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Acidogenic Fermentation of Long Chain Fatty Acids (LCFA) Rich Wastewater

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The main objective of this study was to investigate the fermentation efficacy of Long Chain Fatty Acids (LCFA) containing wastewater by acidogenic microorganisms. The digestibility of the wastes were evaluated up to 120 h using batch anaerobic digestion tests performed in serum bottles with acclimatized anaerobic acidogenic microorganisms at 28±2 °C. The breakdown of various organic constituents resulted in decrease of 62% COD, 72% TOC and 87% sulphate and release of wide range of fermentation metabolites resulted in 29% increase in VFA. In the initial start up hydrolytic phase, the value of redox potential (Eh) was -65mV and in the second fermentative phase it reached up to -242mV within a short retention time of 24 h. In the process of acidogenic fermentation the extracellular Eh reached a maximum of -410mV at 72 h and was maintained throughout the batch digestion.

Key words: Acidogenic Fermentation, Long Chain Fatty Acids, Wastewater.

Tanneries and slaughterhouses can produce effluents highly rich in long chain fatty acids (LCFA) and lipid wastes (Li *et al*., 2002). An appropriate effluent treatment processes cause very less environmental damage and it was required to reduce the pollution load which is possible only by enhancing the efficiency of treatment technologies. Nature of the lipid and LCFA makes them much difficult for degradation by conventional means of treatment technologies. Anaerobic treatment processes is a promising technology that have been widely used to the treatment of complex wastewater because of demonstrable performance and cost effectiveness (Maria *et al*., 2008). Sulphides produced during the anaerobic process causes imbalance in the treatment efficacy by disturbing the methanogenic population during the treatment progress. Many researchers consider lipids and LCFA as potential substrates and have applied anaerobic treatment technologies for treatment due to its high theoretical methane yield compared to different organic substrates (Martin-Gonzalez *et al*., 2010). Acclimatized anaerobic microorganisms used in the treatment process also have the advantage of simultaneous waste reduction and methane/hydrogen generation. Researchers have isolated and identified microorganisms that degrade LCFA in co-culture (Chunyang *et al*., 2004; Diana *et al*., 2007, Hatamoto *et al*., 2007). Many anaerobic treatment processes with LCFA wastewater are operated at very lower organic loading rates and long term operation founds to be much unstable. This is mainly attributed to the acute toxicity of LCFA; it gives rise to substrate toxicity in anaerobic

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microbial consortium and resulting in complete biomass washout (Alves *et al*., 2001). A broad review of literature elucidates that many treatment process have been investigated for LCFA rich wastewaters (Masse *et al*., 2002; Pitk *et al*., 2013). Many of these biological treatment process includes upflow anaerobic reactors, sequencing batch reactors, completely stirred tank reactor, single- and two-phase continuously stirred tank reactors and expanded granular sludge bed reactors. However, due to slow growth rates of anaerobic microorganisms and its highly prone to external shock induced by LCFA, thus the reactor has to be operated at low dilution rates to avoid loss of biomass. This causes difficult in reactor operation as well as process instability at long term operation. Based on varying strength of the wastewater, there is a need for efficient technology that will be cost effective and eco-friendly. Therefore, a pre-treatment process using acidogenic fermentation process was necessary to reduce the toxic effect and increase the anaerobic treatment efficiency. The process and pathway of product formation by acidogenic microbial population is very complex and is greatly influenced by factors such as substrate concentration, pH, temperature, Eh and nutritional requirements for enzymatic activity. Although extracellular metabolites play a main role in anaerobic digestion, the studies focusing on their role on anaerobic degradation are very limited or perhaps nil. The principal objective of the present study was to focus on the utilization of LCFA as a substrate for studying some valuable factors influencing the acidogenic process. The data obtained from this study are expected to provide basic information for the enhancement of anaerobic treatment process aimed to treat complex LCFA rich wastes.

MATERIALS AND METHODS

LCFA substrate

The wastewater rich in LCFA was collected at a tannery plant, Chennai, approximately once a month, in 20 L cans. At the plant, preliminary screening of the wastewater was carried out to remove any solid particles more than 1mm in size. In the laboratory, the wastewater was mixed with mineral salts and pH was adjusted before the start up of the reactor. The quality of the wastewater during the study period is provided in results section.

Anaerobic sludge adaptation to LCFA

In order to acclimatize the microbial community to LCFA, a batch test of anaerobic digestion was performed with the anaerobic sludge sampled from UASB treatment plant used for tannery wastewater treatment. The specific activity tests were carried out in 1L vials filled with 500 mL of anaerobic sludge (5.0 gVSS/L) with LCFA rich wastewater supplemented with mineral salts. Strength of the wastewater was increased at weekly intervals to acclimatize the biomass for a period of 30 days. The process of acclimatization was established by the biogas production amount.

