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Performance of Acidogenic Microbial Treatment Process of Tannery Wastewater in Batch Digester and Packed Bed Reactor

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To enhance the treatment of tannery wastewater a combined system consisting of acidogenic batch digester and packed-bed bioreactor, using wood chips activated carbon (WCAC) material as immobilization support has been designed. In combined treatment, the COD removal percentage reached a maximum of 80-90% with high organic loading rate (OLR). Acidogenic microbial consortium developed on WCAC was investigated using scanning electron microscopy (SEM). Fourier transform infrared spectroscopy (FTIR) and Gas chromatography–mass spectrometry (GC-MS) analyses elucidated the degradation of complex organics and metabolites formed during treatment process. The potential of acidogenic consortium to treat complex tannery effluent provides significant insight into possible application in treatment of effluent containing diverse groups of organics.

Key words: Acidogenic microorganisms, Packed bed reactor, Tannery wastewater, Long Chain Fatty Acids, degradation.

Development of industries plays a vital role in overall economic strategy. Some important industries such as oil/ fat refineries, food processing industries, tanneries and slaughterhouses generate pollutants containing long chain fatty acids (LCFA), lipids and complex organics. Triacylglicerides are the major constituent of lipids which can be converted to LCFAs and glycerol (Fernandez et al., 2005). Nature of LCFAs in wastewater is mainly attributed on the origin of the lipids; however these compounds have short or long detrimental effect on the environment. Tannery wastes are considered as one of the main polluting source in contaminating the water environment. The effluent from the tanneries contains complex mixtures of both organic and inorganic constituents. The amount of wastes generated during the treatment process depends on technologies applied in each process. Pre-tanning process is one of the major sources of generation of solid wastes in tannery industries. The solid wastes consists of fats, proteins, blood and salts having the potential of generating high methane and can be advantageous to be used as carbon source in an anaerobic digestion process. Production of complex mixtures of LCFA and lipid wastes makes the treatment progress very difficult through conventional anaerobic treatment process. Most tannery organics resist degradation and anaerobic treatment processes is the most opted technology that have been widely used to treat wastewater and to remove toxic metabolites (Ganesh Kumar et al., 2005).

The embedded carbon reserves in LCFA and lipid wastes are considered as viable substrate

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and researchers consistently have used anaerobic treatment technologies for treatment due to its high theoretical methane yield in comparison with that of other organic substrates (Martin-Gonzalez et al., 2010). The potential of anaerobic digestion in LCFA treatment and by-product generation is considered as highly advantageous. Many research findings highlighted the anaerobic microbial diversity involved in degradation of LCFA (Hatamoto et al., 2007). To efficiently treat and produce biogas from lipid rich wastewaters the anaerobic degradation of LCFA is very vital (Sousa et al., 2013). In the process of anaerobic digestion, acidogenic microorganisms play a preliminary role in hydrolyzing and fermenting the complex organic materials into volatile fatty acids (VFA). The produced VFA are then slowly utilized by acetogenic and methanogenic bacteria. Thus VFA is the major intermediate metabolite generated in the pathway of methane generation. Therefore, acidogenic microorganisms are considered as much important for good performance of treatment system. Although anaerobic microorganisms are known to degrade various toxic organics, the compositions of LCFA rich tannery effluents offer resistance to degradation, thus there is a need to isolate new treatment options to utilize and completely mineralize major components of effluents. Therefore, acidogenic microorganisms acclimatization and utilization with broad complex organics substrate speciûcity is of current importance. In the present study, tannery effluent treatment with acidogenic microorganism enriched batch digestor and packed bed reactor with activated carbon matrix was studied in detail.

MATERIAL AND METHODS

Sources of Tannery wastewater

The wastewater employed in the present study was collected from a local tannery plant and stored under dark. Before the start of the reactor the pH of the wastewater was adjusted and mixed with mineral salts (g l”1): NH_4HCO_3 - 5.0; $NaHCO_3$ - 6.5; K_2HPO_4 - 0.125; $MgCl_2.6H_2O$ - 0.1; $MnSO_4.6H_2O$ - 0.015; $FeSO_4.7H_2O$ - 0.025; $CuSO_4.5H_2O$ - 0.005; and $CoCl_2.5H_2O$ – 0.0001.

