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Patent No.	:	**229515**
Application No.	:	**758/MUM/2006**
Date of Filing	:	**18/05/2006**
Patentee	:	**NORTH MAHARASHTRA UNIVERSITY, JALGAON**

It is hereby certified that a patent has been granted to the patentee for an invention entitled A METHOD FOR DECOLORIZATION OF DISTILLERY SPENT WASH USING DEAD FUNGAL BIOMASS as disclosed in the above mentioned application for the term of 20 years from the 18 day of MAY 2006, in accordance with the provisions of the Patents Act,1970.

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Controller of Patents

Note.-The fees for renewal of this patent,if it is to be maintained , will fall / has fallen due on 18 day of MAY 2008 and on the same day in every year thereafter.

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PCT Conventions:

#	PCT Application Number	Date of Convention	Priority Country
1		NA	

Title of Invention	A METHOD FOR DECOLORIZATION OF DISTILLERY SPENT WASH USING DEAD FUNGAL BIOMASS
Abstract	The invention relates to a process for decolorization of molasses spent wash or water or soil contaminated with molasses spent wash using dead biomass of the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial Technology, Chandigarh, India, bearing the reference number MTCC 7691 as biosorbant. The process involves growing the fungal biomass, autoclaving it and processing it to form a fine powder, for use as a biosorbant. The biosorbant can be desorbed to remove the colored component and can be used repeatedly for a number of times for removal of color from molasses spent wash or water contaminated with molasses spent wash.
Full Text	FORM 2 THE PATENT ACT 1970 (39 OF 1970)

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THE PATENTS RULES, 2003
COMPLETE SPECIFICATION
TITLE OF THE INVENTION
A method for decolorization of distillery spent wash using dead fungal biomass
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The following specification particularly describes the invention and the manner in which it is to be performed

FIELD OF THE INVENTION

The invention relates to a biosorption process for the decolorization of distillery spent wash using dead biomass of the fungus, Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference number MTCC 7691.

BACKGROUND OF THE INVENTION

Large volume of effluent wastewater is released into the environment as a result of industrial activities. Distilleries, alcohol-producing industries are listed among the most environment polluting industries. In India, where sugarcane is a major cash crop, distilleries use molasses - byproduct of sugar industries, as a raw material for the alcohol production. After fermentation by yeast, alcohol is recovered by distillation and a dark brown colored liquid, with acidic pH remains behind. It is called distillery spentwash. It is a major environmental hazard to land or aquatic sources as it contains colored pigments, high-suspended solids, a high concentration of BOD and COD, besides causing aesthetic damage to sites. It contains a large amount of a dark brown pigment called melonoidin, which is not broken down by usual biological treatments. Molasses spentwash pollutes aquatic ecosystems due to its intense brown color, which cuts off light, prevents photosynthesis and causes anaerobic conditions. It is recalcitrant in nature and hence remains persistent in soil and water bodies for long time.

However, increasing awareness of harmful effects of these pollutant on ecology as well as human health, is leading to significant increase in research to develop those technologies, which may be applied to remove the environment contaminants from industrial waste effluent.

Among the various sustainable techniques being developed for the treatment of different types of wastewaters, biosorption technology is gaining attention of scientists, as an easy and quick method for recovery of environmentally toxic pollutants from the waste effluent. Particularly, it can be applied for those effluents that contain mixture of various polymeric, inorganic & organic compounds, including distillery spentwash.

Anaerobic digestion, which is a popular method for treatment of spentwash, results in formation of a dark brown sludge. The effluent after such treatment has reduced Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) but is still dark brown in color and is a major problem with distilleries.

Conventionally used color removing technologies include secondary treatments such as

enzyme pre-treatment, resin separation and ion exchange, adsorption on wood, membrane processes, irradiation, electrolytic processes, etc. However, conventional biological treatment systems are not successful in removing color.
Commercially available polyelectrolytes are used for removing color from pulp mill effluents. However, the cost-benefit analysis of this treatment has not yet been worked upon and hence this type of technology is not viable. However, since the polyelectrolytes rely on ionic charge of the effluent, the color reducing ability will be highly variable, considering the enormous fluctuations occurring in the composition of the wastewater.
The majority of color removal techniques work either by concentrating the color into a sludge or by the partial breakdown or complete breakdown of the colored molecule. However, the color and chemical composition of the pulp mill effluents are usually subject to both daily process as well as seasonal variations. A single, universally applicable end-of-pipe solution has therefore not emerged till date.
General physico-chemical color removal methods such as chemical precipitation,

