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Multi-Criteria Analysis of Lignocellulose Substrate Pre-Treatment

Ilze VAMZA[1*], Karlis VALTERS[2], Dagnija BLUMBERGA[3]

[1–3]*Institute of Energy Systems and Environment, Riga Technical University, 12-k1 Āzenes street, Riga, LV-1048, Latvia*

Abstract **– Due to growing topicality of indirect land use change, greater shift towards second generation biofuels should be observed. In order to help smaller biogas and bioethanol producers, multi-criteria analysis of lignocellulose pre-treatment is conducted to elucidate the most preferable approach for lignocellulose pre-treatment. There are four main pre-treatment groups – biological, chemical, physical and physochemical pre-treatment. In this article three pre-treatment types were described by highlighting their specific approaches; using multi-criteria analysis a conclusion was reached that the most preferable pre-treatment option for lignocellulose biomass like corn stover or sugarcane is microbiological pre-treatment, as it showed the closest proximity to ideal solution.**

Keywords **– Biological pre-treatment; corn stover; microwave pre-treatment; simple sugars; sugarcane bagasse**

1. INTRODUCTION

Lignocellulose substrate has been used for commercial biofuel production for around a decade, during this time government incentives have been implemented to stimulate faster biofuel entrance into market and moving economy towards bio-economy [1]. Biofuels are beneficial not only to environment, but national economy as well. Nations without fossil fuel reserves can provide their own fuels ensuring energy independence [2], [3]. In case of nation's farmland exploitation for energy crop production, food safety cannot be compromised, so second generation biofuels is a better choice, in this case food crop lignocellulose residue like stalks can be used as substrate. Second generation biofuels are more sustainable choice as it does not cause indirect land use change when food crop land is used for energy crop cultivation, as indirect land use change cause huge greenhouse gas emissions from deforestation [2] and increase national carbon footprint. Running argument against lignocellulos substrate is the end product cost, as historically it has been quite higher than ethanol produced from corn starch [4]. Increasing concern about sustainable bioresource use and growing demand for biofuels are stimulating development of various lignocellulose pre-treatments varying between physical, chemical and biological in nature [5]. All methods have shown good results and plenty of pilot pre-treatment plants have been built [6]–[8]. Nevertheless, constant development of new pre-treatment methods is taking place. This

[*] Corresponding author.
E-mail address: Ilze.Vamza@rtu.lv

analysis can elucidate the most promising method. In this study we are conducting multi-criteria analysis for comparing all three types of pre-treatment methods – biological, chemical and physical, used for lignocellulose pre-treatment. As the amount of acquired free sugar directly impacts the yield that could be acquired from microorganisms, results from this study would be relevant to both - small biogas and bioethanol producers. In addition to glucose yield, retention time, operational costs and temperatures are considered for overall evaluation.

2. LIGNOCELLULOSE

Lignocellulose is most abundant biomass, typically being the leftovers in agriculture and forestry industries. With growing demand for microbial feed for second generation biofuel production, lignocellulose has become a sympathetic substrate due to amount of cellulose it contains. There are various options for pre-treatment of lignocellulose substrate for greater reducing sugar yield. Lignocellulose main purpose in plants is to ensure structural rigidity and its main components are natural polymers – carbohydrates in form of cellulose and hemicellulose and lignin consisting of multiple aromatic compounds. Although these three polymer groups are crucial to lignocellulose, ratios of these polymers vary from plant to plant [9] and even between parts of one plant – stems can contain different lignin amount from leaves [10]. In addition to variations in proportions – types of lignin vary between plants. Hard woods are associated with syringyl lignin, but softwoods with guaiacyl lignin. This aspect should be taken into account when choosing the right pre-treatment method, as guaiacyl compared to syringyl lignin restricts enzyme accessibility [11].

