High rate biogas production from waste textiles

Karthik Rajendran

&

Gopinath Balasubramanian

This thesis comprises 30 ECTS credits and is a compulsory part in the Master of Science with a Major in Resource Recovery: Industrial Biotechnology, 120 ECTS credits No. 5/2011
High rate biogas production from waste textiles

Karthik Rajendran, karthik.1988@gmail.com
Gopinath Balasubramanian, gopitech17@gmail.com

Master thesis
Subject Category: Technology

University of Borås
School of Engineering
SE-501 90 BORÅS
Telephone +46 033 435 4640

Examiner: Prof. Mohammad Taherzadeh.
Supervisor name: Azam Jeihanipour, Solmaz Aslanzadeh.
Supervisor address: School of Engineering,
University of Borås.
Date: 2011-09-02
Keywords: biogas, waste textiles, NMMO-pretreatment, two-stage anaerobic digestion, UASB, CSTR.
Abstract

Textile is a global product used by all people in the world. These textiles after the use are thrown into the trash for incineration or land filling. However an efficient way that can be used to produce more energy, in an environmentally friendly process is anaerobic digestion. Waste textiles which contain cellulosic fibers (e.g. Cotton and viscose) can be converted to biogas. In this study, the performance of a two-stage anaerobic digestion process for biogas production from four different materials, including untreated jeans, treated jeans, cotton, and starch was studied. Starch was used as an easy-to-digest material to compare its digestion with that of cellulosic materials.

The two-stage processes were composed of a CSTR (for hydrolysis) and a UASB (for methanogenisis) which were investigated in two different configurations, namely (closed and open systems). In the closed system, the outlet of UASB was completely returned back to the CSTR, while in the open system the UASB outlet was sent to sewage. In a stepwise progress, the OLR was aimed to increase from 2 to 20 g Vs per L per day along with reduction in hydraulic retention time from 10 days to 1 day.

The results showed that the closed system was more stable when compared to the open system. The pre-treatment of jeans by NMMO helped to produce methane as that of cotton. The hydraulic retention time was decreased to less than 9 days for treated jeans and less than 5 days for starch. The overall methane yield at OLR of 4 gVS per L per day for starch and treated jeans was 98.5% and 97.4% in the closed system, whereas in the open system the yield was 77.0% and 35.5%, respectively.

Another experiment was conducted to compare the performance of two-stage process with that of a single stage process of anaerobic digestion of textiles containing polyester and cotton or viscose. Viscose textiles produced more gas compared to the cotton textile; it may be due to the higher crystalline of cotton which makes it hard to be degraded by the microorganisms. Furthermore, two-stage process could able to produce more methane than the single stage process.

The parameters like total solids, volatile solids, pH, gas production, gas composition, concentration of nutrients, and COD were also analyzed for both of the experiments.

Keywords: biogas, waste textiles, NMMO-pretreatment, two-stage anaerobic digestion, UASB, CSTR.
## Contents

1  **Introduction** ............................................................................................................... 1  
1.1  Need for sustainability .............................................................................................. 1  
1.2  History ....................................................................................................................... 1  
1.3  Biogas ......................................................................................................................... 3  
   1.3.1  Methane and carbon dioxide .............................................................................. 4  
   1.3.2  Nitrogen and Oxygen ....................................................................................... 5  
1.4  Substrate .................................................................................................................... 5  
1.5  Textile waste ............................................................................................................. 6  
1.6  Process of formation of biogas .................................................................................. 7  
   1.6.1  Hydrolysis ........................................................................................................... 7  
   1.6.2  Acidogenesis ..................................................................................................... 8  
   1.6.3  Acetogenesis ..................................................................................................... 8  
   1.6.4  Methanogenesis ............................................................................................... 9  
1.7  Process and Bioreactors ............................................................................................ 11  
   1.7.1  Batch process .................................................................................................... 11  
   1.7.2  Continuous process .......................................................................................... 11  
   1.7.3  Fed batch process ............................................................................................. 11  
   1.7.4  CSTR ................................................................................................................ 12  
   1.7.5  UASB ............................................................................................................... 12  
   1.7.6  Two-stage Process ............................................................................................ 13  
1.8  Parameters .................................................................................................................. 13  
   1.8.1  Temperature ....................................................................................................... 13  
   1.8.2  pH ..................................................................................................................... 13  
   1.8.3  Nutrients .......................................................................................................... 14  
   1.8.4  C/N/P ratio ....................................................................................................... 14  
   1.8.5  Retention Time ................................................................................................. 14  
   1.8.6  Mixing .............................................................................................................. 15  
   1.8.7  Water content ................................................................................................... 15  
1.9  Pretreatment ............................................................................................................. 15  
   1.9.1  Acid hydrolysis .................................................................................................. 16  
   1.9.2  Alkali hydrolysis ............................................................................................... 16  
   1.9.3  Hydrolysis by NMMO ...................................................................................... 16  
1.10 Applications of biogas ............................................................................................. 16  
1.11 Objective ................................................................................................................... 17  

2  **Materials and Methods** .......................................................................................... 18  
2.1  Materials ................................................................................................................... 18  
2.2  Methods ..................................................................................................................... 18  
   2.2.1  Pretreatment of Jeans ..................................................................................... 18  
   2.2.2  Total solids (TS) and Volatile solids (VS) measurement ................................. 19  
   2.2.3  Continuous anaerobic digestion process ......................................................... 20  
      2.2.3.1  Start up ...................................................................................................... 20  
      2.2.3.2  Experimental setup .................................................................................. 21  
   2.2.4  Batch experiments ............................................................................................. 25  
      2.2.4.1  Experimental setup .................................................................................. 25  
   2.2.5  Chemical Oxygen Demand ............................................................................. 26  
   2.2.6  Ion exchange chromatography ....................................................................... 27  
   2.2.7  Gas production ................................................................................................. 28  
   2.2.8  Gas Chromatography ...................................................................................... 28  
   2.2.9  Optical Microscopy ......................................................................................... 29
3 Results ..................................................................................................................................................30
  3.1 Continuous process ..........................................................................................................................30
    3.1.1 TS and VS ..................................................................................................................................30
    3.1.2 Gas production .........................................................................................................................30
    3.1.3 Effect of pH ..............................................................................................................................34
    3.1.4 COD and COD removal efficiency ............................................................................................34
    3.1.5 CH₄:CO₂ ratio ..........................................................................................................................37
    3.1.6 Effect of nutrient concentration ...............................................................................................38
      3.1.6.1 Concentration of ammonium ............................................................................................38
      3.1.6.2 Concentration of potassium ..............................................................................................40
    3.1.7 Optical microscopy ..................................................................................................................42
  3.2 Closed system after feeding stopped ...............................................................................................43
    3.2.1 Gas Production ..........................................................................................................................44
    3.2.2 Effect of pH ..............................................................................................................................45
    3.2.3 COD and COD removal efficiency ............................................................................................46
    3.2.4 Ratio CH₄:CO₂ in a closed system ............................................................................................48
    3.2.5 Effect of nutrients .....................................................................................................................49
  3.3 Batch process ....................................................................................................................................51
    3.3.1 TS and VS ..................................................................................................................................51
    3.3.2 Gas production ..........................................................................................................................51
    3.3.3 Effect of pH ..................................................................................................................................53
    3.3.4 COD ............................................................................................................................................53
    3.3.5 CH₄:CO₂ ratio ............................................................................................................................54
    3.3.6 Effect of nutrients .....................................................................................................................56
4 Discussion ...........................................................................................................................................59
  4.1 Continuous process ..........................................................................................................................59
  4.2 Continuous process after feeding stopped .....................................................................................61
  4.3 Batch process ....................................................................................................................................61
5 Conclusions ........................................................................................................................................63
  5.1 Advantages .......................................................................................................................................64
  5.2 Drawbacks .......................................................................................................................................64
  5.3 Future Work ......................................................................................................................................65
Acknowledgements ..................................................................................................................................66
References ...............................................................................................................................................67
List of Figures

Figure 1 Closed cycle pathway of methane ......................................................... 3
Figure 2 Correlation of the methane percentage with carbon chain length [1] .......... 4
Figure 3 Amount of textile waste in the UK .......................................................... 7
Figure 4 Electron micrograph of methanogenic bacteria [11] ................................. 9
Figure 5 Formation of granules in UASB ............................................................... 12
Figure 6 Two-stage reactors showing closed system .............................................. 22
Figure 7 Two-stage reactors showing open system ................................................ 22
Figure 8 UASB and CSTR reactors ................................................................. 23
Figure 9 Two-stage reactors with a filter in CSTR ................................................. 26
Figure 10 Automatic Methane Potential Testing System ..................................... 28
Figure 11 Methane production in the UASB Closed system ............................... 31
Figure 12 Methane production in CSTR closed system ........................................ 32
Figure 13 Total Methane production in the closed system ...................................... 32
Figure 14 Methane production in the UASB open system .................................... 33
Figure 15 Methane Production in the CSTR open system ..................................... 33
Figure 16 Total methane production in the open system ...................................... 33
Figure 17 COD in the CSTR closed system ............................................................ 35
Figure 18 COD in the UASB closed system ............................................................ 35
Figure 19 COD in the CSTR open system ............................................................. 36
Figure 20 COD in the UASB open system ............................................................ 36
Figure 21 Effect of ammonium concentration in an UASB closed system ............. 39
Figure 22 Effect of ammonium concentration in CSTR closed system .................. 39
Figure 23 Effect of ammonium concentration in an UASB open system ............... 40
Figure 24 Effect of ammonium concentration in a CSTR open system ............... 40
Figure 25 Effect of potassium concentration in UASB closed system .................... 41
Figure 26 Effect of potassium concentration in CSTR closed system .................... 41
Figure 27 Effect of potassium concentration in an UASB open system .......................... 41
Figure 28 Effect of potassium concentration in a CSTR open system .......................... 42
Figure 29 Granules from Starch UASB closed system ............................................ 43
Figure 30 Granules from the Starch UASB open system .......................................... 43
Figure 31 Methane production in an UASB closed system after feeding stopped .......... 44
Figure 32 Methane production in a CSTR closed system after feeding stopped .......... 45
Figure 33 Total Methane production after feeding stopped ...................................... 45
Figure 34 Effect of pH in UASB closed system after feeding stopped ....................... 46
Figure 35 Effect of pH in the CSTR close system after feeding stopped ..................... 46
Figure 36 COD in the CSTR close system after feeding stopped .............................. 47
Figure 37 COD in UASB after feeding stopped ...................................................... 47
Figure 38 COD removal efficiency after feeding stopped .......................................... 48
Figure 39 Methane to Carbon dioxide ratio in UASB after feeding stopped .......... 48
Figure 40 Methane to Carbon dioxide ratio in CSTR after feeding stopped .......... 49
Figure 41 Effect of ammonium in UASB closed system after feeding stopped .......... 49
Figure 42 Effect of ammonium in CSTR closed system after feeding stopped .......... 50
Figure 43 Effect of potassium in UASB closed system after feeding stopped .......... 50
Figure 44 Effect of potassium in CSTR closed system after feeding stopped .......... 50
Figure 45 Methane production in the UASB batch process ...................................... 52
Figure 46 Methane production in CSTR for batch process ....................................... 52
Figure 47 Total methane production comparison for single stage and two-stage process 52
Figure 48 Effect of pH in CSTR for batch process .................................................. 53
Figure 49 COD in UASB closed system for batch process ........................................ 54
Figure 50 COD in CSTR closed system for batch process ...................................... 54
Figure 51 Ratios of methane to carbon dioxide in UASB for batch process ............. 55
Figure 52 Ratios of methane to carbon dioxide in CSTR for batch process ............. 55
Figure 53 Effect of ammonium in UASB for batch process ..................................... 56
Figure 54 Effect of ammonium in CSTR for batch process ................................................. 56
Figure 55 Effect of potassium in UASB for batch process .............................................. 57
Figure 56 Effect of potassium in CSTR for batch process .............................................. 57
Figure 57 Effect of calcium in the UASB for batch process ........................................... 58
Figure 58 Effect of calcium in CSTR for batch process ............................................... 58
Figure 59 Beneficial aspects of a closed system ............................................................ 60
List of Tables

