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Improved Cellulose and Organic-Solvents based Lignocellulosic Fractionation Pre-treatment of Organic Waste for Bioethanol Production

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Abstract: - This study investigates the performance of the Cellulose and Organic-Solvents based Lignocellulosic Fractionation (COSLIF) method for the pretreatment of Source-Separated Organic (SSO) waste. An improvement on the standard method of COSLIF pre-treatment was developed based on lower enzyme loading and using an ethanol washing instead of acetone. It was demonstrated that a much higher glucose yield (90% after 72 hours) was possible with this improvement, as compared to the original method, which yielded 70% in the same time frame. Evaluation of the enzymatic hydrolysate obtained from the modified COSLIF pre-treatment was further examined by anaerobic fermentation with Zymomonas mobilis 8b strain. At 48 hours, ethanol concentration reached to 140 g/L, which is equivalent to 0.48 g of ethanol produced per gram of SSO biomass. This study demonstrated that the modified COSLIF pretreatment provides a substantial improvement over the standard method in terms of enzyme savings, glucose formation, and ethanol production.

Keywords: - *lignocellulose, organic waste, pre-treatment, ethanol, enzyme, bacteria*

I.   INTRODUCTION

Pre-treatment is considered one of the most expensive processing steps in the bioconversion of lignocellulosic biomass, often accounting for up to 40% of the total processing cost [1]. In addition, it greatly affects the downstream cost of operations such as enzymatic hydrolysis and fermentation. Additional costs resulting from inefficient pre-treatment include detoxification, limited enzymatic hydrolysis rate, high enzyme loading, low product concentration, and complicated product purification. Therefore, pre-treatment can be seen as a key step in limiting the feasibility of bioconversion. Pre-treatment, together with enzymatic hydrolysis, is the central task of the entire bioethanol production process [1]. Evidently, all the lignocellulosic pre-treatment processes experience sugar degradation and inhibitor formation. The shortfalls of the current leading lignocellulosic pre-treatments can be mainly attributed to: 1) inefficiency in breaking up the orderly hydrogen bonds in crystalline cellulose, resulting in slow hydrolysis rates and low cellulose digestibility, which compromises the overall sugar yields, and 2) the presence of lignin and hemicellulose on the surface of cellulose, which is commonly thought to have the effect of restricting the accessibility of enzymes to the biomass [2].

Cellulose and Organic-Solvents based Lignocellulosic Fractionation (COSLIF) is a promising technology, recently developed to overcome these problems. The COSLIF pre-treatment is a technology that can effectively fractionate lignocelluloses into amorphous cellulose, lignin, hemicelluloses, and acetic acid [2], [3]. This technology has been applied successfully to a broad range of substrates from agricultural to industrial waste, with inclusion of organics such as: food, paper, cardboard, plastics and yard wastes [1], [2], [4], [5], [6]. The COSLIF technology has many advantages over traditional lignocellulosic pre-treatments, most notably the following: modest treatment conditions at 50°C and atmospheric pressure; minimized degradation of sugars; no inhibitor formation; co-utilization of different sugars increasing potential output; high sugar yields; fast hydrolysis rates; efficient solvent recycling; low usage of enzymes; and low energy consumption [2].

The objective of this paper is to evaluate the performance of the COSLIF pre-treatment on an innovative feedstock for ethanol production, namely, source-separated organic (SSO) household waste. Due to its potential for high energy content and environmental implications, SSO has been proposed as a suitable feedstock for bioethanol production [7]. It was demonstrated that the overall process of lignocellulose fractionation with the use of cellulose solvent (phosphoric acid) and organic solvent (acetone/ethanol) as pre-treatment reagents is effective in hydrolysing the sugar content of the waste [2]. In order to successfully deal with the causes of the SSO recalcitrance - breaking up orderly hydrogen bonds in crystalline cellulose chain and removing lignin and hemicelluloses from the surface of cellulose, a standard COSLIF process was modified by using ethanol washing solvent instead of acetone and lowering enzyme loading. It allowed to increase the concentration of glucose released after enzymatic hydrolysis and to achieve highest ethanol yield in fermentation step. The enzymatic hydrolysis behaviours of the original and modified pre-treatment methods were also investigated and compared in terms of their glucose yield. A scanning electron microscopy (SEM) was used to examine the supra-molecular structures of COSLIF-pretreated SSO samples for qualitative comparison.