Acidogenic microbial consortium

The anaerobic sludge obtained from a local UASB treatment plant and cow dung was mixed. The tannery wastewater mixed with mineral salts and 0.01% dextrose was supplemented to sludge at weekly intervals up to 45 days in order to acclimatize acidogenic microorganisms for treatment.

Acidogenic batch digestion with WCAC

Acidogenic digestion is first step in anaerobic degradation and it was studied in 100 mL serum bottles fitted with rubber stoppers and aluminium seal with syringe fitting to monitor gas production. The experiments were started using tannery effluent at pH 7.0, 5% WCAC and 10% acclimatized sludge. Two experimental conditions were set up to elucidate the acidogenic potential (with and without WCAC). Analysis was carried out every 3 days for over 30 days.

Operations of packed-bed bioreactor

The effluent from acidgenic digestor was treated in packed bed reactor. The packed-bed columns were packed with 90 % WCACs as the support for acidogenic microbial growth. The acclimatized anaerobic sludge was mixed along with WCAC and microbial cells stabilized within a brief period of 5 days. The effluent was passed in upflow direction and the top was fitted with gas collector. The reactor was operated at HRT of 4 to 24 h and whole operation was carried out at room temperature at 6.5-7.0 pH.

Analytical methods

WCACs were prepared by pre-carbonization and chemical activation process according to our earlier reports (Selvam and Ganesh Kumar, 2015). Characterizations of wastewater are measured according to Standard Methods (APHA). The microorganisms grown WCAC surface morphology is visualized, using Leo-Jeol Scanning Electron Microscope at the magnification of 2500 – 10000X. The IR spectra of the solvent extracted tannery effluent before and after treatment were measured in the spectrometer after preparation without any moisture under ambient conditions using the transmission mode (%T) and the measurements were carried out in the range 4000 to 400 cm^{-1} with IR Affinity-1 Schimadhzu spectrometer. The solvent extract of tannery effluent was analyzed by GC–MS. The whole analysis was performed on a GC 7890 A, 240-MS/4000 mass spec, External Ionization - EI mode (Agilent, USA).

RESULT AND DISCUSSION

Substrate and support matrix characterization

The basic physic-chemical characterization of the wastewater used in this study has COD 1000–5500 mg/L, pH 6.0–8.0, Total suspended solids 1500–2500 mg/L and kjeldal nitrogen 10-25 m/L. The pore surface area, pore size and pore dia. are detailed in our previous studies (Selvam and Ganesh Kumar 2015).

Enrichment of LCFA utilizing acidogenic microbes

The enrichment was carried out under mesophilic conditions using anaerobic sludge obtained from tannery treatment plant. Lipid rich tannery waste was given as sole carbon source at initial concentration of 1% w/v. Overall growth and mild gas production was observed in enrichment cultures after 1 to 2 months of incubation. This sludge (10% v/v) was successively transferred into fresh medium each month. In the progress of enrichment, the growth rate of acidogenic cultures showed increase in measurable growth concentration and gradual decrease in LCFA. Microscopic observation of the enrichment culture elucidated the presence of two morphologically different microorganisms (Fig. 1). This enriched culture was used as culture for the process of batch digester, hybrid digester and packed bed reactor experiments.

Hybrid Batch digester treatment

As this is a controlled acidogenic digestion the generation of gaseous end products is less during the initial stages. During the start up phase the batch hybrid digester was fed with approximately 1-2 g of VS/L for a short period. The following parameters were monitored at regular intervals: COD removal percentage, TDS, conductivity, ORP, temperature and pH. Biogas production was very less at both the starting and the end stages of digestion. Initial pH in the effluent was in the range of 7.5-8.5 due to high organic load. However, the acidogenic digestion reduced the pH to 5.5-6.0 sharply. The concentration of VFA was decreased to 900 mgL^{-1} during the final stage. Hydrolysis of complex organics and LCFA in the hybrid batch digester resulted in formation of breakdown metabolites which were converted to gaseous end products. This stage results in removal of readily available organics and leaving recalcitrant COD in the reactor. It is noteworthy that in the normal batch anaerobic digestion process, the cumulative gas production is less compared to hybrid digester. The total COD degradation in the hybrid digester was in the range of 60-80% after 2 weeks of digestion while in the conventional system the degradation was around 20-35%. Therefore, much short retention time can be attained in the hybrid digester.

