Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Bioethanol Production from Leather Solid Waste through Anaerobic Digestion

Kumar Omprakash*[1], Bhagat S. K.[1] and Ahmad Md. Tazim[2]

[1]Deptt. of Civil Engg.,Sri Balaji College of Engineering and Technology, Jaipur, India
[2]Biotechnologist, GenPro Biotech & Labex Corporation, New Delhi, India

ABSTRACT

Leather industry is one of the oldest cottage industries in India. Although tanning has been in existence for a long time, the problem of environment pollution received serious consideration only in recent years. The leather industry produces a significant amount of chromium bearing hazardous waste. This work has two aspects one is the management of such huge generation and another is the production of energy in terms of Bioethanol. On production of biofuel in the form of ethanol from tannery solid wastes was made using selective anaerobic microorganism isolated form rumen. Chromium is present at the chemical composition of solid wastes and the protein concentration was at higher percentage (79%) followed by fat (7.57%). The screened microorganism was subjected for zymogram and the different bands were found on SDS-PAGE and on compared with standard collagenase bands, it was confirmed the production of collagenase enzyme from anaerobic microorganism. In the Anaerobic digestion of formic, acetic and propionic acid, production of ethanol was observed only with the acetic acid and with not formic and propionic acid. Volatile fatty acids production determines the rate of ethanol production; it was found that after 48 hours of incubation, more than 75 % of substrate reduced. The maximum yield of ethanol was observed after 48 hours of incubation. It was found that maximum ethanol was produced with digestion of 0.30gram of substrate and concentration of ethanol is 3.53 g/lit. This work can solve the problems of leather waste management as well as bring the fulfillment of energy requirement in terms of Bioethanol.

KEYWORDS: Bioethanol, Anaerobic Microorganism, SDS-PAGE, Collagenase Bands, Volatile Fatty Acids, HPLC.

Corresponding Author-

Omprakash Kumar
M.Tech* (Environmental Engineering),
Department of Civil Engineering,
Sri Balaji College of Engineering and Technology, Jaipur, India
E-mail: opkumar12@gmail.com ,
Mob: 09694209302

INTRODUCTION

General

The world's primary source of energy for the transport sector is oil. With oil prices near an all time high and with few alternative fuels from transport, Brazil, the European Union, the United States, and several other countries are actively supporting the production of liquid biofuels (bioethanol and biodiesel). As a renewable energy source, biofuels could help mitigate climate change and reduce dependence on oil in the transportation sector[1].

In 1925, Henry Ford had quoted ethanol as "the fuel of future". He furthermore stated, "The fuel of the future is going to come from apples, weeds, sawdust – almost anything".

According to the Renewable Fuel Association (RFA), in 2010 the global production of ethanol was 12 billion US gallons, 89% of which was produced in USA and Brazil[2] figure 1. The technology used for produce this ethanol, referred to as "first-generation bioethanol" is relatively mature and based upon traditional brewing techniques involving the fermentation of sugars, such as sucrose and glucose, by variants of the yeast *Saccharomyces cerevisae*. Now second generation of bioethanol is more powerful than first generation (FAO, 2008)[3].

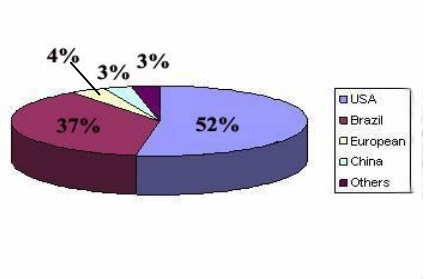


Fig 1 World fuel Ethanol Production, 2010 (Data from RFA)

Although carbon dioxide is released during both fermentation of biomass to ethanol and combustion of ethanol and it removes the pollution imparted by tannery wastes. Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to green house gas effect. The reduction of green house gas (GHG) pollution is the main advantage of utilization biomass conversion into ethanol[4].

