Bioremediation of Ayurvedic Hospital Wastewater Using Bacillus Cereus: Effect of Agitation Speed and Time

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Abstract— *Ayurveda is an inevitable medicinal treatment process for Indian community particularly in Kerala. Kerala has a number of Ayurveda treatment centers, which generate large quantity of wastewater to the nature. A clear protocol has not defined for the effective treatment of oily contaminated wastewater coming from Ayurvedic hospitals. Bioremediation of ayurvedic wastewater using bacillus cereus is being studied to overcome this problem. Bacillus cereus was isolated from the ayurvedic wastewater contaminated soil and applied to the wastewater for bioremediation studies. The ability of the microbe to degrade the wastewater was observed by investigating the effect of agitation speed and time on the removal of oil & grease, COD and BOD. The results showed that bacillus cereus could reduce 88%, 92% and 90% of oil & grease, COD and BOD respectively at an optimum agitation speed of 180rpm, at 72hrs at a temperature of 37◦C. The results of this study showed that bioremediation of wastewaters from Ayurvedic hospital with native microorganisms isolated from oily residues can be efficiently used for the removal of oil and grease, COD & BOD.*

***K*eywords—** Bioremediation, Ayurveda, oil and grease, microorganism, wastewater

I. INTRODUCTION

Ayurveda is an inevitable medicinal treatment process for Indian community particularly in Kerala. Kerala has a number of Ayurveda treatment centers, which generate large quantity of effluents to the nature. Wastewater treatment of ayurvedic hospital is a major task all over the world. A clear protocol has not defined for the effective treatment of oily contaminated wastewater coming from Ayurvedic hospitals. A large amount of ayurvedic oil is discharged from these hospitals each day causing heavy pollution in fresh water and seawater [1]. After bathing and washing, water will be contaminated with oil and grease, particulate solids, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and suspended solids (SS), which may cause tedious pollution to the water body near by the hospitals. Parameters for bioremediation such as nature of pollutant, moisture content, pH, Temperature, nutritional state, microbial diversity of the site and oxidation-reduction potential affects the bioremediation process [2]. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions [3] may permit microbial organisms to degrade otherwise recalcitrant molecules [4]. Strategies for inexpensive and natural bioremediation include natural attenuation, biostimulation, bioventing, bioaugmentation, landfarming, composting, and phytoremediation. Compared to traditional methods such as incineration, bioremediation is an economical technique and since its a natural process , bioremediation is well accepted among the public. Microorganisms either present naturally in the environment or introduced to the site are the prime agents for bioremediation process [5]. Microorganisms can be isolated from almost any environmental conditions and these microbes can adapt at subzero temperature, extreme heat, in the presence of oxygen, anaerobic condition, in the presence of hazardous contaminants, or at any waste stream. The main requirements for microorganisms are the energy source and the carbon source. Bioremediation technology consist of promoting the growth of specific microbial consortia that are naturally present at the contaminated site are able to perform desired activities [6]. Biodegradation of oil depends on the nature of the oil, the type of microbial community and a variety of environmental factors. A variety of treatment processes are used to remove the oil impurities from oily wastewater which includes electrochemical treatment, membrane filtration, use of biological treatment, adsorption, flotation , chemical coagulation etc. Green oils are being used as consumer goods and as raw materials in industries such as food processing, pharmaceutical and cosmetic. Spills of non-petroleum hydrocarbons including vegetable oils, ayurvedic oil and fish oils are of environmental concern because of their potential to cause serious effects on marine life and coastal environments. Biodegradation by indigenous microorganisms is an important and potentially omnipresent process which affect both the chemical composition and physical properties of contaminant oils. Ayurvedic medicines involves a large number of ingredients in their preparation which makes them difficult to study. For example Grahanimihiratailam (GRM) is a liquid preparation which uses 34 ingredients with Sesame oil as the basic ingredient. The GC-MS analysis of the saponified and methylated sample lead to the identification of 14 fatty acids. In this, linoleic acid (43%) was present in a higher amount when compared to others. Palmitic acid was the next major fatty acid (20.6%) in sample. The other major fatty acids are respectively myristic acid (8.9%), lauric acid (5.9%), eicosanoicacid (5.7%) and capric acid (3.4%). 99.18% of the total fatty acids were identified and 0.820% was left unidentified [7]. Hence there should be a specific microbial degradation of these wastewater before discharging to the nature.

