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Physico-Chemical Analysis and Microbial Degradation of Spent Wash from Sugar Industries

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Abstract

Distilleries are responsible for the generation of ecotoxic wastewater spent wash. Spent wash contains caramel, melanoidin and much more toxic chemicals. Melanoidin is a recalcitrant pigment responsible to give brownish colour to spent wash. In this study the physicochemical properties of spent wash effluent are studied. The isolated indigenous bacterial strain K_5 was selected for the study of melanoidin degradation. Spent wash dilution up to 20% was used to study the degradation of melanoidin by means of isolated bacterial strain. The maximum degradation of about 55% was observed by use of isolated strain K_5. This study shows that by analyzing physicochemical parameters and using indigenous bacterial strains biodegradation of spent wash contaminated land or water can be possible.

Keywords: Spent wash, melanoidin, decolorization, degradation.

Introduction

Spent wash is very rich in organic component and possess high BOD value. Due to its richness in nutrients its use for the agricultural purposes is very common. Upon application of spent wash increase in organic matter, nutrient and soluble carbon has been previously reported[1]. Use of spent wash for the agriculture purposes also avoid many environmental risks associated with the water pollution[2]. Use of spent wash for agriculture purposes not only prevent the water pollution but also serve as a good source of fertilizer[3]. The spent wash is useful as a fertilizer for production of crops like wheat, maize, sugarcane and rape seed. As far as the bioremediation part is concern use of microorganism for the degradation and decolourization process is a suitable way other than chemical decomposition processes[4]. But so far there is a limited success in decolorization of spent wash by fungi and bacteria has been reported[5].

The problem of using distillery spent wash is that due to its long term use for agriculture purpose increase in quantity of salt has been reported[6]. Due to improper guidance and knowledge to use spent wash as a fertilizer, its excess use by farmer's results into stunted growth of crops and increase in salinity of soil. Therefore understanding of chemical parameter of spent wash is very necessary to avoid the dangerous impact on crop ecology.

Most of the industrial effluents are insusceptible to degradation. Toxic metals are extremely lethal for living beings. Melanoidin is one of such recalcitrant compound present in spent wash which is responsible to induce toxicity in soil as well as in aquatic habitat[7]. Spent wash also contains mutagenic and carcinogenic compounds as well as other chemical compounds like dioxins, phenols, lignins etc[8]. Bioremediation of low molecular weight chemical compounds is possible but high molecular weight chemical compounds remains unaffected and can pass into waterbodies[9].

Isolation and identification of indigenous microorganism possessing biodegradation potential is the key step for the development of efficient effluent treatment in bioremediation technology[10]. Bioremediation technology involving application of aerobic and anaerobic bacteria is get considered as a efficient method of detoxification of spent wash. By considering these points related with the ecotoxicity we investigate here the physico-chemical analysis and microbial degradation of spent wash from sugar industries.

Material and Methods

Collection of samples: Spent wash samples were collected in sterilized plastic bottles. Temperature and pH of the samples at the time of collection were measured and kept in controlled environment.

Physicochemical analysis: All solutions are prepared in pure distilled water with AR grade chemicals. pH was determined by pH meter (PICO, Lab India). Other parameters were measured titrimertically and volumetrically by standard method[11].BOD measurement was carried out by means of Winkler method[12].

Isolation of spent wash decolorizing microorganisms: Microorganisms having the capability to decolorize spent wash were isolated by means of enrichment techniques[13]. For the isolation of spent wash decolorizing bacteria minimal salt glucose medium containing 0.5% glucose, 0.01% ammonium phosphate monobasic, 0.05% sodium chloride, 0.02% magnesium chloride, 0.01% potassium phosphate, dibasic, was

supplemented with 1% of spent wash. Different dilutions of spent wash are prepared to isolate the bacteria showing efficient decolorization. Bacterial isolate showing highest decolorization potential was used to further study and inoculated into minimal salt glucose medium containing 20% of spent wash. By means of spread plate technique screening of bacterial isolates were carried out. The obtained bacterial isolates were numbered from K_1-K_5.