Operations of acidogenic digesters

LCFA rich tannery waste water with mineral salts including ($g\ l^{-1}$): 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 are used in digestion studies. The acclimatized acidogenic anaerobic bacterium rich sludge in the concentrations of 5.0 $g\ l^{-1}$ was added to the digester and tested up to 120 h. The acidogenic digestion was carried out at room temperature and initial pH of the medium was controlled at 6.0-6.5.

Analytical methods

Characterizations of wastewater are measured according to Standard Methods (APHA). A Shimadzhu infrared spectrometer was used for the investigation of the metabolites generated during the stage of acidogenic fermentation. Finely powdered samples were scanned in the spectral range of 4000-400 cm^{-1}. The Eh of the fermented LCFA rich waste water in minimal medium was measured using an ion selective electrode, Orion 920 A1 (Thermo Electron Corporation, USA). The pH, conductivity, TDS, salinity and temperature are tested using PCSTestr 35, Eutech Instruments (Thermo Scientific).

RESULTS AND DISCUSSION

Wastewater characterization

The effluent concentration various at time based on the nature of raw materials used. Broad range of the physico-chemical characterization of the wastewater used in this study was given in Table 1. The wastewater characteristics and COD

Table 1. Physico-chemical characterisation of the LCFA rich wastewater

Parameter	Value
pH	6.0–7.5
COD (mg/L)	3000–4500
BOD5 (mg/L)	650–1500
Total solids (mg/L)	2000–4500
Total dissolved solids (mg/L)	2500–4000
Total suspended solids (mg/L)	1200–1500
Volatile suspended solids-VSS (mg/L)	1250–1450
Kjeldahl nitrogen (as N, mg/L)	7.5–20

Table 2. Characterizations of LCFA rich wasterwater before and after acidogenic treatment

Parameters	Untreated	Acidogenic treatment
pH	6.8-7.5	6.1-6.5
COD, mg/L	3000-4500	1100-1700
TOC, mg/L	1200-1600	285 -500
Sulphide, mg/L	17-56	9-15
Sulfate, mg/L	910-975	118 -120
VFA, mg/L	520 -705	675 -910
TDS, ppt	3.46-3.58	3.12-3.32
Salt, ppt	2.15-2.54	2.3-2.6
Conductivity, mv	4.7-4.8	4.83-4.86

(in term of LCFA) elucidate the possibility of high level of metabolites production. Moreover, the concentration range in the wastewater was not inhibitory to acidogenic fermentation.

Anaerobic sludge adaptation to LCFA

At the beginning, the batch anaerobic digester was fed with around 3-5g of VSS/L for a time interval of 2-3 weeks. The duration at which the anaerobic microbial consortia are being acclimatized to LCFA substrate is the start-up period. The VS quantity added to the digester was regularly increased till the gas production reached around 1-2mL/min. Slowly a equilibrium is formed between the acclimatized microorganisms and the substrate. However, a much long start up period of 15-30 days for gas production was observed in the anaerobic sludge due to high time required for microbial acclimatization towards LCFA. The adaptation to LCFA by the acidogenic microbial consortia was confirmed primarily by the constant production of gaseous end products, release of acidogenic fermentation metabolites in the extracellular medium and a rapid shift in pH. Hwu (2001) evaluated the potential of acclimatized sludge to enhance the anaerobic biological treatment of lipid containing wastewaters. The addition of acclimatized microorganisms can reduce the inhibition due to LCFA and can withstand high ammonia concentration. Furthermore, they were adapted to utilize lipids at high concentrations.

LCFA degradation

The characterizations of LCFA rich wasterwater before and after treatment were given in Table 2. The acidogenic fermentation was carried out for 20 days using acclimatized sludge where the pH was maintained in the range between 6.0–6.5. The breakdown of various organic constituents resulted in removal of 62% COD, 72% TOC and 87% sulphate and release of wide range of fermentation metabolites resulted in 29% increase in VFA. The VFA concentration in the effluent was in the range of 675-910 mg/L. These levels of VFA during the acidogenic digestion steps process indicate the prevalence of dominant acidogenic microorganisms. Many authors have elucidated that in any process of anaerobic degradation pathway, the complex organics are hydrolysed and fermented initially resulting in increase of VFA (Ganesh Kumar *et al*., 2005).

