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Isolation of biomethanated effluent (BME) Degrading Bacteria and Fungi

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Abstract

Biomethanated effluent (BME) has low DO, high BOD, COD, TSS which has adverse impact on environment if this waste effluent were not properly treated and dumped directly in water source; the quality of water gets badly affected and in turn affects aquatic life. This will cause a serious hazard to environment; hence treatment of distillery waste becomes essential. Thus this work aimed at isolation of microorganisms that can degrade BME. Physical and chemical analysis of effluent collected from Padmashree vithalrao Vikhe patil SSK Ltd. Pravaranagar Ahemadnagar, and from Sanjivani Takali SSK Ltd. Sahajanandnagar kopergaon India, was carried out to observe. Bacterial strains isolated from the soil collected from distillery effluent exposed land were identified as *Acenetobacter* spp, *Staphyloccous spp.* and *Acitobacter spp*. and the Fungal strains as *Aspergillus*, *Wardomyces*, *Rhizopus*. All the bacterial and fungal strains were found to reduce pollutant majorly, TDS, COD, BOD. Bacteria reduce TDS up to 70%, COD 40%, BOD 20% and fungal isolates reduce TDS 75%, COD 74%, BOD 80%. Thus bioremediation with the indigenous organisms is a possible cost effective solution for the degradation of BME.

Keywords: Biomethanated effluent (BME), bioremediation, Bacteria, Fungi

1. Introduction

Molasses spent wash or distillery effluent is the wastewater generated after distillation of alcohol in the distillery plant. The dark brown colour of distillery effluent with low pH and high concentration of organic and inorganic matter (Wedzicha and Kaputo, 1992) is mostly due to the presence of coloring pigments like melanoidin and caramel. In distillery industries the raw spent wash is directed to the effluent treatment plant for anaerobic digestion to reduce the organic and inorganic loads of the wastewater. The pH of the treated spentwash increases from 4.5 to 8.0 after treatment from anaerobic digester and is called as Biomethanated effluent. The BOD and COD load also decreases significantly from 65 to 80%. The remaining load after biomethanation process is still high enough, which cannot be released in the water bodies. Chemical methods for BME treatment are expensive and generate large amount of sludge, causing secondary pollution. Hence, in the recent past, microorganisms capable for decolorization and mineralization of distillery effluent have become the center of attraction. The effect of pH on color removal from molasses wastewater by *Aspergillus niger* was studied by Miranda *et al*. (Miranda *et al*., 1996). Various basidiomycetous fungi have showed promising result for melanoidin decolorization (Sirianuntapiboon *et al*., 1988; FitzGibbon *et al*., 1995). Sirianuntapiboon *et al*. (2004) used an acetogenic bacterium to obtain a decolourization yield of 76.4% under optimal nutrient conditions. Thus the current work focused on treatment of BME with indigenous microorganisms.

2. Material and Methods

2.1 Collection of samples

The biomethanated distillery effluent sample and soil from same area, was collected from M/s Padmashri Dr. Vithalrao Vikhe Patil SSK Ltd. Pravaranagar, Ahmednagar and was assigned as Sample 1 and from M/s Sanjivani Takli SSK Ltd. Sahajanandnagar, Kopergaon which was assigned as Sample 2. The effluent samples were collected in sterile plastic cans from the outlet of anaerobic biodigesters and were stored at 4 degree until further analysis.

2.2 *Isolation and Characterization of BME Degrading Bacterial Isolates*

Soil samples were collected from above areas. 1gm of soil was added to 10ml of sterile distilled water and serially diluted up to 10^{-6} dilution. Using spread plate technique, 0.1ml of last three dilutions was spread on sterile Nutrient Agar with 30% BME. The plates were incubated at 37^0C 48 hrs. Isolates showing significant BME discoloration were

identified and characterized on basis of morphological, cultural, and biochemical characteristics using identification scheme of Bergeys Manual of Determinative Bacteriology (9th EDITION) that identifies bacteria.

2.3 *Isolation and Characterization of BME Degrading Fungal Isolates*

Soil sample was collected from above areas. 1gm of soil was diluted with 10ml of distilled water and serial dilution of all samples was carried out till 10^{-6}. Using spread plate technique, 0.1 ml of each dilution was spread on sterile PDA plates containing 30% effluent. Plates were incubated at 30^0C for 48-72 hrs.