Table 1 Composition of biogas ................................................................. 4
Table 2 Theoretical methane and carbon dioxide percentage of organic materials [5] ........ 6
Table 3 Macronutrients added during the startup of UASB reactors [16] ....................... 20
Table 4 Micro nutrient concentrations [16] .................................................................. 21
Table 5 Start up concentration of the CSTR ................................................................. 21
Table 6 schedule of the process at different OLR ........................................................ 24
Table 7 Outlet volume from reactor every day ............................................................. 24
Table 8 Concentrations maintained at different stages for the nutrients added .............. 25
Table 9 Concentrations added to make up for the next stage ....................................... 25
Table 10 Comparison of the TS and VS before and after the continuous process .......... 30
Table 11 COD removal efficiency in the closed system ............................................... 35
Table 12 COD removal efficiency in the open system ................................................. 37
Table 13 \( \text{CH}_4 : \text{CO}_2 \) ratio of the Closed system .................................................. 37
Table 14 \( \text{CH}_4 : \text{CO}_2 \) ratio of the Open system ....................................................... 38
List of Equations

Equation 1 theoretical equation for biogas production [4] ...................................................................... 3
Equation 2 Formula to calculate TS and VS of a material ........................................................................ 19
Equation 3 COD removal efficiency ........................................................................................................ 34
1 Introduction

1.1 Need for sustainability

Globally the major share of the energy requirement is fulfilled by burning the crude oil. The contribution of renewable sources is almost negligible that this would lead to an energy crisis in the future and increase in the pollution [1]. Environment pollution is one of the most important consequences faced by human beings in day to day life. The climate change due to dumping of waste materials to the environment and global demand for the fossil fuels is the big hassle to humans. Biotechnology approaches are the most sustainable, environmental friendly and cost effective way of converting the pollutants to the value-added materials. Among that anaerobic digestion is one of the best approaches to deal with these problems by converting the waste to biogas, and fertilizer as a byproduct. The waste materials including municipal waste and cellulosic material waste (e.g. wood and waste textiles) are examples of biowaste that can be used as substrate for anaerobic digestion for biogas production [2].

1.2 History

The history of biogas begins before the birth of Christ. Even around 3000 BC, anaerobic cleansing of waste was done by Sumerians. The property of explosion exhibited by gas was first described by Alessandaro Volta in 1776 when he collected gas from the Lake Como, and found that gas was produced due to the fermentation process. Dalton, Davy and Henry in 1800 described the structure of methane. However the final structure of methane (CH$_4$) was given by Avogadro in 1821. In 1859 a leprosy hospital in Mumbai, India inaugurated the biogas plant for treating their waste water and to provide biogas for lightening purposes [1].

Depth of science in biogas increased in the 19$^{th}$ century. Béchamp in the year 1868 identified that several microorganisms are involved in the biogas production and large amount of byproducts are formed in the fermentation process depending on the substrate [1]. In the year 1881 a French journal, Cosmos, designed the first airtight container for the treatment of the domestic waste called “Mouras Automatic Scavenger” [2].

Generation of energy from the waste was first initiated by Louis Pasteur, in the year 1886. He along with his student Gavon, collected horse dung from the street of Paris and produced 100 L of biogas from 1 m$^3$ of horse dung. The energy thus obtained was enough to lighten a street light of Paris. In 1906 a two-stage process was developed by Travis to purify waste water with biogas production [1].
In 1906 Sohgen found methane to be made of three main types of materials namely formate, carbon dioxide and hydrogen. In 1920 a Chinese scientist Guorui developed an 8 m³ digester and established SanzouGuorui Biogas Lamp Company. In 1923, Germany was the first country to sell methane to public gas work. Around 1930, methane gas was purified in order to remove carbon dioxide and sulfides, and was compressed in a bottle to be used as a fuel for vehicles [1].

In 1940, Germany and France started to use agricultural waste products for the biogas production. Later in 1950 Imhoff found that cow waste produces 100 times more biogas than sewage waste. Hence he continued his research in generating biogas from cow and horse waste. Pobel in 1950 was the first scientist to conduct co-fermentation in order to increase the yield of biogas by combining various household organic waste [1]. Stander in 1950 recognized the importance of biogas using Sludge Residence Time (SRT) by the success of the anaerobic process that gave rise to the high rate digestion of waste water [2].

In the same year 1950 Germany first opened the large biogas plant in Celle. About 50 plants were installed in the next couple of years. Around 1955 due to the low price of fuels and availability of mineral fertilizer, importance of biogas got reduced and many plants were shut down. After a few years during 1970, again the demand for biogas rose due to severe oil crisis. Hence, the biogas plants were established again. In the year 1956, an Indian scientist, JashuBhai J Patel developed a floating cup bioreactor known as “Gobar Gas plant” [1].

At the end of the year 1990, United States of America established a large number of biogas plants and developed a mechanical and biological treatment for the garbage with the technology mainly focused on anaerobic digestion and few aerobic composting [1]. In the year 1997, more than 400 agricultural biogas plants were built in Germany [3]. By 21st century, the law of “Renewable Energies” which states the rules for gain of power from the biogas plant, became more effective [1].
### 1.3 Biogas

Biogas is generated by the decomposition of organic materials under anaerobic conditions with the help of anaerobic bacteria. Further, methanogens degrade organic matter and transfer biogas to the environment. In this way, natural production of biogas plays an important role in the biogeochemical carbon cycle. Biogas is a renewable source of energy and has a completely closed cycle pathway [3]. The general reaction for biogas production under complete digestion of biomass is as following [4]:

\[ C_{c}H_{h}O_{o}N_{n}S_{s} + yH_{2}O \rightarrow xCH_{4} + nNH_{3} + sH_{2}S + (c-x) \text{ CO}_{2} \]

\[ x=1/8 \ (4c+h-2o-3n-2s) \]
\[ y=1/4 \ (4c-h-2o+3n+2s) \]

The overall reaction for the biogas production from the carbohydrates is:

\[ C_{6}H_{12}O_{6} \rightarrow 3\text{CH}_{4} + 3\text{CO}_{2} \]

**Equation 1 theoretical equation for biogas production** [4]

![Figure 1 Closed cycle pathway of methane](http://2.bp.blogspot.com/)

---

1 [http://2.bp.blogspot.com/](http://2.bp.blogspot.com/)
Biogas mainly consists of methane and carbon dioxide with several amounts of impurities. The composition of biogas varies depending on the substrate. Biogas with more than 45% of the methane content are flammable [1].

<table>
<thead>
<tr>
<th>Components</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane (CH₄)</td>
<td>50-75</td>
</tr>
<tr>
<td>Carbon dioxide (CO₂)</td>
<td>25-50</td>
</tr>
<tr>
<td>Nitrogen (N₂)</td>
<td>0-10</td>
</tr>
<tr>
<td>Hydrogen (H₂)</td>
<td>0-1</td>
</tr>
<tr>
<td>Hydrogen sulfide (H₂S)</td>
<td>0-3</td>
</tr>
<tr>
<td>Oxygen (O₂)</td>
<td>0-2</td>
</tr>
</tbody>
</table>

1.3.1 Methane and carbon dioxide

The composition of methane and carbon dioxide varies due to the various factors. The long chain hydrocarbon compound like fat materials can increase the quality of the gas, but many large chains may lead to acidity.

Figure 2 correlation of the methane percentage with carbon chain length [1].

---

1 [http://en.wikipedia.org/]
In the anaerobic decomposition long time exposure of material to decompose may lead to increase the methane content and decrease the amount of carbon dioxide proportion. The homogeneous materials would lead to increase the fermentation process and shorten the exposure time. Higher amount of liquid phase in the reactor would lead to increase in dissolving of carbon dioxide in the liquid phase. Hence, lowest amount of carbon dioxide is found in the gas phase. The higher temperature maintained in the reactor leads to the lower the concentration of dissolved carbon dioxide in the liquid. The higher pressure inside the digester eventually leads carbon dioxide to dissolve of in the liquid, so that there is less amount of the carbon dioxide in the gas phase [1].

1.3.2 Nitrogen and Oxygen

The ratio of nitrogen to oxygen in the biogas is usually 4:1; these two components are added due to supply of the air to remove the sulfide in the biogas [1].

1.4 Substrate

The substrate which has carbohydrates, proteins, fats, cellulose and hemicelluloses as their main components is suitable for the biogas production. Some of the points to be considered while selecting biomass are:

- The content of organic substances should be suitable for the fermentation process to provide high yield.
- The substrate should have an appropriate nutritional value; hence the ability of the reactor to produce gas will be as high as possible.
- The substrate should be free of pathogenic substance and other organisms should aid the fermentation process.
- The amount of hazardous substances should be low in such a way that does not affect the fermentation process.
- The percentage of methane content of the biogas should be appropriate for the further application.
- The residue after anaerobic digestion should be beneficial to the environment e.g., as a fertilizer [1]
Table 2 Theoretical methane and carbon dioxide percentage of organic materials [5]

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Liter gas per Kg of dry substance</th>
<th>CH₄%</th>
<th>CO₂%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>700</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>Fat</td>
<td>1200</td>
<td>87</td>
<td>33</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>800</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

These three main organic substances in waste materials are classified under three main categories:

- Land based resource: It mainly covers the agricultural waste such as sugar cane trash, weeds, straw, spoiled fodder, forest litter like roots and leaves, textile waste. The various by-products of these agro based industries are oil cakes, bagasse, waste from vegetable and food processing industry, cotton dust from textile mills.

- Animal based resources: Cattle sheep wastes, poultry litter, Fish wastes, Slaughter house wastes, wool waste, human waste (urine, excrete).

- Marine waste materials: marine algae, sea-weeds, water hyacinth [5].

Starch, also known as amylum, is a carbohydrate consisting of many glucose units linked together by glycosidic bonds. It is commonly present in food products like potatoes, maize, cassava, rice and wheat. Starch mainly consists of two types of molecules; amylose and amylopectin. Generally starch composes of 20-25% of amylose and 75-80% of amylopectin\(^1\). Starch can be easily degraded by microorganisms. It is an easy-to-digest material and can produce biogas in a much faster rate\(^2\). In this study, starch was used in the continuous process for both configurations (closed and open) as a reference material to be able to compare to the rate of digestion of cellulosic materials.

1.5 Textile waste

The textile production and its application have been increasing drastically worldwide in the past few years. The total fiber production was more than 68.7 million tons in 2007, where cotton was more abundant, followed by polyester [6].

