thiosulphate and starch was used as an indicator. The end point was the disappearance of blue colour.

Estimation of Nitrate

To 10ml of water sample in an Erlenmayer flask 2ml of sodium chloride was added; shaken well and placed in cooling water bath. 10ml of H_2SO_4 and 0.5ml brucine sulphanilic acid were added and shaken well. The flask with contents was kept in a hot water bath for 30 minutes and allowed to cool. Finally the absorbance was measured at 410nm in spectronic 20 against reagent blank. The amount of Nitrate (mgl-1) was calculated by using a standard graph.

Estimation of Nitrite

To 10ml of filtered sample, 0.2ml of sulphanilamide solution was added. After 10 minutes 0.2ml of NED was added. The pink colour appeared was measured at 543nm in spectronic 20 against reagent blank. The amount of Nitrite (mgl-1) was calculated from the standard graph prepared by using sodium nitrite.

Estimation of Ammonia

To 10ml of filtered sample 0.4ml phenol reagent was added and stirred, then 0.4ml of nitroprusside reagent was added and mixed. Finally, 1ml of the oxidizing reagent was added and mixed thoroughly and then incubated at room temperature for an hour. Absorbency was measured at 630nm in spectronic 20. The amount of ammonia was calculated by using a standard graph prepared by using Ammonium chloride.

Estimation of total phosphorus

To 10ml of filtered sample, 2ml of mixed reagent and 2 ml of 5% Potassium persulphate reagents were added and diluted to 15ml with distilled water. The reading was taken after 10 minutes in spectronic 20 against the reagent blank at 882 nm. The amount of inorganic phosphate was calculated using a standard graph prepared by using K_2HPO_4.

Estimation of inorganic phosphate

To 10ml of filtered sample, 2ml of mixed reagent was added and diluted to 15ml with distilled water. The reading was taken after 10 minutes in spectronic 20 against the reagent blank at 882 nm. The amount of inorganic phosphate was calculated using a standard graph prepared by using K_2HPO_4.

Estimation of Organic phosphate

Organic phosphate was estimated by subtracting inorganic phosphate from the total phosphorus.

Estimation of Hardness

To 10ml of filtered sample 0.2ml of buffer solution and 50mg of Eriochrome black T were added. The solution turned wine red. This was treated against EDTA (0.01 M) solution. At the end point, colour change from wine red to blue was noted.

Estimation of Calcium

To 10 ml of filtered sample, 0.4 ml of 0.1 N NaOH and 50 mg of murexide indicator were added. This was titrated against EDTA (0.01 M) solution until the pink colour solution changed to purple.

Estimation of Magnesium

Amount of magnesium was estimated by the following methods

$$Mg^{++}\,(mgl^{-1}) = \frac{Y - X \times 400.8}{\text{Volume of sample}}$$

Estimation of chloride

To 10ml of filtered sample in an Erlenmeyer flask, few drops $K_2Cr_2O_4$ were added and titrated against silver nitrate (0.0141 N) solution until a persistent red tinge colour appeared. The amount of chloride was calculated as follows.

Determination of Biological Oxygen Demand

pH of the water sample was adjusted to neutrality using 1 N acid or 1 N alkali. BOD bottles were filled with water sample without air bubbles. One ml of allylthiourea was added to each bottle. Dissolved oxygen content for 3 of the bottles were estimated by Winkler's method (DO estimation) and the mean of the three bottles were taken (D1). Rest of the bottles (3) was incubated at 27°C in a BOD incubator for 3 days. DO concentration was estimated after 3 days in these incubated bottles

Chemical Oxygen Demand (COD)

10ml of sample in 100ml conical flask was taken in triplicate. A blank was run for each using COD free water (Sample is diluted when COD is more than 16 mgl^{-1}). The flasks were kept in boiling water bath for 1 hour after adding 1ml of 0.1 N $KMnO_4$ solutions. After cooling for 10 minutes, to each flask, 1ml of KI solution and 2ml of H_2SO_4 were added. Then the sample was titrated with 0.1N sodium thiosulphate and 1ml of starch was added when the solution becomes pale yellow. Titration was continued until the colour disappeared completely. Finally the titre values were noted.

Estimation of decolourizing activity

Melanoidin degradation activity was assayed by the measurement of the decrease in optical density as absorbance at 475 nm after diluting with 0.1 M acetate buffer (pH 5.0). The decolourization yield was expressed as the degree of the decrease in absorbance at 475 nm against the initial absorbance at the same wavelength.

