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Studies on the Generation of Biogas from Collagen Hydrolysate obtained from Chrome Shavings by Alkaline Hydrolysis: A Greener Disposal Method

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Abstract

The leather industry produces a significant amount of chromium bearing hazardous waste. Solid waste disposal is increasingly becoming a huge challenge to tanners due to paucity of landfill sites and strict environmental legislations worldwide. Hence, finding a holistic solution to the tannery solid waste disposal problem is a challenge for researchers. Protein based solid wastes acquire much attention due to its high value. In this work, proteinous matter recovered from chrome shaving through chemical process was feed to the anaerobic digester to generate biogas. Two different modes of alkaline chromed shaving dust hydrolysis i.e. using lime and KOH followed by neutralization with HCl and H_3PO_4 subsequently. The Full scale investigations conducted to evaluate the performance of anaerobic digestion of collagen hydrolysate. The feed and overflow of both digesters were monitored for various parameters such as total solids %, ash content %, volatile fatty acids and chemical oxygen demand (COD). COD reduction efficiency 34-46 % and 45-54% was observed in lime-HCl and KOH-H_3PO_4 digesters respectively. Gas generation is increased 30% in KOH-H_3PO_4 digester as compared to Lime-HCl and CO_2 % in KOH-H_3PO_4 digester was in the range of 19-20% as compared to Lime-HCl were it was 30-40%. Hence, it shows that CO_2 produced by the acidogens is effectively utilized by the methanogens in producing methane gas and potassium and phosphates are the macronutrients to the microorganisms.

Keywords: Chrome shavings, hydrolysis, collagen hydrolysate, biogas, leather processing.

Introduction

The global environment is gradually deteriorating as a result of the socio-economic activities of mankind. Among them, processing industries causes adverse changes in the environment, where, leather industry is one of them. Leather and environment can be described as two sides of the coin despite the leather industry making traceable and visible impacts on socio-economic through employment and export earning; the industry has gained a negative image in society owing to the resulting pollution[1].

In the tanning industry, raw skin is transformed into leather by means of a series of chemical and mechanical operations. Environmental challenges from leather processing arise from both the nature and the quantum of wastes discharged [2-5]. There are about 1600 tanneries located in India and are distributed mainly in four states, viz. Tamil Nadu, West Bengal, Uttar Pradesh and Punjab with a processing capacity of 0.9 million tons of raw hides and skins[6]. The leather-making process generates substantial quantities of solid, liquid, and gaseous wastes[7] figure 1. Many cleaner processing approaches aimed at the reduction of liquid and solid wastes proved to be economically and environmentally beneficial [8-15].

Chromium has been used as a predominant tanning agent approximately 85% of all leather produced in the world. However, due to the uniqueness of the chromium tanning and properties that chrome tanning confers to the resulting products [16-18]. It has been estimated that about 0.8 million tons of chrome shavings could be generated per year globally. This waste is partly used in the manufacture of leather board, but most are normally disposed of in landfill sites, wasting all the contained resources[19-20]. This waste presents low compaction ability and density, therefore presenting high land filling costs per mass unit. Furthermore, the cost of disposal increases as the hazardous nature of the waste increases. The literature suggests that chromium-tanned leather waste may be managed through more sustainable technological alternatives, namely by wet treatments. Hydrolysis of shavings with alkalis such as CaO, NaOH and MgO at moderate temperatures and oxidative de-chroming were studied to recycle chrome tanned solid waste[21-30].

In present study, process developed to separate collagen hydrolysate and chromium by alkaline hydrolysis. Collagen hydrolysate is feed for anaerobic digester, and chromium after further processing is recycled as basic chromium sulphate and reused in tanning operation. The obtained hydrolysate is protein rich and has an approximate. C:N ratio of 3:1. Experiments were conducted to evaluate the anaerobic digestion of collagen hydrolysate after two different modes of alkaline hydrolysis i.e. using lime and KOH followed by neutralization with HCl and H_3PO_4 subsequently. The efficiency of protein separation from chrome shaving by both the method has been studied. Collagen hydrolysate from both the process was used as feed in anaerobic digester and gas generation was measured.