The morphological characteristics of the fungus were studied by slide culture technique. From the cultural, morphological, microscopic characteristics, these isolates were identified up to genus level using the Fungal Atlas and Compendium of soil fungi (Domsch *et al.*, 1980).

2.4 *Treatment of BME with isolated microorganisms (Bacteria and Fungi)*

Bacterial treatment

Isolated bacteria were grown by inoculating the culture in 100ml Nutrient broth for 24 hrs at 37˚C in conical flask. 10ml of 24 hrs old bacterial cultures were added to both BME sample 1 and sample 2 in respective flasks, incubated on shaker incubator for 7-10 days. After 10 days BME samples were filtered with the help of Whatman filter paper No.1 and were centrifuged at 4000 rpm for 10 min. Supernatant was collected for post-treatment chemical analysis.

Fungal treatment

Isolated fungi were grown in 100ml Sabourauds dextrose broth at room temperate for 7 days. After heavy growth of fungal mycelia, 100 mL BME (S1, S2) was added to the respective flask and incubated at room temperature in static condition for 7 to 10 days. Samples were filtered with Whatman filter paper No.1 and centrifuged at 4000 rpm for 10 min. Supernatant was collected for post-treatment chemical analysis.

2.5 *Physiochemical analysis*

All solutions for analysis were prepared with distilled water and analytical grade chemicals. All tests were done as per international and national standards (Manivasakam, 1996). Parameters like pH, Odor, Color, Total solids, Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Sulphate, Calcium, Copper, Iron, Potassium, Sodium, and Phosphate were analyzed before and after treatment.

3. Results

3.1 *Identification and characterization of BME degrading bacteria*

The bacterial isolates were subjected to a set of biochemical test which included: Oxidase, Catalase, Citrate utilization, Nitrate, Carbohydrate utilization. Isolates 1, 2 & 3 (named as B1, B2 & B3 respectively) were identified from cultural, morphological (Table 1), biochemical characteristics (Table 2) & Bergey's manual of Determinative Bacteriology (9th Edition). These isolates may belong to B1- ***Acinetobactor*** spp. *B2*-***Staphylococcus*** spp. & *B3*-***Acetobactor*** spp.

Table 1: Colony Characters and Enzyme Reactions

Colony characters	B1	B2	B3
Size	1mm	2mm	4mm
Shape	Pin point	Circular	Elliptical
Colour	Yellowish	Light brown	Creamish
Margin	Entire	Regular	Smooth
Elevation	Convex	Convex	Flat
Opacity	Opaque	Opaque	Opaque
Consistency	Sticky	Sticky	Dry
Gram character	Gram negative bacilli	Gram Positive cocci	Gram negative rods
Motility	Non motile	-	Non motile
Oxidase	-	-	-
Catalase	+	+	+
Gelatinase	-	+	-
Nitrate	-	+	+
Citrate	+	+	-

3.2 *Identification and characterization of BME degrading fungi*

Isolate 1, 2 & 3 (named as F1, F2 & F3 respectively) were identified from the cultural, morphological (Table 3), microscopic characteristics (Fig 1) & by using Fungal Compendium. These isolates may belong to F1-***Aspergillus*** F2-***Wardomyces*** & F3-***Rhizopus*.**

Table 2: Sugar fermentation for Bacterial isolates

Sr. No.	Sugars	B1	B2	B3
1	Glucose	+	-	+
2	Sucrose	+	+	+
3	Fructose	-	-	-
4	Lactose	-	-	-
5	Dextrose	+	-	+
6	Maltose	-	+	+
7	Mannitol	+	-	+

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Table 3: Colony morphology of the fungal isolates

Isolate Colony characteristics	**F1**	**F2**	**F3**
Color	Light green	Dark green	White
Size	2 cm to 2.5cm	2 cm to 2.5cm	1cm to 1.5cm
Shape	Spherical	Spherical	Spherical

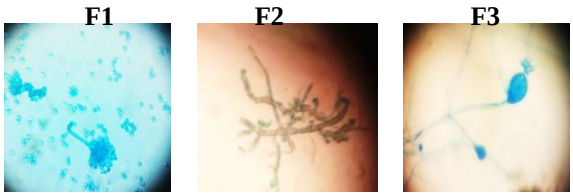

Fig 1: Microscopic observation of fungal isolates

3.3 *Post-treatment chemical analysis of BME treated with bacteria*

Treatment of BME with bacterial isolates showed that the dark color of the BME was decolorised to light brown. Similarly, reduction in many other parameters was observed due to the bacterial treatment (Table 4).