II.　　　MATERIALS AND METHODS

The SSO waste utilized in this work was initially pre-processed mechanically, under high temperature (of ~120OC) and pressure (over 50 bars) with a thermal screw press to form a dry stable mass. Samples were prepared as a heterogeneous substrate by blending with 20% of woodchips waste from construction before pre-processing [8]. Optimum Waste Recycling Systems, Toronto, Canada, supplied the biomass feedstock used in this work. The general flowchart of the experimental investigation is shown in Fig. 1.

-Fig. 1 about here –

It started with the SSO waste supplied to thermo-screw press and to make it homogenous. After this, the SSO samples underwent lignocellulosic fractionation with the use of a cellulose solvent (85% phosphoric acid) and an organic solvent (either acetone or ethanol). Next step in the flowchart above is enzymatic hydrolysis with addition of commercial available enzyme, Accellerase 1500, to mediate enzymatic hydrolysis process and release fermentable sugars as much as possible. Accellerase 1500 is Genencor's new generation of enzyme product, a significant step forward towards more cost effective, commercial scale production developed for second generation of biorefineries. It has been shown to successfully hydrolyze a wide range of lignocellulosic feedstocks [9]. Accellerase 1500 enzyme used in this research was supplied by Genencore Inc., a Denisco Division, Rochester, New York, USA, as well as the Sigma Aldrich Corp., USA.

Prior to testing, the SSO samples were oven-dried at 45-50° C for 72 hours accordingly to [10]. Five grams of dry lignocelluloses was placed in a 250 mL centrifuge bottle and then mixed with 40 mL of 85% concentrated phosphoric acid using a glass rod. The solid/ liquid slurry was placed in a benchtop shaking incubator at 150 rpm and 50 ^{o}C $\pm$ 0.2^{o}C for 2 hours. One hundred mL of ethanol was then added and mixed well. After centrifugation at 7000 rpm at room temperature for 15 minutes, the supernatant was decanted. The solid pellet was then re-suspended by 150 mL of ethanol and centrifuged. The supernatant again was decanted. Next, the solid pellet was re-suspended by 150 mL of distilled water and centrifuge for two times.

Enzymatic hydrolysis experiments were conducted next in sequence in the chosen SHF approach in a benchtop shaking incubator. The separate hydrolysis and fermentation (SHF) approach was used in this study to avoid interference of samplings. The procedure for enzymatic cellulose hydrolysis was adopted from a procedure developed by the National Renewable Energy Laboratory, as described in [11], [12]. After thawing, the treated solid pellet containing amorphous cellulose was neutralized to pH 4.8-5.0 by NH4OH. Upon diluting to 20 g glucan/L based on the 27% glucose content from [11], the sample was then brought to 50°C before adding 30 FPU/ g glucan or 60 FPU/g glucan of Accellerase 1500. The incubator was set at 250 rpm to keep solids in constant suspension with the temperature of 50°C for 72 hours. Sampling was carried out at 0, 12, 24, 48 and 72 hour and glucose yield was measured.

Following enzymatic hydrolysis, batch soluble sugar fermentation was carried out to determine the ethanol yields. The Zymomonas mobilis 8b recombinant strain was chosen for its capability to ferment glucose and to produce ethanol at high yields [13] and was donated by the National Renewable Energy Laboratory, Golden, Colorado, USA. Soluble sugars batch fermentation was performed in 250-mL serum bottles with 100-mL working volume and purged with nitrogen before being autoclaved. Temperature was maintained at 30-37oC and pH was controlled at 5.0-6.0 by 1M potassium hydroxide (KOH) as suggested by previous studies [14]. Each batch sugar fermentation process was carried out in triplicates on the pre-treated biomass for both the standard and modified COSLIF methods.

