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Conditions of the Anaerobic Digestion of Biomass

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Abstract**—**Biological conversion of biomass to methane has received increasing attention in recent years. Grasses have been explored for their potential anaerobic digestion to methane. In this review, extensive literature data have been tabulated and classified. The influences of several parameters on the potential of these feedstocks to produce methane are presented. Lignocellulosic biomass represents a mostly unused source for biogas and ethanol production. Many factors, including lignin content, crystallinity of cellulose, and particle size, limit the digestibility of the hemicellulose and cellulose present in the lignocellulosic biomass. Pretreatments have used to improve the digestibility of the lignocellulosic biomass. Each pretreatment has its own effects on cellulose, hemicellulose and lignin, the three main components of lignocellulosic biomass. Solid-state anaerobic digestion (SS-AD) generally occurs at solid concentrations higher than 15%. In contrast, liquid anaerobic digestion (AD) handles feedstocks with solid concentrations between 0.5% and 15%. Animal manure, sewage sludge, and food waste are generally treated by liquid AD, while organic fractions of municipal solid waste (OFMSW) and lignocellulosic biomass such as crop residues and energy crops can be processed through SS-AD. An increase in operating temperature can improve both the biogas yield and the production efficiency, other practices such as using AD digestate or leachate as an inoculant or decreasing the solid content may increase biogas yield but have negative impact on production efficiency. Focus is placed on substrate pretreatment in anaerobic digestion (AD) as a means of increasing biogas yields using today's diversified substrate sources.

Keywords**—**Anaerobic digestion, Lignocellulosic biomass, Methane production, Optimization, Pretreatment.

I. Introduction

ANAEROBIC digestion (AD) methods decompose organic substrates under oxygen-free conditions using anaerobic microorganisms. Biogas and organic nitrogen compounds are the end-products of AD. Enhancing the digestibility of lignocellulosic biomass leads to more efficient conversion of cellulose and hemicellulose to ethanol, methane, and hydrogen. Methane production is energy-efficient. Even with successful pretreatments, the characteristics of lignocellulosic biomass and inhibitory products are still not completely known. Different pretreatment methods can improve the digestibility of lignocellulosic material. The anaerobic processes are hydrolysis, acidogenesis, acetogenesis, and methanogenesis are all important. Hydrolytic and acidogenic bacteria use enzymes to catalyze hydrolysis of complex organic materials into smaller units. Then acidogenic bacteria utilize these smaller substrates. Methane and carbon dioxide are produced by methanogenic bacteria after metabolizing acetate, hydrogen and carbon dioxide. Alcohol and volatile fatty acids are oxidized by acetogenic bacterial symbiotically with methanogens [1]. The AD process depends on parameters such as pH, temperature, C/N ratio, organic loading rate, reactor design, inoculums and HRT (hydraulic retention time) in relation to kinetics of microorganisms. Design of anaerobic digestion processes, therefore, should consider optimization of these parameters. Pretreatment can be considered concurrently with reactor design. Design must also be optimized to account for low gas production rates from agricultural residues, large hydraulic retention times and reduced gas production due to colder temperatures during winter. This review presents the effects of various pretreatment methods upon efficiency of methane production. It is focused on anaerobic degradation of lignified biomass using various pretreatment. Fundamental requirements for optimization of operating parameters and major constraints are discussed with. Conclusions regarding promising pretreatment techniques are developed.

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II. The Composition of Lignocellulosic Material

Lignocellulosic material consists primarily of three different types of polymers, i.e. cellulose, hemicellulose and lignin.

A. Cellulose

Cellulose contains D-glucose subunits that are linked by α-1,4glycosidic bonds. Plant cellulose is partially crystalline (organized), and partially amorphous (unorganized). Cellulose fibrils or bundles are found in strands which are wound together. Individual cellulose fibrils are independent, but are weakly bound by hydrogen bonds [2].

B. Hemicellulose

Hemicellulose is a complex carbohydrate. It consists of polymers of various compounds, e.g., pentoses, hexoses, and sugar acids. Xylan is the largest component of hemicellulose in hardwoods and agricultural residues. In softwoods, glucomannan is the major component [3]. Hemicelluloses are easy hydrolysable polymers. They have lower molecular weights than cellulose. Hemicelluloses connect lignin and the cellulose fibers. They give the whole plant matrix more rigidity [2].