RESULTS

For the present study, the microbial flora and physico-chemical analysis of molasses were carried out. For molasses treatment *Pseudomonas* putida, Aspergillus niger and Saccharomyces cerevisiae was selected based on screening process.

Bacteria

Bacteria were isolated from the molasses by serial dilution techniques. Then the isolated bacteria were identified through number of various biochemical tests (Table. 1). Totally 5 species of bacteria such as *Pseudomonas* putida, Escherichia coli, Staphylococcus aureus, Bacillus subtilis and *Micrococcus sp* were identified from the effluent sample (Table. 2).

Mould

Totally 6 species of mould belongs to 5 genus from the molasses were recorded (Table.3 and Fig.1). Among the genus multicellular fungi *Aspergillus* was recorded as dominant genus with 3 species such as *A.niger, A.flavus* and A.terreus. The remaining genus such as Penicillium, Fusarium, and Rhizopus were recorded single species each (Table 3).

Yeast

Totally 4 species of unicellular fungi such as Saccharomyces cerevisae, Torulopsis glabrata, Candida glabra and C.albicans were isolated from the molasses (Table.4 and Fig.2).

Physico -chemical characteristics of molasses

pH:

The pH was recorded in the effluent initially 6.3 and it was brought down to 6.8 in treated molasses (Table 5).

Free CO_2:

The initial free CO_2 was recorded 33 mg l^{-1} and it was reduced in treated molasses. The maximum reduction was observed in *A. niger* (8 mg l^{-1}) in treated effluent (Table 5).

Alkalinity:

The alkalinity was recorded 108 mg l^{-1} initially. It was brought down in all the treated molasses. The maximum (90%) percentage reduction was observed in *A.* niger in treated effluent (Table 5).

DO:

The dissolved oxygen level was increased in treated molasses when compared to control. The DO level was increase 1.4 to 2.75 mg l^{-1} in the treated molasses (Table 5).

Nitrate, Nitrite and Ammonia:

The Nitrate, Nitrite and Ammonia were recorded initially 95, 50 and 45 mg l^{-1} respectively (Table 5). It was reduced nearly 55% in *A.* niger treated molasses when compared to control.

Total, inorganic and organic phosphate:

Total inorganic and organic phosphate levels observed and recorded in both control and treated molasses. All the forms of phosphates were reduced in treated molasses (Table 5). The maximum amount of reduction was observed in *A.* niger treated molasses when compared to S.cerevisiae and P.putida treated molasses.

Calcium:

The calcium level was recorded initially 68 mg l^{-1}. It was reduced to 25 mgl^{-1} in *A.* niger treated molasses (Table 5). The percentage reduction was nearly 60% than control.

Magnesium:

The similar trend was noticed in magnesium level as in calcium. The 48% reduction was observed in *A.* niger treated molasses when compared to control (Table 5).

Chloride:

Chloride level was observed in 35 mg l^{-1} initially (Table 5) and it was slightly decreased in all the treated molasses. When compared to other parameters the chloride level was reduced very less amount.

BOD and COD

The BOD and COD levels were recorded 245 mg l^{-1} and 350 mg l^{-1} initially It was reduced to 62 mg l^{-1} and 110 mg l^{-1} respectively in *A.* niger treated molasses (Table 5).

Decolourization activity

Decolourization activity was found to be higher in the molasses inoculated with *A.* niger (44 %) followed by *P.* putida (31 %) and *S.* cerevisia*e* (25 %).

DISCUSSION

The abundant variation in the types of all living organisms taken together in any geophysical area is called biodiversity. With reference to large organisms (plant, animals etc.) the distinctive morphological and anatomical features visible to the naked eye enable us to identify the different genera and species and. therefore; we can easily assess the extent of their diversity. However, most fungi are not visible to the naked eye and they need to be studied under the microscope. The culturable fungi can be identified provided they sporulate but the biotrophs which are not amenable to culturing in growth media go unnoticed. It is, therefore, imperative that there is a huge gap in our knowledge of the diversity of all microbes including fungi. Fungi are better known than bacteria, viruses and other smaller forms of life such as viroids. There are about 72,000 named species and new species are being added at the rate of about 1500 each year. The total number of

fungal species both known and unknown are estimated at 250,000. The 72,000 known species exist in different culture collection centres in the form of several subspecies, pathovars and strains, the total cultures going upto 170,000

The mycoflora of unique ecological niches have some common features and it will be very rewarding to explore fungal diversity in habitats such as thermophilic environments, e.g., hot springs, thermal vents, sun-heated soils, compost pits, self-heated coal refuse piles, steam line discharge sites, etc. The thermophilic fungi and bacteria grow at temperatures between 40-60 C are the sources of thermostable enzymes. Other ecological niches to be explored are western ghats, the Himalayan ranges, marine ecosystems, mangroves, coral reefs, sand dunes, industrial effluent contaminated soils, ant hills, refineries, activated sludge, insects and several other natural sources. Endophytes of plants and animals are very meagrely understood.