Concentrations of glucose in hydrolysates from the COSLIF pre-treated biomass and ethanol from in fermentation broths were analyzed by high performance liquid chromatography (HPLC), Bio-Rad HPX-87P column quipped with the appropriate guard column. All concentrations were reported as per liter volume basis. Percent theoretical ethanol yield was calculated as in [15]:

$$\% \text{ Theoretical ethanol yield} = \frac{[EtOH]f - [EtOH]i}{0.51(f[Biomass]1.111)} \times 100$$

where: [EtOH]f - ethanol concentration at the end of fermentation, (g/L)
[EtOH]i - ethanol concentration at the beginning of fermentation, (g/L)
[Biomass] - dry biomass concentration at the beginning of fermentation, (g/L)
f - cellulose fraction of dry biomass (g/g)
0.51- conversion factor for glucose to ethanol
1.111- converts cellulose to equivalent glucose

Supra-molecular structures of the intact and pretreated SSO samples were examined by scanning electron microscope, as described elsewhere [16], [17]. A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a rectangular pattern of image, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer. Samples can be observed in high and low vacuum, and in wet conditions. A SEM was kindly provided by the Ryerson University Analytical Center, Toronto, Canada.

III.   RESULTS AND DISCUSSION

A detailed quantitative assessment on the composition of SSO waste was carried out in [11] and adopted for further investigation in this study. The SSO samples, contained 20% woodchips, were already pretreated by the thermal screw machine. The woodchips were typically Douglas fir wood waste originated from home construction furniture, flooring, cabinet, and doors. All sharp foreign matter such as metal needles, plastic and rubber wastes, and broken glasses were collected and removed, as much as it was possible. The dried SSO biomass was sent to MBI International, the Michigan State University Foundation, for grinding and determination of polymeric sugars content. The results are summarized in Table 1.

-Table 1 about here -

It turned out that those essential polymeric sugars made up 41.3% in oven dried SSO samples, including: 27% glucan, 5.4% xylan, 5.7% mannan, 1.2% arabinan, and 1.2% of galactan, which were a good starting point for enzymatic hydrolysis followed by fermentation. It was found that the SSO samples were acidic (pH of 5.0-5.5) and had the lowest content of the food waste, just about 10% of total waste of samples. Comparison between pretreated and non-treated SSO validated the high recalcitrant nature of lignocellulosic fraction of biomass as suggested in [2], and which was in agreement with other works [1], [3], [14].

3.1. Glucose Yield

Results obtained from COSLIF washing with concentrated phosphoric acid and acetone reagent generated a significant glucose yield of about 70%, in the first few trials (Fig. 2).

-Fig 2 about here –

But, acetone is a more toxic reagent and it is less safe to use than ethanol. The cost of using acetone is higher than that of ethanol and during the recovery of the reaction's by-products more energy is consumed when acetone is used as the reagent. In addition, pre-treatment with acetone must be performed under extremely stringent and efficient conditions due to the volatility of acetone. Ethanol, on the other hand, is less corrosive and can be easily recovered by distillation under milder conditions. Therefore, after extensive trials and investigations, some changes were made to further improve the efficiency of COSLIF pre-treatment to obtain a higher glucose yield. The major change made to the original standard method of COSLIF pre-treatment was to omit acetone altogether and use 95% (v/v) ethanol as the organic solvent instead. Another was changing enzyme loading from 60FPU to 30FPU. As a result of these changes, it was found that the glucose yield increased to approximately 90% (Fig. 2). It was also observed that only 50% of the original volume of ethanol was needed to replace the acetone.

3.2. Enzymatic Hydrolysis

Fig. 3 shows the glucose digestibility profiles over a course of 72 hours for the SSO samples treated by the standard and modified COSLIF methods as well as non-treated samples.

-Fig 3 about here -

High glucan digestibility of the pretreated SSO was accredited to drastic changes in the supramolecular structure of the biomass before and after the COSLIF pre-treatment, observed by the SEM in this study. Typical COSLIF pretreatment conditions were used, namely $50^{\circ}C$ and atmospheric pressure with a pretreatment time from 30 to 60 minutes, depending on the type of feedstock. Although diverse feedstocks showed great variations in enzymatic digestibility, suggesting that their different recalcitrant structures confer variable resistance to enzymes, the use of concentrated phosphoric acid at $50^{\circ}C$ can efficiently dissolve them so to erase their inherent structure difference and result in an amorphous biomass with similar high-accessibility [3], [6]. As a result, COSLIF-pretreated biomass feedstock exhibited similar enzymatic glucan digestibility regardless of their sources [6]. When concentrated phosphoric acid was used as the cellulose solvent, it should be used at $50^{\circ}C$ or lower to avoid extensive hydrolysis of polymeric carbohydrates and sugar degradation.