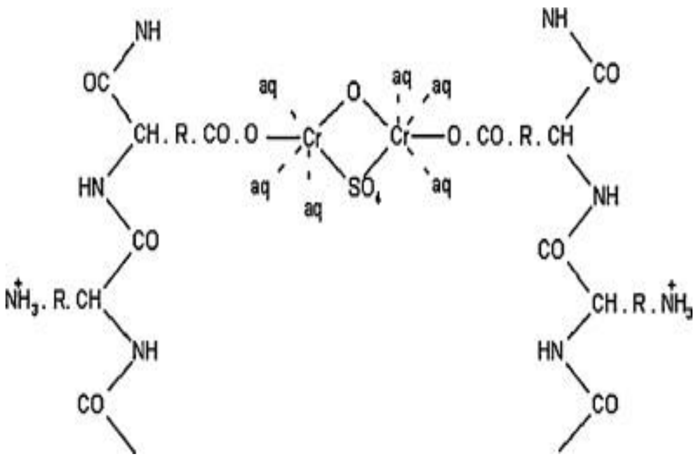

Figure-1
Molecular structure of chrome-tanned leather and shavings

Material and Methods

Fresh chrome leather solid waste – chrome shaving was collected from a leading commercial tannery in India. All the chemicals used for the analysis were of analytical grade.

Characterization of Chrome shavings: The collected chrome shavings were characterized for moisture, chrome content, inorganic ash, nitrogen, oils and fats, pH and apparent density as per standard procedures [31-37] table 1.

Table-1
Characteristics of chrome shaving*

Parameters	Values
% Moisture content	24.1±0.6
% Cr_2O_3	3.12±0.12
% Inorganic ash	5.21±0.11
% Nitrogen	16.4±0.1
% Oils and fat	1.5±0.05
pH (10% aqueous leather)	3.4±0.2
Apparent density	0.91 g/mL

*based on dry weight, Number of replicates 3

Hydrolysis of chrome shavings: Collagen hydrolysates were prepared by treating shaving dust with lime and KOH separately in aqueous media at 90-95^{0}C temperature for 4 hrs. The hydrolysates after cooling were filtered and neutralized to 6.8-7.2 pH with HCl and H_3PO_4 respectively. To maintain uniformity both hydrolysates were diluted to 3 % total solids. Analysis results of both the hydrolysates are summarized in table-2.

Biomethanation of collagen hydrolysate: Two separate anaerobic continuous digesters of 20 liters effective volume each started with starter culture of bottom sludge of existing biomethanation plant. After incubation for 3 days KOH-H_3PO_4 and lime-HCl hydrolysate were charged in lab scale anaerobic digester. Initially, 500 ml feeding/day started andtemperature maintained at around 32-35^{0}C throughout the experiments. Gas volume measured every day using water displacement columns. Input and output samples of both digesters monitored for pH, chemical oxygen demand, total solids, % ash content, volatile fatty acids and density of microbial population. Analysis result of feed and overflow of both digesters were given in table-3.

Table-2
Analysis result of KOH-H_3PO_4 and Lime-HCl hydrolysates

Sr. No.	Test Parameter	KOH-H_3PO_4 Hydrolysate	Lime-HCl hydrolysate
1.	pH	7.12	7.06
2.	% Total solids	3.23	3.12
3.	% Ash content	20.1	16.3
4.	VFA (as such)	1260	1138
5.	SS mg/L (as such)	1240	971
6.	VSS mg/L (as such)	240	155
7.	Sulphate mg/L (as such)	2163	1025
8.	COD mg/L (as such)	27190	26241

Table-3
Analysis result of feed and overflow of both hydrolysates digester

Test Parameter	KOH-H_3PO_4		Lime-HCl	
	Feed	Overflow	Feed	Overflow
PH	7.12	7.76.	7.06	7.5
% Total solids	3.23	1.02	3.12	1.24
% Ash content	20.1	52.6	16.3	46.5
VFA (as such)	1260	3726	1138	2178
COD mg/L (as such)	27190	14388	26241	17220
Microbial population	Moderate growth	Heavy growth	Scanty growth	Moderate growth

Microbial Study: Microbial population in both samples varied in terms of number and type as seen under microscope and was confirmed through biochemical tests of hydrolysis, decarboxylation and deamination by bacteria.

Recovery and reuse studies: Protein and chromium derived from chrome shavings were used as feed in anaerobic digester and chrome tanning in re-chroming process of leather making. The chromium bearing residue of chromium (III) hydroxide was treated with sulphuric acid till pH 2.8, left overnight and the basicity of the chromium (III) sulphate estimated by standard procedures[37-38].

