

CBSE-2014 [2nd and 3rd April 2014]

Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Effect of initial pH on biodegradation of distillery wastewater by batch process using anaerobic mixed consortia

K. Sridevi* and P. Mullai

Pollution Control Research Laboratory Department of Chemical Engineering
Annamalai University, Annamalai Nagar 608002, Tamilnadu, India

*Corres.author:sridevi.kothandapani@gmail.com

Abstract : Biodegradation of distillery wastewater is dependent on initial pH. In this present research, the effect of initial pH from 5.0 to 6.5 on biodegradation of distillery wastewater using anaerobic mixed consortia by batch process was investigated. The parameters like chemical oxygen demand (COD) removal efficiency, oxidation reduction potential (ORP), final pH, total phenol content, total protein were determined to study the biodegradation of distillery wastewater. From the experimental results, maximum COD removal efficiency of 91.96 % was obtained an initial pH 6.0. Maximum phenol removal, protein removal and decolorization percentage recorded were 88.76%, 71.04% and 55.23% respectively at initial pH 6.5. It could be concluded that initial pH 6.5 are favourable for maximum biodegradation of distillery wastewater.

Introduction

Molasses-based distilleries generate around 8–15 L of wastewater with high chemical oxygen demand (COD) (80–160 g/L) for every litre of the alcohol produced¹. It ranks high amongst the pollutants produced by industries and its treatment had been a challenge for a long time. It contains high concentration of biodegradable organic material, such as sugars, lignin, hemicelluloses, dextrin, resins and organic acids². The high COD, nitrogen and phosphates content contribute to eutrophication of lakes and rivers³. The brown colour of the distillery wastewater is due to the water soluble recalcitrant colouring compound called melanoidin that are formed during Maillard reaction between a sugar and amino acid^{2,4}. They are acidic, polymeric with highly dispersed colloid and are negatively charged due to the dissociation of carboxylic acids and phenolic groups. They possess antioxidant properties, which are toxic to aquatic organisms⁵. Biological (both aerobic and anaerobic treatment), activated sludge treatment, clarification, physico-chemical treatment and filtration are some of the treatment technologies being carried out. Anaerobic treatment of spent wash ensures high degree of treatment and also recovery of renewable resources, which serves as dual purpose⁴. Anaerobic digestion is more attractive for tropical countries like India, where temperatures are suitable for anaerobic digestion^{6,7,4}. Suitable microbes are available for biodegradation of distillery wastewater. Application of bacteria, yeast, fungi, etc used for the biodegradation of distillery effluent^{1,3,8}. Besides, anaerobic mixed consortia can also be used for biodegradation which can degrade the pollutants. In this research work, the effect of initial pH on biodegradation of distillery wastewater was investigated.

Materials and methods

Anaerobic mixed consortia

The anaerobic mixed consortia were collected from anaerobic digester of distillery unit, Tamil Nadu State, India. It was heat pretreated at 102°C for 1 h to inhibit the methanogens and also to speed up hydrolysis, the rate limiting step in anaerobic digestion^{9,10}.

Substrate

Distillery wastewater was used as substrate.

Nutrient solution

One litre of nutrient solution was prepared using the following composition (g/L) NH₄Cl - 0.5; K₂HPO₄ - 0.25 ; MgCl₂.6H₂O - 0.3 ; NiSO₄ - 0.016 ; CoCl₂-0.025 ; ZnCl₂-0.0115; CuCl₂-0.0105; CaCl₂-0.005 and MnCl₂ - 0.015⁹.

Batch tests for biodegradation of distillery wastewater

Batch tests were carried out in 1 L Erlenmeyer flask with a working volume of 700 mL. The initial substrate concentration was maintained at 5000 mg COD/L. The effect of pH was studied by varying from 5.0 to 6.5 The culture medium was inoculated with 1 g VSS/L of heat pretreated mixed anaerobic sludge. After pH adjustment and inoculation, the flasks were capped tightly with a rubber cork and the conical flasks were incubated at 35°C for fermentation.

Analytical methods

COD was recorded for every 24 h and measured according to standard methods¹¹. Volatile suspended solids (VSS) concentration was measured at steady state conditions. pH was measured using a pen type pH meter (Eutech, India). The total phenolic content was estimated by folin-ciocalteau reagent¹² and was expressed as mg of gallic acid equivalent per liter (GAE mg/L). Gallic acid was used as standard compound and the absorbance was read at 750 nm. The total protein content was determined by folin-phenol reagent method¹³. The absorbance was read at 660 nm and the total protein was determined against a standard curve of bovine serum albumin.

