



Spent wash: Concomitant Pollution Abatement Potential Employing the Fungus *Aureobasidium pullulans*

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 26/11/2010
Accepted: 30/12/2010

Key Words:

Pollution abatement
Spent wash
Aureobasidium pullulans
Pullulan

ABSTRACT

Spent wash is a typical end product of distillery. It pollutes soil and water due to its characteristics and mixture of pigments, i.e., melanoidins. Various microbial and chemical methods have been designed for reduction of melanoidins. The microbial method has been mainly anaerobic. The objective of the present study was two pronged, first to reduce pollutant value of spent wash by abating COD and BOD using biomass of *Aureobasidium* sp., and next to use spent wash as a growth medium for Aureobasidia. One representative species *Aureobasidium pullulans* has been deployed for this. It produces pullulan, which has wider industrial significance. We supplemented spent wash with various nitrogen and carbon sources to enhance process efficiency, which was essentially aerobic.

INTRODUCTION

Distilleries are one of the major polluting industries. A typical end product is spent wash which is responsible for soil and water pollution. Spent wash is strongly acidic, dark brown coloured, hydrophilic viscous liquid waste with strong objectionable odour. (Jain et al. 2002). Dark brown colour of spent wash is mainly because of presence of brown polymeric melanoidin pigments which are highly recalcitrant. (Chavan et al. 2006). Melanoidins are generated through the Maillard reaction between amino and carbonyl group in organic substance (Nayoki 2000). Antioxidant nature of the pigments make them toxic to many microorganisms.

Spent wash is hazardous to aquatic ecosystems because melanoidins present in spent wash form layer on water surface and reduce photosynthetic activity and decrease dissolved oxygen in water bodies (Kuma et al. 1997). Disposal of spent wash in river and on land leads to pollution of natural water bodies and agricultural lands which decreases fertility of land (Agarwal & Pandey 1994). So it is necessary to decrease pollutant value of spent wash before discharging it into any water body.

Various methods like reduction of melanoidins or decolourisation of spent wash by using microorganisms like *Flavodon flavus* (Chandralata et al. 2004), *Pseudomonas* sp. (Chavan et al. 2006, Ghosh et al. 2009), soil bacteria (Patil & Kapadnis 1995) and *Coriolus hirutus* (Naoyuki et al. 2000) have been reported. Some other biological methods like activated sludge (Naoyuki et al. 2000), anaerobic digester (Patil & Kapadnis 1995) and biodegradation using anaerobic

hybrid reactor (Gupta et al. 2007) have been reported. These are useful for reduction of COD and BOD of spent wash.

The objective of the present study was to reduce pollutant value of spent wash by lowering the key COD and BOD parameters employing biomass of yeast like fungus, viz. *Aureobasidium pullulans*. This is a ubiquitous saprophyte which is found worldwide, commonly in phyllosphere of many crops and other plants (Deshpande et al. 1992). It occurs on the leaves of wide range of crops where it can be an indicator organism of environmental pollution, being able to withstand pollutants. It also grows on the surface layers of many types of soil, where it increases in abundance following nitrogen fertilization (Deshpande et al. 1992). *Aureobasidium pullulans* produces pullulan, an exopolysaccharide. Pullulan is a homopoly saccharide with many attractive properties like non-toxic, non-immunogenic, non-mutagenic and non-carcinogenic. It is transparent, colourless viscous and tasteless (Lachke & Rale 1994, Deshpande et al. 1992). Because of all these attractive properties pullulan is achieving eminence in exploring various biomedical applications, which include targeted drugs and gene delivery, and surface modification. It also has wide applications in food and cosmetic industry (Lachke & Rale 1994, Anna Chelebowska 2007). Our approach in present study was exclusively focused on maximization of aerobic growth on spent wash and concomitant reduction in BOD and COD values. Interestingly, Aureobasidia also produce pigments like melanin and eumelanins (Prezemyslaw & Maja 2006).

MATERIALS AND METHODS

Organism: *Aureobasidium pullulans* (NCIM 1050) was obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. Organism was maintained on Potato Dextrose Agar slants and grown on sterilized Potato Dextrose Broth, distributed in 250 mL flasks, sterilized at 121°C for 15 min. Subsequent to inoculation, flasks were incubated at room temperature (30±2°C) for 48 hours.

Feed stock: Spent wash was obtained from Yashwantrao Sahakari Sakhar Karkhana (YSSK) Ltd., Theur, District Pune. Spent wash was supplemented with various nitrogen sources (ammonium chloride, ammonium sulphate, potassium nitrate) at a concentration of 0.25g/100mL and appropriate control (without nitrogen sources). Spent wash was also supplemented with various carbon sources (cellulose, starch, glucose, lactose, sucrose, xylose, maltose and fructose) at a concentration of 1g/100mL. Supplemented spent wash was distributed into flasks (50mL per 250 mL flask) and sterilized at 121°C for 15 min. Inocula of *Aureobasidium pullulans* were prepared in Potato Dextrose Broth, incubated at (30±2°C) for 48 hours on incubator shaker (150 rpm) and inoculated into spent wash containing flasks at 5% w/v.