Results and Discussion

Characterization of Chrome shavings: The characteristics of chrome shaving from a commercial tannery are given in table 1. The amount of moisture, chromium (as Cr_2O_3 on dry weight basis), % inorganic ash, % nitrogen, %oils and fat present in the chrome shavings are 24.1, 3.12, 5.21, 16.4 and 1.5, respectively. The pH of aqueous leather and apparent density of chrome shavings used in this study was 3.4 and 0.94 g/ml.

Optimization of hydrolysis of collagen hydrolysates: It was observed that under aerobic conditions putrefaction of KOH-H_3PO_4 gelatin is faster than Lime-HCl gelatin. Both hydrolysates were kept in a thermostat at 30-35^oC temperature for almost two weeks and changes in parameters like pH, volatile fatty acids, suspended solids and volatile suspended solids were studied after every four days figure 2-a, 2-b, 2-c, 2-d. Spectrophotometric turbidity analysis confirmed that the growth of bacteria is faster in KOH-H_3PO_4 hydrolysate. Increase in volatile suspended solids also confirms faster growth of micro-organisms in KOH-H_3PO_4 hydrolysates as more and more total dissolved solids gets hydrolyzed and converted to volatile suspended solids by bacteria. Analysis results are given in table-4.

Gas Analysis: Gas volume was monitored for almost 6 months and an increase of average 25-30% biogas in KOH-H_3PO_4 digester was observed. One month analysis results of both digesters are shown in figure 3. Gas samples of both digesters checked for CO_2 and H_2S % using Gastec Standard Tube Detector System from Gastec Corporation 6431 Fukaya, Ayase-Shi, Kanagawa 252, Japan using tube no. 2HH for CO_2 and 4HH for H_2S. Throughout the experiment CO_2 % in KOH-H_3PO_4 digester was found 25-35% against Lime-HCl route were it was 30-40%, whereas H_2S was 2-3% and 1-2% respectively. The CO_2 result of KOH-H_3PO_4 digester further confirmed in a 1 ton digester of similar feed with improved digester design having more surface area contact and was observed the similar result as mentioned above. Increase of average 25-30% biogas observed in KOH-H_3PO_4 digester as compared to Lime-HCl operated under same experimental conditions.

Table-4
Analysis of both KOH-H_3PO_4 andLime-HCl hydrolysates

Sr. No.	Parameter	KOH-H_3PO_4 gelatin					Lime-HCl gelatin				
		1st day	4th day	8th day	12th day	16th day	1st day	4th day	8th day	12th day	16th day
1	pH	7.12	7.85	7.96	8.15	8.11	7.06	7.48	7.46	7.48	7.33
2.	VFA	1260	2377	1985	1462	40	1138	183	400	610	1340
3.	SS	1240	1340	1691	1821	5691	971	1014	1116	1343	1431
4.	VSS	240	1120	1744	1785	1791	155	217	288	290	301

Table-5
Microbial Study of both digester overflow

Bacterial arrangements	Enzyme produced	Chemical nature
Gram positive rods in chains	Protease producing	*Deaminating*
Gram positive rods singly arranged	Protease producing	*Deaminating*
Gram positive very short rods with spores	Protease producing	*Deaminating*
Gram positive very thin long rods	Protease producing	*Deaminating*
Gram positive rods in clusters	-	*Deaminating*
Gram positive short rods with rounded ends	Protease producing	*Deaminating*