Out of 1000 kg of raw hide, nearly 850 kg is generated as solid wastes in leather processing only 150 kg of raw material is converted into leather. Tannery generates huge amounts of solid waste

as follows: Fleshing 50-60; chrome shaving, chrome splits and buffing dust, 35 – 40; skin trimmings, 5 – 7; and hair, 2 – 5 % solid wastes in the leather processing constitute, beam house, 80; tanning, 90; finishing, 1 %. If these protein and other chemicals, which are present in the chemical treated protein, are not utilized properly it will pose hazardous pollution problem to the environment.

In many Indian cities, Tannery waste is disposed in unscientific manner or discarded which causes the public health hazards and diseases like malaria, cholera, typhoid. Inadequate management of wastes like uncontrolled dumping bears several adverse consequences. This unscientific dumping is not only leads to polluting surface and groundwater through leachate and further promotes the breeding of flies, mosquitoes and other disease bearing vectors. Also, it creates aesthetic problems, mean while it produces green house gases such and contributing to global warming[5].

Anaerobic digestion (AD) is a hopeful method to treat the wastes. Anaerobic digestion leads to the overall gasification of organic wastewaters and wastes, and produces methane and carbon dioxide; this gasification contributes to reducing organic matter and recovering energy from organic carbons[6].

Need of study

One side, Municipal cooperation or managing committee is facing the challenge in terms of disposal of Leather waste, which is poisonous in nature. Tannery generates huge amounts of solid waste. If these protein and other chemicals, which are present in chemical treated protein, are not utilized properly it will pose hazardous pollution problem to the environment. Other side, energy demand is increasing day by day which is also challenge has been faced by government. Bioethanol is one kind of renewable energy, which could help mitigate climate change and reduce dependence on oil in the transportation sector. This study help to optimize the dimension of bioethanol to utilized ample of tannery waste and also to get benefitted in terms of electricity and reduce dependence on oil in transportation sector. The need of study is also due to the problem associated by the waste, it includes such as the waste treated or disposal off in unsatisfactory way causes serve aesthetic nuisance in terms of smell and appearance. If the solid wastes are not treated in proper way than it attracts flies and flies spread diseases. We are know that leather industry, one of the polluting industries because of huge amount of liquid and solids wastes such as raw trimmings fleshing, chrome shaving, buffing dusts and keratin wastes. Accumulation of these wastes leads to sludge problem and choking of treatment pipes and finally result in efficiency of treatment plant. This study is beneficial for the area which has

appropriate climate of leather industry to manage the waste and follow the production of bioethanol in ample amount per day.

Objectives

There are following objectives of the work which has been accomplished.

i. To isolate anaerobic microorganism from sewage and rumen.
ii. To estimate growth of anaerobic microorganism.
iii. To perform Zymogram for collagenase activity.
iv. To determine the concentration of Volatile Fatty Acids.
v. To estimate the optimum substrate and microbial concentration for biodegradation.
vi. To determine the concentration of ethanol after biodegradation by HPLC analysis.

MATERIAL AND METHOD

Sources of Waste

There are different types of solid waste generated in tannery industry such as Chrome shavings, Veg-tanned, trimmings and fleshing. This research work was done with Chrome shavings.

Tannery waste

Chrome shavings were collected directly from the Tannery Industry "Habeeb Tanning Company" located at Naraina Vihar, New Delhi. Waste contains Chrome shavings as shown in figure 2. This waste brought into the Microbiology Laboratory of the Allele Life Sciences (P) Ltd, Noida in clean polythene bags. Collection time was 12:00 PM.

Fig 2 Collection of Tannery waste

Isolation of bacteria and Sub culturing

The anaerobic bacteria was serially diluted in order to reduce the population of microbes and transferred to selective media (Blood agar medium). The organisms present in the sample are further confirmed by the haemolysis of RBCs. The anaerobic microbes produce extra toxic protein haemolysin that cleave the RBCs and formed zone of clearness. For sub culturing FTM medium was used.

