CHAPTER 3

Literature review

This chapter is the literature from several papers. The details refer to previous papers which are concerning for pretreatment method, material preparation, toxicity and others which are advantageous to continuous study.

Piyarat Boonsawang (2009) had studied about feasibility of ethanol production from cellulose and hemicellulose of palm fiber by pretreating palm fiber with alkaline. Decaying palm fiber in reducing sugar and ethanol fermentation effect to experiment with palm fiber, trailing by pretreating palm fiber with any solvent such as 10% NaOH boiled at 100 ^{o}C. Comparing with using 10% NaOH, combined with microwave and 10% NaOH using along with steaming at 121 ^{o}C for 15 mins long. It was found that fiber pretreatment affected the increasing proportion of cellulose while proportion of hemicellulose and lignin decreased. All these three pretreatments produced the amount of cellulose and hemicellulose as cellulose was equal to 56.5% - 60.4%, hemicellulose was equal to 11.0%-12.2% and lignin was equal to 18.6%-20.4%, when pretreated fiber was decayed by any methods with enzyme and xylenes resulted in palm fiber pretreating with alkaline and boiled at 100 ^{o}C are the best proper method and state for degradation was enzyme using cellulose 8 FPU/g. Initial substances combined with xylenes enzyme 6 U/g. Initial substances were ripened at 40 ^{o}C. The experiment found that the great amount of sugar in the third date was equal to 12.845 g/lL. After that trailing to produce ethanol from palm fiber hydrolysate liquid that made from palm fiber degradation with Rhizopus oryzae STR 3099, Saccharomyces cerevisiae TISTR 5055 and Candida shehate TISTR 5834 at shaking state 150 rpm. and without shaking. Besides that remaining to study the feasibilities of prehydrolysate liquid made from palm fiber. It was found that this did not have efficiency for using in ethanol industrial production because of ethanol product was in small quantity. All these Microorganisms

inhibited substance in prehydrolysate liquid and when using pretreated palm fiber then decaying combined with fermented ethanol by using Mucor indicus STR 3237, M. hiemalis STR 3047 and R. oryzae STR 3099 found R. oryzae STR 3099 effected to quantity of Ethanol was the highest without shaking within 24 hrs. of fermentation was equal to 0.24 g/l.

Teeraphat Srinorrakrut et al. (2008) studied about proper conditions for sugar production from top and leaf of sugar cane for producing ethanol. Tops and leaves of sugar cane used in this research are composed of cellulose and hemicellulose equaled to 38% and 30.6% by dried weight respectively and lignin quantity was quite as little as 12.8%, with dried weight made the tops and leaves of sugar cane were not hard and easy for downsizing process. Sugar production process from the top and leaf consisted of materials reduction. To pretreat using chemical process combined with heating and Hydrolysis with enzyme, from experiment got the proper states for sugar production as the following; sizes of the top and leaf of sugar cane were ranging from 0-10 mm., quantity 14% with the weight per volume of preparing with concentrated sulfuric acid 1.5% by weight per volume, heated by autoclave sterilization at steam pressure at the temperature 121 ^{o}C and pressure was 15 psi. for 15 mins long. After that, adjusted pH of substrate which made from pretreatment was 5 and hydrolysis with accellerase enzyme 1000 quantity 40 FPU/g, Substrate at 50 ^{o}C. Shaking rank was at 160 rpm. It was found that reduced sugar was equal to 59 g/l or 386.38 mg per substrate, accounted an efficiency of hemicellulose degradation to be sugar was equal to 50.69% during 12 hrs only.

Jirasak Kongkiatkajorn (2008) had developed the increasing products of bioethanol by fermentation process from scrap materials made of cassava and water hyacinth. The purpose of this research is to produce ethanol by converting it to be sugar before fermentation process and process of changing to be sugar together with fermenting water hyacinth and cassava. Water hyacinth leaves and roots were grinded finely and then concentrated sulfuric acid 0.1M was added by steam baking and decaying with enzyme. Glucose sugar and reduce sugar in hydrolysis were 5.81 g/l and 8.52 g/l respectively while glucose sugar and reduce sugar in hydrolysis of water hyacinth root was equal to 4.83 g/l and 5.99 g/l respectively. Ethanol production by SHF process form leaf and root of water hyacinth by yeast mixed between S. cerevisiae TISTR 5048 and C. tropicalis

TISTR 5045, greatest produced ethanol. From studying the production of ethanol by SSF method from root and leaf of water hyacinth, it was found that S. cerevisiae KM 1195 produced ethanol per substrate weight and highest ethanol product. From studying by SSF method from all parts of Water hyacinth, it was also found that S. cerevisiae TISTR 5048 and C. tropicalis TISTR 5045 produced Ethanol per substrate weight and quality of Ethanol was equal to 0.19 g substrate 2.81 g/l respectively. To ferment by SHF method, it was found that cassava leaf and stem use S. cevisiae KM1195 yeast highest rank to produce ethanol per hour and to use cassava stem for producing Ethanol product higher than cassava leaf 2 times on average. From studying ethanol produced by SSF method from cassava leaf, S. cerevisiae KM1195 provided ethanol per substrate weight and highest quantity of ethanol product. From studying ethanol produced by SSF method from cassava stem, it was found that to use S.cerevisiae KM1195 mono yeast produced ethanol per substrate weight and highest quantity of ethanol product. From experiment found that cassava stem using for Ethanol producing by SSF method produced much more than cassava leaf using to be substrate 2 times on average, to use S.cerevisiae KM1195 mono yeast produced highest Ethanol both with cassava and water hyacinth.