rapid sand filtration, membrane processes and adsorption have been developed. These processes, although are efficient, but expensive.
Application of electrochemical methods is another way to treat the wastewaters from the cellulose paper production. This method guarantees high treatment efficiency but its effectiveness depends upon the types of electrodes, the construction of electrocoagulators and the conditions under which the process is run.
Chemical precipitation, using alum, ferric chloride and lime has also been studied extensively. In spite of short retention times and low capital costs, there are some drawbacks reported, such as high cost of chemicals for precipitation as well as for pH adjustment, voluminous sludge production due to heavy dosages, problems associated with dewatering and disposing of generated sludge and high residual cation levels, so that their color remains in the supernatant.
In theory, biological treatment gives the ideal solution to color removal as less sludge is produced as compared to chemical treatments. Raghu Kumar et al., 2001 (US patent, No. 6,613,559), showed that fungi could also be utilized for color removal from bleached plant effluent. Moghe, et al., 2002 (US patent, No. 6,929,942) have reported the decolorization of pulping effluents using mixed culture algae.
Various other organisms have also been tried for degradation of molasses spent wash in distillery wastewater and bioremediation. However, the time required for maximum decolorization was more, the process was expensive or there were practical limitations on a commercial scale.
Till date, there are almost no reports regarding the use of biosorption for removal of color from spentwash. It is a process in which solids of natural origin are employed for the sequestration or separation and isolation of coloring matter from an aqueous environment. It is more advantageous to use this technique for

treatment of spent wash for two main reasons. Distillery spent wash is the mixture of various polymeric, inorganic and organic recalcitrant compounds. All the methods used so far are found to be insufficient to decolorize this spent wash completely. Also microbial biodegradation of the color components is reported to take 8-10 days, resulting very less decolorisation, due to recalcitrant nature of melanoidins. Another reason is that in case of

microbial biodegradation external nutrient supply is very necessary, as, easily available source of carbon, nitrogen, enzyme activators etc.
Considering all this, the present invention aims at the application of dead biomass of pre grown fungal culture as a biosorbant, for removing color.

OBJECTS OF THE INVENTION

Accordingly, main object of the present invention is to develop a simple and rapid method for decolorization of molasses spent wash using dead fungal biomass of the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference number MTCC 7691.

SUMMARY OF THE INVENTION

To meet the above object, the present invention provides a process for the decolorization of the molasses spent wash using dead fungal biomass of the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference number MTCC 7691.

DETAILED DESCRIPTION OF THE INVENTION

Accordingly, the present invention provides a process for the decolorization of distillery spentwash using dead fungal biomass of the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India,

bearing the reference number MTCC 7691 and characteristics such as described herein, said process comprising:

1. Inoculating the spore suspension of the fungus Aspergillus oryzae in suitable medium and incubating on a rotary shaker for at least 72 hours.
2. Collecting and autoclaving the biomass by conventional method
3. Drying and crushing the biomass to get finely powdered form.
4. Contacting the powdered biomass with the spentwash for specific interval
5. Removing the powdered biomass from the spentwash by any conventional method known person skilled in the art.
6. Desorbing the colored component of the spentwash from the dead biomass for reuse of the biomass.

Particularly, the present invention relates to removal of colored pigment from molasses spent wash by the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference number MTCC 7691.
In a preferred embodiment of the present invention, the medium for growing the fungus comprises of potato infusion, 20 % and dextrose 2 %.
In another preferred embodiment of the present invention, the spore suspension for growing the biomass is at a concentration of 106 -108 spores/ml.
In still another preferred embodiment of the present invention, the said fungal culture is to be grown for at least 72 hours to get maximum biomass.
In another preferred embodiment of the present invention, the biomass is removed from the growth medium by filtration
In one more preferred embodiment of the present invention, the fungal biomass is autoclaved by any conventional method known to person skilled in the art.

In one more preferred embodiment of the present invention, the dead fungal biomass is directly contacted with the raw molasses spent wash.

In yet another preferred embodiment of the present invention, the concentration of molasses spent wash is between 10 to 50 percent.
In still another preferred embodiment of the present invention, the color of distillery spent wash is removed by directly contacting the dead fungal biomass.
In a preferred embodiment of the present invention, the color of the distillery spent wash is reduced by 60 percent.
In yet another preferred embodiment of the present invention, the contact period required for decolonization is 15 minutes per gram of biomass.
In an alternate embodiment of the present invention, the said fungus can also be grown in conventional media such as potato dextrose broth or large biomass of the fungus can also be obtained readymade from any antibiotics manufacturing unit where such biomass is a waste product.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

Figure 1 relates to sorption profile of spentwash at varied concentrations and different time intervals by Aspergillus oryzae by 1gm biosorbent.
Figure 2 relates to sorption profile of spentwash at varied biosorbent cone, of A. oryzae after 20 minutes.
Figures 3 and 4 relate to desorption studies carried out on the fungal biomass

DETAILED DESCRIPTION WITH RESPECT TO EXAMPLES AND DRAWINGS

The invention is described in detail in the examples given below which are provided to illustrate the invention and therefore should not be considered to limit the scope of the present invention.