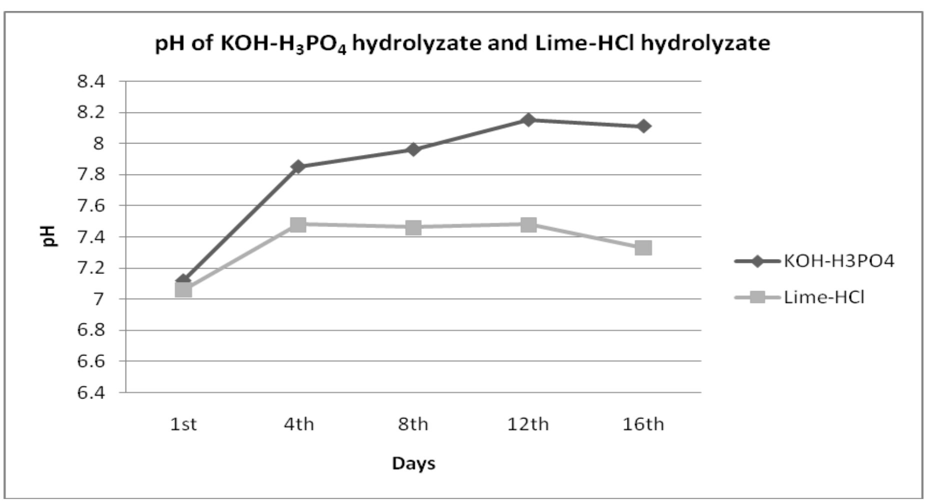

Figure 2-a
pH of KOH-H_3PO_4 and Lime-HCl digester

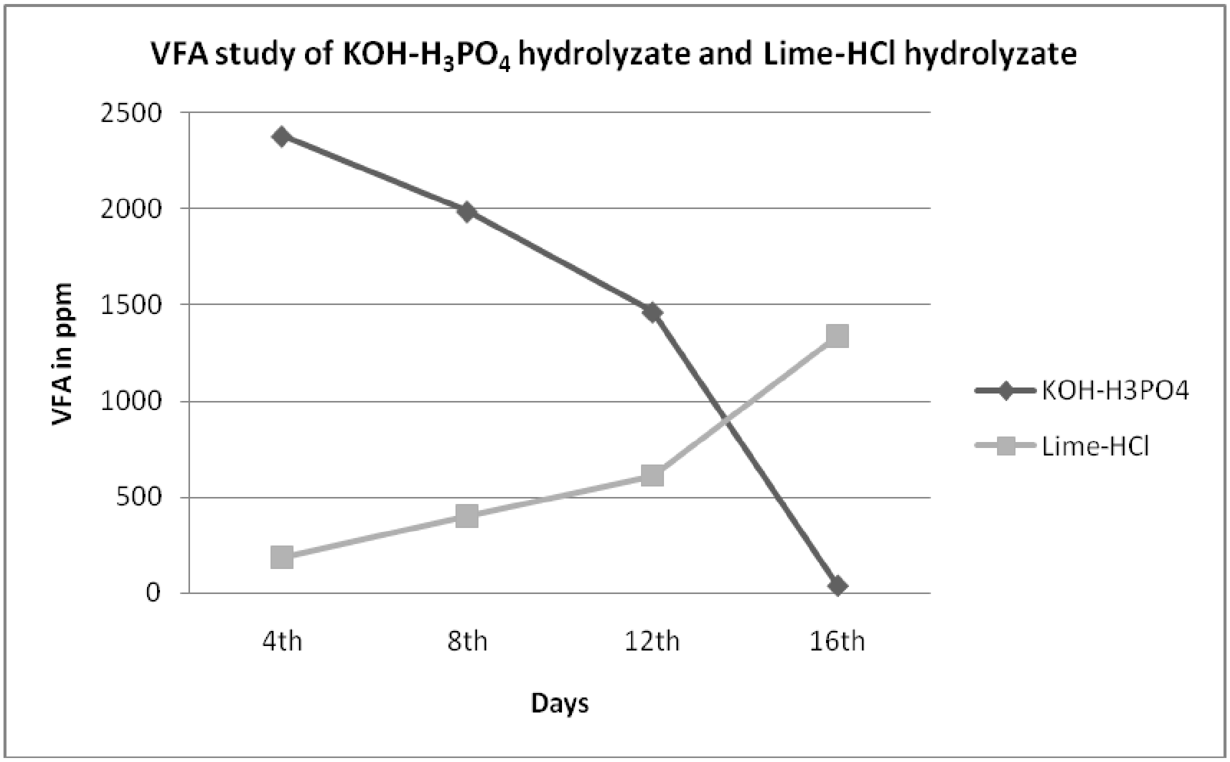

Figure 2-b
VFA of KOH-H_3PO_4 and Lime-HCl digester

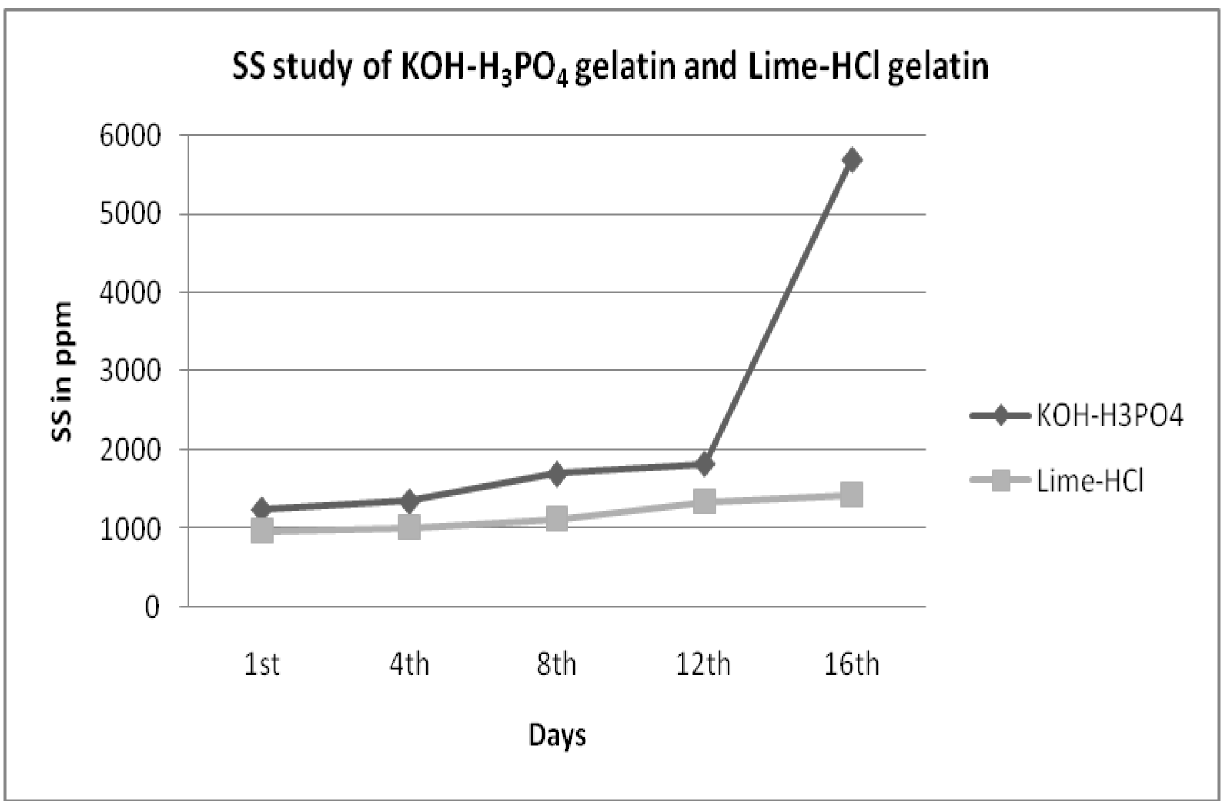

Figure 2-c
SS of KOH-H_3PO_4 and Lime-HCl digester

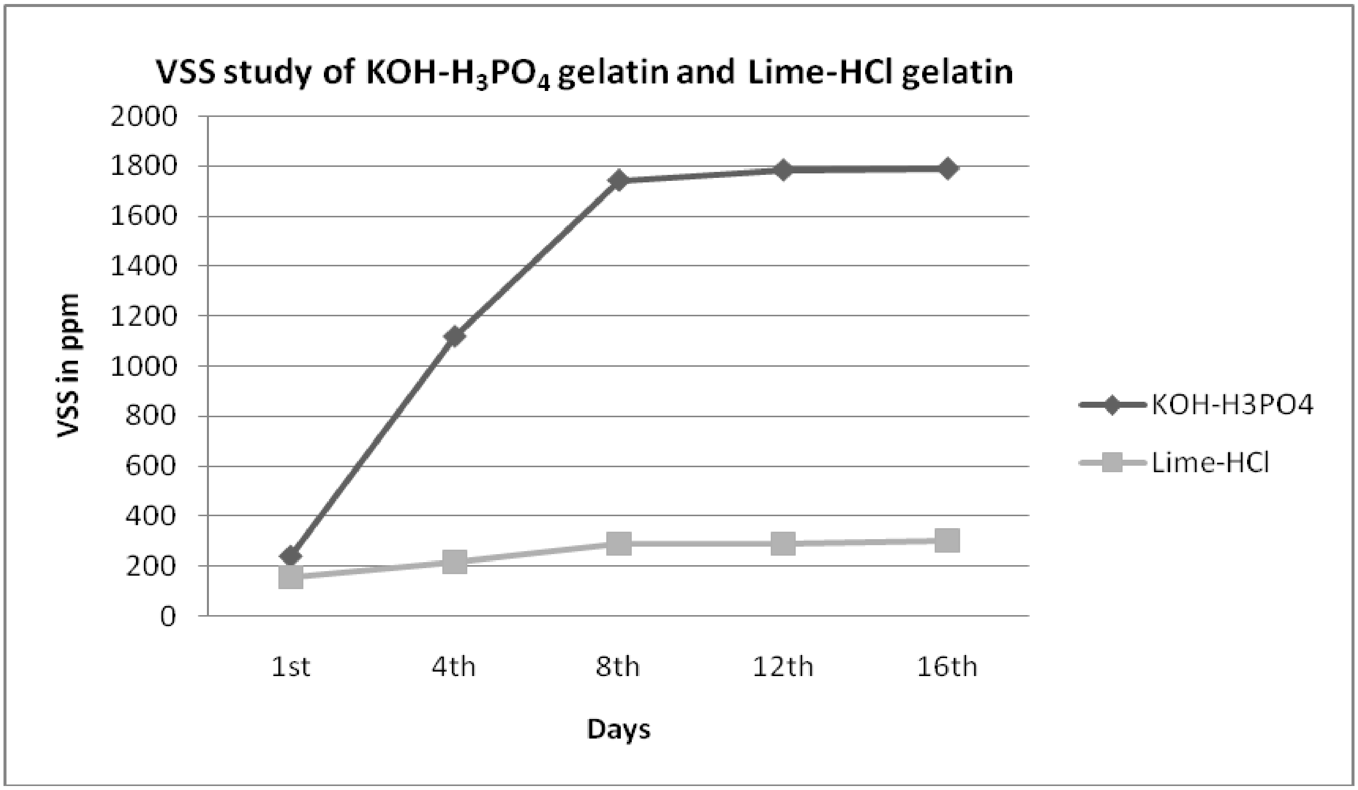

Figure 2-d
VSS of KOH-H_3PO_4 and Lime-HCl digester

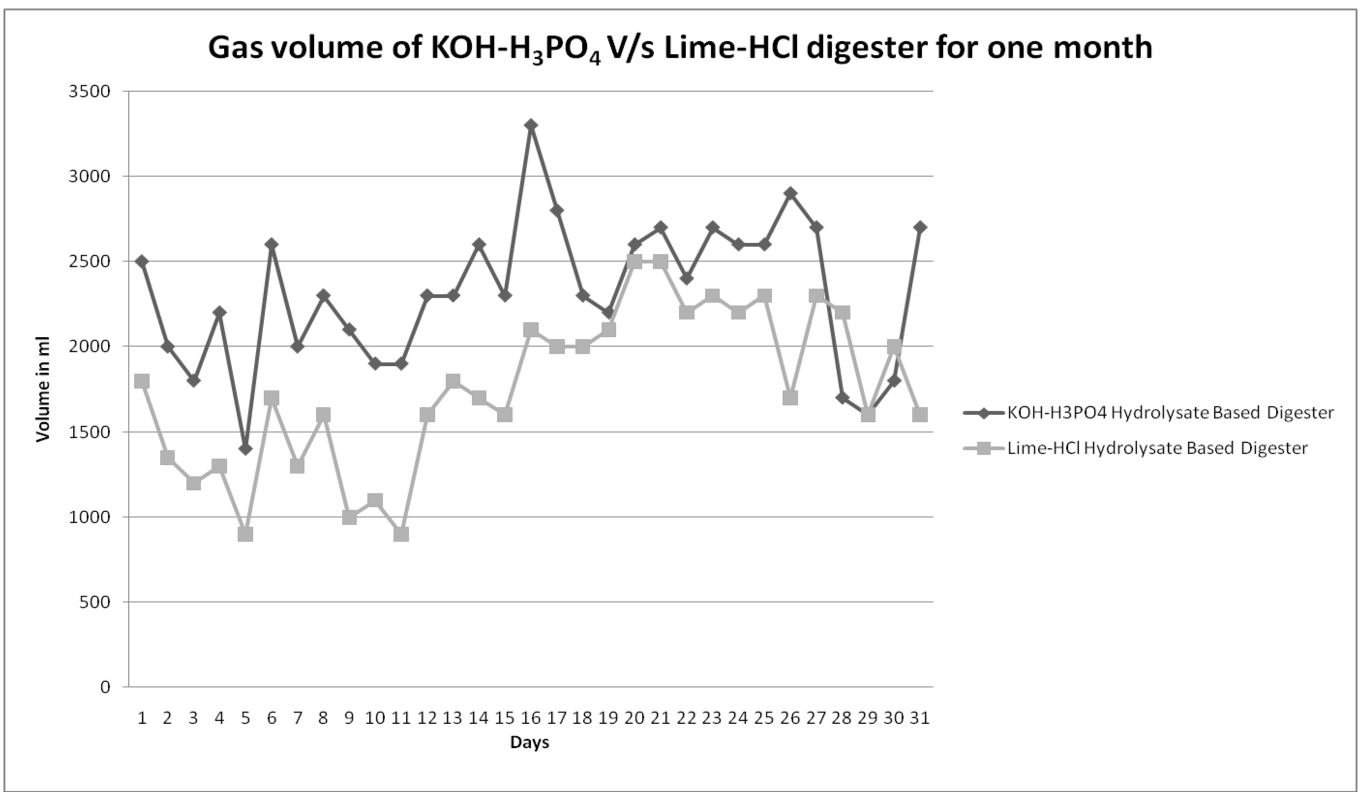

Figure 3

Volume of gas in KOH-H_3PO_4 and Lime-HCl digester

Microbial Study: Analysis confirmed the growth of bacteria is faster in KOH-H_3PO_4 hydrolysate. This is further confirmed by increase in Volatile Suspended Solids value. It seems negatively charged phosphate ion plays an important role in energy metabolism. It is an important part of biologically important molecules ATP and nucleic acid. The amount of phosphate that dissolves depends primarily on the amount of acid that themicroorganisms produce in their metabolic