3. GENERAL PRE-TREATMENT METHODS

There are four pre-treatment types – biological, physical, chemical and physochemical [12], [13]. Each of these types include vast array of pre-treatments that have been studied extensively. Most of these approaches can be combined and all lead to reducing sugar yield. However, all methods vary in cost, retention time and consumed energy. The purpose of pre-treatment is to break down hemicellulose and free up cellulose from lignin, simultaneously reducing particle size to increase surface area to volume ratio [7]; by comparison, feedstock with smaller lignocellulose about, could be simply shredded [14]. There are multiple scientific publications investigating pre-treatment effects on reducing sugar yield from different lignocellulose substrates like rice husks, corn stover, corn cobs, leaves and others [8]–[11].

3.1. Biological pre-treatment

All biological processes require further hydrolysis of cellulose to release reducing sugars used for microbial feed to produce primary or secondary metabolites. Fermenting yeast like *Saccharomyces cerevisiae* cannot hydrolase nor starch, nor lignocellulosic substrate, hence pre-treatment was necessary even for first generation biofuel production where starch was the substrate. In case of ethanol production, even *S. cerevisiae* cofermentation with *Aspergillus niger* was tested [15].

Hydrolysis is not exclusive step for fermentation; anaerobic digestion (AD) requires the substrate to be hydrolysed as well. Hydrolysis is first of four processes taking place in anaerobic digestion (AD) followed by acidogenesis, acetogenesis and methanogenesis [16]. Although hydrolysis is a crucial process in AD, efficiency of hydrolysis is higher in aerobic environment [17]. Hence, conveying biological pre-treatment using microorganisms or enzymes to hydrolase organic polymers ensure higher AD's efficiency in bioreactor. As fungi hyphae are growing through substrate, enzymes are secreted to break down organic polymers like lignin and cellulose [18]–[20]. This is creating challenges as fungi are making substrate more available for further digestion and consumes it at the same time reducing glucose yield. Hence microbial pre-treatment for cellulose is not as popular. Usually white-rot fungi are considered for pre-treatment process, due to their natural ability to break down the lignin by acting upon it with specific enzymes [21] – mainly lignin-peroxidase. Although this process is already happening in nature and can be utilised for production needs, there are active studies finding ways to stimulate specific fungi to produce greater amounts of ligninaze enzymes [20]. Other factors, like the amount of nitrogen, should be taken into account when biological pre-treatment is considered. When white-rot fungus is used for pre-treatment, nitrogen limitation would be preferable, as higher glucose consumption is associated with excess nitrogen [22]. Although fungi as decomposes are well known, there has been proof that bacteria could be used for decomposition of lignin as well. Due to natural abilities of bacteria to break the lignocellulose down fast, they have been used for enzyme production itself [23]. Moreover, in addition to lignin peroxidase, manganese peroxidase and laccase can be found in bacteria. All three enzymes can decompose lignin [24].

There are various benefits for using bacteria instead of fungi to conduct pre-treatment – bacteria usually grow with more rapid speeds; in addition, medium can be constantly mixed as tearing fungal mycelium is not a problem. Mixed medium means that the pre-treatment can be done in a more homologous way and there are less "blind spots" where pre-treatment is not taking place due to lack of microbial activity in certain parts of substrate. Though mixing approach means that more energy input is required for this process, alternatively, fungi are capable of invasive growth and apply physical force by penetrating substrate [19].

Another type of biological pre-treatment is an application of enzymes without the use of microorganisms at all [25]. This pre-treatment can be used to hydrolyse cellulose and other carbohydrate polymers into reducing sugars [25], as in this case microorganisms that could use these sugars are not present, it increases the sugar yield. Although this benefits the sugar yield, enzyme price and re-usability of them needs to be taken into account when considering this approach. Enzymes can be free flowing in medium or they can be immobilized on surface or in gel. This approach lets them to be re-used and even protects them from inactivation from medium conditions [26]. Usually enzymatic pre-treatment is combined with various other methods analysed in this work. Using enzymes or specific microorganisms as pre-treatment is often associated with higher price due to extra investments in pre-treatment reactor building [11] and enzyme price itself.