\(^1\) http://en.wikipedia.org/
\(^2\) http://www.goodnewsindia.com/
Figure 3 Amount of textile waste in the UK\textsuperscript{1}

All these used textiles ends up as a waste sooner or later in a waste collection station, and these wastes are generally incinerated or land filled [7]. In many countries including European nations, land filling of organic waste is forbidden (The council of European nations) [6]. However, converting the textile waste into useful product such as fuel can be done by pyrolysis technique. Treating of these textile wastes by pyrolysis would lead to production of large amounts of smoke and fumes [8]. In order to obtain both economic and environmental friendly products from cellulosic waste, the best approach is anaerobic digestion [9]. The textile waste consists of 40% cellulose; these cellulosic wastes could be converted to biogas and bioethanol [6].

1.6 Process of formation of biogas

There are several processes involved in the biogas production:

1.6.1 Hydrolysis

Hydrolysis is the primary step in the formation of biogas. In this process, complex organic (long chain) materials such as carbohydrates, proteins and lipids are broken down into simplest (small chain) compounds like sugars, amino acids and fatty acid using hydrolytic

\textsuperscript{1} http://dl.dropbox.com/
bacteria. Hydrolysis is also known as liquefaction and this is the limiting step of the overall anaerobic process [2].

1.6.2 Acidogenesis

Acidogenesis is the second process followed by hydrolysis in biogas formation. In this process, all the monomers are converted into VFAs (volatile fatty acids). Mainly acidogenic bacteria are responsible for producing VFAs such as Valeric acid, Butyric acid, Propionic acid, Caprionic acid, Isovaleric acid etc., These VFAs compounds can be analyzed using HPLC (High Pressure Liquid Chromatography) technique [2].

Many groups of bacteria are responsible for the hydrolysis and acidogenesis process. They mainly belong to the family Streptococcaceae and Enterobacteriaceae and genera of Bacteroides, Clostridium, Butyribrio, Eubacterium, Bifidobacterium, and Lactobacillus. Some other families such as Bacillaceae, Lactobacillaceae and Enterobacteriaceae are present in the digested sludge. Clostridium species is mainly responsible for the acidogenesis process for the production of VFAs [2].

1.6.3 Acetogenesis

In the acetogenesis process all the VFAs compound, ethanol and aromatic compounds like benzoate are converted into the acetic acid, hydrogen and carbon dioxide using acetogenic bacteria [10]. In order to be thermodynamically favored for the acetogenesis, the partial pressure should be below $10^{-3}$ atm through the efficient removal of hydrogen of the hydrogen-consuming organisms such as hydrogenotrophic methanogens. The low partial pressure of hydrogen would lead to acetate, carbon dioxide and hydrogen formation. Whereas high partial pressures would lead to the formation of propionate [2].

Propionate to acetate
(i) $\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + \text{HCO}_3^- + 3\text{H}_2$

Butyrate to acetate
(ii) $\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2$

Benzoate to acetate
(iii) $\text{C}_7\text{H}_5\text{CO}_2^- + 7\text{H}_2\text{O} \rightarrow 3\text{CH}_3\text{COO}^- + 3\text{H}^+ + \text{HCO}_3^- + 3\text{H}_2$

Ethanol to acetate [2]
(iv) $\text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2$
1.6.4 Methanogenesis

In the methanogenesis process, methanogenic bacteria use acetate, hydrogen and carbon dioxide as a substrate for the production of the methane. On the basis of the chemical oxygen demand (COD) 72% of methane is produced by the decarboxylation of acetate using acetotrophic or an aceticlastic methanogens type of bacteria. The remaining methane is produced from the hydrogen and the carbon dioxide using hydrogenotrophic methanogens. Since methane is mainly produced from the acetate, homoacetogens bacteria are mainly responsible for the synthesis of acetate from hydrogen and carbon dioxide [2].

Acetate to methane

\[ \text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + 2\text{HCO}_3^- \]

Hydrogen and carbon dioxide to methane [2]

\[ 4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \]

There are mainly three groups of bacteria involved in the methane production.

*Methanosarcina* genus (spherically shaped), *Methanothrix* bacteria (long and tubular), Bacteria that catabolize furfural and sulfates (short and curved rods) [3].
Figure 5 Process of methane production

1.7 Process and Bioreactors

Three different processes involved in fermentation namely batch process, continuous process and fed-batch process.

1.7.1 Batch process

Batch process is also known as a closed system process because inoculum, substrate and enough amounts of nutrients are added at the beginning of the process and do not get replenished once the process is started. After the completion of batch process, all the slurry and the digested materials are removed. And the reactor is cleaned for further use. During this process, gas production increases initially and then gets decreased. In the beginning stage microorganisms get adapted to the environment and start growing; later biomass growth remains constant. This in turn increases the amount of biogas yield. At the final stage biomass start dying due to lack of nutrients and other factors like decrease in substrate and pH, so the biogas yield start decreasing [5].

Batch process generally proceeds for 30 to 180 days. It is a simple and easy to handle process, with little attention during loading and unloading the reactor [5].

1.7.2 Continuous process

In the continuous process, the reactors are continuously fed and withdrew simultaneously in order to stabilize the gas production. The substrate and nutrient are added continuously to maintain the constant growth of the biomass, and this in turn increases the duration of exponential phase due to constant growth and death of biomass. Mixing is done with the help of the agitator and the flow is maintained. High flow would lead to washing out of the biomass. Temperature and pH are maintained in order to stabilize the gas production. The gas which is constantly produced from the reactor is collected in a gas holder [5].

1.7.3 Fed batch process

In the fed batch process the inoculum, substrate and nutrients are added at the beginning of the process. Later nutrients and substrate are added at the regular intervals. Duration of this process usually depends on digester volume and not with the utilization of substrates. In fed batch digester, materials cannot be drawn continuously. It is done periodically. Hence, fermentation has to be stopped for some time to empty the digester [5].
1.7.4 **CSTR**

CSTR stands for Continuous Stirred Tank Reactor; it is most commonly used anaerobic digester for the production of the biogas. CSTR is a cylindrical vessel with agitator. Agitation provides complete mixing and prevents the settling down of substrate at the bottom and scum formation at the top layer. It is mainly used to decompose the solid substrate in the biogas production\(^1\).

1.7.5 **UASB**

UASB stands for Upflow Anaerobic Sludge Blanket. In this bioreactor, the fed solution passes through the granules of biomass [12]. Biogas is produced at the surface of the granules, and due to the adhering gas bubbles, the granules rise to the liquid phase and release the gas that is collected with the help of the gasholder. After this process, the granules again settle down to the sludge blanket. This process does not require sludge recycling and can operate high organic rate. The efficiency of this process is about 97% [9]. UASB is mainly used in the waste water treatment plant [13].

The main drawback of the UASB is its relatively long start up time due to the formation of bacterial granules [9]. The microscopic analyses of these sludge granules during the process reveals the size of the granules and long multi-cellular filaments of rod shaped organisms present in the granules [14]. The filamentous microorganisms are entangled to form a pellet and this theory is called as spaghetti theory.

![Formation of granules in UASB](http://www.uasb.org/)


\(^2\) [http://www.uasb.org/](http://www.uasb.org/)
1.7.6 Two-stage Process

Two-stage processes are the combination of two single stage processes. This is to increase the rate of biogas production and to decrease the retention time. This process involves the separation of the solid component in the first stage and feeding of liquid fraction (VFA) in the second stage, to increase the yield of biogas. Most commonly a two stage process utilizes the combination of a CSTR and a UASB in which the high amount of solid content is digested in CSTR and the liquid part is pumped into UASB for the high rate of biogas production [9].

1.8 Parameters

1.8.1 Temperature

Temperature plays an important role in the biogas production. It affects the rate of the reaction, the solubility of the heavy metals and carbon dioxide, and the composition of gas. The rate of the reaction can be improved by increasing the ambient temperature. This would lead to an increase in the biogas production. The magnitude of this increase in biogas production is given by the temperature coefficient ($Q_{10}$) [5]

$$Q_{10} = \frac{\text{Rate at (T °C+10 °C) /Rate at T °C}}{\text{Rate at T °C}}$$

There are three temperature ranges where the bacteria can have high activity.

Psychrophilic less than 15 °C

Mesophilic (15-45 °C)

Thermophilic (45-65 °C)

In the biogas reaction, generally mesophilic and thermophilic range is more important (at low temperature below 10 °C anaerobic reaction does not take place) [5].

1.8.2 pH

pH is one of the significant factors playing a key role in the biogas production. Even slight changes in pH would lead to adverse effects in the optimum results. The microorganisms involved in the production of biogas can be classified into two main class based on pH. One is an acidogenic species and the other is methanogens. Methanogens are more sensitive to pH when considering the other microbial community. Their optimal pH ranges between 7.8 -8.2. Acidogenic bacteria are less sensitive to pH and it lies in the range of 5.5 -6.5. In the combined culture of both methanogens and acidogenic species, the optimum pH ranges
between 6.8 - 7.4. Since methanogens is considered as the rate limiting step, it is necessary to maintain the pH close to neutral [2].

1.8.3 Nutrients

Microorganisms need a certain amount of mineral nutrients along with carbon source for their growth. Other than carbon and hydrogen it also requires major nutrient components such as nitrogen, phosphorous, potassium, magnesium, calcium and some trace elements like iron, manganese, molybdenum, zinc, cobalt, selenium, and nickel. However the highest concentration of these nutrients would lead to the inhibition of biogas production. The concentration of the nutrient in the reactor can be analyzed using Ion Exchange Chromatography [3].

1.8.4 C/N/P ratio

When the C/N ratio is too low it would lead to decrease in the biogas production. On the other hand if the C/N ratio is high, it would lead to lack of nitrogen and will have a negative impact on the protein formation. This would result in the structural metabolism of the microorganisms. A high ratio will have nitrogen starvation by biomass. This causes the microorganisms to die returning nitrogen to the process, but decreases the rate of methane production. On the other way, a low C/N ratio results in high nitrogen concentration in the digestate. This would affect the digestate being used as a fertilizer.

The optimum range of C/N ratio for anaerobic digestion is 16:1 - 25:1 [1]. Since the aim is to achieve low biomass production and high biogas production, small amount of nutrient is sufficient for this process. The optimum range of C/N/P/S ratio of the nutrient is 500-1000:15-20:5:3 and the optimum organic matter ratio of COD: N: P: S is 800:5:1:0.5.

1.8.5 Retention Time

Digestion of organic substance in the anaerobic process is slow and therefore, the substrate is maintained at different length of the time for the complete decomposition or digestion of the organic matter. Retention time is described as the time span spent in the reactor for a complete digestion. It is also known as residence time. The retention time for liquid is called as HRT (Hydraulic Retention Time) and solid is called SRT (Solid Retention Time [5].

---

The retention time for a continuous process is given by the digester volume divided by influent rate. It depends on the vessel geometry and pumping in the case of two-stage reactors [3].

1.8.6 Mixing
Mixing is the most important factor in order to keep the substrate in contact with microorganisms. The key advantages of mixing are

- The mixture is homogeneous to maintain the uniformity of substrate concentration, temperature and other environmental factors.
- To minimize the scum formation at the surface.

Generally mechanical stirring is used for mixing the substrate. There are also other types of mixing such as circulation pump, gas compression and the gear valve for complete stirring of substrate in the digester [5].

1.8.7 Water content

Water is an essential material for the survival of microorganisms. It is necessary for the movement of organisms, extracellular activity and to facilitate the breakdown of the substrate. Higher water content would lead to decrease in the slurry temperature and eventually lead to a decrease in the biogas production. On the other hand, lower water content would lead to excessive acid formation which would affect the fermentation process. The ideal ratio for the feed to water is 1:1. Hence it is necessary to calculate the amount of water content in the substrate. The optimum TS (Total solids) concentration is 7 to 9%. The amount of organic substance can be measured by measuring the VS (Volatile substance) content [5].