Growth estimation

The growth of anaerobic microorganism was key indicator in the synthesis of Bioethanol. Bacterial growth can be modeled with four different phases: lag phase, exponential or log phase, stationary phase, and death phase. Growth was measured by spectrophotometer. The optical density was measured everyday at 600 nm.

Estimation of collagenease

Zymography is an electrophoretic technique, based on SDS-PAGE, which includes a substrate copolymerized with the polyacrylamide gel, for the detection of enzyme activity. The polyacrylamide gel acts as a molecular sieve, slowing the migration of proteins approximately in proportion to their charge to mass ratio. Stacking gel is used to concentrate the protein sample into a sharp band before in enters the main separating gel. The Triton X -100 solutions was used to remove the SDS from the gel.

Estimation of VFA and confirmation of VFA by TLC

The production of volatile fatty acid was done in FTM medium and checked by titrating against 0.1*N* Sodium hydroxide, using phenolphthalein indicator.

Amount of Volatile fatty acid present in the sample was estimated by TLC. TLC involves a stationary phase consisting of a thin layer of adsorbent material, usually silica gel onto a flat inert carrier sheet.

Confirmation of ethanol at different concentration of VFA by Jone's Test

Most of the chemical oxidation methods are based on the complete oxidation of ethanol by dichromate in the presence of sulphuric acid with the formation of acetic acid. This reaction is popular because potassium dichromate is easily available in high purity and the solution is indefinitely stable in air. The theoretical reaction stoichiometry is shown below

$$2\ Cr_2O_7^{--} + 3\ C_2H_5OH + 16H^+ \rightarrow 4\ Cr^{+++} + 3CH_3COOH + 11H_2O$$

Dichromate ($Cr_2O_7^{--}$, Cr (VI) is yellowish in colour and the reduced chromic product (Cr^{+++}, Cr (III) is intensely green[7].

Estimation of optimum substrate and microbial concentration For biodegradation

The known quantity of substrate (1.5 g) was taken in three different vials. The know quantity of culture (1.01 g, wet weight) was transferred to each vials and incubated for 72 hrs. After every 24 hrs of incubation, the sample was centrifuged at 1000 rpm for 5 minutes. The supernatant was transferred to another vials and stored in freeze for HPLC analysis. Few drops of concentrated acid were added to residue to kill the microbes and weighed the residue. The remaining residue was dried in hot oven till constant weight. The remaining final substrate and culture weight were subtracted with initial weight of substrate and culture respectively. Final weight of substrate and culture were calculated by subtraction with initial weight.

Estimation and analysis of ethanol production by HPLC

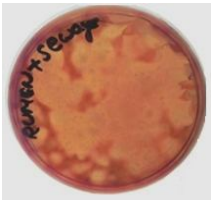
HPLC is used in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvents used.

RESULT

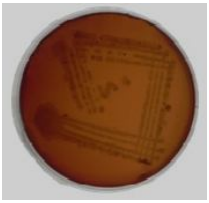
Isolation of Anaerobic Bacteria

Contamination was not found in control Blood Agar plate. RBCs lysis in spread plate indicated the presence of anaerobic microorganism as shown in figure 3 (a), (b) and (c).

Fig 3(a) Control Blood Agar Plate



(b) Spread Plate onto Blood Agar



(c) Quadrant streak Plate onto Blood Agar

Subculture using FTM (Anaerobic Microorganism)

The colour and density change of medium was observed as shown in figure 4.

Fig 4 Subculture using FTM (Anaerobic Microorganism)

Estimation of microbial growth rate

All the four phase of anaerobic microorganism was observed and the maximum growth was observed on 4th day as shown in figure 5.