Packed-bed bioreactor treatment

Most of the odour of the acidogenic treated samples was removed efficiently after treatment in packed bed reactor indicating the consumption of complex organics and LCFAs by anaerobic microbial consortium. The pH of the acidogenic treated water changed from acidic (5.5-6.5 pH) to alkaline (7.3-8.0) resulting in process of reducing acidity of the effluents. In the packed bed bioreactors the COD removal was around 70-

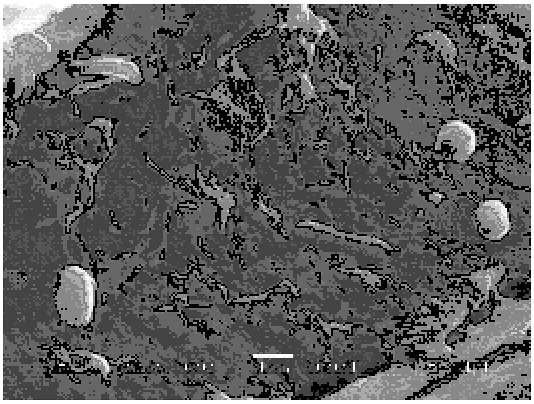

Fig. 1. Scanning Electon Microscopic (SEM) micrographs of morphologically distinct microorganisms in the acclimatized anaerobic sludge.

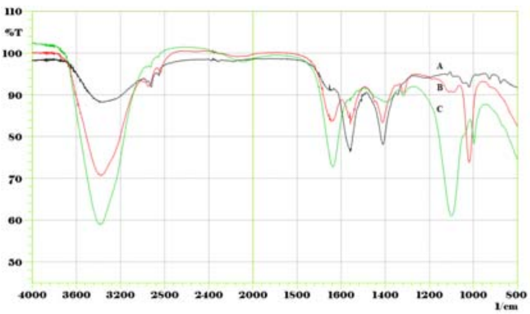

Fig. 2. FTIR analysis of (A) tannery wastewater, (B) acidogenic digested wastewater, (C) upflow packed bed reactor treated wastewater

85%. These results suggested that the acidogenic treatment prior to packed bed reactor treatment could be a good technique for increasing the treatment potential. The residual recalcitrant organics (residual COD) present after initial treatment are completely removed in packed bed system evidencing the complete utilization and mineralization of organics and LCFA in tannery waters during the combined treatment in acidogenic digestion and anaerobic packed bed reactor treatment. Interestingly, TDS reduced significantly, elucidating the complete degradation of complex organics in the effluent by the anaerobic consortium. Nikolaeva *et al*. (2013) carried out pilot scale plant operations with a dairy wastewater using an anaerobic fixed bed reactor packed with a hybrid material composed of tire rubber and zeolite. COD removal percentage varied from 28 to 82 % at hydraulic retention time of 1.0-5.5 days.

FTIR measurements

The FTIR analysis of tannery wastewater, acidogenic batch digested and upflow packed bed acidogenic reactor treated wastewater was performed using an IR Affinity-1 Schimadhzu spectrometer. Tannery wastewater showed a strong C-N stretching vibration of the organics in the region 1404 cm^{-1}. A peak at 2850 cm-1 was attributed to CH_2 symmetric stretching (Fig. 2a). FTIR spectra after acidogenic fermentation showed formation of some new absorption bands at 1000 and 1600 cm^{-1}. These new absorptions suggested that the fatty acids were formed during the acidogenic process (Fig. 2b). Major peaks at 900-1200 cm^{-1} constitutes for C-O, C-C and C-O-C deformations. Many peaks have decreased in intensity upon treatment. This can be attributed to the degradation of the complex organics and LCFA further into their respective gaseous end products (Fig. 2c).