Results and discussion

Removal efficiency of COD

The COD removal efficiencies for different initial pH 5.0, 5.5, 6.0, and 6.5 were 76.11, 90.09, 91.96 and 91.07%, respectively (Fig.1). The minimum COD removal efficiency 76.11% was observed at pH 5.0. The recorded ORP values (108 mV) also proved that lower pH 5.0 did not favour the anaerobic digestion and that resulted in lower biodegradation of distillery wastewater (Fig.2). Thus maximum COD removal efficiency of 91.96 % was obtained at pH 6.0. The final pH profile is shown in Fig.3. It is mentioned that initial pH less than 6.0 did not favour the biodegradation of high strength wastewaters like winery wastewater, molasses wastewater, distillery wastewater, palm oil mill effluent^{2,4}.

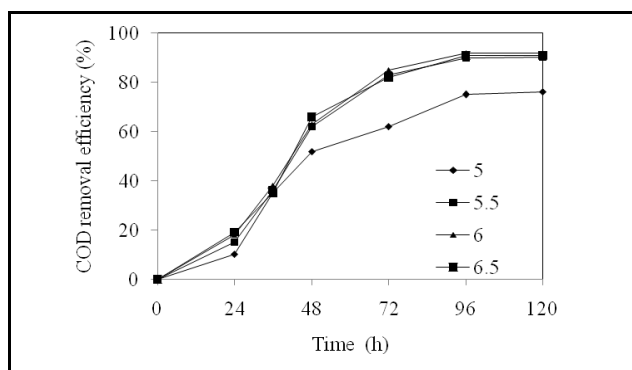


Fig.1 COD removal efficiency in biodegradation of distillery wastewater

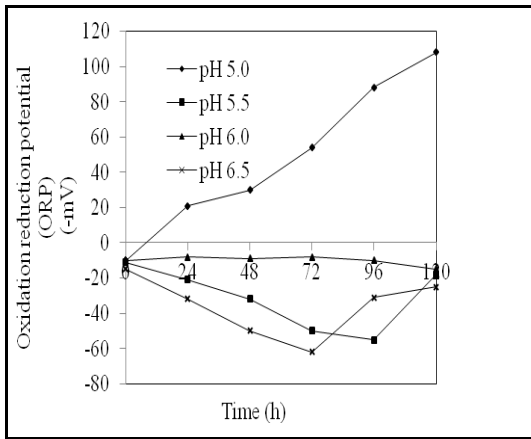


Fig.2 Oxidation reduction potential (ORP) profile observed during biodegradation studies.

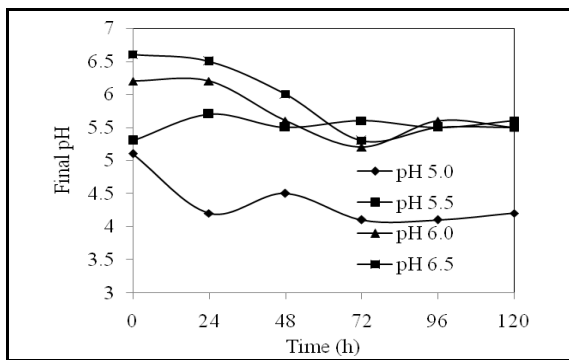


Fig. 3 Final pH profile in biodegradation of distillery wastewater

Total phenol content

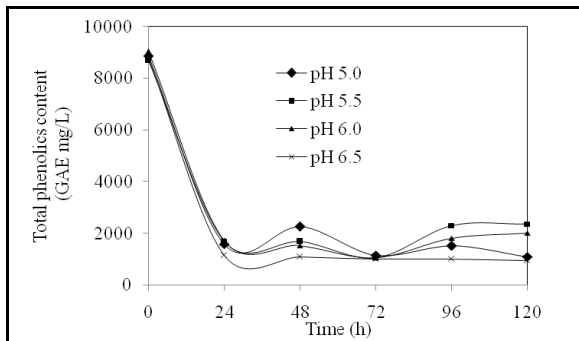


Fig.4 Total phenolic content profile for various initial pHs in biodegradation studies