The feed concentration at COD 1000-1500 mg/L provided a buffering system between the microorganisms and the substrate in controlling excessive VFA formation. Thus, the acidogenic fermented samples at this COD level (Table 2) is opted for further treatment in methanogenic reactor which can able to generate substantial biogas. The structure of the microbial community (acidogenic or methanogenic) is the vital factor controlling the success rate of anaerobic treatment of slaughter house waste (Palatsi *et al*., 2011).

Interestingly, high acidic metabolites generation and less generation of biogas throughout the process confirmed the high activity of acclimatized sludge. The treatment of LCFA rich tannery wastewaters by acidogenic degradation process is constrained by the decrease in the pH that delays further conversion of acidic metabolites to methane. This issue can be addressed by deploying two phase anaerobic reactor having acidogenic fermentation phase and methanogenic degradation in the second phase.

Effect of the hydraulic retention time on redox potential (Eh)

Acidogenic microorganisms decreased the Eh value from -65mV to Eh value of -242mV during fermentation of LCFA rich wastewaters in pH range of 6.0-6.5 at room temperature within a short retention time of 24 h. The Eh profile during the acidogenic fermentation process shows dominant shifts in values at different retention period. The very first stage of LCFA breakdown starts at hydrolysis stage, characterized by rapid change in Eh values of around -200mV within 24 h and during the process of acidogenic fermentation Eh reached the maximum of -410mV at 72 h and maintained throughout the batch digestion. This major second region is due to fermentative process induced by acidogenic microbial consortia. The process and progress of hydrolysis is well characterized by the changes in ORP values of around -300mV (Colmenarejo *et al*., 2004). The ORP values reveal that particulate material is being more hydrolyzed to simple monomers within a short retention time.

FTIR measurements

The FTIR analysis of LCFA rich water (Fig. 1A) showed broad band at 3430-3300 cm^{-1}, this band presented an asymmetry caused by the presence of O-H stretching in alcohol and N-H stretching vibrations in amides group. The bands around 3000-2800 cm^{-1} exhibited the C-H stretching vibrations of -CH2 and -CH3 groups. A strong intensity band around 1450 cm^{-1} was also observed. The lipid chain absorption bands are visible at 3000, 2800, 1500 and 1350 cm^{-1}. The bands in the region of 2925 to 2855 cm^{-1} may be considered for asymmetrical and symmetrical stretching of methyl and methylene groups (Ganesh Kumar *et al*., 2008). The peak observed at 1730 cm^{-1} was attributed to the carboxyl stretching. The peaks recorded at 1790-1500 cm^{-1} were attributable to compounds containing nitrogen. The peaks in the range 1000-1400 cm^{-1} represented alkyl halide groups. The peaks around 1400 cm^{-1}, 1300 cm^{-1} and 1040 cm^{-1} respectively represent the functional groups of amino, carboxylic, phosphate and carbonyl groups in wastewater. FTIR spectra of acidogenic fermented tannery effluent showed new absorption bands (Fig. 1b). Moreover, the intensity of lipid chain absorption bands are much lowered. The band at 1644 cm^{-1} was due to presence of -COOH groups and the peak at 1410 cm^{-1} represents the -C=O group. However, the peaks in the range of 1100-1200 cm^{-1} corresponds to the-C-O groups. These new bands suggested that the acidified products were formed during the acidogenic process.

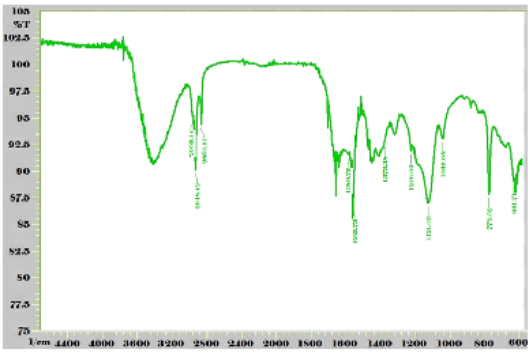

Fig. 1(a). FTIR analysis of LCFA rich wastewater at start up of fermentation

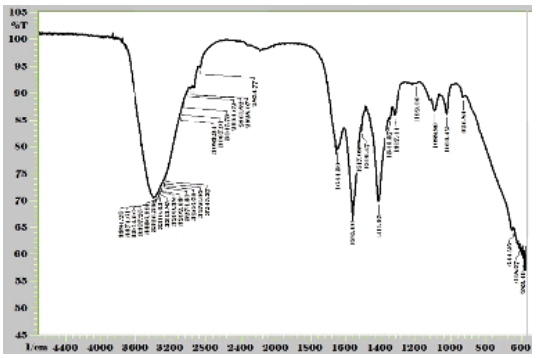

Fig. 1(b).FTIR analysis of formation of anaerobic digestion metabolites at 120 h

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