II. OBJECTIVES

The objectives of this study were

1) To characterize the Ayurvedic hospital wastewater
2) To get the growth curve of *bacillus cereus*
3) To study the biodegradation of Ayurvedic wastewater at different agitation speed and time.

III. MATERIALS AND METHODS

A. Collection of sample and physico chemical analysis of the wastewater

Ayurvedic waste water and soil sample was collected from Government Ayurvedic Hospital Varkala and the collected waste water and soil sample was stored in the refrigerator at a temperature of 4°C. Both the physical and chemical characteristics of the Ayurvedic wastewater was analysed which includes colour, pH, BOD, COD, TSS, TDS, oil and grease (OG), turbidity , conductivity based on Standard Methods.

Fig. 1: Ayurvedic wastewater collected

B. Growth Curve of Bacillus Cereus in the Wastewater

The growth curve of the identified microbe in the Ayurvedic wastewater was analysed using Spectrophotometer at OD600.

C. Preparation of inoculum

The bacterial culture was inoculated in nutrient broth medium and kept in the incubator shaker at 200rpm at 37°C for a period of five days. The growth was recorded depending on the extent of turbidity by nephlometer.

D. Bioremediation studies

50ml of wastewater was diluted to 100ml in a conical flask and inoculated with 1ml of bacillus cereus strain. Conical flasks were kept in a shaking incubator at 100-200rpm for 24hrs to study the effect of agitation speed. Effect of time on treatment was analysed by incubating the conical flasks for 24, 48, 72 and 96 hours. After incubation oil & grease, BOD & COD were analysed.

IV. RESULTS AND DISCUSSION

A. Characteristics of the Wastewater

The wastewater collected from the Ayurvedic hospital was highly turbid and dark brown in colour. The chemical characteristics of the wastewater such as pH, BOD, COD, oil and grease, total dissolved solids, total suspended solids, turbidity, conductivity are given in the table 1.

Parameters tested	Value
pH	4.37
COD	3680 mg/l
BOD	2200 mg/l
Oil and grease	64 mg/l
Total dissolved solids	2000 mg/l
Total suspended solids	3000 mg/l
Turbidity	148.8 NTU
Conductivity	638 µS/cm

Table 1: Characterization Of Ayurvedic Wastewater

B. Growth curve of bacillus cereus

Growth curve of Bacillus cereus in the Ayurvedic hospital wastewater in shown in figure 3. From the growth curve it can be seen that upto 1 hr it was lag phase and after 1hr the log phase started and maximum growth value was at 70hrs. After 70hrs the growth decreased and resulted in decline phase.

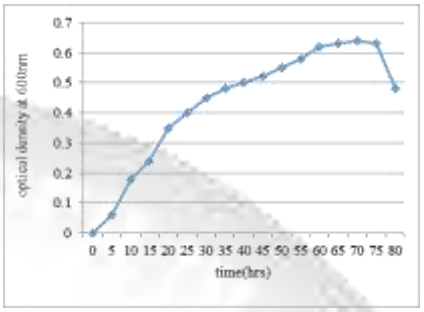

Fig. 2: growth curve of bacillus cereus in ayurvedic wastewater

C. Optimization of Agitation Speed

First the wastewater was treated at different agitation speed of 100, 150, 180 & 200rpm for 24hrs and the decrease in OG, COD & BOD was analysed. It was seen that as agitation speed increased, the degradation rate also increased upto 180rpm. At 200 rpm there was a decrease in percentage degradation. Effect of agitation speed in reduction of OG, COD & BOD is shown in fig 4,5,6. 48.56% OG, 72.28% COD & 80.3% BOD was reduced at 180rpm.