1.9 Pretreatment

Pretreatment is done to facilitate the hydrolysis of cellulose component present in the substrate. Cellulose has a highly crystalline structure due to the presence of an extensive hydrogen bond and inter-chain in the cellulose structure. Different solvent such as heavy metal-amine complex solutions, concentrated metal salts, thiocynate/amine, and LiCl/dimethylacetamide (DMAc) have been used to dissolve the cellulose component. There are various methods of pretreatment to remove the lignin component and to reduce the crystalline nature of the cellulose structure [15].
1.9.1 Acid hydrolysis
Treatment of cellulosic materials with concentrated sulfuric acid and phosphoric acid would decrease the crystalline structure of cellulose. Pretreatment of cotton with concentrated H$_3$PO$_4$ followed by acetone would lead to produce regenerative amorphous cellulose. However these materials are hazardous and there is a lack of efficient recycling technique in the large scale [6].

1.9.2 Alkali hydrolysis
In the textile industry sodium hydroxide is used as a swelling agent in cotton treatment and in viscose production process [7].

1.9.3 Hydrolysis by NMMO
N-methyl morpholine-N-oxide (NMMO) is mainly used to degrade the cellulose component. NMMO is non-toxic and fully biodegradable. Furthermore, it can be recycled and reused. NMMO melts at 70 $^\circ$C and decompose at 130 $^\circ$C, and it is important to use NMMO between these temperatures. The 83-87% composition of NMMO-H$_2$O mixture is suitable for the digestion of cellulosic waste. When there is a decrease in the composition of the mixture, it would lead to ballooning or partial dissolution of cellulose fiber [15].

1.10 Applications of biogas

- Biogas provides an alternative fuel for vehicles. However the quality of biogas should be improved and make it equivalent to that of natural gas. Removal of particulates, carbon dioxide, hydrogen sulfide and moisture can be done to increase the methane percentage up to 95%. The presence of carbon dioxide reduces the fuel value and hydrogen sulfide with water increasing corrosion. Biogas after purification can be blended with natural gas and used as a fuel for vehicles [2].
- Biogas is a source of energy to produce heat and electricity. Traditionally biogas is used as fuel for boiler and provides heat to the anaerobic process. Digester plants produce a large amount of biogas than it is needed, these excess biogas can be used as energy for the other processes [2].
- Biogas can be used in a combustion engine generator and in the turbines to produce heat and electricity for a plant operation. In the biogas plants, the generator uses biogas to produce electricity and waste heat produced by the generator is transferred to heat the digester[2].
Biogas is used for cooking and lightening in many developing nations. Biogas produced from the anaerobic digester of waste material is connected directly to the low pressure stove and used for cooking purposes [3].

In Biogas technology all the waste material is dumped into the anaerobic container, this in turn reduces the spread of pathogens present in the waste to the environment and avoids bad odor. By this way it helps to improve the hygienic condition [3].

The digested organic material after the biogas production is a rich source of nitrogen and other nutrients. It can be used by the farmer as a fertilizer for their crop fields. Through this it saves the economy to the farmers in buying the chemical fertilizer [3].

By the usage of waste it avoids the environmental pollution such as land, water and the air pollution [3].

Biogas provides job opportunities to the local people. Finally it improves the economy standard of people [3].

1.11 Objective

- To achieve a high organic loading rate and decrease the digestion time.
- To compare anaerobic digestion of four different materials (untreated jeans, treated jeans, cotton and starch)
- To compare anaerobic digestion of two-stage process in two different configurations (closed and open).
- To compare single stage and two-stage reactor for viscose and cotton combined with polyester with a filter in CSTR of two-stage process.
- To increase the overall yield of methane production using UASB reactors.
- Analysis of pH, gas volume, gas composition, COD, ions such as calcium, potassium, magnesium and ammonium through ion chromatography.
2 Materials and Methods

2.1 Materials

The anaerobic digestion process of the four different materials was compared: 1. Untreated jeans (bought from Myrona), 2. Treated jeans, 3. Cotton and 4. Starch (bought from the ICA super market). The materials Jeans and Cotton were cut into 3*3 cm² and then milled into fine materials.

With an aim of making a process suitable for industry without any pretreatment procedure, a single stage reactor was compared with the two-stage process closed system in a batch condition. The materials used in this process were textiles with a blend of cellulose and polyester, including a cotton/polyester (50/50 w/w) and viscose polyester (60/40 w/w). These textiles were cut into small pieces of 2.5*2.5 cm².

2.2 Methods

2.2.1 Pretreatment of Jeans

The pretreatment of the material was done with an environmental friendly cellulose solvent N-methylmorpholine N- oxide (NMMO). Commercially available 50% (w/w in aqueous solution) NMMO (BASF, Germany) was evaporated to 85% and stored in glass bottles. The evaporator used for concentrating the NMMO was a rotary vacuum evaporator (Laborota 20, Heidolph, and Schwabach, Germany) fitted with a vacuum pump with (PC 3004 VARIO, Vacuubrand, Wertheim, Germany).

The concentrated NMMO was solid at room temperature and was autoclaved at 121 °C for 20 minutes to get melt before treatment of the material. These materials were treated in a batch process. The ratio of material (jeans) to NMMO was 6% dry matter and for each batch, approximately 150 g of milled materials was treated in an oil bath at 120 °C for 3 h.

The materials were added slowly into the beaker with an agitator providing enough mixing. The beaker with the treated material and NMMO was removed from the oil bath and the suspension was added slowly to the warm water. The ratio of warm water to NMMO added was approximately 2:1. The warm water was added to stop the pretreatment, and then the contents were filtered using a cloth filter bag. The NMMO was washed further using the running tap water and de-ionized water was used for the last wash. The cellulose was then filtered using a vacuum filter to remove excess of water present in the material. The treated
material was stored in a cold room (5 °C) to avoid evaporation of water from the material [15].

2.2.2 Total solids (TS) and Volatile solids (VS) measurement

The water content or dry weight of the material can be measured using total solids measurement. The volatile solids were a measure to determine the amount of carbon or the volatile organic compounds present in the material which was converted to ash. The equipments need for the measurement of total solids and volatile solids were crucibles, desiccator, analytical balance, oven, and muffle furnace. The crucibles were marked for identification. The marked crucibles were then placed in an oven, a day before the analysis was done. The crucibles were then transferred to desiccator and cooled down to room temperature for 20 minutes. Then, the empty weight of crucible was noted using an analytical balance. The material for which the total solids and volatile solids need to be measured was weighed in the crucible.

The crucibles were covered with a lid and then transferred to an oven at 105 °C for one day. The next day the crucibles containing the dry material were allowed to cool in a desiccator for about 20 minutes. The crucibles with dry material were weighed using an analytical balance and then placed in a muffle furnace maintained at a temperature of 550 °C for three hours. The crucibles with ashes in it were transferred to a desiccator and allowed to cool down to room temperature for about one hour. The crucibles were always kept in the desiccator before measurements in order to avoid change in weight due to moisture in the atmosphere. The measurements were repeated thrice and averaged for the precision.

The TS and VS measurements were carried out using Equation 2:

\[
TS: \frac{(\text{Dried crucible} + \text{dried sample} - \text{dried crucible})}{(\text{sample weight})} \times 100
\]

\[
\text{LoI (Loss on ignition): } \frac{(\text{Dried crucible} + \text{sample}) - (\text{Dried sample} + \text{dried crucible})}{(\text{Dried crucible} + \text{sample}) - (\text{Dried crucible})}
\]

\[
\text{VS: LoI} \times \text{TS}
\]

Equation 2 Formula to calculate TS and VS of a material
2.2.3 Continuous anaerobic digestion process

2.2.3.1 Start up

The two-stage process consists of two reactors; Continuous stirred tank reactor (CSTR) for hydrolysis of the materials and Upflow Anaerobic Sludge Blanket (UASB) reactor for the production of biogas. The CSTR was operated at 55 °C and the UASB was maintained at 34 °C. The working volume of CSTR and UASB were 3 L and 2.25 L respectively. CSTR contained a water jacket to maintain temperature around 55 °C; a feed inlet, a sample point, a feed outlet and a gas line to the gas measuring system. There was a sampling point in the gas line to take the gas sample to be analyzed by GC. In addition, CSTR had an impeller to mix the contents well.

Similarly, the UASB reactor had a water jacket, a gas line, a feed inlet, an effluent outlet and a sampling point. The inlet of the UASB reactor had a mesh to avoid big particles entering in. The sedimentation tank was also kept for the big particles to get settled before the liquid passes through the pump. The pump used in this process was a peristaltic pump with a tube diameter of 1.02 mm. The UASB reactor was equipped with water jacket to maintain a temperature of 34 °C. The UASB granules were collected from Hammarby Sjöstadsverk, a pilot plant located in Stockholm (Sweden). The granules were stored in a cold room until it was used. Initially, The UASB reactors were filled with granules for a height of 40±3 cm. The remaining volume of reactor was filled with water. The dead granules floating on top of the water were removed using a filter. A synthetic medium with acetic acid, propionic acid and butyric acid in the ratio of 3: 1: 1 was fed to the granules for their activation. The pH of the synthetic medium was maintained at pH 7 by NaHCO₃ buffer. The tables below shows the concentration of macro and micro nutrients according to [14, 16]. The micro nutrients were prepared as a stock solution of a concentration of 100 times more and each time the stock was diluted in the ratio 1:100.

<table>
<thead>
<tr>
<th>Table 3 Macronutrients added during the startup of UASB reactors [16]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macro nutrients</td>
</tr>
<tr>
<td>NH₄Cl</td>
</tr>
<tr>
<td>K₂H₂PO₄.3H₂O</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
</tr>
<tr>
<td>CaCl₂.2H₂O</td>
</tr>
</tbody>
</table>
Table 4 Micro nutrient concentrations [16]

<table>
<thead>
<tr>
<th>Micro nutrients</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₂·4H₂O</td>
<td>2000</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>50</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>50</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>500</td>
</tr>
<tr>
<td>CuCl₂·2H₂O</td>
<td>38</td>
</tr>
<tr>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>50</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>2000</td>
</tr>
<tr>
<td>NiCl₂·6H₂O</td>
<td>142</td>
</tr>
<tr>
<td>Na₂SeO₃·5H₂O</td>
<td>164</td>
</tr>
</tbody>
</table>

The CSTR was filled with 2.5 L of sludge and 0.5 L of nutrient solution as given in the Table 5 with the C: N: P: S ratio maintained for 1 g/L cellulose. The sludge from Sobacken (Borås energi och miljo, Sweden) was incubated at 55 °C for three days. Then the sludge was fed to the reactor. Avicel at the concentration of 1 g/L was fed to the reactor for the sludge to get activated. It acted as a synthetic carbon source.

Table 5 Start up concentration of the CSTR

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose (Avicel)</td>
<td>1000</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>76.4</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>5.18</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.27</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>10</td>
</tr>
<tr>
<td>Trace metal</td>
<td>1</td>
</tr>
</tbody>
</table>

2.2.3.2 Experimental setup

The two-stage reactors with four different materials were compared with two systems namely 1. Closed system (Fig. 6) 2. Open system (Fig. 7).