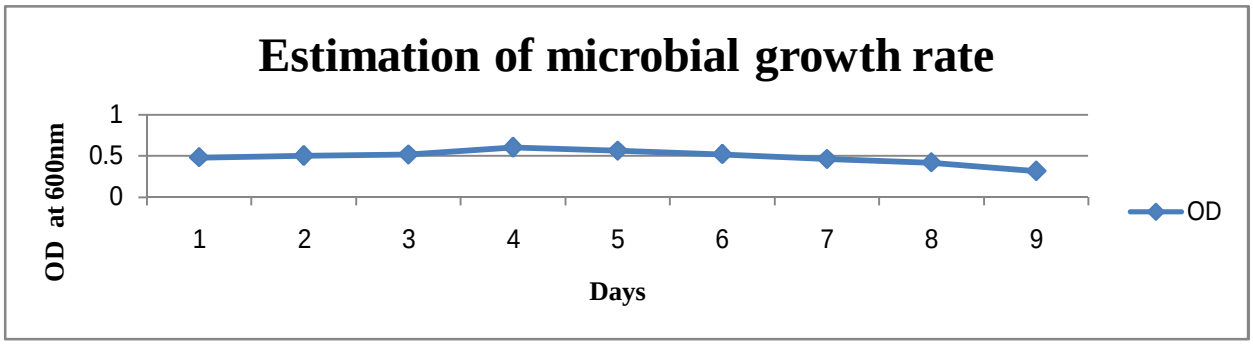


Fig 5 Microbial growth rate

Zymogram (anaerobic microorganism producing collagenase)

The band of collagenase was observed in the sample as shown in figure 6.

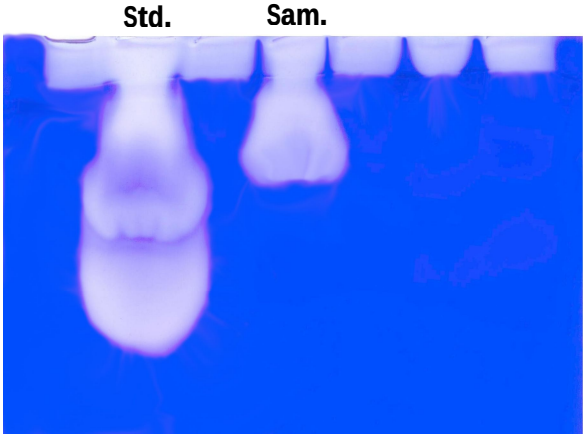


Fig 6 Zymogram (Microbes producing collagenase enzyme)

Estimation of VFA and confirmation of VFA by TLC

The maximum acetic acid concentration was found to be on 96 hours of the incubation as shown in figure 7.