The cost of biological pre-treatment may vary due to chosen approach. Using microbial isolates depends on desirable enzymatic activity and microbial growth rates [27]. Alternatively, only physical or chemical pre-treatment can be used mainly for lignin disruption, easing hydrolysis process in AD reactor. However, in comparison to biological pre-treatment, physical and chemical types are associated with even higher price [28].

3.2. Physical pre-treatment

Lignin is physically intertwined with cellulose fibres – it is cross-linked and surrounds the straight cellulose fibres creating lignocellulose matrix [12]. Lignocellulose physical pre-treatment is associated with mechanical disruption of this matrix dividing lignin from cellulose and hemicellulose. As this could only be done by applying mechanical force, it is energy demanding pre-treatment approach [6], [29]. A few of more popular physical pre-treatments are using microwave, ultrasound and the abovementioned milling [29]. Physical pre-treatment approach is usually non-specific, and disruption is accidental as mechanical force is simply tearing the matrix in the weakest point. Nevertheless, there is evidence that microwave pre-treatment is affecting glucoside bonds in cellulose but is strongly correlated with lignin presence in the material. This means that irradiation could be used as pre-treatment of lignocellulosic materials to acquire oligosaccharides without using cellulase enzymes for glucoside bond disruption. Nevertheless, this method is costly, and more intensive irradiation can even break the ring structure of the glucose down [11], leaving the pre-treated biomass unusable. When considering irradiation pre-treatment, more precise lignin to cellulose ratio in substrate should be known to evaluate the potential yield. Ultrasonification is another method for physical bond disruption, in addition causing free radical cascading reactions from produced hydroxy radicals. Unlike irradiation that cause cellulose glucoside bond disruption, micro-jets produced from cavitation bubbles disrupt cellulose-lignin structure breaking C-C bonds of aromatic rings of lignin [30], [31]. Cavitation bubbles are created in liquid medium by ultrasonic frequency produced by sinus wave generator and cavitation bubble disruption occurs near the liquid-solid medium boundary [31]. Milling in some form as a pre-treatment method is quite popular, as this method can be easily adjusted to specific substrate. It is not rare for biogas producers to use a screw type mill for substrate size reduction before transferring it to the reactor, or to at least chip the material to reduce particle size to around 1 mm. There are various milling techniques, such as hammer mills, disc mills, knife mills and ball mills, and all these methods create friction in substrate; this, in turn, leads to the heating of the substrate and releasing a lot of heat energy, hence creating heat loss [32]. Less friction can lead to lesser energy demand, by comparison – hammer milling acquires less energy than disc milling, but hammer milling produces particles with more variable and bigger particle size [6]. Kratky and Jirout conducted a thorough investigation comparing various mills by their consumed energy. The review illustrates the vast array of energy demand, confirming the abovementioned – hammer milling requires 130 kW h^{-1} to reduce hardwood particles to 1.6 mm, but disc mill consumes around 300 kW h^{-1} to do the same [32].

3.3. Chemical pre-treatment

Lastly, there is chemical pre-treatment. In general, chemical pre-treatment serves the same purpose – to split organic polymers into smaller units that are more available to microorganisms for further utilization. Usually acid or base is used for solvolytical extraction and depolymerization of lignin. If strong acid or base is used for pre-treatment, acquired products are – pulp, lignin oil and sugars [33]. Due to specificity of chemical pre-treatment, polymers are divided into simple sugars straight away and enzymatic treatment for cellulose hydrolysis is not necessary. Diluted sulphuric acid can dissolve almost 50 % of lignocellulose biomass [10]; however, Donghai *et al.* research suggests that diluted acid pre-treatment brings differing results, depending on lignocellulose structure – different parts of one plant can produce different sugar yield [34]. Both diluted alkali and diluted acid pre-treatments hydrolyse cellulose, but conditions of these reactions vary – acid pre-treatment requires higher pressure and temperatures than alkali pre-treatment [12]. As bases and acids are chemically active agents, they are not only assisting on lignocellulose dissolving process, but catalyse other biologically active molecule synthesis like furfuroles that in turn are known for their inhibition of microorganisms [12], hence glucose higher glucose yield does not mean higher biogas or ethanol yield.