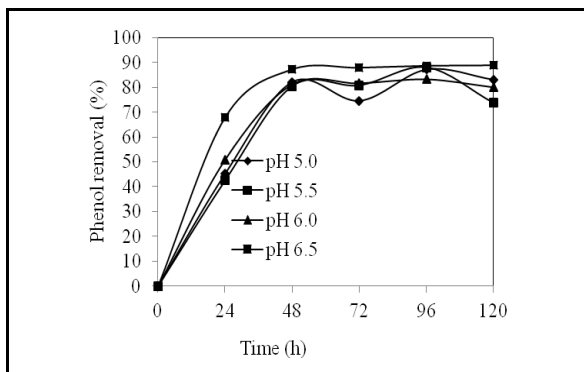


Fig.5 Phenol removal for various initial pHs during biodegradation

Fig. 4 and Fig. 5 show the changes in the total phenol content during the biodegradation of distillery wastewater. The initial total phenol content was present at pH 5.0, 5.5, 6.0 and 6.5 were 8865.01, 8702, 9025 and 8785 GAE mg/L respectively. The total phenol content decreased to 1585 mg GAE/L at 24 h, when the initial pH was 5.0. Similar trend was also observed in other initial pH 5.5, 6.0 and 6.5. At 72, 96 and 120 h, lower phenol content was observed in all the pH ranges. The reduction in total phenol content was attributed to the absorption of phenols on the biomass¹⁴. The increase in total phenol content was due to liberation of simple phenolic compounds after the acid and enzymatic hydrolysis of polymerized phenolic compounds¹⁴. It could be concluded that initial pH 6.5 favoured the maximum removal of phenol from the distillery wastewater. Similarly, in treatment of wine distillery wastewater by high rate anaerobic digestion, 1440 mg/l of phenols was attained after the treatment process¹⁵. The total phenol profile was also reported in treatment of palm oil mill effluent using fungal mycelia¹⁴.

Total protein content

During the biodegradation of distillery wastewater, the protein content profile was studied (Fig.6). The initial protein content was estimated as 8500 mg/L (approximately) in all the initial pH studied. The protein removal at the pH 5.0, 5.5, 6.0 and 6.5 was 3.72, 18.5, 19.69 and 71.04% respectively. From the results, initial pH 6.5 favoured higher protein removal of 71.04% which was also affirmed by the high COD removal efficiency of 91.07% (Fig.7). At initial pH 6.5, the protein concentration at the end of fermentation was found to be 2500 mg/L. The decrease in protein content was attributed to the utilization of protein as substrate from the fermentation medium¹⁶.

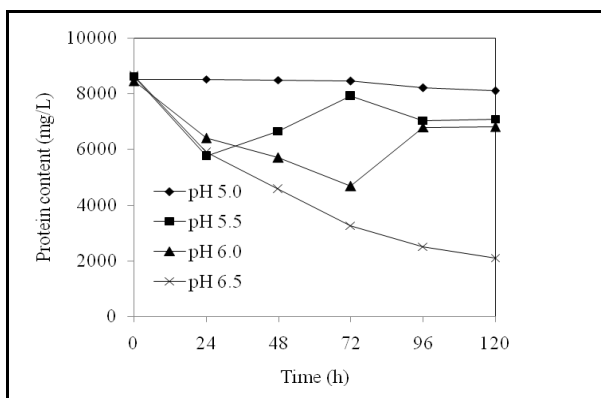


Fig.6 Protein concentration for various initial pHs during biodegradation

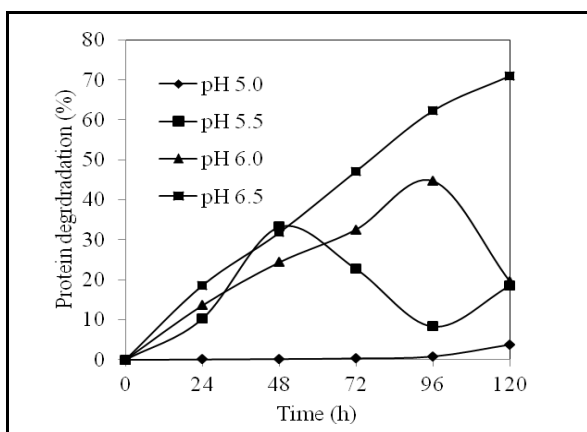


Fig.7 Protein degradation for various initial pHs during biodegradation

Decolourization percentage

pH has a crucial role in melanoidins decolourization^{17, 18}. Molasses wastewater lacks in carbon source, so biodegradation without any additional carbon source was very complex. Hence, supplementation with carbon sources is necessary for decolourization of molasses wastewater by bacterial consortium¹⁸. The decolourization of melanoidins might occur due to the colour adsorption by bacterial cell and the metabolism of facultative and anaerobic bacteria and anaerobic respiration¹⁹.

The decolourization percentage at different initial pH 5.0, 5.5, 6.0 and 6.5 was found to be 23, 45.62, 49.56 and 55.23% respectively. The maximum decolourization of 55.23% at pH 6.5 might be due to the elevated growth of microorganisms and polymerization of melanoidins. It was also confirmed maximum phenol removal and protein removal of 88.76% and 71.04% at initial pH 6.5. High colour removal was observed at acidic pH as the melanoidins are less soluble in acidic pH than alkaline pH and they could be easily precipitated and removed easily. Hence, it could be concluded that protein degradation and biological activity of microbes affected the decolourization percentage¹⁹. Hence, maximum decolourization percentage was achieved in the pH range between 5.5 and 6.5. Acetogenic bacteria strain No.BP103 decolourized 73.5% of molasses pigments in molasses wastewater medium when the medium was supplemented with glucose, yeast extract, and basal mineral salts 20.