3.4 *Percent reduction in physio-chemical parameters by bacteria*

Treatment of BME with bacterial isolates showed that B3 could significantly reduce many of the physio-chemical parameters of the BME sample 2, studied here (Table 4). Overall, it was observed that the BME sample 2 was more degradable than the BME sample 1.

3.5 Percent reduction in physio-chemical parameters by fungi

Treatment of BME with fungal isolates showed that F3 could significantly reduce many of the physio-chemical parameters of the BME sample 2, studied here (Table 5). As seen in the treatment of BME with the bacterial isolates, it was also observed for the fungal treatment that the BME sample 2 was more degradable than the BME sample 1.

Table 4: Percent reduction in physio-chemical parameters by bacterial isolates

Parameter	B1		B2		B3	
In ppm	S1	S2	S1	S2	S1	S2
TDS	55	79	**97**	62	53	**72**
COD	6	17	17	28	10	**40**
BOD	10	5.9	4.7	4.1	**17.2**	5.87
Sulfate	30	20	30	30	23	**46**
Phosphate	12	**39**	10	28	20	10

Table 5: Percent reduction in physio-chemical parameters by fungal isolates

Parameter	F1		F2		F3	
In ppm	S1	S2	S1	S2	S1	S2
TDS	63	71	59	67	64	**75**
COD	71	60	67	57	**74**	71
BOD	48	45	60	58	**80**	**70**
Sulfate	**61**	**70**	46	50	30	30
Phosphate	13	3	-	2	**20**	**46**

Bacterial isolates showed reduction in pollution load like TDS -70%, COD – 40%, BOD – 20%, Sulfate and Phosphate up to 40%.

Fungal isolates showed reduction in pollution load like TDS – 75%, COD – 70%, BOD – 80%, Sulfate – 70% and Phosphate – 46%

4. Discussion

Microbial decolourization and degradation is an environment friendly and cost competitive alternative to chemical decomposition processes (Moosvi *et al*., 2005, Kumar *et al*., 1998). In order to reduce the colour and COD, it is considered highly desirable to exploit the biodegradation potential of soil microorganisms from polluted sites exposed to recalcitrant compounds of distillery spent wash for prolonged periods (Kumar *et al*., 1998). Microbial decolourization and degradation is an environment friendly and cost competitive alternative to chemical decomposition processes (Moosvi *et al*., 2005, Kumar *et al*., 1998). Physical or chemical methods of waste treatment are invariably cost intensive and require high reagent dosages and generate large amount of sludge (Mohana *et al*., 2007, Adikane *et al*., 2006) and cannot be employed in all industries.

An environment friendly and cost competitive alternative to chemical decomposition processes is microbial decolourization and degradation. These microorganisms can be exploited to reduce the colour and COD and other hazardous parameters of the BME. Similar attempt was made in this current study which could isolate three bacterial and three fungal strains that reduced the unwanted parameters of the BME. This could be due to their inherent capacity to breakdown a variety of complex compounds for degradation/decolorization of toxic and recalcitrant compounds present in various industrial wastes for environmental safety (Gonzalez *et al*., 2000; Chowdhary *et al*., 2017; Chowdhary *et al*., 2018). In this present study fungal strains were observed to be more promising organisms that can be used for the treatment of the BME than the bacterial isolates.

Bacterial strains like *Pseudomonas, Aceinetobacter, Enterobacter, Aeromonas* were found to be effective in case of anaerobic digestion process (Ghosh *et al*., 2004). Fungi like *Aspergillus niger, Pleurotus ostreatus* were found to be effective in the

degradation of spent wash as well as in the biological treatment of wastewater of coffee pulp (Rodriguez *et al*., 2003 and Namdhari *et al*., 2012).
However, in this investigation, it has been observed that by using the indigenous bacterial and fungal isolates, the physico-chemical parameters can be reduced and bioremediation of BME is possible.

5. Conclusion

It is becoming increasingly important from environmental and aesthetic point of view to eliminate the pollutants from distillery effluent. The treatment of this stream is rather challenging by conventional methods due to the large volumes of effluent and presence of certain recalcitrant compounds in it. Thus it is very necessary in today's world to use biological methods to remove the hazardous contaminants. With this view, we have isolated indigenous microorganisms (fungi & bacteria) showing reduction of such recalcitrant compound, which could be cost effective and time efficient process too. The consortium of these microorganisms would degrade more effectively and efforts in this direction are a must.

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