The enzymatic glucose digestibility for pre-treated COSLIF samples was calculated as described in [2]. With high enzyme loading (FPU=60) and acetone washing, the glucose digestibility of the pretreated standard COSLIF sample was approximately 70% as presented in Fig. 3 above. With a lower enzyme loading (FPU=30) and ethanol washing, it reached 90% digestibility after 36 hours. This suggests that by removing hemicelluloses and lignin barriers, there was an increase in accessibility to the cellulose change by the cellulobiose, while also reducing the competitive inhibition of xylan to endo-glucanase. Data from this study on the hydrolysis rates and digestibility were comparable to the range (90%-95%) cited in other scientific papers [18], [19].

3.3. Fermentation

Fermentation is the final step in evaluating the overall process of cellulosic ethanol production. The effectiveness of the enzymatic hydrolysis was gauged by assessing the potential inhibitory factors and effects of fermentation. These results can be found in the following section. A genomic DNA-integrated glucose and xylose co-fermenting strain, Z. mobilis 8b recombinant strain was used due to its ability to ferment glucose and xylose to produce ethanol at high yields [13]. The microbe was developed and evaluated by the NREL on a broad range of agricultural biomass and can convert sugars to ethanol more rapidly as compared to other species.

Besides the major changes during the COSLIF pre-treatment process, some minor improvements in the fermentation procedure were also made and they undoubtedly affected overall efficiency of the final ethanol output. These improvements were as follows: a serum bottle with a crimp top was used instead of an Erlenmeyer flask with stopper for better air-tight seal; a flushing serum bottle with nitrogen was used to maintain anaerobic conditions prior to fermentation; a direct transfer technique was exploited to move concentrated Z. mobilis 8b cells from an inoculums tube to a serum bottle; and a growth curve was developed for the Z. mobilis 8b strain prior to fermentation tests which was important in order to identify the OD (optical density) range in the exponential phase of a curve. The OD values in the exponential phase were vital in determining the time to harvest the cells to start the fermentation process. There were two protocols that could be employed for harvesting the cells to start the fermention process: 1) use of a direct transfer (10%) to the main fermentation bottle or 2) use of concentrated cells by centrifuging in a centrifuge tube and then re-suspending the cells in a hydrolysate before transferring it back into the fermentation bottle. The second protocol was chosen because the inoculated seed media contained not only cells but also a large amount of glucose sugar which would be transferred into the fermentation bottle. Unless distilled deionized water (DDW) blank was created, this would result in false and inaccurate HPLC readings of glucose and ethanol concentrations.

The high ethanol yield presented in Fig. 4 indicated that very little inhibitors were present in the hydrolysates that were pretreated by the modified COSLIF method. Depending on feedstock and process, the actual yield could be anywhere from 60% to 100% of the theoretical yield. Achieving a high yield may be costly compared to lower yield processes that are often more cost effective.

-Fig. 4 about here -

The ethanol concentration rate was calculated on the basis of sugars consumed as described in [20], and it yielded in 132.1g/L for the pre-treated samples by the modified COSLIF method after 24 hours. At 48 hours, the ethanol concentration reached 140 g/L, which is equivalent to 0.48 g ethanol/g biomass or 94% of the theoretical ethanol yield. As per this work, percent theoretical ethanol yield was calculated as in [15]. Although the ethanol concentration for some samples seemed to be fluctuating from time to time, over 90% ethanol yield in Fig. 4 can be attributed to the high accessibility of the pretreated cellulosic materials and low presence of lignin.