GC-MS analysis: Chemical composition of the tannery wastewater and metabolites formed due to biodegradation

The total compounds present in tannery wastewater and the metabolites formed during degradation by acidogenic microorganisms were analysed using GC-MS (Fig. 3). The analysis of the test samples revealed the presence of organic compounds including phthalic acid, beneze propanoic acid, benezene carboxylic acids and higher alkanes. Apart from major organic constituents, the undegraded tannery wastewater also contains some fractions of complex mixture which were very difficult to identify. After acidogenic microbial treatment, most of the dominant organic pollutants detected in untreated

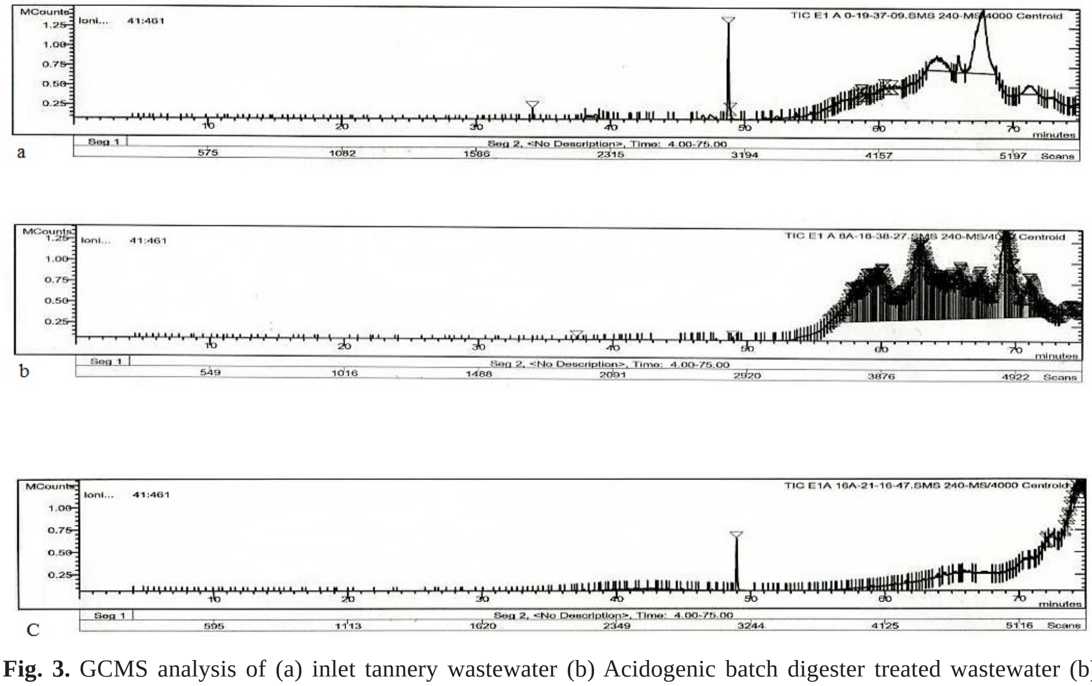

Fig. 3. GCMS analysis of (a) inlet tannery wastewater (b) Acidogenic batch digester treated wastewater (b) upflow packed bed treated wastewater

tannery effluents were almost completely mineralized after reactor stabilization period of 15 days. The metabolites eluted at retention time (*Rt*) of 59.8 matched to the acetic acid and 62.2 is for 2-butenoic acids, respectively. Subsequent cleavage of each of all the complex organics of tannery effluents by microbial degradation led to the accumulation of acetic and butenoic acids in the treatment system (Tripathi and Garg 2014).

CONCLUSION

The acclimatization of acidogenic microorganisms and utilization in batch digestion and upflow packed bed reactor for treatment of tannery wastewater is novel methodology compared to existing technologies available for treatment of tannery wastewater. The FTIR spectra and GC-MS analysis confirmed the breakdown of complex organics and formation of metabolites. This combined system resulted in 80-90% reduction in COD which emphasizes their potential application for treatment of complex tannery effluents.

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