EXAMPLE 1

Culturing of the Fungus:

The culture Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference number MTCC 7691 was maintained on slants of potato dextrose agar containing potato infusion, 200 g; dextrose, 20 g; and agar 15 g per liter water.
For inoculum preparation, the spores were inoculated at a concentration of 106 spores/ml in potato dextrose broth. The pH of the medium was 5.6±0.2. The culture flasks were inoculated at room temperature on a rotary shaker at a speed of 130-140 rpm for 72 hours.

EXAMPLE 2

Preparation of biomass

The biomass of the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference number MTCC 7691, grown as described in example 1 was strained through a muslin cloth, washed twice with tap water and autoclaved at 15 psi for 20 minutes. Dead biomass was dried in sunlight for 3-4 days and then crushed thoroughly in a mortar and pestle to get fine powder.

EXAMPLE 3

Biosorption studies

The biomass of the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference number MTCC 7691 was used for biosorption studies. One gram of dry biomass was added to 100 ml of 30% spent wash (30ml spent wash +70ml water) in a conical flask. Contents were mixed on the

rotary shaker at 130-140 rpm, for 30 minutes and then filtered through Whatman filter paper. Biosorption was tested with decreasing optical density of filtrate, monitored on UV- visible spectrophotometer (Shimadzu, 1601) at 475nm, as the A,max of melanoidin in the spent wash is 475nm.

EXAMPLE 4

Effect of different concentrations and time intervals on biosorption

Sorption profile of spent wash of varying concentration and different time interval was studied by taking spent wash concentration in the range of 10% - 60% and time interval of 10 min. from 10 to 60 minutes. Biosorption was tested with decreasing optical density of filtrate, monitored on UV- visible spectrophotometer (Shimadzu, 1601) at 475nm, as the Amax of melanoidin in the spent wash is 475nm.

EXAMPLE 5

Effect of pH on biosorption

The biomass of the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference

number MTCC 7691 was used for biosorption studies. Sorption profile of spent wash at different pH was studied at a pH range of 2 - 10.5. Biosorption was tested with decreasing optical density of filtrate, monitored on UV- visible spectrophotometer (Shimadzu, 1601) at 475nm, as the Xmax of melanoidin in the spent wash is 475nm. pH range of 2 - 4 (acidic) was observed as most suitable. Increasing pH found to reversibly affect biosorption mechanism as shown in Figure 3. It is a significant observation because original pH of the spent wash is also 2-4, which offers the advantage that there is no need to adjust pH before biosorption treatment.

EXAMPLE 6

Desorption studies

Residual part of fungal biomass was dried at room temperature for 3 days and preserved. Desorption of the spent wash components was tried initially using 25% 50% and 75% ethanol. Sedimented and dried biosorbent in each flask were collected separately and added in 50ml of ethanol and stirred on magnetic stirrer. 5ml aliquots interval were at time interval of 20 minutes and whatever color desorbed was estimated spectrophotometrically at 475nm. Desorption profile of the absorbed spent wash components was maximum by using 75% alcohol in the period of 80-100 minutes. Desorption mechanism points out the recycling of dead fungal biomass to remove the colour. Also reuse of the desorbed components for other purpose can be possible.

We claim

1. A process for decolorization of distillery spentwash using fungus strain Aspergillus oryzae MTCC 7691 said process comprises:

(a) growing the strain of Aspergillus oryzae in a suitable medium containing assimilable C and N source for at least 3 days;

(b) harvesting the biomass and processing it into a fine powdered form;

(c) contacting the resulting fungal biomass with molasses spent wash or water contaminated with molasses spent wash for a minimum period of 20 minutes;

(d) removing the fungal biomass to get the spent wash or water contaminated with molasses spent wash devoid of color imparted by the

molasses spent wash.
(e) desorbing the fungal biomass for reuse using 75% alcohol to remove the colored component

2. The process as claimed in claim 1, wherein the carbon source for growing the fungus is selected from the group consisting of glucose, sugarcane bagasse, sugarcane molasses or mixture thereof and having at least 1% concentration.

3. The process as claimed in claim 1, wherein the concentration of molasses spent wash in the effluent is between 10 to 60 percent vol/vol.

4. The process as claimed in claim 1, wherein melanoidin pigments of the molasses spent wash are removed by contacting the said fungal biomass.

5. The process as claimed in claim 1, wherein the retrieval of fungal biomass after decolorization of molasses spent wash is carried out by filtration.

Abstract

The invention relates to a process for decolorization of molasses spent wash or water or soil contaminated with molasses spent wash using dead biomass of the fungus Aspergillus oryzae, which has been deposited at Institute of Microbial technology, Chandigarh, India, bearing the reference number MTCC 7691 as biosorbant. The process involves growing the fungal biomass, autoclaving it and processing it to form a fine powder, for use as a biosorbant. The biosorbant can be desorbed to remove the colored component and can be used repeatedly for a number of times for removal of color from molasses spent wash or water contaminated with molasses spent wash.

N/A