3.4. Comparison with Constructed Sugar Model

In a further series of experimental evaluations, enzymatic hydrolysate obtained from both COSLIF pre-treatments by batch culture fermentation with Z. mobilis 8b strain were compared with constructed sugar model (glucose/xylose ratio as 5:1) in SSO substrate. In a constructed model, after 24 hours, 100% of glucose and 40% of xylose were consumed. While in the enzymatic hydrolysate, pre-treated by COSLIF with ethanol washing reagent, the fermentation also advanced rapidly and 90% glucose and 40% xylose were also consumed, in the enzymatic hydrolysate, pre-treated by COSLIF with acetone washing reagent, the fermentation advanced slowly and 45% of glucose remained unused in the same period of time. Low bacterial activity in the fermentation process of SSO hydrolysates may be attributed to many factors including: longer lag phase for Z. mobilis 8b strain as the adaptation time to growth condition, low growth rate on SSO hydrolysates, unavoidable contamination during sample preparations, lack of nutrients, and presence of inhibitors.

3.5. Qualitative Analysis

As per qualitative comparison, SEM images of oven-dried SSO substrate before and after preatreatment were conducted in collaboration with [11] and provided in Fig. 5.

-Fig. 5 about here -

These images show the appearance of SSO before grinding – 1-1, after grinding – 2-1, and after COSLIF pre-treatment – 3-1. Each pre-treatment (physical and chemical) process changed the structure of the SSO biomass. It is clear that before the pre-treatment, the plant cell wall structures of the SSO and cellulose fibers were clearly identified. The SEM images from 1-1 and 2-1 present changes in particle size. The image from 3-1 shows all fibrous structures completely disrupted after pre-treatment, indicating that phosphoric acid and ethanol washing not only disrupted all linkages among cellulose, hemicelluloses and lignin, but also disrupted the orderly hydrogen bonds among glucose chains. These qualitative images are consistent with the images from similar studies [1], [17].

IV.   CONCLUSION

The SSO waste samples utilized in this research were pre-processed by the thermal screw press (TSP) and further used as a substrate for all enzymatic hydrolysis and fermentation processes.

COSLIF pre-treatments were applied for cellulose extraction. Results indicate that the percent glucose conversion was considerable for the modified COSLIF method with a significant glucose yield. This study also demonstrated and confirmed that the COSLIF pre-treatment can be carried out on this innovative type of biomass with a relatively high percentage of glucose and ethanol yields, when certain modifications are made to the process.

In conclusion, given the satisfactory results obtained, there are still aspects of the process that need further investigation. For example, biomass size reduction by milling or grinding is energy intensive and costly which will affect the total cost of ethanol production. The extrusion process alone could disrupt the lignocellulosic structure, which would enable enzyme to gain access and attack the carbohydrates [21]. Detailed investigation on ethanol concentration and yield is still required. It was hypothesized that the large variations of ethanol concentration in this study were caused by interference of samplings. However, it has yet to be proven.

V.   ACKNOWLEDGEMENTS

REFERENCES

[1]   Z. Zhu, N. Sathitsuksanoh, T. Vinzant, D.J. Schell, J.D. McMillan, and Y.-H.P. Zhang, Comparative study of corn stover pretreated by dilute acid and cellulose solvent-based lignocellulose fractionation: Enzymatic hydrolysis, supramolecular structure, and substrate accessibility. *Biotechnology and Bioengineering, 103*(4), 2009, 715-724.

[2]   Y.-H. P. Zhang, S.-Y. Ding, J.R. Mielenz, R. Elander, M. Laser, M. Himmel, J.D. McMillan, and L.R. Lynd, Fractionating recalcitrant lignocelluloses at modest reaction conditions. *Biotechnology and Bioengineering, 97*, 2007, 214–223.

[3]   J.A. Rollin, Z. Zhu, N. Sathisuksanoh, and Y.-H. Zhang, Increasing cellulose accessibility is more important than removing lignin: a comparison of cellulose solvent-based lignocelluloses fractionation and soaking in aqueous ammonia. *Biotechnology and Bioengineering, 108*, 2011, 22–30.

[4]   X. Ge, D.M. Burner, J. Xu, G.C. Phillips, and G. Sivakumar, Bioethanol production from dedicated energy crops and residues in Arkansas, USA. *Biotechnology, 6*, 2011, 66–73.

[5]   N. Sathitsuksanoh, Z. Zhu, N. Templeton, J. Rollin, S. Harvey, and Y.-H.P. Zhang, Saccharification of a potential bioenergy crop, Phragmites australis (common reed), by lignocelluloses fractionation followed by enzymatic hydrolysis at decreased cellulase loadings. *Industrial and Engineering Chemistry Research, 48*, 2009, 6441–6447.