In addition to abovementioned pre-treatment types, there are multiple pre-treatment options categorised in between – for example, steam combustion uses physical force and water as chemical agent for bond disruption [12]. Glucose yield and overall lignocellulose disruption success vary from pre-treatment to pre-treatment and each pre-treatment has its peculiarities and it should be chosen accordingly to further substrate use – for AD, alcohol fermentation or any other use. Steam explosion is considered more suitable for AD due to organic acid generation during process [35].

4. Methods

To compare multiple pre-treatment methods, scientific works using similar lignocellulose substrates were selected. Criteria for comparison was chosen based on data availability in literature. Three main criteria were considered – time, cost and yield, additional criteria – operational temperature were chosen, as bigger shifts from ambient temperature means higher costs for insulation and higher energy demand. To conduct MCA Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) method was chosen and supplemented with Analytic hierarchy process (AHP) for criteria ranking. Work algorithm is depicted in Fig. 1.

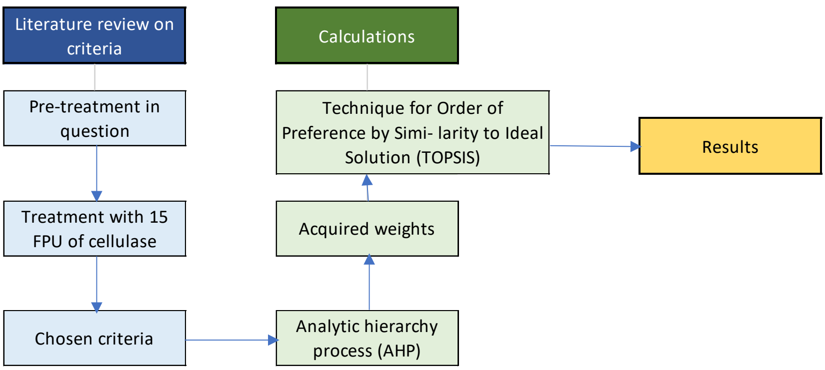

Fig. 1. Overall scheme for work algorithm.

Chosen criteria and corresponding importance are shown in Table 1. Criteria are ranked by AHP method in scale from one to nine, representing importance from equal to extreme respectively. AHP method was used to rank criteria by their importance in relation to one another [36]. In addition, AHP allowed to determine the priority vector for each criterion.

TABLE 1. CHOSEN PRE-TREATMENT CRITERIA AND ASSIGNED DEGREE OF IMPORTANCE

Degree of importance explanation	Degree of importance	Criteria
Moderate plus	4	Retention time, h
Moderate importance	3	Operational temperatures
Extreme importance	9	Glucose recovery, %
Very, very strong	8	Operational costs, low = 1, high = 0

Criteria were chosen after literature analysis by investigating the ones mentioned most frequently. Weights of criteria were assigned by conducting an AHP, and results were following: retention time 0.18, operational temperatures 0.12, glucose recovery 0.37 and operational costs 0.33. Pre-treatment process described in publications had to be corn stover or similar, like sugarcane bagasse due to similarities of these two substrates [37]. After investigated pre-treatments in question, enzymatic treatment was applied to all substrates as this could show the real-life situation as some pre-treatments only free-up cellulose and make it more accessible but does not hydrolase it into simple sugars. As in this work methods for lignin disruption are investigated, publications chosen for multi-criteria analysis are following similar workflow – firstly, there is applied a pre-treatment to separate lignin from cellulose and secondly, cellulase enzymes are applied to hydrolyse cellulose to reducing sugars. All works chosen are using cellulase enzymes with around 15 FPU enzyme activity. Values of chosen corresponding criteria for each analysed pre-treatment method are shown in Table 2.