C. Lignin

Lignin is found in the cell walls of plants. It is one of the most abundant polymers in nature. Lignin has three different types of phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) bound through different linkages. It is an amorphous heteropolymer. Its function is to support the plant structure, making it impermeable and resistant to microbial attack and oxidative stress. Additionally, lignin is water insoluble and optically inactive. Lignin is very resistant to

biological degradation. Under neutral conditions at 180°C, lignin can be dissolved in water. Lignin components control its solubility in acid, neutral and alkaline environments [4].

III. The Biomethanation Process

Biomethanation is a complex biological process that can be divided in four degradation and conversion phases. These are hydrolysis, acidogenesis, acetogenesis, and methanation.

A. Hydrolysis Phase

Cellulose, other carbohydrates, proteins, and fats, are broken down into monomers by hydrolase enzymes of facultative and obligate anaerobic bacteria. Simple carbohydrates can be hydrolyzed in a few hours. Hydrolysis of proteins and lipids may take a few days. Lignocellulose and lignin degrade slowly and incompletely [5].

B. Acidogenic Phase

The monomers from hydrolysis processes are utilized by different facultative and obligatorily anaerobic bacteria. These monomers are further degraded into short-chain organic acids, C1–C5 molecules (e.g., butyric acid, propionic acid, acetate, and acetic acid), alcohols, hydrogen, and carbon dioxide. Intermediate hydrogen affects the fermentation products. Intermediate fermentation products are formed if the partial pressure of hydrogen is high enough.

C. Acetogenic Phase

Acidogenic products of the previous phase serve as substrates for bacteria in the acetogenic phase. Homoacetogenic microorganisms of the acetogenic phase use exergonic H_2 and CO_2 to form acetic acid. Methanogenic bacteria grow concurrently with acetogenic bacteria. Short-chain organic acids and alcohols are converted to acetate. In the conversion of ethanol to acetate, carbon dioxide is used and acetate and hydrogen are produced. Acetate production decreases if hydrogen partial pressure is great enough.

D. Methanogenic Phase

The methane production takes place under strict anaerobic conditions. Not all methanogenic bacteria degrade all substrates. One can divide acceptable substrates acceptable for methanogenesis into the following three groups:

(I) Acetoclastic Methanogenesis

$$\text{Acetate} \rightarrow CH_4 + CO_2$$

(II) Hydogenotrophic Methanogenesis

$$H_2 + CO_2 \rightarrow CH_4$$

(III) Methyltrophic Methanogenesis

$$\text{Methanol} \rightarrow CH_4 + H_2O$$

IV. Optimization of Operational Parameters for Biomethanation

There are basic requirements for anaerobic bacteria. These bacteria utilize particular biomass types depending upon the feed characteristics and environmental conditions. Requirements for effective operation of biogas systems are as follows.

A. Retention Time

Generally, higher retention times result in higher biogas yield since yield is cumulative. Concurrently, total volatile solid mass is reduced. At the initial stage of biomethanation, the gas production rate is high but then it gradually declines as the digestion approaches completion [6]. Digestion of volatile fatty solids is not significant, even when the retention time is more than 12 days. Toxic compounds accumulate when retention time is high. Digested sludge is applied to land or incinerated.

B. Process Temperature

Temperature influences almost all biological activity. Lower temperatures inhibit methane-forming bacteria. There are two temperature ranges in which most methane-forming bacteria are active. The mesophilic range is 30–35◦C and the thermophilic range is 50–60◦C. Methane-forming bacteria are not active at temperatures between 40 and 50◦C. Small scale anaerobic digestion systems, e.g., Imhoff reactors, septic tanks and sludge lagoons, usually operate in the psychrophilic temperature range (10–20◦C). Biomethanation in the psychrophillic range is generally not very productive [7]. In all of these temperature ranges (psychrophilic, mesophilic and thermophilic), organic substrates are converted into methane. Under mesophilic conditions, the biogas production approaches its maximal value if temperature is maintained at around 35◦C [8]. The thermophilic range has an advantage over other ranges. Degradation time is shorter.