The closed system consists of a closed cycle where the liquid was pumped from CSTR to UASB and a natural recirculation was preceded from the UASB to CSTR. In the open system, the liquid was pumped from the CSTR to UASB and from the UASB it was collected in a measuring cylinder. The outlet volume was measured and fed next day with the feed but during the course of time the reactor volume in CSTR will decrease till the next day. Based on the materials, system separation and two-stage process, a total of 16 reactors were used. This includes eight UASB reactors and eight CSTR reactors. These reactors were divided into two for the two systems.
Figure 6 Two-stage reactors showing closed system

Figure 7 Two-stage reactors showing open system
The C: N: P: S ratio was maintained in the reactor as 500: 20: 5: 3. During the beginning, avicel was fed to the reactor in the concentration of 1 g/L and the nutrients were calculated based on the carbon source present.

The objective of the process was to decrease the digestion time. Initially a digestion time of 10 days was maintained as the OLR increased; the digestion time was decreased gradually to 1 day at OLR 20 g VS/L/day. Each OLR was achieved a steady state condition for gas production. HRT was decreased by increasing the flow rate of the pump. Table 6 describes the schedule of the process at different OLR and their respective HRT, flow rate and duration. This process was varied based on the OLR into six different stages.
### Table 6 schedule of the process at different OLR

<table>
<thead>
<tr>
<th>Step</th>
<th>OLR (g VS/L/day)</th>
<th>HRT in CSTR (day)</th>
<th>HRT in UASB (day)</th>
<th>Flow rate (ml/min)</th>
<th>Pump (RPM)</th>
<th>Duration (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>10.0</td>
<td>7.50</td>
<td>0.21</td>
<td>3.79</td>
<td>30.0</td>
</tr>
<tr>
<td>2</td>
<td>2.7</td>
<td>7.5</td>
<td>5.62</td>
<td>0.28</td>
<td>5.05</td>
<td>30.0</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>5.0</td>
<td>3.75</td>
<td>0.42</td>
<td>7.58</td>
<td>15.0</td>
</tr>
<tr>
<td>4</td>
<td>8.0</td>
<td>2.5</td>
<td>1.88</td>
<td>0.83</td>
<td>15.15</td>
<td>8.0</td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>2.0</td>
<td>1.50</td>
<td>1.04</td>
<td>18.94</td>
<td>6.0</td>
</tr>
<tr>
<td>6</td>
<td>20.0</td>
<td>1.0</td>
<td>0.75</td>
<td>2.08</td>
<td>37.88</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Hydraulic Retention Time (HRT) determines the average time that soluble compounds were present in the reactor. The soluble compounds were the digested materials by the microorganisms during the time they spend in the reactor. The HRT was decreased from 10 days to 1 day in CSTR. Based on the HRT, the outlet volume of open system was calculated. For instance, on stage 3 the HRT was 5 days and the working volume was 3 L so that the outlet of every day should be 600 mL. On sampling days, additional 15 mL was taken out from both CSTR and UASB and it was replaced with nutrients and water to maintain a balance in the process. The samples were stored in a freezer for further analysis. Table 7 describes the volume removed from the reactor every day from the closed system and open system.

### Table 7 Outlet volume from reactor every day

<table>
<thead>
<tr>
<th>Stage</th>
<th>Closed System (mL)</th>
<th>Open system (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>400</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>600</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>1200</td>
</tr>
<tr>
<td>5</td>
<td>210</td>
<td>1500</td>
</tr>
<tr>
<td>6</td>
<td>450</td>
<td>3000</td>
</tr>
</tbody>
</table>

The concentration of the nutrients was calculated on the basis of cellulose concentration in the various steps of the process. Initially, 500 mL of nutrients was added for the cellulose concentration of 1 g/L. Then the cellulose concentration was increased to 2 g/L in step 1; for this a difference in nutrient between initial cellulose concentration and the concentration during the process was calculated and added to the reactor as make up for concentration. This was carried out in all stages and added to the CSTR the day before the process was proceeded to the next stage. Tables 8 and 9 describe the desired concentration in the reactor and makeup.
concentration to each reactor for all stages. On all other days, a nutrient solution of the desired concentration was prepared and added to the reactors along with the cellulose.

**Table 8 Concentrations maintained at different stages for the nutrients added**

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Step</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td></td>
<td>2000</td>
<td>2700</td>
<td>4000</td>
<td>8000</td>
<td>10000</td>
<td>20000</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td></td>
<td>152.83</td>
<td>206.32</td>
<td>305.66</td>
<td>611.31</td>
<td>764.14</td>
<td>1528.29</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td></td>
<td>10.38</td>
<td>14.01</td>
<td>20.76</td>
<td>41.51</td>
<td>51.89</td>
<td>103.78</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td></td>
<td>0.55</td>
<td>0.74</td>
<td>1.10</td>
<td>2.19</td>
<td>2.74</td>
<td>5.48</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td></td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>trace metal (ml)</td>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Table 9 Concentrations added to make up for the next stage**

<table>
<thead>
<tr>
<th>Make up (mg/reactor)</th>
<th>Beginning of step</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>573.11</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>38.92</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>2.06</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>22.50</td>
</tr>
<tr>
<td>trace metal (ml)</td>
<td>2.25</td>
</tr>
</tbody>
</table>

2.2.4 Batch experiments

2.2.4.1 Experimental setup

This process was compared between single stage CSTR and closed system two-stage reactors. The process was started with an OLR about 20 g VS/L and the nutrient was fed as the same in table 8 for OLR 20 g VS/L. The start up of this process was similar to the continuous process but the UASB was activated during the continuous process, so feeding with synthetic nutrients was not required. Sampling was done throughout the process every day. A filter was kept in the CSTR of the two-stage process to keep the material in the CSTR and allow the liquid part alone to the UASB; since the material may affect the pumping.

The filter had pores through which the re-circulated liquid from UASB had enough contact with materials present in the reactor. The polyester part of the textile was recovered after the process completion. The total solids and volatile solids of the residual materials were
measured after the process completion. The dry weight of the residual material was calculated to check how much material was degraded. The dry weight was measured by weighing the materials in the crucible and then kept in the oven at 70 °C for a day and weighed it again.

Figure 9 Two-stage reactors with a filter in CSTR

2.2.5 Chemical Oxygen Demand

The samples from both CSTR and UASB were analyzed for soluble Chemical Oxygen Demand (COD). All the samples were centrifuged at 10,000 RPM for 5 minutes and it was filtered using a 0.22 µm filter; then the clear liquid was analyzed. COD vials from HACH, Germany were used. These vials were covered with a tissue paper to avoid any contact with light before analysis. The COD vials were at different ranges; 0-150, 0-1500, and 0-15,000 mg COD/L. The vials 0-150 and 0-1500 mg/L needed a sample volume of 2 mL whereas, 0-15,000 needed a sample volume of 0.2 mL. A blank was prepared with the same required volume of sample with de-ionized water.

The vials were loaded into the COD reactor and heated to 150 °C for two hours. The samples were then cooled down to room temperature, and analyzed in a direct reading spectrophotometer for the concentration of COD with the absorbance at different wavelengths. The absorbance was measured at 620 nm for vials 0-15,000 mg/L. On the other hand, the vials with 0-150 and 0-1500 mg/L were analyzed at 420 nm due to the difference in
concentration of chemicals used. The samples were diluted in the ratio 1:10 if they were out of the range. Generally, the closed system (UASB and CSTR) and the open system CSTR samples were analyzed at the range of 0-15,000 mg/L. The UASB samples of open system were analyzed at 0-150 and 0-1500 mg/L range.

2.2.6 Ion exchange chromatography

The changes in the concentration of the macro and micro nutrients, including ammonium, potassium, calcium and magnesium, were analyzed using ion exchange chromatography.

The principle of ion chromatography is that opposite charged compounds are present in the column. For instance, to separate positive charged ions negative charged compounds are present in the column, which is a stationary phase. A mobile phase which was an eluent runs across the sample. The sample contains cations which get bound to the column. Each ion which gets attached to the column has different charge interactions; based on the charge they are eluted at different times. The concentration of each ion was calculated from the standard curve and the area under the curve of each sample [17,18].

The instrument used for this purpose was a product from Metrohm. The eluent solution was composed of 4 mmol/L tartaric acid and 0.75 mmol/L dipicolinic acid. The operating conditions including the flow of liquid was 1mL/min; pressure was maintained at 7-9 MPa and the temperature at 35-40 °C. The instrument was turned on for 1 hour with the cation column selected to prepare the instrument for the correcting baseline and then the samples were loaded. Before injecting the samples, they were diluted with eluent and to adjust the pH range between 2 to 3. The injection volume was 10 µl and all the samples were diluted with the ratio of 1:10. The samples were centrifuged at 10,000 RPM for 4 minutes and it was filtered through a 0.45 µm filter. The filtering was done to avoid bigger particles entering the column which may ruin the guard of the column.

The instrument was first run with standard for each cation at different concentrations and a standard curve was obtained. To measure the standard of ammonium, potassium, calcium and magnesium ions, the following salts were used as standards: ammonium chloride, potassium chloride, calcium chloride and magnesium chloride. While preparing the standards, the purity of each salt was also taken into account. The standard curve was obtained by running the above mentioned salts at concentrations of 5, 10, 15, 20 and 25 ppm, respectively. The standard solutions as well as the samples were diluted with the eluent. The different ions were
calibrated based on the above mentioned concentration to obtain a standard curve. From the area of the standard curve, the concentration of different samples was determined.

2.2.7 Gas production

The gas production was recorded every day using the gas measuring part of an Automatic Methane Potential Testing System (AMPTS, Bioprocess control (Sweden)). The measuring system was consisted of 15 cells. The gas from the reactor passed through the gas line and gets accumulated in the cell. Each time the cell was opened and it was recorded on the computer. AMPTS also showed the flow in each cell which helped to check the leakage of the line.

![Automatic Methane Potential Testing System](image)

**Figure 10 Automatic Methane Potential Testing System**

2.2.8 Gas Chromatography

The gas chromatography was used to analyze the composition of gas produced by the process. The sampling was done twice a week and daily during the phase transition and during the high OLR (8, 10, 20 gVS per L per day). The composition of methane and carbon dioxide was determined to calculate the gas volume.

The gas chromatography works on the principle that a mobile phase (carrier gas) runs across a stationary phase (column) with the sample need to be analyzed. The sample was vaporized and as it runs through the column they were separated based on the analyte charge and interaction with the stationary phase. The retention time and the peak height were used to determine the compound and its area respectively. The separated samples were detected by a detector and was recorded in a computer [17].
The operating conditions of gas chromatography (Perkin Elmer) including a TCD (Thermal Conductivity Detector) were injection temperature of 150 °C, detection temperature of 200 °C, and oven temperature of 75 °C. The pressure of carrier gas nitrogen was maintained at 10.2 Psi. The flow was usually maintained at 40 mL for both reference flow and carrier flow. A syringe of 0.25 mL was used to analyze the gas samples.

Two bottles, one of them containing methane of 100% and the other one containing 20% carbon dioxide and along with 80% nitrogen were used as standard. The standards were first run for three trials for stabilization and the values were averaged. The samples which were to be analyzed were injected and later the area of methane and carbon-dioxide was noted. The comparison of the area obtained from the sample of the standard gave the gas composition (percentage of methane and carbon dioxide). Based on the gas composition, the gas volume of methane and carbon dioxide was calculated individually from the total gas volume obtained for each reactor.