processes. Microorganisms secrete protease enzymes that hydrolyze proteins and polypeptides to amino acids, which are transported in the cells and catabolized. The first step in amino acid utilization is deamination, the removal of the amino group from an amino acid. This is often accomplished by transamination (amino group is transferred from an amino acid to an alpha keto acid acceptor). The organic acid resulting from deammination can be converted to Pyruvate, Acetyl-CoA, TCA cycle intermediate and eventually oxidized in the TCA cycle to release energy. It can be used as a source of carbon for the synthesis of cell constituents.

Bacterial count of diluted samples of same concentrations of both digesters showed variations with higher count in KOH-H_3PO_4 digester. In both digesters gram positive rod shaped bacteria of varying arrangements are found and it is observed that their growth is faster in KOH-Phosphate digester. This contributes to high speed putrefaction of KOH-Phosphate gelatin. Excess nitrogen from deamination may be excreted as ammonium ions, thus making the medium alkaline. The bacterial arrangements and their chemical nature have given in table5.Since it is difficult to maintain strict anaerobic conditions in Lab. Methanogens are not considered in this study. Inference related to methanogens was made indirectly by methane gas readings.

Reuse of chromium in tanning process: Protein and chromium derived from chrome shavings were used as feed in anaerobic digester and chrome tanning in re-chroming process of leather making. The chromium bearing residue of chromium (III) hydroxide was treated with sulphuric acid till pH 2.8, left overnight. The chromium (III) sulphate solution was used in tanning as a 40% replacement for basic chromium sulphate (BCS) salt as per conventional procedures and the leathers evaluated against control leathers tanned using conventional chromium salts.

Conclusion

Waste management approaches are gaining importance in all the industries for sustainability. In this study, an attempt has been made to extract and reuse protein and chromium from chrome shaving through two different chemical hydrolysis methods. Thus growth of micro-organism is faster in KOH-H_3PO_4 gelatin than the Lime-HCl gelatin during storage for few days in aerobic conditions, which ultimately helped in more gas production during anaerobic digestion. Indirectly, faster the degradation of shaving dust hydrolysate more is the volatile suspended solids produced which is readily utilized my methanogens. This is reflected in the methane gas increase with reduced CO_2 %. Thus it is concluded that chromed leather waste hydrolysis and neutralization using KOH-H_3PO_4 mode is more feasible than Lime-HCl method.Extracted chromium is reused in tanning process.

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