C. pH

pH is an important parameter in biomethanation processes. It affects the growth of various microorganisms involved at all stages [7]. To maintain pH within a desired range, organic loading is done at an optimal feeding rate. A reduction in the population of methanogenic bacteria occurs at pH values between 6.0-8.5. Intermediate biomethanation products are formed during biological digestion. These products influence pH. A pH below 6.6 adversely affects the methanogenic activity. Additionally, toxicity occurs at pH 6.2. For acid-forming bacteria, there is acceptable enzymatic activity at pH values above 5.0. In contrast, enzymatic activity of the methane forming bacteria occurs at pH values greater than 6.2. Methane-forming bacteria produce well within a pH range of 6.8–7.2.

D. Solids Concentration

Solids concentration is the degradable part of feed material in a unit volume of slurry. Total solids (TS) affect the pH, temperature and effectiveness of the microorganisms in digestion processes. Reactor design considers the solids concentration. A solid concentration of 7–9% is suitable for floating dome reactors [9]. A CSTR (completely stirred tank reactor) was simulated over a range of 4-10% TS [10]. The hydraulic retention time (HRT) and capital cost generated by

size of digester were reduced by high organic loading rates (OLR).

E. Organic Loading Rate

The organic loading rate (OLR) is the concentration of volatile solids (VS) or chemical oxygen demand (COD) concentration loading per day per digester reactor volume. The digester's size and the capital cost are reduced at high organic loading rates. However, conversion of organic material to biogas is based upon the retention time [11]. The specific carbon loading affects the growth response of methanogens. Normally 8-10% TS content in the reactor feed is desirable for optimum gas yield in biomethanation processes [12]. Ammonia toxicity and trace nutrient limitations occur under conditions of high total solids in anaerobic digestion.

F. C/N Ratio

High levels of nitrogen (>80 mg/l) as undissociated ammonia at low C/N ratios can cause toxicity. In addition, low levels of nitrogen at high C/N ratios can slow the rate of digestion. It is important to keep the C/N ratio in the desired range. The microorganisms in biomethanation processes utilize 25-30 times more carbon than nitrogen. The type of reactor selected is partially determined by the C/N ratio of the intended feedstock. A two-stage reactor is reliable with C/N ratios less than 20:1 [13].

G. Fatty Acids

The intermediate VFA (volatile fatty acid) products (acetic acid, propionic acid and butyric acid) in the biomethanation process are capable of inhibiting methanogensis at high concentrations [7]. A high organic loading rate causes accumulation of VFAs. This occurs since acetogens grow more slowly. The rate of acidogensis is reduced at high VFA concentrations because of inhibition by acid producing bacteria. Total VFA concentrations above 4 g/l inhibit fermentation of sugars [14]. VFA levels above this level inhibit methane production [15].

H. Inoculation

Lignocellulosic degradation in an anaerobic digester requires a variety of acids, alcohols, carbon dioxide and hydrogen from carbohydrates, lipids and proteins. Some anaerobes are strong acid producers and are active in absence of oxygen. Strict anaerobes in anaerobic digestion, such as *Methanobacterium*, and *Methanococcus*, convert acetate, alcohol, carbon dioxide and hydrogen into methane. Biomethanation by several groups of microorganisms is necessary for efficient degradation of waste [16].

I. Reactor Designing

Different digester configurations are used. These include one-stage and two-stage digesters, wet and dry/semi-dry digesters, batch and continuous digesters [17]. Others use combinations of different approaches [18]. Various gases are produced over time. To maintain a constant gas supply, several reactors must be operated simultaneously [10]. In wet fermentations, normally the solid content is 6-10% TS. In dry fermentations, a higher solid content is used, usually more than 20%. Continuous flow stirred tank reactors (CSTRs) are used for substrates with high total solid concentrations.

J. Mixing

Mixing can create uniformity in fluids and eliminate concentration and temperature gradients [13]. Intimate contact between microorganisms and substrate while stirring the digester can enhance the biomethanation process. Excessive mixing can reduce biogas production. Efficiency of biogas production requires gentle mixing because it results in less shock [13]. In addition, excessive mixing causes disruption of granular structures. It reduces the rate of oxidation of VFA leading to digester instability [19]. The German Federal Agricultural Research Centre investigated anaerobic digestion using several submersible mixer impellers. These included paddle, long shaft, and central types as well as and a combination of these [19]. The studies of the University of Natural Resources and Applied Life Sciences, Vienna showed an average mixing time of 3-4 h/day with 10-20 rpm is useful for high solid contents [19].