Melanoidin degradation studies: The melanoidin degradation studies were carried out by means of analyzing decrease in optical density of the supernatant medium at 475nm, inoculated with test organisms whereas the growth of bacterial strain was monitored at 660nm by using UV-Vis spectrophotometer (Cyberlab UV 100). The percent degradation of melanoidin was calculated by using the formula as[14],
Percent decolorization = Ab – Aa /Ab X 100,

Where Ab is absorbance of compound at 475nm before degradation and Aa is absorbance at same wavelength after degradation.

Statistical analysis: All the experiments were carried out in triplicate. Analysis of the variants was carried out on all data at P< 0.05 using Graph Pad software. (Graph Pad Instat version 3.00, Graph Pad software, San Diego, CA, USA).

Results and Discussion

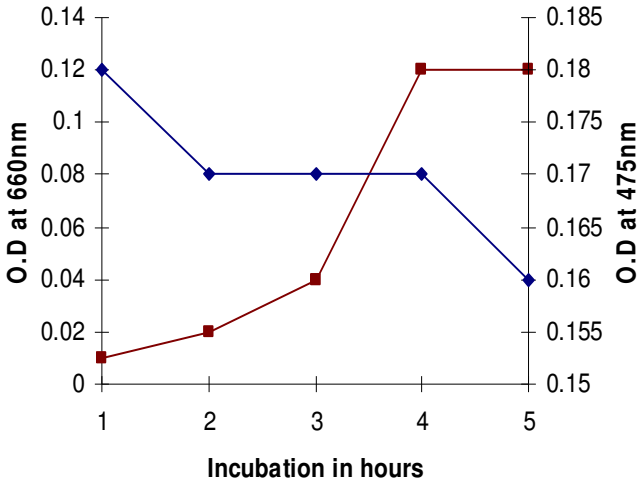
Physicochemical analysis: Table 1 show physical and chemical properties of spent wash sample obtained from sugar industries as,

Table 1
Physico-chemical properties of spent wash samples

Parameters	Effluent No.1	Effluent No.2	Effluent No.3
pH	6.1	6.2	6.7
Temperature	32.1	30.7	32.5
Colour	Brownish	Slightly brownish	Slightly brownish
TS (ppm)	0.6	0.4	1
TDS (ppm)	220	320	518
Total hardness (ppm)	1420	1804	960
Cholride (ppm)	127	161	195
Sulphate (ppm)	2.5	2.7	2.4
Nitrate (ppm)	0.3	0.21	0.15
Nitrite (ppm)	1.7	1.5	1.4
BOD (ppm)	157	370	430
COD (ppm)	152	368	432

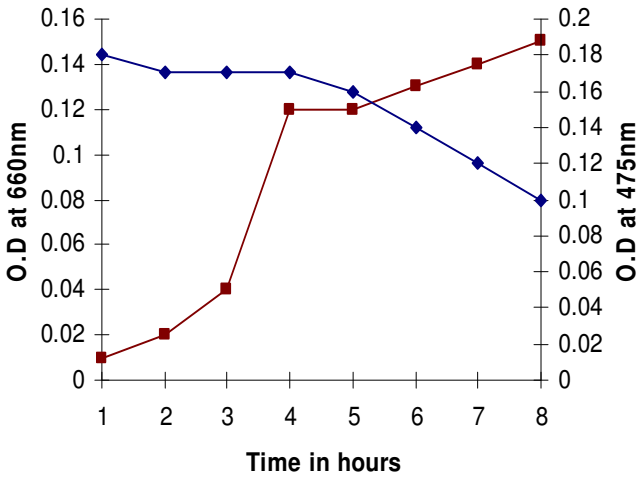
Isolation of spent wash decolorizing microorganisms: The spent wash decolorizing bacteria were isolated on minimal salt glucose medium containing 5% of spent wash. The bacteria showing highest decolorization in minimum incubation time was studied for further study.

Melanoidin degradation studies: Among the isolates obtained bacterial strain no. K_5 was found to decolorize spent wash effectively which was confirmed by means of analysis by using UV-Vis spectrophotometer. The degradation capability of strain K_5 was tested on all three effluent samples. Strain K_5 was found to decolorize all three effluent samples at the same percentage after 12hr of incubation. Figure 3 shows melanoidin degradation of about 55% after 12hrs of incubation. Figure 1 indicates 16% of degradation by strain K_5 after 5hrs of incubation in presence of 20% of spent wash whereas, Figure 2 indicates 44% of degradation was observed after 8hrs of incubation.