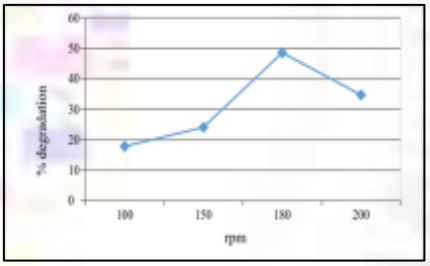

Fig. 3: Percentage degradation of oil & grease at different rpm

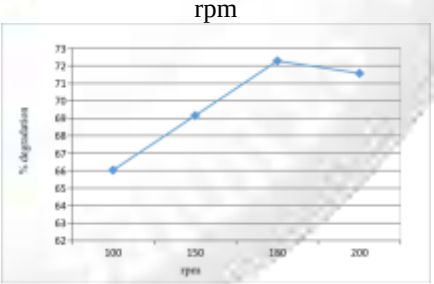

Fig. 4: Percentage degradation of COD at different rpm

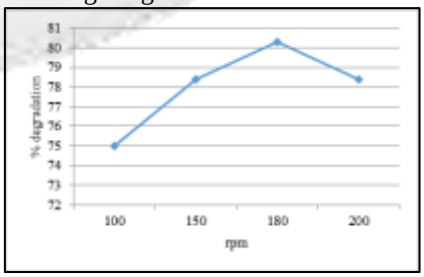

Fig. 5: Percentage degradation of BOD at different rpm

D. Optimization of time

Wastewater was incubated at 37°C with *Bacillus cereus* at 180rpm for different time interval of 24, 48, 72 & 96 hrs. Effect of time on reduction of OG, COD & BOD is shown in fig 7,8,9. In this study treatment with Bacillus cereus at 72hrs gave maximum reduction in OG by 88.23% , COD by 92.01% and BOD by 90.66%.

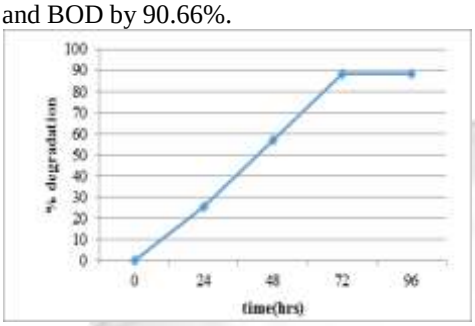

Fig. 6: Percentage degradation of oil & grease with time

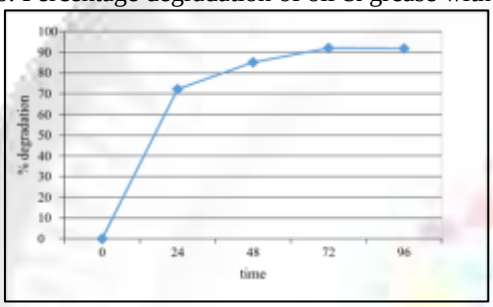

Fig. 7: Percentage degradation of COD with time

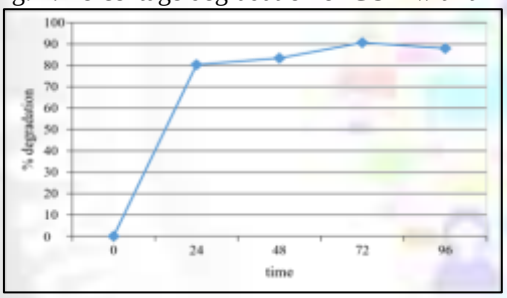

Fig. 8: Percentage degradation of BOD with time

V. CONCLUSION

The present treatment proposal using a selected aerobic bacterial strain isolated from ayurvedic wastewater exhibited high efficiency for removal of oil & grease as well as COD & BOD. Bacterial strain was successfully isolated and identified as *Bacillus cereus* .The results shows that *bacillus cereus* is capable of effectively reducing 88%, 92% and 90% of oil & grease, COD & BOD of Ayurvedic wastewater at an agitation speed of 180 rpm at 72hrs.

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