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Full Length Research Paper

Biodegradation of solid waste molasses from Aranthangi sugar industry, Tamilnadu by using *Aspergillus niger*

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ABSTRACT

In order to study the efficiency of *Aspergillus niger* in treating waste molasses effluent from sugar industry, Tamilnadu, South India. *A.niger* was selected for treating the molasses effluent based on the screening process. The effluent was tested for all the physicochemical parameters before inoculation of microbe. After incubation the physicochemical parameters results showed significant decrease of the parameters such as BOD (75%) and COD (65%) in the effluent treated with *A.niger*. From the findings, it is evident that *A.niger* could effectively be used for the treatment of molasses. The understanding of the mechanisms of the study may widely used in bioremediation.

Keywords: *A.niger*, Bioremediation, molasses.

INTRODUCTION

Across the world 125 to 130 million tons of sugar is produced every year. About 2/3rd of this is produced from sugarcane and 1/3rd 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 or 40% on the sugar produced. Molasses is one of the major components of growth media used in industrial process. Due to its unique physical and chemical properties molasses has traditionally been used as a major component in compound feeds, livestock feeds and silage additives and most widely used in various industrial processes. Molasses-based distilleries are one of the most polluting industries generating large volumes of high strength wastewater **(**Y. Satyawali and M. Balakrishnan, 2007).

Melanoidins are major pollutant when discharged into a water resource system. They prevent the penetration of sunlight and affect the photosynthetic activity of aquatic plants. The high organic load of the effluent causes eutrophication. This will therefore create anaerobic conditions thereby killing most of the aerobic aquatic fauna (Bernardo et al., 1997; Raghukumar et al., 2004).

Control of pollution is one of the prime concerns of society today. With economic constraints on pollution control processes, affordable and effective methods have become a necessity. Untreated or partially treated wastewaters and industrial effluent discharges into natural ecosystems pose a serious problem to the ecosystem and the life forms. Among the many types of organics present, the most difficult to remove is colour. As melanoidins are recalcitrant to biodegradation, the elimination of colored effluents in molasses-based distillery wastewater treatment system is mainly based on physical or chemical procedures such as adsorption, coagulation, precipitation and oxidation.

Although these methods are effective, they suffer from such short coming as requiring high reagent dosage, high cost, formation of hazardous byproducts and intensive energy consumption. These methods also generate large amount of sludge. Therefore, as an alternative, biological treatments with microbe are drawing attention.

Although decolorization of industrial effluents has been achieved by degradation using bacterial (Chaturvedi et at., 1986; Dupouy et al., 1988; Subramaniam et al., 2002 and Suhuttaya Jiranuntipon, 2009) and fungal (Subramaniam and Carpentor, 1994; Madhupratap, 1999; Sarangi et al., 2001, Suhuttaya Jiranuntipon, 2009) isolates, increasing demands for effective and economical technologies for colour removal have led to research into a biosorption – based process that utilizes the absorption capacity of biological material for the removal of pollutants (Desa, 2000). The biosorption techniques have been used effectively in the effluent treatment processes mainly for the heavy metals (Carpentor and Price, 1976; Borstad et al., 1992) and dyes (Chauhan et al., 2002; Sarangi et al., 2001). 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 biostimulation), or through addition of microorganisms (bioaugmentation) (Ashoka et al., 2002). The ability of microorganisms to transform has since been studied to unfold their degradative abilities in remediation of pollutants.

Molasses-based distillery wastewaters generation and characteristics

Alcohol production from molasses generates large volumes of high strength waste water that is of serious environmental concern. The aqueous effluent stream from distilleries known as sugarcane molasses wastewater is approximately 12–15 times the volume of the produced alcohol. The wastewater from distillery is characterized by extremely high chemical oxygen demand (COD) (80,000–100,000 mg/L) and biochemical oxygen demand (BOD) (40,000–50,000 mg/L). However, the amount and the characteristics of the sugarcane molasses wastewater are highly variable and dependent on the raw material used and on the ethanol production process (Pant and Adholeya, 2007; Satyawali and Balakrishanan, 2008). Washing water used to clean the fermenters, cooling water and boiler water further contribute to its variability (Pant and Adholeya, 2007).

MATERIALS AND METHOD

For the present study the samples were collected from Sugar industry at Aranthangi taluk, Pudukottai district, 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 sample was brought to the laboratory and the analysis for the physicochemical parameters was carried out as per APHA (1999) procedures.