2.2.9 Optical Microscopy

The optical microscope was used to study the granules from the UASB after the process was completed. The optical microscopy works on a simple principle of lens system to view a magnified object. The microscopy consists of two lens objective and eyepiece. The objective zooms out the object present and the eyepiece will give a further magnification to the viewer.\(^1\) The latest microscopes have the ability to capture the picture and store it on a computer. The granules were also focused and compared for different reactors. The optical microscope used in this process was NIKON optical microscopy.

\(^1\) [http://www.molphys.leidenuniv.nl/](http://www.molphys.leidenuniv.nl/)
3 Results

3.1 Continuous process

3.1.1 TS and VS

The TS and VS were analyzed before the process in order to calculate the amount of feed required. This measurement was also done at the end of the process to see whether the process was effective enough to degrade the materials during the course of the period.

Table 10 lists the TS and VS of different materials before and after the process for both closed and open systems. The treated jeans had a low TS and VS before the process when compared to the untreated jeans because the treatment of jeans had a loss due to washing and filtering and also the treated jeans contained a large volume of water in it.

The measurement after the process revealed that the closed system was much more efficient that of the open system because the TS and VS of the closed system was much lower than that of open systems. The treated jeans and cotton had very low TS of less than 1% whereas the open system had a higher value. The untreated jeans of both open system and closed system was high after the process which showed that an untreated jean was not a good substrate to work with.

<table>
<thead>
<tr>
<th>Material</th>
<th>Before the process</th>
<th>Open system after the process</th>
<th>Close system after the process</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TS (%)</td>
<td>VS (%)</td>
<td>TS (%)</td>
</tr>
<tr>
<td>Untreated jeans</td>
<td>96.1</td>
<td>94.01</td>
<td>18.28</td>
</tr>
<tr>
<td>Treated jeans</td>
<td>11.56</td>
<td>11.34</td>
<td>6.84</td>
</tr>
<tr>
<td>Cotton</td>
<td>95.6</td>
<td>95.61</td>
<td>13.72</td>
</tr>
<tr>
<td>Starch</td>
<td>82.9</td>
<td>75.34</td>
<td>15.35</td>
</tr>
</tbody>
</table>

3.1.2 Gas production

The gas production of the two-stage process was measured using an AMPTS. The gas production was calculated based on per gVS per day. The closed system possessed a more steady state condition when compared to the open system. The CSTR of closed system for untreated jeans initially had a gas production around 100 mL per gVS per day. This was low when compared to other materials due to the effect of dyes present in the jeans. Methanogens in the CSTR was affected because of the dye in untreated jeans. This effect can be identified with the lowest gas production in CSTR, followed by a high gas production in UASB at OLR.
2 gVS per L per day. So, initially the UASB of untreated jeans produced a more methane because the dissolved materials in CSTR could not be converted to methane therefore. Further while increasing the OLR, the gas production in CSTR of untreated jeans increased. The reason may be because the microorganisms get acquainted to the dye effect. The other materials in the closed system-CSTR almost produced the same volume of gas even after increasing the OLR.

A sharp decrease in the gas production was observed when the OLR was increased at each stage. The starch was easy to degrade and hence the total gas production of starch was much higher when compared to the other materials. The cotton and treated jeans almost produced the same volume of methane which showed that the treatment of jeans helped to work better like cotton than the untreated jeans.

The high OLR was applied just for starch. The CSTR produced a high acid content which caused a decrease in pH and along with the low gas production. So, the high acid from CSTR led to an increase in gas production of UASB. All UASB reactors in the closed system were almost in a steady state condition even after increasing the OLR which shows that a higher OLR could be possibly achieved.

![Figure 11 Methane production in the UASB Closed system](image-url)

Figure 11 Methane production in the UASB Closed system
The open system was maintained in a steady state condition initially, but the steady state was affected once it reached a high OLR. The volume of CSTR was decreasing till the next day, increasing the concentration of volatile fatty acids and with a pH decrease, causing the death of methanogens. To maintain the pH at 6.5 in CSTR, buffer was added but it could not be maintained every day due to the continuous process.

At high OLR the starch was observed to be in more steady state than the other materials and the gas production in UASB was also increased. The decrease in volume of the open system CSTR makes it difficult to work with high OLR. Total gas production chart revealed that the gas production in treated jeans was slightly lesser when compared to cotton and starch.
Figure 14 Methane production in the UASB open system

Figure 15 Methane Production in the CSTR open system

Figure 16 Total methane production in the open system
3.1.3 Effect of pH

The drop in the pH trend was observed in all CSTR. The closed system was able to maintain pH due to the recirculation of liquid from UASB, whereas open system cannot. The open system required a large amount of buffer to maintain the pH at 6.5. When the OLR was increased, the starch material CSTR was adversely affected in both configurations.

3.1.4 COD and COD removal efficiency

The COD was an important measure to know the amount of material available for the microorganisms to degrade. The COD analyzed was a soluble COD, whereas the un-dissolved materials need to be accounted. The COD removal efficiency was calculated based on the inlet and outlet COD of the UASB. The following equation shows the calculation of the COD removal efficiency of the UASB.

\[
\text{COD removal efficiency (\%)} = \frac{(\text{COD}_{\text{CSTR}} - \text{COD}_{\text{UASB}})}{\text{COD}_{\text{CSTR}}} \times 100
\]

\text{Equation 3 COD removal efficiency}

The COD from the CSTR for all materials other than starch lied in the range of 5000 mg/L, whereas starch alone had a peak of 30,000 mg/L. The starch was easily dissolved and degraded; hence a higher COD was observed. The closed system UASB could able to remove the COD in the range of 2000-4000 mg/L throughout the process. The COD removal efficiency was less in a closed system when compared to the open system. The reason may be that in an open system the liquid along with granules was moved out from the UASB, whereas in the closed system the outlet granules could circulate to CSTR.

The granules were a consortium of biomass. When they go to the CSTR, which was operated at 55 °C, an autopyrolysis process could take place and the granules will be degraded. The degradation of these granules or the cell wall of the microbes causes a production of polymer in the process which inhibits the methanogens in the UASB to consume the volatile fatty acids\(^1\). Hence the COD removal efficiency of the UASB was low. The efficiency of the UASB closed system for starch had a high efficiency around 85%, but the other reactors had efficiency around 65-70%. The untreated jeans had a higher efficiency when compared to other materials because of the dye effect only hydrolysis was taking place in CSTR and had made UASB to consume all the COD making it more efficient.

\(^1\) http://www.lemvigbiogas.com/
Table 11 COD removal efficiency in the closed system

<table>
<thead>
<tr>
<th>Closed System</th>
<th>Untreated jeans</th>
<th>Treated jeans</th>
<th>Cotton</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>66.8±9.7</td>
<td>53.4±14.4</td>
<td>49.2±21.6</td>
<td>37.5±21.7</td>
</tr>
<tr>
<td>Stage 2</td>
<td>72.0±3.3</td>
<td>42.2±11.5</td>
<td>39.4±16.5</td>
<td>24.3±7.0</td>
</tr>
<tr>
<td>Stage 3</td>
<td>56.6±10.5</td>
<td>22.9±14.5</td>
<td>45.6±15.9</td>
<td>21.8±22.3</td>
</tr>
<tr>
<td>Stage 4</td>
<td></td>
<td></td>
<td>83.3±5.1</td>
<td></td>
</tr>
<tr>
<td>Stage 5</td>
<td></td>
<td></td>
<td>83.7±1.0</td>
<td></td>
</tr>
<tr>
<td>Stage 6</td>
<td></td>
<td></td>
<td>75.8±9.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 11 and 12 shows the COD removal efficiency in closed and open system respectively. The efficiency of open system was around 90% for all materials. The efficiency showed a decrease initially because of the low OLR and then showed an increasing trend. This shows that UASB was more efficient in high OLR than low OLR. The open system efficiency was
around 95% in high OLR. The COD in the UASB open system was very low when compared to the UASB closed system because the open system outlet was collected in a measuring cylinder whereas the closed system recirculates back to the CSTR. The autopyrolysis effect in a closed system was not observed because of any recirculation. Also the closed system recirculation contains dyes and other materials which increase the COD when compared to open system. The UASB of an open system for starch was able to reduce the COD up to 90% efficiency, even when the COD in CSTR reached around 35,000 mg/L which proved the high efficiency of the process.

Figure 19 COD in the CSTR open system

Figure 20 COD in the UASB open system
Table 12 COD removal efficiency in the open system

<table>
<thead>
<tr>
<th>Stage</th>
<th>Untreated jeans</th>
<th>Treated jeans</th>
<th>Cotton</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>74.4±12.3</td>
<td>63.1±25.4</td>
<td>61.9±16.3</td>
<td>66.7±20.4</td>
</tr>
<tr>
<td>Stage 2</td>
<td>95.9±2.0</td>
<td>93.9±1.3</td>
<td>93.3±3.5</td>
<td>94.9±4.3</td>
</tr>
<tr>
<td>Stage 3</td>
<td>92.7±3.5</td>
<td>84.7±2.7</td>
<td>93.9±1.8</td>
<td>98.1±0.9</td>
</tr>
<tr>
<td>Stage 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.1.5 CH₄:CO₂ ratio

The methane to carbon dioxide ratio was an important phenomenon and it was very interesting to compare for UASB and CSTR as well as in a closed system and open system. The UASB always had a high ratio when compared to CSTR. The reason was that in CSTR the hydrolysis takes place leading to the formation of more carbon dioxide. The hydrolyzed material was sent to the UASB making it more favorable for the granules present in UASB to produce more methane in the biogas production rather producing carbon dioxide for the growth of biomass.

The ratio in all UASBs of closed and open system was between 4 and 7 whereas in the CSTR it was between 1 and 2.5. If the ratio in CSTR gets decreased, more VFA’s getting accumulated increases the ratio in UASB (a readymade food for granules in UASB). Another important phenomenon which was observed in the ratio of methane to carbon dioxide was in open system-CSTR. The ratio was getting decreased with an increase in OLR. This trend showed that closed system was more stable than the open system. Further increase in OLR for starch revealed that hydrolysis was not taking place in CSTR leading to a low ratio in UASB at OLR 20 gVS per L per day.

Table 13 CH₄: CO₂ ratio of the Closed system

<table>
<thead>
<tr>
<th>Stage</th>
<th>Untreated jeans UASB</th>
<th>UASB</th>
<th>UASB</th>
<th>UASB</th>
<th>UASB</th>
<th>UASB</th>
<th>CSTR</th>
<th>CSTR</th>
<th>CSTR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSTR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.3±0.5</td>
<td>1.4±0.1</td>
<td>4.7±0.5</td>
<td>1.9±0.2</td>
<td>4.4±0.4</td>
<td>1.9±0.2</td>
<td>4.1±0.6</td>
<td>2.1±0.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.4±0.4</td>
<td>1.9±0.2</td>
<td>4.1±0.1</td>
<td>2.1±0.1</td>
<td>4.2±0.2</td>
<td>2.0±0.1</td>
<td>3.4±0.2</td>
<td>2.2±0.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.0±0.2</td>
<td>2.1±0.2</td>
<td>3.6±0.3</td>
<td>2.1±0.4</td>
<td>4.1±0.1</td>
<td>2.1±0.0</td>
<td>3.4±0.1</td>
<td>1.8±0.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.2±0.3</td>
<td>0.9±0.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.7±0.4</td>
<td>0.8±0.1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.3±0.8</td>
<td>0.7±0.1</td>
<td></td>
</tr>
</tbody>
</table>
Table 14 CH\textsubscript{4}: CO\textsubscript{2} ratio of the Open system

<table>
<thead>
<tr>
<th>Stage</th>
<th>Untreated jeans</th>
<th>Treated jeans</th>
<th>Cotton</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UASB</td>
<td>CSTR</td>
<td>UASB</td>
<td>UASB</td>
</tr>
<tr>
<td>1</td>
<td>4.9±1.4</td>
<td>1.7±0.2</td>
<td>4.1±0.5</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>2</td>
<td>5.6±0.5</td>
<td>1.7±0.3</td>
<td>4.4±0.6</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>3</td>
<td>5.3±0.8</td>
<td>1.9±0.2</td>
<td>3.9±0.3</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>4.2±0.2</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>3.5±0.8</td>
</tr>
</tbody>
</table>

3.1.6 Effect of nutrient concentration

The concentration of nutrients was analyzed using ion exchange chromatography for ammonium and potassium. The results revealed a decrease in nutrients during the course of increasing OLR.