V. Pretreatment

A. Mechanical Pretreatment

Milling is a mechanical pretreatment of lignocellulosic biomass done by cutting the material into smaller pieces. Smaller particle sizes lead to increased available specific surface area and reduce the degree of polymerization (DP). It also causes shearing of the biomass. These factors can enhance the total hydrolysis yield of lignocellulose by 5–25% depending on kind of biomass, type of milling, and duration of milling [20]. It also reduced the digestion time by 23–59% due to an increased hydrolysis rate [20].

B. Thermal Pretreatment

The lignocellulosic biomass is heated during the pretreatment. The hemicelluloses and lignaceous parts of biomass solubilize when the temperature increases above 150–180◦C. The thermal, acid and alkali stability of the hemicellulose affects the composition of the hemicellulose backbone and the branching groups. Xylans and glucomannan are the two dominant components in hemicelluloses. The xylans are the least thermally stable. However, differences between xylans and glucomannans are quite small. Hemicelluloses are solubilized when temperature is above 180◦C by an exothermal reaction. High temperature is an indication of this exothermal reaction. The thermal reactivity of lignocellulosic biomass is largely based on its composition. Hemicellulose is hydrolyzed during thermal processes and acid is produced.

C. Acid Pretreatment

Acid pretreatment of lignocellulose at ambient temperature can enhance its anaerobic digestibility. The goal of acid pretreatment is to solubilize hemicellulose, making cellulose more accessible. Either dilute or strong acid can be used in such pretreatments. Hydrolysis of hemicellulose is promoted

by acid. The hydrolytic reactions upon hemicelluloses produce monomers such as furfural and other volatile products in acidic environments [21]. Lignin is solublized during acid pretreatment [22]. Strong acid pretreatment solubilizes hemicellulose and lignin to a greater degree than dilute acid pretreatments.

D.Alkaline Pretreatment

Alkaline pretreatment affects hydrolysis of hemicellulose and lignin. Degradation of hemicellulose is advantageous for cellulose solubilization. However, hemicellulose degradation products and solubilized lignin formed during the alkaline heat pretreatment have an inhibitory effect. In producing ethanol, alkaline pretreatments are less attractive since fermentable sugars are lost and inhibitory compounds produced. For ethanol production, methanogens are less influenced by inhibitory compounds than yeasts. This is because methanogens can adapt to these compounds.

E. Oxidative Pretreatment

An oxidative pretreatment uses an oxidizing compound such as hydrogen peroxide or peracetic acid. These compounds are in aqueous solution. Oxidative pretreatments can remove hemicellulose and lignin to improve the accessibility to cellulose. In oxidative pretreatments, several reactions occur. These include electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkages and oxidative cleavage of aromatic nuclei [23]. Hemicellulose and cellulose are lost because oxidants are not selective. Lignin is oxidized to produce soluble aromatic compounds. There is a high risk of formation of inhibitors. Use of peracetic acid at ambient temperatures as an oxidant for sugar cane bagasse was investigated by Teixeira [24]. Using a 21% peracetic acid pretreatment, the enzymatic hydrolysis of the cellulose increased from 6.8% in untreated samples to a maximum of about 98% [24].

F. Combinations of Ammonia and Carbon Dioxide Pretreatments

1. Thermal Pretreatment in Combination with Acid Pretreatment

External acid positively affects the performance of thermal steam or liquid hot water (LHW) pretreatments. It catalyzes solubilization of hemicellulose. The optimal pretreatment temperature is low. In addition, they give a better enzymatically hydrolysable substrate. Using SO_2 or H_2SO_4 for lignocellulose acid pretreatment is possible. SO_2 is converted to H_2SO_4 within the first 20 seconds of the process. After that catalytic hydrolysation of hemicellulose starts. Hemicellulose is gradually removed and lignin can trigger a reorientation of cellulose to a more crystalline form. For steam pretreatment at 160°C and higher with 0.5% sulfuric acid addition, an appreciable production of furfural is observed [25].

2. Thermal Pretreatment in Combination with Alkaline Pretreatment

An external alkali can improve thermal pretreatment. Lime pretreatment is one very common alkaline pretreatment. Usually the temperature is in the range of 100–150◦C with approximately 0.1 g $Ca(OH)_2$ per g of substrate [26]. The effect of lime pretreatment is to promote the production of acetyl compounds and decomposition of lignaceous compounds. It makes the substrate more accessible to hydrolysis [27]. Lime with thermal pretreatment can increase the digestibility of substrates with low lignin contents. It is not sufficient for substrates with high-lignin contents [28]. Other researchers showed that lime pretreatment of switch grass and corn stover did not inhibit enzymatic saccharification and fermentation [26].