Phanwilai Gingsuwannarat (2002) performed the experiment regarding usability of dried cassava root and other agriculture scraps for producing ethanol. Drying cassava rootstock has been degraded to reducing sugar and then fermented to be ethanol, with 3 processes as following; 1) Pretreating cassava rootstock with NaOH, 2) Cassava rootstock degradation, and 3) Ethanol production. From the experiment, it was found that in NaOH solution pretreatment process would dissolve bonds of molecule between lignin and cellulose, effected to lignin and hemicellulose had burst from cassava rootstock and helped cellulose fiber had been swelled. This caused more diffusion of enzyme into cell wall when concentration of hydroxide was increasing so it could dissolve hemicellulose and lignin more. All these proper states were concentration of NaOH solution as 2.0 M when at the temperature which boiled solution was increasing. It affects NaOH solution to be able to spread into Cellulose structures faster. This made forming and swelling rapidly. Thus, there could be more dissolved and separated hemicellulose and lignin by the best proper state was at the temperature 50 ^{o}C. Concentration of NaOH affected to lignin and hemicellulose, separating more results than from boiling temperatures. The best proper state was when cassava rootstock was grinded and drained through small

colander size 0.25 mm, then dipped into sodium hydroxide solution concentrated 2.0 M for 24 hrs long, then filtered the solution then added again as ratio of cassava rootstock weight and solution equaled to 1 per 10 then it was boiled at 50 ^{o}C for 90 mins long and produced cellulose 96.46% hemicellulose 1.85% and lignin. For the cellulose degradation in cassava rootstock, the proper state for cellulose degradation in cassava rootstock with Celebrex enzyme was found at the best proper condition was when the cellulose of pretreated cassava rootstock was reacted in citrate buffer solution that was pH is equal to 4.8 and enzyme quantity was equal to 4.079 FPU/g. Cassava rootstock reacted at temperature 50 ^{o}C for 24 hrs and then comparative study of decaying cellulose in wet cassava rootstock would produce reducing sugar more than dried cassava rootstock because Cellulose fiber in wet and swelled cassava rootstock affected enzyme spreading more into cell wall.

Raweewan Kleawkla (1995) had studied Ethanol production from rice straw, the purpose was to take cellulose from rice straw to be decayed to be reducing sugar and then was fermented to be ethanol. In ethanol production has 3 processes 1) rice straw pretreatment, 2) rice straw cellulose degradation, and 3) ethanol production. From the study, it is found that the first process, pretreatment with dipping the straw in concentrated NaOH solution 2.0 M for 24 hrs and then boiled at temperature 70 ^{o}C for 90 mins was the best method.This would produce straw sediment which was cellulose 94.46%, hemicellulose 1.24% and lignin 2.16% while was not passed pretreated process would cellulose 59.47%, hemicellulose 4.31% and lignin 21.73%. The second process, decayed straw with Lulase enzyme with cellulose was equal 500 µl/g. Dried weight cellulose in sodium acetate solution concentrated 0.05 M at pH was equal 4.0, temperature 55 ^{o}C for 16 hrs degradation-long, could producing reduce sugar 557.07 mg which averaged the changing value with cellulose quantity was equal to 49.58% and the third process by taking producing reduce sugar was to add nutrient and adjusted PH rank to be proper rank for Saccharomyces cerevisiae yeast growing, species TISTR 5013 and then had fermented at temperate room in non-Oxygen state for 4 days. This would produce concentrated Ethanol 1.3% by volume which could be more concentrated when taking extract.

Kittiya Main (2009) had produced ethanol by using dried tectona grandis L.f., that had been decayed by crude enzyme from Bacillus sp. CM12 in order to produce reducing sugar. when did dipping test the leaves with acid before bacteria degradation found that lactic acid decayed cellulose most to be reducing sugar, when cultured bacillus sp. CM12 through leaf medium that was ripped with 1% (v/v) lactic acid. It was found that it had cellulose degradation to be a bit reducing sugar, when fermented reducing sugar to be ethanol with saccharomyces cerevisiae, found that cell weight fermenting crowded 1% (w/v) and 2% (w/v) as maximum ethanol 0.75% (v/v) and 0.74% (v/v) respectively. However, could find that dried tectona grandis L.f., leaf using as initial substance produced ethanol depreciably, process developing was in need.