Hence, the present study was undertaken to know the bacterial, fungal and yeast species in industrial molasses. For the present investigation molasses sample was collected from sugar industry. The bacterial, mould and yeast species were isolated and identified from molasses sample were recorded.

In order know the bacterial diversity in molasses, sample was collected from this totally 4 species of bacteria were isolated. Bacteria were isolated from the molasses by serial dilution techniques. Then the isolated bacteria were identified through number of various biochemical tests (Table. 1). Totally 5 species of bacteria such as Pseudomonas putida, Escherichia coli, Staphylococcus aureus,Bacillus subtilis and Micrococcus sp were identified from the effluent sample (Table. 2). It is confirmed and supported by earlier finding of Ramlake and Bhattacharjee (1992)[12]. They also suggested the polluted habitats found mostly *Pseudomonas* because it is having ability to degrade various pollutants from water samples.

Totally 6 species of mould belongs to 5 genus from the molasses were recorded (Table.3). Among the genus Aspergillus was recorded as dominant genus with 3 species such as A.niger, A.flavus and A.terreus. The remaining genus such as Penicillium, Fusarium, and Rhizopus were recorded single species each (Table 3). Totally 4 species of yeast such as Saccharomyces cerevisiae, Torulopsis glabrata, Candida glabra and C.albicans were isolated from the molasses (Table.4) performed a similar study except they tested seven fungal species isolated from a wastewater stabilization pond. The major goal of the study was to maximize biomass production of fungi as a food source for animal or human consumption.

Increase in population, development, diversification of individual consumption, urban concentration, increase of the number of industrial units and the lack of a strategy of treatment of garbage by satisfactory techniques can cause big problems for the environment and human health.

The present work is essentially focused on the physical, chemical and microbiological characterization of molasses after fermentation. Also, this work provides a biological process for the treatment of this molasses. The treatment of unwanted products of agro-food waste has been the subject of the previous work on sugar cane, the breakdown of the cellulose and fermentation of olives.

Of all the technologies investigated in waste cleaning, bioremediation has emerged the most desirable approach for cleaning up many environmental pollutants. Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation of or transformation of various toxic chemicals to less harmful forms. The general approaches to bioremediation are to enhance natural biodegradation by native organisms (intrinsic bioremediation), to carry out environmental

modification by applying nutrients or aeration (biostimulation), or through addition of microorganisms (bioaugmentation). Bioremediation is similar to the use of plants to restore contaminated sites (Phytoremediation). The ability of microorganisms to transform a variety of chemicals has led to their use in bioremediation processes. A number of microorganisms have since been studied to unfold their degradative abilities in remediation of pollutants.

Most studies on the metabolism of organic contaminants have been performed with bacteria especially in the context of bioremediation. Bacteria generally are easier to culture and they grow more quickly than fungi. They are more amenable to molecular genetic manipulations. They are able to metabolize chlorinated and other organic contaminants such as oil and mineralize chemicals using them as carbon or energy source. Diverse fungal cultures have been investigated recently for bioremediation processes By virtue of their aggressive growth, greater biomass production and extensive hyphal reach in the environment, fungi have been seen to perform better than bacteria. The high surface-to-cell ratio of filamentous fungi makes them better degraders under certain niches

The fungi technology is very different from other well-established methods of bioremediation (e.g. bacterial systems). The differences are primarily due to the unusual mechanisms which nature has provided them with and several advantages for pollutant degradation. One distinct advantage these fungi have over bacterial systems is that they do not require preconditioning to the particular pollutant. Bacteria usually must be pre-exposed to a pollutant to allow the enzymes that degrade the pollutant to be induced. The pollutant also must be in a significant concentration; otherwise, induction of enzyme synthesis cannot occur. Thus, there is a finite level to which bacteria can degrade pollutants. Also because the induction of the degrading enzyme is not dependent on the pollutant in the fungi, the pollutant can be degraded to a near non-detectable level. In contrast to the bacterial system, the degradative enzymes of fungi are induced by nutrient limitation. Thus, cultivation of the white rot fungi on a nutrient-limited substrate, will initiate the process

The primary purpose of wastewater treatment is to remove the suspended and soluble organic constituents measured as chemical oxygen demand (COD) or biochemical oxygen demand (BOD). Biological treatment processes are used to degrade the organics in the wastewater before it is discharged. The most common biological process for wastewater treatment, the microbes is suspended with the wastewater. In order for this process to work effectively, the biomass must be separated from the water and this is accomplished by gravity settling in a ‘final clarifier’. To effectively settle, the microbes must flocculate, and then aggregate into units large enough and dense enough to settle out of solution. If the biomass does not flocculate well, some microbes will end up in the effluent (supernatant turbidity). Furthermore, the characteristics of the flocculated biomass will have important impacts on the biomass (sludge) disposal process.