3. Thermal Pretreatment in Combination with Oxidative Pretreatment

Saccharification of cedar soaked in peracetic acid and treated at a temperature 231◦C for 10 min was proportional to the amount of peracetic acid adsorbed by the wood. Another oxidative pretreatment method is wet oxidation where the oxidizer is oxygen. In wet oxidation pretreatments, soluble sugars were produced from wheat straw. In contrast to steaming or acid hydrolysis pretreatments, its products were monomers. The end products of wet oxidation of phenolic monomers are carboxylic acids.

4. Thermal Pretreatment in Combination with Alkaline Oxidative Pretreatment

Using oxygen as oxidizer during alkaline pretreatment has a limitation in the case of lime pretreatment. The enzymatic digestibility of high lignin substrates is not improved by lime pretreatment [26]. At a relatively low temperature of 150°C, low sugar degradation was observed during alkaline oxidative pretreatment. Alkaline oxidative pretreatment improved enzymatic digestibility of biomass to a level 13 times higher than for untreated material. Pretreated biomass was washed to remove inhibiting soluble lignin compounds [26]. About 21% of the added lime can be recovered using carbon dioxide after oxidative lime pretreatment.

5. Ammonia and Carbon Dioxide Pretreatment

Ammonia and carbon dioxide pretreatments are other possible processes. Ammonia loading is done at a ratio of around 1:1 (kg ammonia/kg dw biomass) at ambient temperature for durations of 10–60 days. Using a temperature of 120◦C, the duration of this process was reduced to several minutes [29]. Alizadeh et al. [29] reported a six-fold increase in enzymatic hydrolysis yield and a 2.5-fold ethanol yield after this pretreatment. Kim and Lee [30] suggest swelling of the cellulose and delignification as the factors responsible for this increased yield. High-pressure carbon dioxide and high temperatures of up to 200◦C for a duration of several minutes were used for carbon dioxide pretreatments.

VI. Conclusion

Biological digestion of lignocellulosic biomass is influenced by crystallinity of cellulose, available surface area and lignin content. Pretreatments significantly improve these processes. Several factors influence the overall economy of pretreatment. It is necessary to avoid production of inhibitors

in these processes. The liquid fraction may need detoxification. This fraction should be removed in order to minimize the overall costs of the process as well as allowing the process to continue. Some pretreatment methods are effective but expensive, e.g., use of concentrated acids, wet oxidation, solvents and metal complexes. Considering economic effectiveness and potential, the most attractive pretreatment methods are steam pretreatment, lime pretreatment, LHW systems and ammonia. Carbon dioxide pretreatment has not been well studied and the potential of this method is largely unknown. Pretreatment efficiency depends on biomass characteristics and operating conditions. Research on process optimization is an area that is open for future research. Production of ethanol and methane are two attractive bio-fuel production processes. However, their conversion efficiencies are not large. Low tolerance of inhibiting compounds is a disadvantage of the ethanol production.