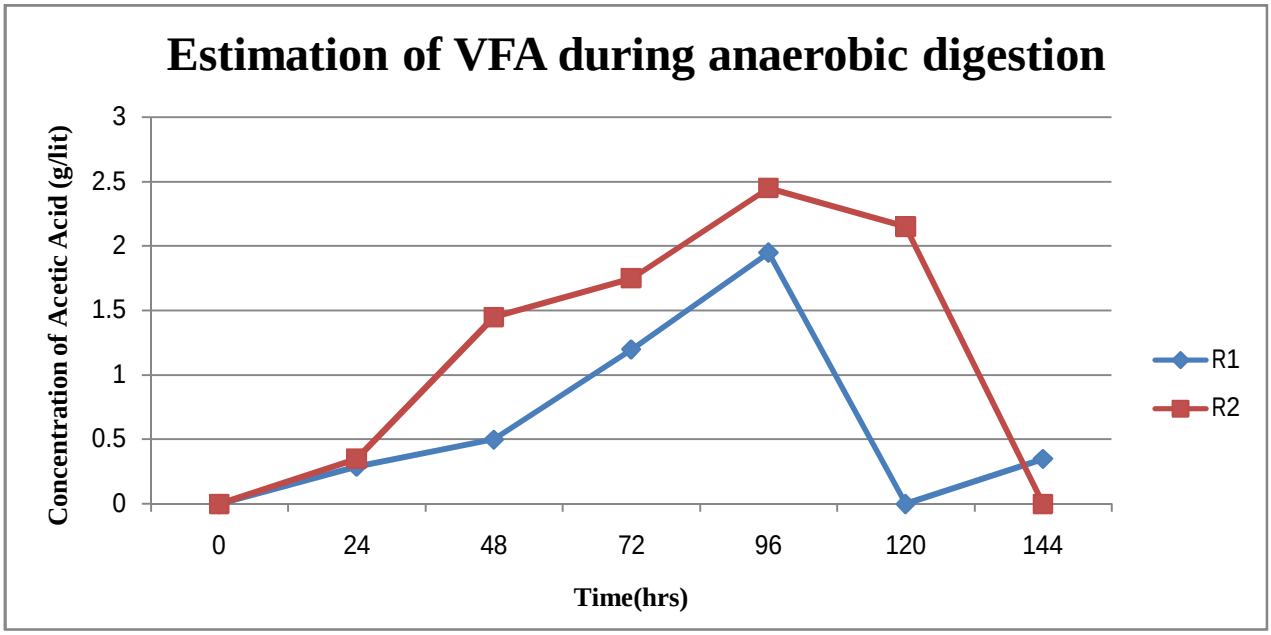


Fig 7 Estimation of VFA during anaerobic digestion

Figure 8 reveals that there is no Band formation from the sample spot with respect to standard VFA

Fig 8 VFA identification

Table 1 Confirmation of Ethanol at different concentration of VFA by Jones Test

Conc. Of VFA (%)						Vol. of Culture (mL)	Incubation for 72 hrs	Ethanol (Jones test)
Formic acid	0.05	0.5	1	3	5	5		- ve (all conc.)
Acetic acid	0.05	0.5	1	3	5	5		+ve (3 & 5 %)
Propionic acid	0.05	0.5	1	3	5	5		- ve (all conc.)

Confirmation of ethanol at different concentration of VFA by Jone's Test

The microorganism was able to convert only Acetic acid to Ethanol. Table 1 reveals the peak and least value of VFA (%).

Estimation of optimum substrat and microbial concentration For biodegradation

On increasing the number of days, the substrate digestion by microorganism was observed to be increased as shown in figure 9.

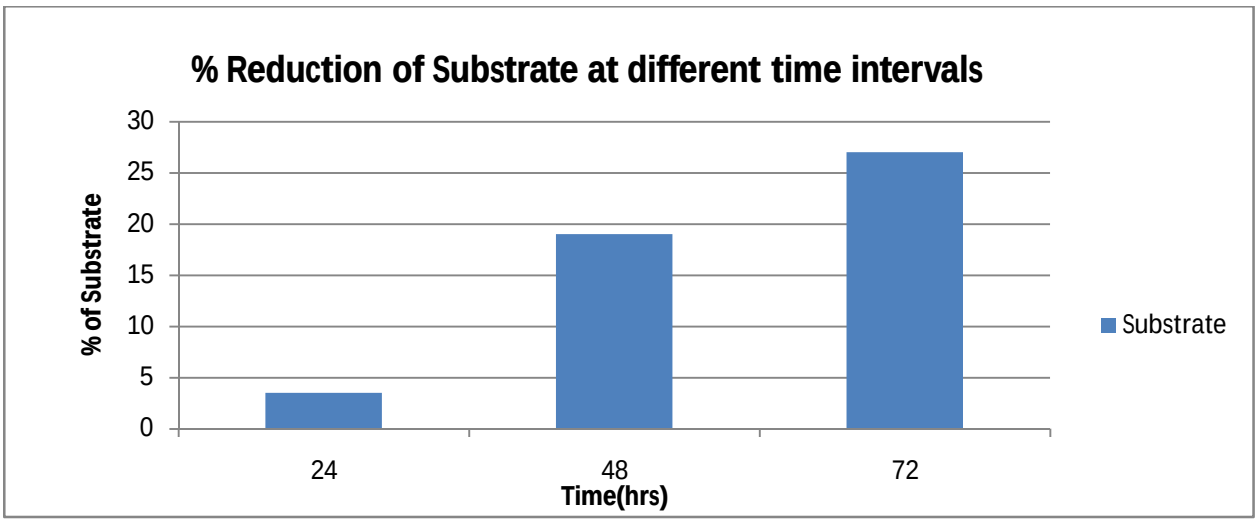


Fig 9 % Reduction of Substrate at different time intervals

Estimation and analysis of ethanol production by HPLC

HPLC is used in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules.