Anaerobic treatment in general does not lead to the low pollution standards of COD and BOD that can be met with aerobic systems and which are required by environmental laws. Anaerobic treatment of wastes and wastewater is often considered a pretreatment process to minimize the oxygen demand and surplus sludge formation in a subsequent aerobic post-treatment stage. Only after a final aerobic treatment can the COD and BOD concentration limits stated in the environmental laws be met. If limiting concentrations for nitrogen and phosphate also have to be achieved, further treatment steps such as nitrification, denitrification, and biological or chemical phosphate removal, must be considered.

For the evaluation of the pollution load of industrial or domestic wastewaters, a measure of

oxygen requirement of pollutional matter has been developed as standard parameters, which is known as Biochemical Oxygen Demand (BOD). For the present investigation the BOD level was recorded 240 mg/l initially. Before discharging any industrial effluent the BOD should be removed the BOD because it adversely affect the aquatic organism. In this present study the BOD level was reduced on 15th day to nearly 75% it is inconformity with the previous reports. He reported the bacterial culture removed BOD almost completely on two weeks from the date of inoculation in the water samples.

A great deal of effort has been made during the last three decade to establish methods of treatment which will leads to the elimination of much or most of the nitrogen present in waste water. From the present study a successful removal of nitrate, nitrite and ammonia were observed nearly 50%. They reported nearly 60% of removal of nitrate and ammonical nitrogen from the fertilizer industry wastewater by using bacterium *Pseudomonas* sp.

The total phosphorus and inorganic and organic phosphate levels were reduced in treated molasses. The similar study was done by various workers reported the efficiency of removal of phosphates was more in the immobilized condition than free cells of bacteria. It supports the present investigation.

Chlorides are generally considered to be one of major pollutant in effluents, which are difficult to be removed by conventional biological methods. In the present study 9-18% removal of chloride from the molasses was observed due to the inoculation of microbes.

The dyes present in textile effluent impart persistent color to the receiving streams and interfere with photosynthesis of the phytoplankton. Other physical characteristics of the wastewater include odor, change in dissolved oxygen, presence of insoluble substances and corrosive properties. The colloidal and suspended purities cause turbidity in the receiving streams.

The dissolved minerals may increase salinity of the water and thus may render it unfit for irrigation or consumption. Toxic chemicals such as chromium and sulphites may destroy fishes and microorganisms responsible for self-purification of water in streams. Immediate oxygen demand due to the impurities such as starch, sulphites, nitrites, deplete the dissolve oxygen content of water. Starch cotton debris constitute organic wastes which are oxygen demanding. They can undergo decomposition/degradation by bacterial activity. The chemicals use in the processes may change pH of the effluent and once disposed into the water body affects aquatic lives. Dissolved solids can also form incrustations on the surfaces of sewers and chemicals may cause corrosion of the metallic parts of the sewage treatment plants. Thus, an effluent that emanates from the production process of textiles, if not properly disposed, can cause serious environmental pollution, sometimes to levels that can threaten human health, livestock, wildlife, aquatic lives and indeed the entire ecosystem. Every production process goes with wastes generation. Various treatment options are available for treatment of textile wastes before disposal.

Traditional disposal method such as ocean dumping is now out of place following numerous incidents of severe negative impacts on the environment after years of disposal. Typical examples are the Love Canal episode of the Niagara Falls in the United States of America and the Mina Mata Bay experience in Japan where several tons of mercury was discharged through effluent into the bay and the inhabitants suffered the effect after over thirty years. There are physical and chemical methods, which, inspite of costs, do not always ensure that the contaminants are completely removed. In recent times there has been a tremendous upsurge in the search for cost-effective and environmentally friendly alternatives to traditional methods for dealing with wastes.