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REFERENCES

[1] Sotirios K, Boukis I, Kontopoulos G. Development of an investment decision tool for biogas production from agricultural waste. Renew Sustain Energy Rev 2010;14:1273–82.
[2] Laureano-Perez, L., Teymouri, F., Alizadeh, H., Dale, B.E., 2005. Understanding factors that limit enzymatic hydrolysis of biomass. Appl. Biochem. Biotechnol., 1081–1099.
[3] Saha, B.C., 2003. Hemicellulose bioconversion. J. Ind. Microbiol. Biotechnol. 30, 279–291.
[4] Grabber, J.H., 2005. How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. Crop Sci. 45, 820–831.
[5] Gerardi MH. The microbiology of anaerobic digesters, waste water microbiology series Hoboken, New Jersey: John Wiley & Sons, Inc; 2003.
[6] Yadvika, Santosh S, Sreekrishnan TR, Kohli S, Rana V. Enhancement of biogas production from solid substrates using different techniques – a review. Journal of Bioresource Technology 2004;95:1–10.
[7] Pain BF, Philips VR, West R. Mesophilic anaerobic digestion of dairy cow dung slurry on a farm scale energy consideration. Journal of Agricultural Engineering Research 1988;33:123–9.
[8] Zeeman Z, Koster-Treffers ME, Halm HD. Anaerobic digestion of dairy cow slurry. In: European symposium on anaerobic waste water treatment. TNO Corporate Communication Department; 1983. p. 492–510.
[9] Ong HK, Greenfield PF, Pullammanappallil PC. An operational strategy for improved biomethanation of cattle–manure slurry in an unmixed, singlestage, digester. BioresourTechnol 2000;73:87–9.
[10] Rao P, VandBaral SS. Attribute based specification, comparison and selection of feed stock for anaerobic digestion using MADM approach. J Hazard Mater 2011;186:2009–16.
[11] Keri BC, Thomas D, Kyoung SR, Hunt PG. Livestock waste-to-bioenergy generation opportunities. Journal of Bioresource Technology 2008;99: 7941–53.
[12] Hobson PN, Bousfield S. Summers, methane production from agricultural and domestic waste. England: Applied Science Publishers; 1981.
[13] Gomez X, Cuetos MJ, Cara J, Moran A, Garcia AI. Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes – conditions for mixing and evaluation of the organic loading rate. Renew Energy 2006;31(12):2017–24.
[14] Siegert I, Banks C. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. Process Biochem 2005;40:3412–8.
[15] Pandey PK, Bhattacharya D. Biogas engineering, regional biogas development and training centre. India: Chemical Engineering Department. Indian Institute of Technology Kharagpur; 2005.
[16] Vinner٦s B, Schٮnning C, Nordin A. Identification of the microbiological community in biogas systems and evaluation of microbial risks from gas usage. Sci Total Environ 2006;367:606–15.
[17] Chowdhury RBS, Fulford DJ. Batch and semicontinuous anaerobic digestion systems. Renew Energy 1992;2(4–5):391.
[18] De baere LD, Mattheeuws B. State of art-anaerobic digestion of solid waste. Waste Manage World 2008;9(5):1–8.
[19] Stroot PG, McMohan KD, Mackie RI, Raskin L. Anaerobic codigestion of municipal solid waste and biosolids under various mixing condition in digester performance. Water Res 2001;35:1804–16.
[20] Garrote, G., Dominguez, H., Parajo, J.C., 1999. Hydrothermal processing of lignocellulosic materials. HolzRohWerkst. 57, 191–202.
[21] Ramos, L.P., 2003. The chemistry involved in the steam treatment of lignocellulosic materials. Quim. Nova. 26 (6), 863–871.
[22] Liu, C., Wyman, C.E., 2003. The effect of flow rate of compressed hot water on xylan, lignin and total mass removal from corn stover. Ind. Eng. Chem. Res. 42, 5409–5416.
[23] Hon, D.N.S., Shiraishi, N., 2001. Wood and Cellulosic Chemistry, second ed. Dekker, New York. explosion (AFEX). Appl. Biochem. Biotechnol., 1133–1141.
[24] Teixeira, L.C., Linden, J.C., Schroeder, H.A., 1999. Alkaline and peracetic acid pretreatments of biomass for ethanol production. Appl. Biochem. Biotechnol., 19–34.
[25] Tengborg, C., Stenberg, K., Galbe, M., Zacchi, G., Larsson, S., Palmqvist, E., Hahn-Hٮgerdal, B., 1998. Comparison of SO2 and H2SO4 impregnation of softwood prior to steam pretreatment on ethanol production. Appl. Biochem. Biotechnol., 3–15.
[26] Chang, V.S., Kaar, W.E., Burr, B., Holtzapple, M.T., 2001. Simultaneous saccharification and fermentation of lime-treated biomass. Biotechnol. Lett. 23 (16), 1327–1333.
[27] Chang, V.S., Holtzapple, M.T., 2000. Fundamental factors affecting enzymatic reactivity. Appl. Biochem. Biotechnol., 5–37.
[28] Kaar, W.E., Holtzapple, M.T., 2000. Using lime pretreatment to facilitate the enzymatic hydrolysis of corn stover. Biomass Bioenergy 18 (3), 189–199.
[29] Alizadeh, H., Teymouri, F., Gilbert, T.I., Dale, B.E., 2005. Pretreatment of switchgrass by ammonia fiber
[30] Kim, T.H., Lee, Y.Y., 2005. Pretreatment of corn stover by soaking in aqueous ammonia. Appl. Biochem. Biotechnol., 1119–1132.