Typical analysis of spent wash was done for necessary comparison. Total carbohydrate was estimated using anthrone method. Estimation of nitrogen content was made by Nessler's method. Total solids, total suspended solids, COD and BOD were estimated according to APHA methods (APHA 1976). All these methods were used to analyse the spent wash before and after fermentation. After fermentation, contents were centrifuged at 6000 rpm using cooling centrifuge at 4-6°C for 20 min. Residues were washed and dried at 105°C for 18-20 hours to constant dry weight. Ethyl acetate was added to supernatant and kept at room temperature overnight. Ethyl acetate was removed and ethanol was added (1:1 proportion) and kept overnight. The mixture was centrifuged at 6000 rpm for 20 min. The residue was dried to constant dry weight at 60°C and considered as pullulan. Supernatant was dried for estimation of residual sugar by anthrone method. Pullulan present in residue was analysed for confirmation and compared with standard pullulan (Hayashibara Co. Ltd., Okayama, Japan) using I.R. spectroscopy (FTIR Schimatzu).

RESULTS

After complete fermentation (144 hours), growth of *Aureobasidium pullulans* was observed on spent wash. The fungus grew well on spent wash with substantial reduction in COD (33%), BOD (50%) and TS (33.36%). Carbohydrate and nitrogen contents of spent wash were decreased after

fermentation. The results are presented in Table 1.

Spent wash based media showed that *Aureobasidium pullulans* has maximum production of pullulan using ammonium sulphate as nitrogen source (50g/L). Residual sugar in spent wash based media was the least (340mg/L) with ammonium chloride. I.R. spectroscopy data confirmed that exopolysacchride produced by *Aureobasidium pullulans* was indeed pullulan. Wave numbers of standard pullulan and pullulan from *Aureobasidium* sp. were compared.

DISCUSSION

Observations in this study have recorded reduction in COD, BOD and TS values of spent wash. COD was reduced by 33%, BOD by 50% and TS by 33.36% with the help of an aerobic process. Anaerobic process for reduction of pollutant value of spent wash have been reported (Gupta et al. 2007, Bhavik et al. 2008). Reduction in COD and BOD value with the help of anaerobic process like UAFF method (COD was reduced upto 64%) (Bhavik et al. 2008) and by anaerobic hybrid reactor (COD was reduced upto 79%) (Gupta et al. 2007) have shown to achieve comparatively better results. But *Aureobasidium* sp. used in present study was unable to reduce TSS content of spent wash. Reduction of pollutant value of spent wash by aerobic process has been difficult.

Various organisms have been reported till date which are responsible to decrease melanoidins present in spent wash (Naoyuki et al. 2000, Chandralata et al. 2004, Chavan et al. 2006, Ghosh et al. 2009, Patil & Kapadnis 1995). However spent wash has not been shown to be used for cultivation of any microorganisms. In the present study, we have successfully cultivated *Aureobasidium* in spent wash supplemented with nitrogen and carbon sources. Concomitantly, the organism was able to produce pullulan.

CONCLUSION

Concomitant to growth, the values obtained for COD, BOD and TS were significant in the present study with *Aureobasidium pullulans*. The study establishes that *Aureobasidium* can be used to decrease pollutant value of spent wash and mass production of pullulan. It will be interesting to take forward this study in the direction to reduce TSS and melanoidins in spent wash employing *Aureobasidium* spp.

ACKNOWLEDGEMENT

We are thankful to the authorities for allowing us to work at Department of Microbiology, Fergusson College, Pune, and for providing us laboratory facilities. We wish to thank Hyashibara Co. Ltd., Okayama, Japan for providing pure pullulan. We also thank Dr. Miss Shobhna Bhide, Department of Biochemistry, Fergusson College for help in vari-

Table 1: Analysis of spent wash before and after fermentation using *Aureobasidium pullulans*.

Parameter	Method	Value before fermentation	Value after fermentation (Percent reduction in parentheses)
pH	-	4-5	5-6
COD	Open reflux method	2,64,000 mg/L	88,000 mg/L (33%)*
BOD	5-day oxygen difference method	1,60,000 mg/L	80,000 mg/L (50%)*
Total solids	Total solids dried at 103°C-105°C	151.06 g/L	50.4 g/L (33.36%)*
Carbohydrates	Anthrone method	100 g/L	30 g/L (30%)*
Nitrogen	Nessler's method	8 gm/L	0.29 g/L (3.62%)*

All values are averages of triplicate analyses.

ous analyses. We are also thankful to Sanjog Nagarkar, Department of Chemistry, University of Pune for providing data of I.R. analysis of purified pullulan.

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