[6]   N. Sathitsuksanoh, Z.G. Zhu, S. Wi, and Y.-H.P. Zhang, Cellulose solvent-based biomass pretreatment breaks highly ordered hydrogen bonds in cellulose fibers of switchgrass. *Biotechnology and Bioengineering, 108*, 2011, 521–529.

[7]   M. Mirzajani, *The amenability of pre-treated source separated organic (SSO) waste for ethanol production,* Master's thesis, Ryerson University, Civil Engineering Dept., Toronto, Canada, 2009.

[8]   Vartek Ltd Company, *Vartek ATS Technology Compost Pilot Test*. Toronto, Ontario, Canada, 2005.

[9]   S.S. Retka, Genencor, Novozymes launch new ethanol enzymes. *Ethanol Producer Magazine*, 2009. Last Accessed May 7, 2014 http://ethanolproducer.com/article.jsp?article_id=5466

[10]   B. Hames, R. Ruiz, C. Scarlata, A. Sluiter, J. Sluiter, and D. Templeton, *Preparation of samples for compositional analysis. Laboratory Analytical Procedure (LAP).* NREL/TP-510-42620. Technical Report. National Renewable Energy Laboratory, Golden, Colorado, USA, 2008. Last accessed May 5, 2014 http://www.nrel.gov/docs/gen/fy08/42620.pdf

[11]   M. Ehsanipour, *Acid pretreatment and fractionation of source separated organic waste for lignocellulosic sacharification,* Master's thesis, Ryerson University, Civil Engineering Dept., Toronto, Canada, 2010.

[12]   L. Brown, and R. Torget, *Enzymatic saccharification of lignocellulosic biomass. Chemical and Testing Task: Laboratory Analytical Procedure LAP-009*. Technical report. National Renewable Energy Laboratory, Golden, Colorado, USA, 1996. Last accessed May 15, 2014 http://www.p2pays.org/ref/40/39116.pdf

[13]   A. Mohageghi, N. Dowe, D. Schell, Y. Chou, C. Eddy, and M. Zhang. Performance of newly developed integrant of *Zymomonas mobilis* for ethanol production on corn stover hydrolysate. *Biotechnology Letters, 26*, 2004, 321-325.

[14]   J. Zhang, X. Shao, O.V. Townsend, and L.R. Lynd, Simultaneous saccharification of paper sludge to ethanol by Saccharomyces cerevisiae RWB222. *Biotechnology and Bioengineering, 104*(5), 2009, 920-931.

[15]   N. Dowe, and J.D. McMillan, *SSF experimental protocols - lignocellulosic biomass hydrolysis and fermentation*. Laboratory analytical procedure (LAP NREL/TP-510-42630). Technical report. National Renewable Energy Laboratory, Golden, Colorado, USA, 2008. Last accessed May 5, 2014 http://www.nrel.gov/docs/gen/fy08/42630.pdf

[16]   M.J. Selig, S. Viamajala, S.R. Decker, M.P. Tucker, M.E. Himmel, and T.B. Vinzant, Deposition of lignin droplets produced during dilute acid pre-treatment of maize stems retards enzymatic hydrolysis of cellulose. *Biotechnology Progress, 23*(6), 2007, 1333–1339.

[17]   Y.-H. P. Zhang, J-B Cui, L. R. Lynd, and L.R. Kuang, A transition from cellulose swelling to cellulose dissolution by o-phosphoric acid: Evidences from enzymatic hydrolysis and supramolecular structure. *Biomacromolecules 7*(2), 2006, 644–648.

[18]   N. Mosier, C.E. Wyman, B.E. Dale, R.T. Elander, Y.Y. Lee, M. Holtzapple, and M. Ladisch, Feature of the promising technologies for pretreatment lignocellulosic biomass. *Bioresource Technology, 96*, 2005, 673-686.

[19]   C.E. Wyman, B.E. Dale, R.T. Elander, M. Holtzapple, M. Ladisch, and Y.Y. Lee, Coordinated development of leading biomass pretreatment technologies. *Bioresource Technology, 96*, 2005, 1959-1966.

[20]   S.R. South, D. Hogsett, and K. Lynd, Modeling simultaneous saccharification and fermentation of lignocellulose to ethanol in batch and continuous reactors. *Enzyme Microbial Technology, 17*, 1995, 797-803.