It is clear that the biodegradative activity of fungi is a complex one. Understanding the mechanisms of the biodegradation role of this fungus is very important if one must explore the unique enzyme system in it for remediation of colored and complex, toxic effluents. The stability of the enzymes in relation to the physicochemical nature of the effluents is an important factor in evaluating both technical and economic feasibility of using this organism commercially in bioremediation projects.

SUMMARY AND CONCLUSION

In order to study the microbial diversity and treatment of molasses by using bacteria and fungi, sample was collected from sugar industry Aranthangi, Tamil Nadu, South India.For treating the molasses, *Pseudomonas* putida, Aspergillus niger and Saccharomyces cerevisiae were selected based on the screening process. From the present investigation the following observation were made. Totally 5 species of bacteria such as *Pseudomonas* putida, Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Micrococcus sp were isolated from the molasses.Totally 6 species of mould belongs to 4 genus from the molasses were recorded.Among the genus Aspergillus was recorded as dominant genus with 3 species such as Aspergillus niger, Aspergillus flavus and Aspergillus terreus.Totally 4 species of yeast such as Saccharomyces cerevisae, Torulopsis glabrata, Candida glabra and C.albicans were isolated from the molasses.Except dissolved oxygen, all other parameters showed decreased level when compare to control.The BOD and COD levels were reduced nearly 70% and 55% respectively in Aspergillus niger treated molasses when compared to control. The color removal was observed maximum in Aspergillus niger treated effluent than Pseudomonas putida and Saccharomyces cerevisiae treated effluent..Aspergillus niger was marginally better in treating the molasses than Pseudomonas putida and Saccharomyces cerevisiae.From the above results it was inferred that Aspergillus niger could effectively be used for the treatment of molasses. The understanding of these mechanisms has actually been a drawback in the technology and the deployment of this fungus widely in bioremediation. However, continuous research will eventually close the present gap in knowledge about the use of this organism. This present work is written in the hope that it would stimulate interest and investigations into the development of biotreatment of colored and toxic effluents in developing countries using the model system of Aspergillus niger. Mechanism of microorganisms in control of environmental pollution is still being explored. However, it is argued that organisms during bioremediation either eat-up/gobble the contaminants especially organic compounds or assimilate heavy metals themselves, thus effectively degrading specific contaminants/ harmful compounds and converting them to non-toxic useable byproducts.

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TABLES:

Table 1: Biochemical characteristics of isolated bacteria

S. No	**Biochemical Test**	***P. putida***	***E. coli***	***S. aureus***	***B. subtilis***	***Micrococcus***
1	Mac Conkey agar test	+	-	-	-	-
2	Indole test	-	+	-	+	-
3	Methyl red test	+	+	+	+	+
4	Voges Proskauer test	+	-	+	-	+
5	Citrate utilization test	-	-	-	-	-
6	Starch hydrolysis test	+	-	+	-	+
7	Urea hydrolysis test	-	-	-	-	-
8	Nitrate reduction test	-	+	+	+	+
9	H_2 S production test	+	-	+	-	+
10	Cytochrome oxidase test	+	+	-	+	-
11	Catalase test	-	+	-	+	-

Table. 2. Bacteria in the molasses

Si.No	**Name of the Bacteria**
1	*Pseudomonas putida*
2	*Escherichia coli*
3	*Staphylococcus aureus*
4	*Bacillus subtilis*
5	*Micrococcus sp*

Table. 3. Mould in the molasses

Si.No	Name of the fungi
1	Aspergillus niger
2	*A.flavus*
3	*A.terreus*
4	*Penicillium citrinum*
5	*Fusarium sp*
6	*Rhizopus sp*

Table. 4. Yeast in the molasses

Si.No	**Name of the Yeast**
1	*Saccharomyces cerevisiae*
2	Candida albicans
3	*C. glabra*
4	*Torulopsis glabrata*

Table 5. Physicochemical characteristics of molasses

S. No	**Parameters**	**Control**	**Treated molasses**		
			S.cervisiae	***P. putida***	***A.niger***
1	pH	6.3	6.8	6.8	6.8
2	Free CO_2	33	18	13	8
3	Total Alkalinity	108	75	45	18
4	DO	1.4	2.4	2.45	2.75
5	Nitrate	95	74	45	40
6	Nitrite	50	33	29	23
7	Ammonia	45	30	27	21
8	Total phosphate	90	65	56	44
9	Inorganic	55	36	30	26
10	Organic	35	29	26	18
11	Calcium	68	54	35	25
12	Magnesium	39	30	22	16
13	Chloride	35	31	26	22
14	BOD	245	168	135	62
15	COD	350	250	195	110

Except pH all values are expressed in mg l^{-1}

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