3.1.6.1 Concentration of ammonium

The ammonium concentration in both CSTR and UASB of closed and open system showed a decreasing trend. The closed system was more stable when compared to the open system. The decreasing ammonium showed that it had been consumed by the microorganisms for the division of cells. The closed system was stable because of the reason that no volume was removed from the system during the process whereas in the open system ammonium went almost near zero.

The result of analyzing the ammonium concentration in CSTR of closed system was interesting. An interesting observation was observed in the starch-closed system at high OLR. The concentration of ammonium was decreasing steeply, but at high OLR, an increase in concentration was observed. The flow rate was high during this period which made the bed of the UASB move up because of the biogas production which cannot escape in between the granules at the high flow rate. Due to the recirculation of the liquid from UASB to CSTR in a closed system the granules were escaped to the CSTR. The granules were a large consortium of methanogens (biomass). This biomass was operated at 34 °C when they were moved to 55 °C in CSTR; they were degraded liberating the nitrogen present inside the cell. This phenomenon was like an autopyrolysis.

The nutrients were fed to the CSTR and the volume of CSTR was high when compared to the UASB making it to have a high nitrogen concentration when compared to UASB. The process
parameters in UASB were not affected as CSTR. So, in CSTR the biomass production was more, which means consumption of ammonium was high.

**Figure 21** Effect of ammonium concentration in an UASB closed system

**Figure 22** Effect of ammonium concentration in CSTR closed system
3.1.6.2 Concentration of potassium

The potassium also had the same trend as that of nitrogen in both closed systems and open systems. The closed system had a less consumption of potassium when compared to the open system. The CSTR closed system showed a decreasing trend initially, but was stable when preceded. The open system was not able to maintain this trend due to nutrient removal through the UASB effluent. The concentration of potassium in open system UASB and CSTR almost reached around zero after 30 days.
Figure 25 Effect of potassium concentration in UASB closed system

Figure 26 Effect of potassium concentration in CSTR closed system

Figure 27 Effect of potassium concentration in an UASB open system
Figure 28 Effect of potassium concentration in a CSTR open system

3.1.7 Optical microscopy

The optical microscopy pictures of the granules of both closed and open systems of starch revealed a spot of white color attached to its surface. This spot was not observed in granules from other reactors. This spot may be due to polymers which were produced by the microorganisms or starch material by itself. The white spot may be visible in starch because only the starch was operated at high OLR till 20 gVS per L per day. This spot also may be due to the excess materials which were not degraded. The starch only could pass through the mesh kept in the UASB, so the white spot could be starch.

The spot in the open system was much more clear when compared to the closed system and the granules were selected at random for the pictures in optical microscopy.
3.2 Closed system after feeding stopped

The closed system had lots of material that were not degraded, so the system was continued even after the stop of feeding, to monitor the performance of the process during no feeding. The materials untreated jeans, cotton and treated jeans were stopped feeding after OLR 4 gVS/L/day whereas starch was continued till 20 gVS per L per day. The samples were analyzed every day and nutrients were added for maintaining the balance in the process.
3.2.1 Gas Production

The gas production was summarized for each day in order to maintain a uniform unit. Starch was operated until 20 gVS per L per day; and a lot of material was left without degraded. This had left a high gas production in the starch when compared to the other materials. The structure of starch was also making it favorable for a higher gas production after the stoppage of feeding (not in the figure).

The gas production in UASB was in a steady state throughout this period for materials other than starch which had some fluctuations. The trend in CSTR showed that all the materials were degraded during the course of time. So, a decrease in gas production was observed after 15 days of feeding stopped. The CSTR treated jeans was almost utilized by the microbes converting it to methane. This showed a decreasing trend in both UASB and CSTR. The treated material was almost easy to degrade hence it was degraded easily after the feeding was stopped.

![Figure 31 Methane production in an UASB closed system after feeding stopped](image)

Figure 31 Methane production in an UASB closed system after feeding stopped
3.2.2 Effect of pH

The reactors were not buffered after feeding stopped, the pH was maintained because of the closed recirculation to the CSTR. The methane was produced continuously with the available leftover material, but this material will get decreased every day. This decrease in material reduces the load of microorganisms to work faster. So the pH was maintained constant in the CSTR whereas in UASB a decreasing trend was observed. This was because the UASB had a high acid content in the inlet which was converted to methane. The pH of close system CSTR was almost maintained at 6.4 to 7. The pH in the CSTR of the treated jeans increased after all the material was degraded.
3.2.3 COD and COD removal efficiency

The COD of the CSTR which was the inlet COD to the UASB was almost in the range of 5000 mg/L whereas starch alone had a higher COD around 25000 mg/L. The starch was fed till 20 gVS per L per day OLR; so the COD of starch was much higher when compared to the other materials (not in the figure).
The COD of the UASB was almost in the range of 1000-2500 mg/L. The UASB could not take care of the inlet COD because the granules from UASB were circulated to the CSTR. The granules were broken down which led to an autopyrolysis, this may have led to the formation of inhibitors which affect the COD removal in UASB. The COD removal efficiency was continued to be higher than that of other materials. The COD removal efficiency of the materials was almost similar throughout the period after feeding. The decrease in COD in UASB treated jeans was observed and it indicated that all the material was degraded in the course of time.
3.2.4 Ratio $\text{CH}_4:\text{CO}_2$ in a closed system

The ratio of methane to carbon dioxide was same throughout the unfed period. The ratio in UASB was decreased when compared to fed days. This may be due to a decrease in concentration of VFA from CSTR during that period. The ratio in CSTR was maintained at 1.5-2.0 whereas in UASB it was a clear margin between the materials. The cotton UASB (4.5) had a high ratio followed by treated jeans and untreated jeans.

The untreated jeans could not maintain a high ratio in UASB and CSTR because of the dye present in the material whereas the treated material could maintain a high ratio. The pretreatment of jeans helped to remove the dyes, which affect the microorganisms from the optimum conditions to produce biogas.
3.2.5 **Effect of nutrients**

The nutrients concentration for ammonium and potassium remained the same after the stoppage of feeding. The consistency of ammonium concentration during this period revealed that there was no microbial formation. This may be due to the fact that enough biomass was present in the reactor to degrade the leftover material. The potassium concentration was also similar to ammonium which was not affected during unfed period. The consistency of the nutrient concentration can be clearly observed.

![Figure 40 Methane to Carbon dioxide ratio in CSTR after feeding stopped](image)

![Figure 41 Effect of ammonium in UASB closed system after feeding stopped](image)
Figure 42 Effect of ammonium in CSTR closed system after feeding stopped

Figure 43 Effect of potassium in UASB closed system after feeding stopped

Figure 44 Effect of potassium in CSTR closed system after feeding stopped
3.3 Batch process

The batch process was carried to check the feasibility of anaerobic digestion of waste textiles without any pretreatment in order to make it cost effective in the industry. The results from the previous experiments showed that closed system was more stable. In this process, a conventional single stage process was compared to the two-stage process closed systems.

3.3.1 TS and VS

The Total solids and Volatile solids were measured before and after the process. Since a filter was installed inside the CSTR in two stage processes, it was possible to recover the polyester material after the process. From the TS of the material the VS was calculated based on the proportion of cellulose. The TS and VS of the sludge and material were calculated after the processing time of 25 days. Since the material was degraded during the process the TS and VS were decreased. The viscose was much more effective in degradation when compared to the cotton. The amount of gas produced was more in the two-stage process compared to the single stage process. This proved that the rest of the degraded materials in the single stage process were used for the growth of biomass. The TS and VS sludge of the CSTR reactors were almost similar in single stage and two-stage process.

3.3.2 Gas production

The gas production of the materials, cotton and viscose, were compared. The two-stage process produced more gas when compared to the single stage process in both cotton and viscose. Since viscose was easy to degrade, the gas production was high in both single stage and two-stage processes. Additionally, the gas production was high in UASB at the start up stage because the inlet COD from CSTR was high during this period. The single stage cotton gas production started after 15 days; because the textile surface contact with the microorganisms was low. The two-stage cotton produced more methane compared to a single stage because the material was kept inside a filter allowing it to have more contact with microorganisms.
Figure 45 Methane production in the UASB batch process

Figure 46 Methane production in CSTR for batch process

Figure 47 Total methane production comparison for single stage and two-stage process
3.3.3 Effect of pH

There were no buffering and pH controlling in this part of the experiments. The pH of the UASBs was almost constant, between 7 and 8. However, in the CSTR fed by viscose, a drop in pH was observed initially. It can be due to the structure of viscose which was easy to degrade and the rate of hydrolysis was high. The fatty acids were converted to methane and the balance of the process was maintained throughout the process.

![Figure 48 Effect of pH in CSTR for batch process](image)

3.3.4 COD

The chemical oxygen demand was analyzed for both UASB and CSTR. The COD of CSTRs fed by viscose increased initially because the material were degraded easily whereas in case of cotton the COD was increased on further days. The viscose which had a higher COD initially revealed that it was easier to degrade. On these days, a high gas production was also observed. Since the material contact with the sludge was less, the COD and COD removal efficiency of the process were low. The COD removal efficiency was little higher in comparison of cotton to the Viscose. The following graphs show the COD for UASB and CSTR reactors.
3.3.5 \( \text{CH}_4: \text{CO}_2 \text{ ratio} \)

The ratio of methane to carbon dioxide was calculated based on the methane and carbon dioxide volume produced every day with the gas composition from gas chromatography. The ratio was higher in UASB when compared to the CSTR; since it was a batch process the ratio was almost constant between 4 and 5 in UASB and 2-3 in CSTR of two-stage processes. The \( \text{CH}_4: \text{CO}_2 \) ratio in single stage, CSTR, was higher when compared to the two-stage CSTR but it was lesser when compared to the UASB of the two-stage process. This shows that two-stage processes were more efficient to produce methane.
The CH₄: CO₂ ratio in UASB was higher, as the material was broken down to acids in CSTR and sent to UASB. In the UASB, the present microorganisms were almost methanogens which were fond of the volatile fatty acids; so all the acids were converted to methane than utilizing for the growth of the cell. The first two steps (hydrolysis and acidogenesis) of anaerobic digestion were performed in CSTR, which liberated a lot of carbon dioxide. In the last two steps (acetogenesis and methanogenesis) the carbon dioxide production was low. Hence higher methane to carbon dioxide ratio was observed.