[21]   P. Alvira, E. Tomás-Pejó, M. Ballesteros, and M.J. Negro, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis. A review. *Bioresource Technology, 101*(13), 2010, 4851-4861.

Table 1: Compositional analysis of source-separated organic samples

Parameters	Value
A. Physical Properties	
Biomass as received	
pH	5 @ 25°C
Total Solids (TS)	33.14%
Moisture content	66.86%
VOC per dry mass	28.00%
Ash per dry mass	5.14%
Oven-dried and homogenized biomass	
pH	5.5 @ 25°C
Moisture content	6.60%
TS	93.40%
VOC	83.40%
Ash	16.60%
B. Structural Carbohydrate and Lignin (per oven-dried and homogenized biomass)	
Starch	NS
Free Sugar	NS
Glucan	26.80%
Xylane	5.40%
Arabinan	1.20%
Mannan	5.70%
Galactan	2.20%
Total sugars	41.26%
Acid Insoluble Lignin (AIL)	25.40%
Acid Soluble Lignin (ASL)	1.20%
Total Lignin	26.60%
Acetic acid, Lactic acid, and Formic acid	NS
C. Others	
Total Kjehldahl Nitrogen (TKN)	5450 µg/g
Extractives	11.00%
Digestibility	12.70%
Biodegradability	82.00%

NS - not significant
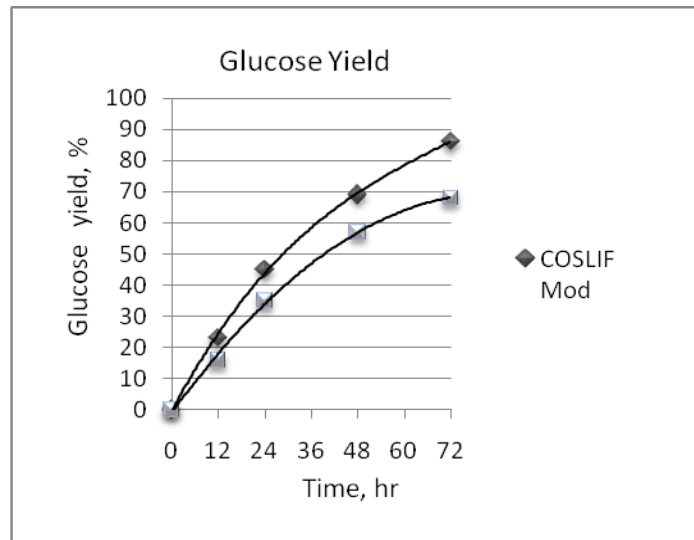Source: Ehsanipour, 2010
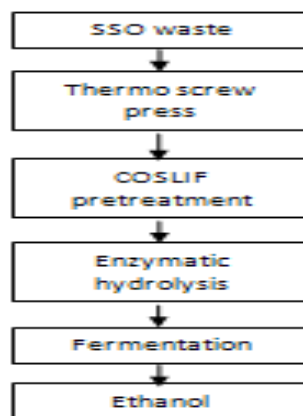
Figure 1: Experimental flowchart

Figure 2: Glucose yields of standard and modified COSLIF pre-treatment performed at 50°C for 72 hours
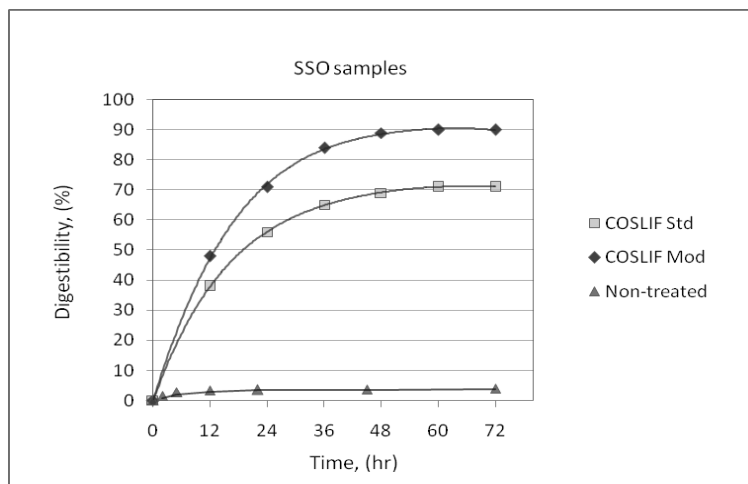