Sample 1

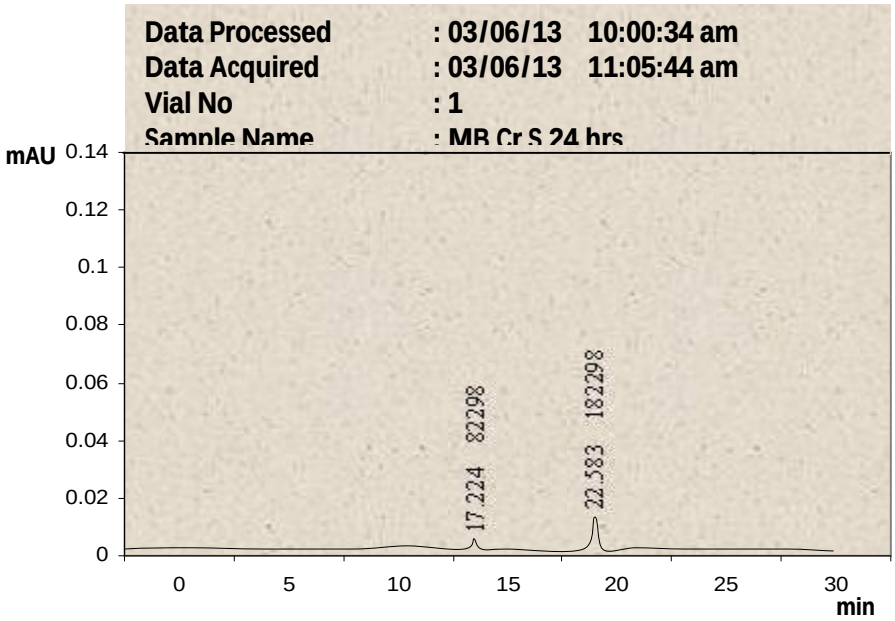


Fig 10 Chromatogram of sample 1

Sample 2

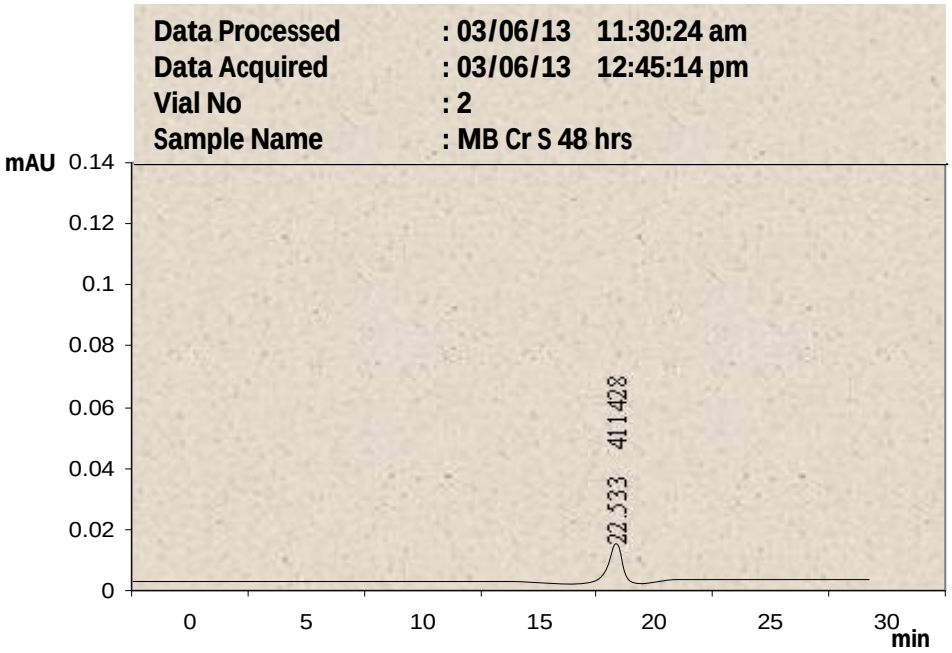


Fig 11 Chromatogram of sample 2

Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvents used as shown in sample 10, 11 and 12 with its peak values.