![Figure 51](image1.png)  
**Figure 51** Ratios of methane to carbon dioxide in UASB for batch process

![Figure 52](image2.png)  
**Figure 52** Ratios of methane to carbon dioxide in CSTR for batch process
3.3.6 Effect of nutrients

This process was operated in a batch condition where no additional carbon source was given, so nutrient consumption was maintained through the process. The nitrogen concentration was almost constant in both UASB and CSTR.

![Graph showing nitrogen concentration in UASB and CSTR](image1)

**Figure 53** Effect of ammonium in UASB for batch process

The consumption of nitrogen in single stage was lesser when compared to the two-stage process in CSTR. The reason might be that some nitrogen has been consumed by UASB reactors due to the presence of methanogens. If the nitrogen concentration was maintained constantly in the continuous process, a better production of methane might have been observed.

![Graph showing nitrogen concentration in CSTR](image2)

**Figure 54** Effect of ammonium in CSTR for batch process
The effect of potassium was also similar to that of nitrogen. The potassium and nitrogen were only used by the microorganisms to produce more biomass. The constant nutrient concentration showed that the production of biomass was less. This low production of biomass should favor biogas production but, it was not so. The reason was that the material was neither milled nor pretreated which led to a very slow process.

In viscose, the concentration of calcium was very interesting to analyze. The viscose textile itself had calcium when produced in the industries. This effect could be seen initially with a high concentration of calcium. This had led to the increase in the calcium concentration in the reactors fed by viscose. The calcium concentration in the single stage process for viscose was observed to be much higher than that in the CSTR of two-stage process. The increase in
calcium concentration was observed only during the starting period of the process. In the later period the microorganisms might got adopted and utilized the excess calcium for the growth and development of the cells.

![Figure 57 Effect of calcium in the UASB for batch process](image1)

![Figure 58 Effect of calcium in CSTR for batch process](image2)
4 Discussion

4.1 Continuous process

The continuous process showed that the closed system was more effective when compared to the open system. When comparing the untreated jeans to the treated jeans, the treated jeans could produce more biogas. The effect of dye in the untreated jeans had been removed during the pretreatment. The pretreatment could also open up the high crystalline structure of cellulose. The gas production of treated jeans was almost similar to the gas production of cotton that didn’t contain any dye. The treated jeans worked like pure cotton.

Starch was used as a reference material as to be able to compare waste textiles digestion with starch digestion. It was possible to continue the feeding of starch at high OLR (8, 10 and 20) g VS per L per day, but not for the other materials due to technical issues. The amorphous nature of starch made it easy to degrade when compared to high crystalline structure in cotton and jeans. Also, the intra molecular hydrogen bonding of cellulose makes it hard to digest.

The comparison of closed systems and open systems is also an interesting discussion. It helps to see how the effect of recycling helps to achieve a high OLR. On the other hand, the open system was more efficient in handling the COD inlet. Another perspective revealed that closed system was much more stable and steady when compared to the open system, which made it an edge over a better process.

The efficiency of removing COD in UASB was higher at higher OLR. A pressure drop in UASB at high OLR was observed. This pressure drop caused the methane to stick in between and pushed the bed to top. The granules were forced to move out of the reactor, causing some advantages and drawbacks in the system. The effect of autopyrolysis in a closed system had an advantage as well and disadvantage. The advantage was that the pyrolysed granules could provide nitrogen and maintain the balance of the process whereas the dark side was the production of inhibitory substances from the cell wall of the bacterial consortium.

A high ratio of CH₄ to CO₂ was observed only in the UASB, because the materials were degraded in the CSTR and only volatile fatty acids were sent to the UASB. In a single stage process, the accumulation of VFA increased the acid concentration, causing the death of methanogens. Whereas, in a two-stage process the produced VFA was converted to methane in UASB. This could help to maintain the process parameters compared to the single stage
process. The acids were on care by the UASB which actually acts like a scrubber to purify the biogas making a high ratio of methane to carbon dioxide.

The concentration of ammonium and potassium ions revealed that both had a decreasing trend. The closed system could overcome it, since the volume of the reactor was always constant through the process. In the open system the concentration of nutrients was almost zero which makes the condition not optimized for the biogas production. The decreasing trend in the concentration of nutrients revealed the consumption of nutrients by the microorganisms for the biomass production. The closed system could able to maintain a nutrient concentration leading to a steady state gas production.

The closed system was more stable than the open system in many perspectives. The solid inlet fed to the reactor was converted to its gaseous form as an outlet. A new technology to build a system for the conversion of solid feed to methane as a fuel in a stable condition at a relatively high OLR and short HRT was developed.

Figure 59 Beneficial aspects of a closed system
4.2 Continuous process after feeding stopped

The continuous process was investigated further as the feeding was stopped in the closed system. The non-degradable materials during the feeding period were degraded in this period. The pH of the system was not buffered during the unfed time and it was increased because the acidic substances were degraded completely and converted to methane. The balance of the process was more stable during this period. The ratio of methane to carbon dioxide decreased in UASB which revealed a fact that there were no enough fatty acids which could get converted into methane. The CSTR could handle the fatty acids produced during this period.

Since OLR of starch was increased till 20 gVS per L per day the gas production during unfed periods was also more when compared to other materials. The concentration of the nutrients was also constant during this period of time when compared to the feeding period.

The COD removal efficiency was almost similar to that during the feeding period. The closed system could not remove much of the COD when compared to the open system. The completely degraded material can be identified using the TS and VS measurement after the process and comparing with that before the process.

4.3 Batch process

The batch process was performed on a view to make it more economical for the industry. The experiments were performed without any pretreatment and milling of materials. The process in itself was very slow as the surface contact of material to sludge was very low. The structure of material also must be considered. The viscose was arranged like a mesh and it was easy to degrade by the microorganisms present, whereas the cotton was a more complex structure in the polyester combination; hence the cotton was not easy to degrade.

The comparison of single stage and two-stage reactors revealed that two-stage reactors produced more biogas when compared to single stage process. The TS measurements after the process showed that single stage had fewer solids compared to two-stage. The gas production revealed two-stage produced more gas. This confirms that the production of biomass was more in a single stage. Since the process was operated in a batch condition, the consumption of nutrients was not more. An interesting result was the concentration of calcium which was higher in the viscose process. This could be due to the presence of calcium in the viscose/polyester textile that was released during the process.
The ratio of methane to carbon dioxide showed that the single stage CSTR had a higher ratio when compared to the CSTR of two-stage processes. On the other hand, the ratio in UASB of two-stage process was more than that in CSTR of the single stage process. This result showed that the volatile fatty acids were sent to the UASB and converted to methane with a high yield. The viscose was easily broken down and hence it resulted in the increase in volatile fatty acids which decreased the pH but then the system could able to recover it and maintain the pH. The lag phase before starting gas production in case of Cotton/polyester textile was long which could be due to the less contact surface between fibers and sludge present in the reactor. The need of filter in the two-stage process was promising to recollect the polyester remained after the process. The recollected polyester could be recycled.
5 Conclusions

The production of biogas from waste textile could be possible from different materials with and without pretreatment. The pretreatment helps to open up the structure of the material and makes easier for the microorganisms to degrade it. The untreated and pretreated materials by NMMO, an environmental friendly cellulose solvent showed more methane and the stable condition could be achieved. The two-stage process was helpful in handling high OLR. The comparison between the closed system and open system revealed a high steady state condition which was observed highly in a closed system rather than in open system.

This process was succeeded to reach high OLR and decrease the digestion time from the conventional biogas production. The digestion time was decreased as less than 9 days for treated jeans and less than 5 days for starch. The closed system was more stable and efficient than the open system.

High methane to carbon dioxide ratio was observed improving the yield of biogas. The closed system was able to maintain a nutrient concentration leading to a steady state condition but the open system cannot. The effect of autopyrolysis was observed in a closed system at high OLR.

The two different experiments (batch and continuous) showed a high margin of difference in biogas production. The batch experiments which revealed the rate of biogas production will be low without pretreatment or milling. The two-stage process produced more methane compared to the single stage process. Viscose was easier to degrade than the cotton. The polyester part of the textile could be recovered at the end of the process.

A dual goal can be achieved by the treatment of waste textiles. One is being the conversion into value-added products like biogas and bioethanol and other being the waste management of the textiles which cause pollution by incineration. The development in resource recovery would lead in developing a sustainable society.
5.1 Advantages

- The high organic loading rate can be achieved in UASB compared to the conventional organic loading rate.

- The comparison of closed systems and open system showed that the closed system was more effective. The volume of closed system was constant whereas the open system was decreasing till the next day feed, which increased the acid content; affecting the equilibrium of the process.

- The granules at high OLR when recirculated to CSTR from UASB, the pyrolysis effect could help the CSTR to obtain nitrogen from the degraded granules that makes the concentration of nitrogen more in CSTR which stabilized the decreasing trend of ammonium in the system, which will be an optimum condition.

5.2 Drawbacks

- The main drawback of the process was pumping. The solid (cellulose) inside the reactor was also pumped through the tube from hydrolysis reactor which makes it stuck all the way to UASB. This problem could be overcome at the industrial scale as it is a technical problem.

- At the high organic loading rate, there was a pressure imbalance in the closed system causing the liquid from UASB to not pass to CSTR. Instead, it moved through the gas line.

- The granules which travel at high OLR from UASB to CSTR will be pyrolysed causing a production of inhibitor for the UASB which led to the high COD outlet in UASB.
5.3 Future Work

- The impeller in the CSTR was very small which did not provide uniform mixing inside the reactor, allowing some solids to settle at the bottom of the reactor. If the impeller was axial, with long blade and also if movement happens in both vertical and horizontal directions, then the degradation of cellulose will be more effective.

- The economic feasibility of the process should be studied to get a view on the industrialization of the process.

- A method need to be improved to keep the sludge inside the CSTR and only the liquid which contain the VFA has to be pumped out of the reactor.

- The bacterial community inside the CSTR can be immobilized to tolerate high acid content which will make it favorable to achieve an even higher organic loading rate.

- The UASB can be operated at different temperatures to obtain an optimum condition to operate.

- In this process, synthetic macro and micro nutrients were added. Instead, a co digestion (municipal sewage or agricultural residue) can make it more economic on an industrial scale.

- This process should be also analyzed how it works on a mixture of textile materials because an industry cannot work on the particular raw material throughout the year. Alternative raw material or combined raw material can also be much more advantageous.
Acknowledgements

We want to take this opportunity to express our credit to many lending hands, motivating personalities, aspiring role models, die-hard team players. We thank them all for their valuable support.

First of all, we would like to thank our examiner Prof. Mohammad Taherzadeh for his concern towards us during our studies and project work. His teaching helped us to understand the basic concepts of bioprocess engineering in an industrial perspective.

We thank our supervisors Azam Jeihanipour and Solmaz Aslanzadeh for their kindness towards us. Azam extended her support even a day before her PhD defense. Solmaz helped us to learn each instrument professionally.

We extremely thank Jonas Hanson, our lab supervisor. He helped us every time with the materials that we need to use during our project. Also our special thanks for Haike for helping us with optical microscopy.

We wish to state our warm gratitude to other PhD students Patrick, Gergely, Johan for their support and suggestions.

We also express our grateful thanks to Michael for his support to learn ion exchange chromatography.

Last but not least, this thesis is dedicated to our parents. Their belief in us made us to move forward every day in the life. Thank you very much!
References