Figure 3: Time trend of glucose digestibility from the non-treated to standard and modified COSLIF pretreated samples
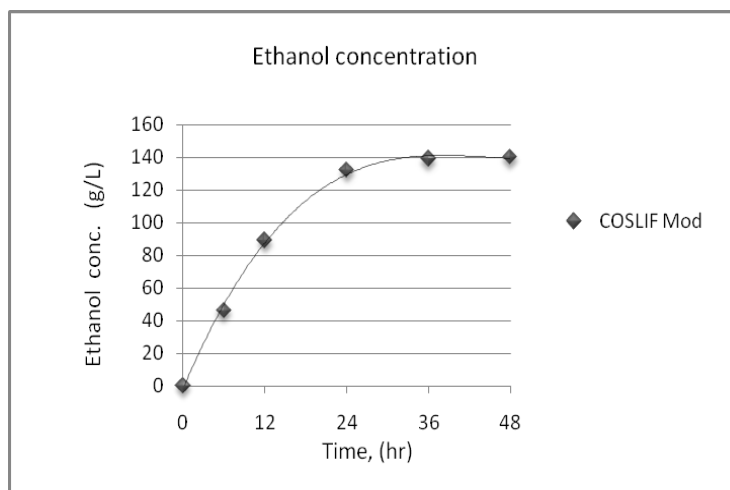

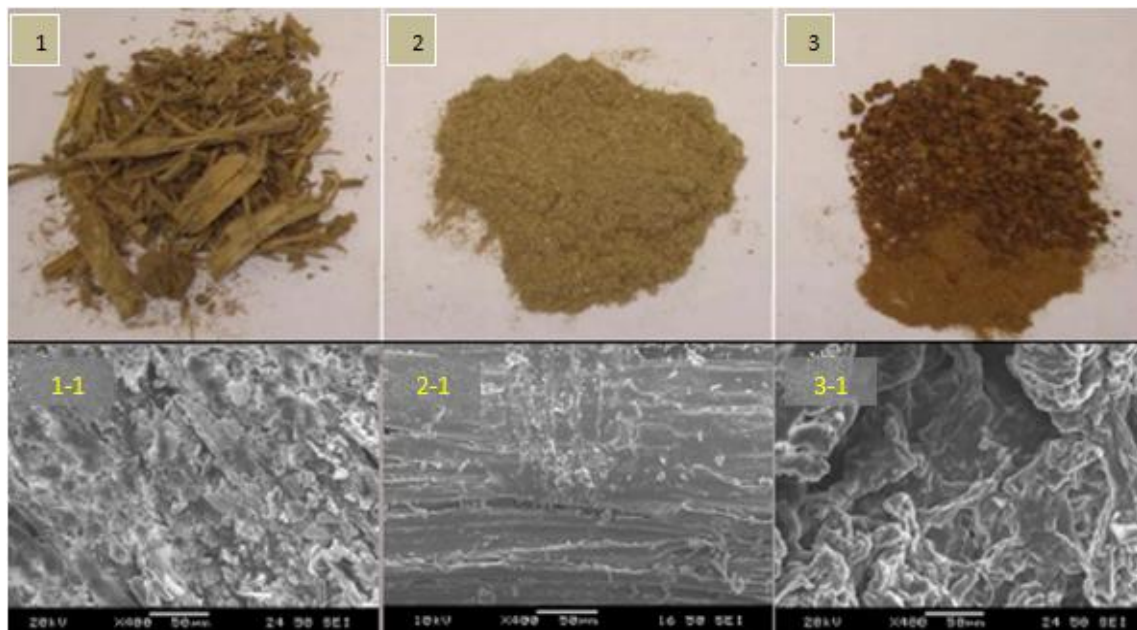**Figure 4:** Ethanol concentration from modified COSLIF pre-treated samples

Figure 5: Scanning electron microscopy images of source-separated organic waste *Source:* Ehsanipour, 2010