Sample 3

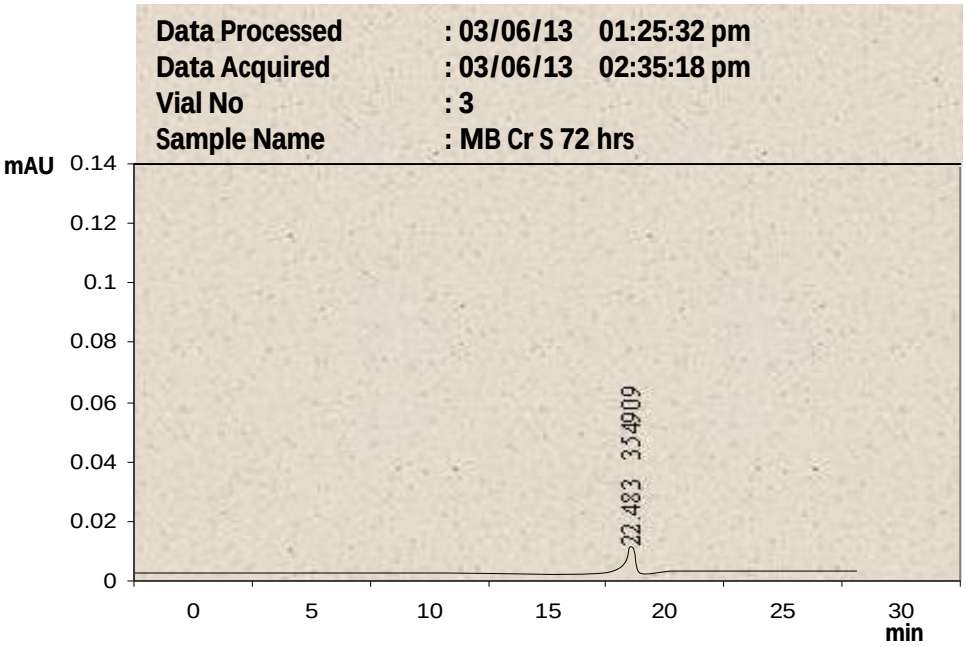


Fig 12 Chromatogram of sample 3

Co-relation between Substrate digested and Ethanol production

The maximum production of ethanol was observed on 48 hours at the substrate concentration of 0.30g as shown in figure 13.