Conclusions

The biodegradation of distillery wastewater was dependent on initial pH as the wastewater contained high organic content. The initial pH affected the COD removal efficiency, final pH, ORP, phenol content, protein content and decolourization percentage. The maximum biodegradation was observed in the pH 6.5.

Acknowledgement

The authors are thankful to the authorities of the Annamalai University, India for facilities offered and DBT, New Delhi for funding the research project (No. BT/PR12051/PBD/26/213/2009).

References

1. Kumar, G.S., Gupta, S.K. and Gurdeep, S., 2007. Anaerobic hybrid reactor – a promising technology for the treatment of distillery spent wash. *JISM*, 2007, 11, 25–38.
2. Venkatamohan, S., Mohanakrishna, G., Ramanaiyah, S.V. and Sarma, P.N. Simultaneous biohydrogen production and wastewater treatment in biofilm configured anaerobic periodic discontinuous batch reactor using distillery wastewater. *Int. J. Hydrogen Energy*, 2008, 33, 550–558.
3. Pal, S. and Vimala, Y. Bioremediation and decolorization of distillery effluent by novel microbial consortium, *J. Exp. Biol.*, 2012, 2, 496-504.
4. Sridevi, K., Sivaraman, E. and Mullai, P., 2014. Back propagation neural network modelling of biodegradation and fermentative biohydrogen production using distillery wastewater in a hybrid upflow anaerobic sludge blanket reactor. *Bioresource Technol.* 165, 233–240.
5. Deepak, P., and Adholeya, A., 2007. Biological approaches for treatment of distillery wastewater: A review. *Bioresource Technology*, 98, 2321 -2334.
6. Moletta, R., Winery and distillery wastewater treatment by anaerobic digestion. *Water Sci. Technol.*, 2005, 51, 137–144.
7. Mullai, P., Arulselvi, S., Ngo, H. H. and Sabarathinam, P. L. Experiments and ANFIS modelling for the biodegradation of penicillin-G wastewater using anaerobic hybrid reactor, *Bioresource Technol.*, 2011, 102, 5492–5497.
8. Mohana, S., Desai, C. and Madamwar, D. Biodegradation and decolourization of anaerobically treated distillery spent wash by a novel bacterial consortium. *Bioresource Technol.*, 2007, 98, 333–339.
9. Mullai, P., Yogeswari, M. K. and Sridevi, K. Optimisation and enhancement of biohydrogen production using nickel nanoparticles - A novel approach. *Bioresource Technol.*, 2013a, 141, 212-219.
10. Mullai, P., Eldon, R. R. and Sridevi, K. Biohydrogen production and kinetic modeling using sediment microorganisms of Pichavaram mangroves, India. *BioMed Res. Int*, 2013b, Article ID 265618, 9 pages. <http://dx.doi.org/10.1155/2013/265618>.
11. APHA, 1995. *Standard Methods for the Examination of Water and Wastewater*, sixteenth ed. American Public Health Association, New York.
12. Singleton, V.R., Orthofer, R. and Raventos, R. M. L. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.*, 1999, 299.
13. Lowry, O.H., Rosebrough, N.H., Farr, A.D. and Randall, R. J. Protein measurement with the folin reagent. *J. Biol. Chem.*, 1951, 193, 265–273.
14. Idris, Z. M., Jamal, P. and Md. Zahangir Alam, Evaluation of Palm Oil mill effluent treatment with concomitant phenolics production by *Aspergillus niger* IBS-103ZA, *Aust.J. Basic Appl. Sci.*, 2012, 6, 55-61.

15. Melamane, X. L. Treatment of wine distillery wastewaters by high rate anaerobic digestion and submerged membrane systems, 2006, Ph.D Thesis, Rhodes University.Pp. 133.
16. Raghavarao, K.S.M.S., Ranganathan, T. V. and Karanth, N.G. Some engineering aspects of solid-State fermentation, *Biochem. Eng. J*, 2003, 13, 127-135.
17. Kambe, T.N., Shimomura, M., Nomura, N., Chanpornpong, T. and Nakahara, T., Decolourization of molasses wastewater by *Bacillus* sp. Under thermophilic and anaerobic conditions, *J. Biosci. Bioeng.*, 1999, 87, 119-121.
18. Kumar, P. and Chandra, R., 2006. Decolourisation and detoxification of synthetic molasses melanoidins by individual and mixed cultures of *Bacillus* spp. *Bioresource Technol*, 2006, 97, 2096-2102.
19. Jiranuntipon, S. Decolorization of molasses wastewater from distilleries using bacterial consortium, 2009, Ph.D thesis, Chulalongkorn University. Pp.198.
20. Sirianuntapiboon, S., Phothilangka, P. and Ohmomo, S. Decolourization of molasses wastewater by a strain no. BP103 of acetogenic bacteria, *Bioresarch Technol*, 2004, 92, 31-39.