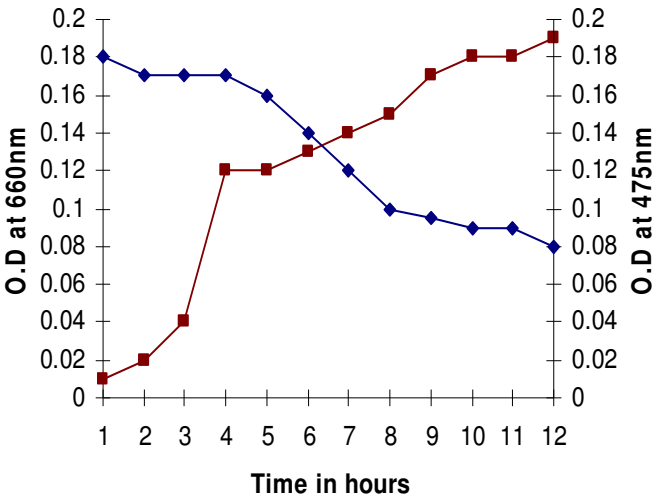
- Growth at 660nm (—■—) and at 475nm (—◆—)

Figure-1
Melanoidin degradation by strain K_5 after 5hrs of incubation



- Growth at 660nm (—■—) and at 475nm (—◆—)

Figure-2
Melanoidin degradation by strain K_5 after 8hrs of incubation



- Growth at 660nm (—■—) and at 475nm (—◆—)

Figure-3
Melanoidin degradation by strain K_5 after 12hrs of incubation

The results obtained after degradation by strain no.K_5 are summarized in table 2.

The potential ecotoxicity of spent wash gave concern about environmental pollution today. Distillery spent wash is the major recalcitrant compound because of the melanoidin, caramel and alkaline degraded products[13]. Physical and chemical methods used for the remediation of spent wash contaminated area are not so effective on industrial scale[15]. Use of biological methods to overcome the problem of contaminated land and water is the most convenient and cost effective method of choice available today. Bacterial strains like *Pseudomonas, Aceinetobacter, Enterobacter, Aeromonas* are found to be effective in case of anaerobic digestion process[16]. Fungi like *Aspergillus niger, Pleurotus ostreatus* are found to effective in the degradation of spent wash as well as in the biological treatment of wastewater of coffee pulp[17,18].

Bacteria like *Bacillus megaterium, Bacillus cereus* and *Xanthomonas fragarie* are also found to be effective in immobilized and free form in case of spent wash degradation[19]. Spent wash degradation by means of alteration in genetic improvement in the strain also been reported[20]. Biodegradation of xenobiotic and recalcitrant compound get studied and concluded that its removal from environment can possible by using genetically modified bacteria[21]. It has been studied that by optimizing different parameters degradation of recalcitrant compound in soil is possible[22]. Biodegradation of xenobiotic compounds by means of bacteria was also get studied and concluded that by using biological treatment toxicity of such chemicals can be decreased [23]. Removal of colour of spent wash by activated charcoal adsorption and electrocoagulation get studied previously and it was concluded that by optimization of various conditions colour removal of spent wash is possible[24,25]. Similarly attempt has been made for the photocatalytic degradation, ultrasound assisted semiconductor of dye degradation[26-28].

However, in this investigation, it has been observed that by understanding the physico-chemical parameters and using the indigenous bacterial isolates bioremediation of spent wash contaminated area is possible.

Conclusion

Due to the composition of various chemicals, spent wash is considered as an ecotoxic effluent. The melanoidin content of spent wash is generally get considered as unsafe for the purpose of agriculture and hazardous to aquatic ecosystem too therefore, use of biological methods to remove these contaminants from environment is very necessary step should be taken today. By using indigenous microorganisms, removal of such recalcitrant compound is possible and it is found to be cost effective and time efficient process too.

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Table 2
Percent degradation of spent wash after 5, 8 and 12hrs of incubation by strain no. K_5

-	Before incubation	After 5hr of incubation	After 8hr of incubation	After 12hr of incubation
Wavelength maxima	475nm	475nm	475nm	475nm
Percent degradation	0%	16.6±0.333	44.2±0.333	55.6±0.333

Results obtained are mean of ±SEM of three experiments

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