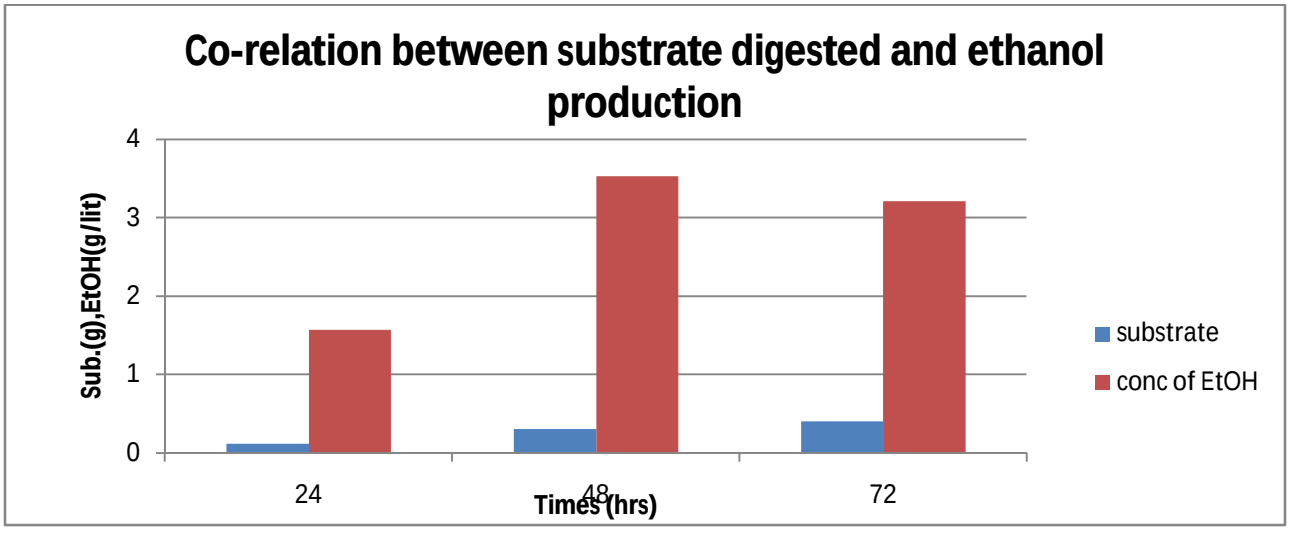


Fig 13 Co-relation between substrate digested and ethanol production

CONCLUSION

Importance of Waste management is sustainability in all the industries also. Chrome shavings are one of the different tannery wastes. It is hazardous to both human health and the environment. Cr (III) may leak from landfill and causes pollution to the drinking water in water supply system or ground water, and also it is to be oxidized [as Cr (VI)] during chlorination process. This study is concluded that, ethanol can be produced from solid leather waste containing chromium. In this study, an extraction and reuses of protein and chromium from chrome shavings has been done. Extracted chromium is reused in tanning process. As a term of renewable energy source, ethanol could help mitigate climate change and reduce dependence on oil in the transportation sector.

REFERENCES

1. Chris Lester."Biofuel: The promise and the risk". Agriculture for Development. World development report. The World Bank: Washington, DC; 2008:70-71.
2. Lichts, F.O. Industry statistics: 2010 World Fuel ethanol production. Renewable Fuels Association. Available at: http://www.ethanolrfa.org/pages/statistics#E. 2011.
3. Taylor, Mark P, Kristen L Eley, Stev Martin, Marla I Tuffin, Stephanie G Burton and Donald A Cowan et al. Thermophilic ethanol genesis: future prospects for second generation bioethanol production, Trends in Biotechnology,2009; 27:398-405.
4. Naomichi Nishio and Yutaka Nakashimada et al. Recent Development of Anaerobic Digestion Processes for Energy Recovery from Wastes, Journal of Bioscience, 2007; 103:105-112.
5. Pierre Germain, Fatiou Toukourou, and Luiz Donaduzzi et al. Ethanol production by anaerobic thermophilic bacteria: regulation of lactate dehydrogenase activity in *Clostridium thermohydrosulfuricum*. Apply Microbiology and Biotechnology, 1986; 24:300-305.
6. Zerdani, I., M. Faid and A. Malki et al. Digestion of Solid Tannery Waste by Strain of Bacillus Sp Isolated From Compost in Morocco. International Journal of Agriculture & Biology, 2004; 6:758-761.
7. Kristen J.J. Steinbusch, Hubertus V.M. Hamelers and Cees J.N. Buisman et al. Alcohol production through volatile fatty acids reduction with hydrogen as electron donor by mixed culture. Water Research: A Journal of the International Water Association, 2008; 42:4033-4214.