TABLE 2. PRE-TREATMENT ALTERNATIVES (BIO – BIOLOGICAL; CHEM – CHEMICAL; PHY – PHYSICAL) AND CORRESPONDING DATA FOR CHOSEN CRITERIA

Pre-treatment	Lignocellulose substrate	Retention time, h	Operational costs	Operational temperatures, °C	Glucose recovery, %	Source
Bio: microbial	Corn stover	432	Low	28	57.65	[28], [38]
Chem: 1N NaOH	Corn stover	60.00	High	25	42	[28], [39]
Chem: $Ca(OH)_2$	Corn stover	2856.00	Low	25	80	[40]
Phy: microwave + $CaCl_2$	Corn stover	0.17	High	160	69.94	[28], [41]
Phy: wet milling + 0.005M H2SO4	Sugarcane bagasse	0.50	High	180	13	[28], [42]

One biological, two chemical and two physical pre-treatment methods were analysed by MCA. As price is very specific to the scale of production and chosen technical solutions, only – high and low price were assigned to pre-treatments by analysing available literature. Some pre-treatments are used in combination with another pre-treatment, like wet milling with diluted acid pre-treatment [42]. Inhibitions and other adverse effect were not considered as this work is aimed on determining the most preferable pre-treatment method for general purpose.

5. Results

According to multi-criteria analysis, microbial pre-treatment showed the best results, being the closest to most preferable option. Microbial and other pre-treatment proximity to most preferable method can be seen in Fig. 2.

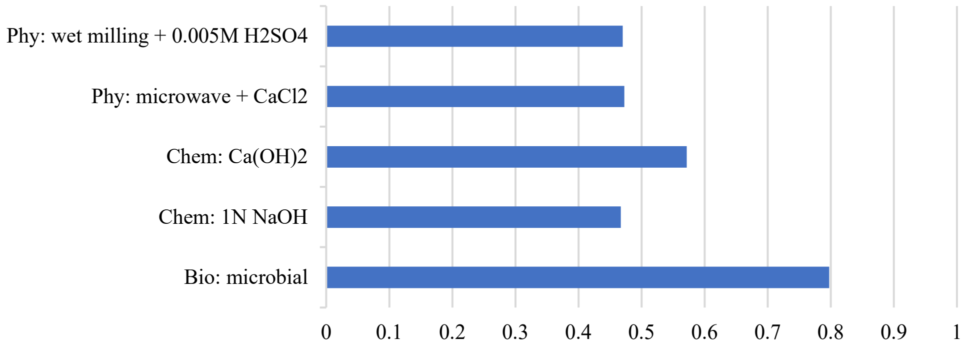

Fig. 2. Multi-criteria analysis results.

According to conducted multi criteria analysis, biological pre-treatment using *Ceriporiopsis subvermispora* shows the highest results. This could be explained by biological pre-treatment not requiring such high temperatures and still ensuring relatively high glucose yield. Results show that physical pre-treatment like irradiation and wet milling are equally good pre-treatment methods, only biological pre-treatment shows considerably better results than all other pre-treatment methods. Further research could be conducted to evaluate specific adverse effects of each pre-treatment as in this work such effects as insoluble residues using $Ca(OH)_2$ and produced inhibitors in diluted base pre-treatment method have not been considered.

6. Conclusions

After conducting MCA, biological pre-treatment was elucidated as the most preferable method. This might be due to high weights of price and glucose recovery criteria. This work was limited to pre-treatments that were followed by comparable treatments with equal cellulase activity, as the final glucose recovery was measured after it. Another limitation was the lignocellulose substrate. Hence only four pre-treatment options were analysed. In order to compare more pre-treatment methods, they would need to be followed by the same enzymatic treatment of cellulose, substrate would need to be consistent. As there are plenty of pre-treatment options available, specific requirements for comparison would mean that most effective way for acquiring all the necessary data for comparison would be by collaboration between multiple research institutions. Additionally, data on costs should be acquired. Despite the laboratory setup cost incongruity to full scale project, data on costs would be beneficial to MCA.

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