Isolation of fungi

100 ml of the molasses sample was taken in a 250 ml conical flask containing 90 ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and transferring serially 10 ml of the molasses suspension to 90 ml 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).The pH of the medium was adjusted to 5.6.

Streptomycin sulphate (100 mg/L) was added to the media to prevent the bacterial growth. The plates were incubated at 25 ± 2^0C 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 photomicrographs (Fig.1) were also made.

Experimental Design

The molasses was filtered through cotton before use and the initial physiochemical analysis of molasses was made following the standard (APHA, 1999). The following treatments were employed. Molasses before inoculation was taken as control for physiochemical analysis.

Aspergillus niger was inoculated to molasses (20 ml) contained in conical flask.

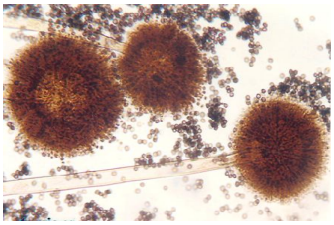

Figure 1: Photomicrograph of *A.niger*

The experiment was conducted in duplicates, under controlled conditions (temperature 27 ± 2^0C with light intensities of 1500 lux provided from 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.

RESULTS AND DISCUSSION

Physico-chemical analysis of molasses were carried out before inoculation with *A.niger* and also after inoculation and 20 days incubation were carried out and results are tabulated (Table.1). 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. One of the important ecological niches to be explored is industrial effluent which is very meagerly understood.

Hence, the present study was undertaken to know the fungal speices with special reference to *A.niger* in industrial molasses of sugar industry.

Decolorization activity

Decolorization activity (Fig. 2) was found to be significant in the molasses inoculated with *A.niger* (42%). The dyes present in textile effluent impart persistent color to the receiving streams and interfere with photosynthesis of the phytoplankton (Cunningham and Saigo, 2001). Removal of melanoidins from molasses waste of 84.16% using *Aspergillus niger* in the presence of glucose has also been reported (Gomaa et al., 2003).

Color elimination by *Aspergillus niger* from wastewater from molasses alcoholic fermentation was studied by Peña Miranda *et al* (1996) and also analyzed the influences of the nutrient concentrations, initial pH and carbon source on this color elimination. It worked in a discontinuous process in shaken cultures and in a continuous process in a bubble reactor. During the batch process, through all experiments the maximal color elimination was attained after 3 or 4 days in the culture. Batch processes showed a maximal color elimination of 69% when $MgSO_4$, KH_2PO_4, NH_4NO_3 and a carbon source were added to the wastewater. The continuous process, with the same nutrient concentrations, showed less color removal and the decolorization activity was maintained for only 4 days. Increasing attention has been directed towards utilizing microbial activity for decolorization of molasses wastewater. Several reports have indicated that some fungi in particular have such a potential (Kumar et al., 1998). One of the most studied fungus having ability to degrade and decolorize distillery effluent is *Aspergillus* such as *Aspergillus fumigatus* G-2-6, *Aspergillus niger*, *Aspergillus niveus*, *Aspergillus fumigatus* $U_{B2}60$ brought about an average of 69–75% decolorization along with 70–90% COD reduction (Ohmomo et al., 1987; Miranda et al., 1996; Jimnez et al., 2003; Shayegan et al., 2004; Angayarkanni et al., 2003; Mohammad et al., 2006).

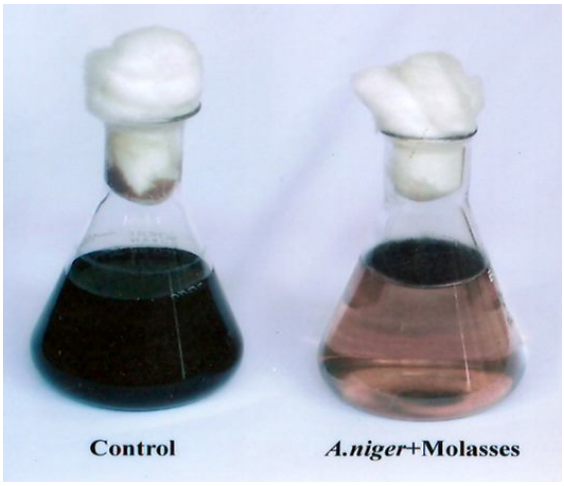

Fig.2 Decolarization of molasses after treating with *A.niger*

Fate of Physico-chemical parameters of Molasses waste after incubation:

After inoculation and incubation the physic-chemical parameters showed significant variations as that of control. Diverse fungal cultures have been investigated recently for bioremediation process (Aust, 1990; Bumpus and Aust 1993; Akamatsu et al., 1990). 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 (Ashoka et al., 2002). pH of the molasses was reduced to 1.47% acidic than that of control, while the free carbon dioxide was reduced to 80.64 % as that of control. Total alkalinity was reduced from 108 to 18 mg/L (83.33 %) after treating with the *A.niger* on the other hand dissolved oxygen was slightly increased from 1.34 to 1.75 (23.42 %). There is a 48.88 % t reduction of nitrate was noticed while, the nitrite was reduced to 21 mg/L from 48 mg/L (56.25 %). The ammonia was reduced to 22 mg/L from 42 mg/L showing 47.61 % of reduction as that of control sample.

Total phosphate also showed significant reduction from 90 mg/L to 44 mg/L (51.1%). The degradation of inorganic phosphate (52.72%) by *A.niger* was noticed to be more with contrast to organic phosphate (48.57 %). The total phosphorus and inorganic and organic phosphate levels were reduced in treated molasses. 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%. The similar study was done by various workers (Banu et al., 2001) reported the efficiency of removal of phosphates was more in the immobilized condition than free cells of bacteria. It supports the present investigation.

The minerals Ca and Mg also showed the reduction in their concentration (65.62% and 62.16% respectively) after treating with *A.niger*. Biological Oxygen Demand was reduced to 60 mg/L from 240 mg/L (75 %) and Chemical Oxygen Demand was reduced to 120 mg/L from 320 mg/L (61.29 %). A study of the aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50% (chemical oxygen demand, COD: 82 g/l) was carried out using the following fungi: *Penicillium* sp., *Penicillium decumbens*, *Penicillium lignorum* and *Aspergillus niger* by Antonia M *et al*,. (2003).

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.

It is clear that the bio-degradative activity of fungi is a complex one. Understanding the mechanisms of the bio-gradation 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.

Table 1. Comparison of Physicochemical characteristics of raw and *A.niger* inoculated molasses effluent

Sl. No	Parameters	Raw Effluent (Control)	Inoculated Effluent with *A.niger*
1	pH	6.8	6.7
2	Free CO_2	31	6
3	Total Alkalinity	108	18
4	DO	1.34	1.75
5	Nitrate	90	46
6	Nitrite	48	21
7	Ammonia	42	22
8	Total Phosphate	90	44
9	Inorganic	55	26
10	Organic	35	18
11	Calcium	64	22
12	Magnesium	37	14
13	Chloride	30	24
14	BOD	240	60
15	COD	310	120

*

Except pH all parameter are in mg/L

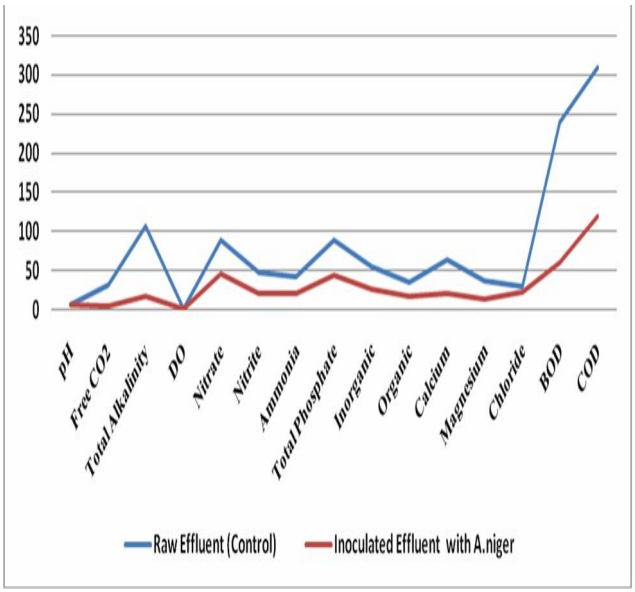

Figure 3: Degradation of Physico-chemical parameters of molasses effluent by *Aspergillus niger*

CONCLUSION

In order to study the treatment of molasses by using *A.niger* molasses effluent sample was collected from sugar industry, Tamilnadu, South India. From the present investigation the following observation were made.

The BOD and COD levels were reduced nearly 75% and 65% respectively (Fig 3) and the color removal was observed maximum in *Aspergillus niger* treated molasses when compared to control.

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 closet 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 by products.

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