Sun and group (1995) said that NaOH and lithium hydroxide ($Li(OH)_2$) solution preparation has more efficiency to decompose lignin and hemicellulose more than potassium hydroxide(KOH) preparation. For NaOH solution preparation, when extended the time of preparation to 144 hrs. This was able to decompose hemicellulose and lignin reached to 82.6% and 59.2% respectively. Concentration of NaOH solution at 1.5% and increases the temperature from 20 ^{o}C to be 40, 60 and 80 ^{o}C could decompose lignin 34.2%, 53.8% and 63.2% respectively and could decomposed hemicellulose 65.2%, 74.7% and 75.5% respectively. The time for all experiment is within 6 hrs. It could be found that hemicellulose was decomposed at higher temperature and number of lignin could be decomposed higher but to add more concentrated of NaOH solution (0.5 to 10%) would be more hemicellulose decomposition.

Krishna and group (1998) found out the proper states for hydrolysis of sugar cane leaf with cellulose enzyme that was pretreated by alkaline peroxide. It was found that the temperature 50 ^{o}C was the proper temperature for hydrolysis with cellulose enzyme but the temperature increase to 60 and 65 ^{o}C would affect to reducing sugar because of the too much temperature affected to enzyme lose condition in hydrolysis activities. After that proper pH studying for hydrolysis among 3.5-6.0 resulted the proper pH was 4.5 and could see the pH among 3.5-5.5 would be not much similar production but pH at 6.0 would reduce product obviously. Because fission at proper positions as catching up initial substance were up to pH rank, for pH 6.0 would affect enzyme was not fissionable thus could catch up initial substance badly.

Shinzo Yokota and group (2006) studied the way for lignin removal from the cell walls with the combined methods of both ozone oxidation and dioxane-water extraction using thin part of wood to determine the appropriateness for the production of recyclable cellulose-based materials. From the experiment, the visible-light absorption spectra of the treated wood parts showed that the lignin removal from the cell walls with ozone increased along with increasing time of ozonization process. Lignin removal using ozone began from the lumen side to the middle lamella within the secondary cell wall, and this process could be accelerated in early wood than in late wood. It appeared that mild ozonization for 10 to 30 mins was sufficient for the lignin removal from the cell wall when some parts were extracted with dioxane after ozonization process. It can be demonstrated that using both Microspectrometry and Wiesner reaction is beneficial for the qualitative analysis of lignin in the cell walls.

Carlo Bonini and group (2007) studied about method of steam explosion using corn stalks along with 3% H_2SO_4 at 200 ^oC for 5 mins can give the highest lignin recovery rate. Apparently, lignin has M_W = 2640 and M_Z = 93,994 in the UV spectrum absorption rate at λ = 231 and 280 nm respectively. ^1H NMR spectrum of lignin show signals that are attributable to cinnamaldehyde, guaiacyl and syringyl units. Particularly, syringyl and guaiacyl units are in the proportion of 1:1. ^{13}C NMR spectrum also showed signals units for guaiacyl, syringyl and p-hydroxyphenyl. The spectrum illustrated a prevalence guaiacyl units. The ^{13}C NMR spectrum is in accordance with the existence of cinnamic units. The same characterization was performed on lignin removed from pine. The irradiation of lignin from pine derived from steam explosion at the existence of oxygen, in conditions described for the formation of superoxidide ion, for different irradiation time was followed by isolating lignin and determining the average molecular weight. The experiments showed that not until 8 hrs irradiation, the value of M_N decreases, while M_W and M_Z increase. After 8 hours of irradiation which inverse behavior was inspected with an increase of M_N and a decrease of M_W and M_Z. These results are according to a process of initial polymerization followed by a photo induced degradation. Ozonization process was conducted in acetonitrile-methanol solution. The reaction found here showed a zero-order kinetics. After 50 mins the average molecular weight of the linen is halved. For this, the reaction mixture was analyzed by using GC-MS while oxalic acid was determined.

M. Teresa and group (2008) In this study they try to experiment that wheat and rye straws were pretreated with ozone to increase the enzymatic hydrolysis extent of potentially fermentable sugars. Through a 2^{5-1} factorial design, this work studies of five operating parameters which are moisture content, particle size, ozone concentration, type of biomass and air/zone flow rate on ozonization pretreatment of straw in a fixed bed reactor under room conditions. The acid insoluble lignin content of the biomass was reduced in all experiments involving the degradation of hemicellulose. Near negligible losses of cellulose were inspected. Yields of enzymatic hydrolysis are up to 88.6% and 57% compared to 29% and 16% in non-ozonated wheat and rye straw. Moisture content and type of biomass revealed the most significant effects on ozonolysis. Additionally ozonolysis experiments in basic medium with sodium hydroxide evidenced a reduction in solubilization and/or degradation of lignin, reliable cellulose and hemicellulose.

Puneet Dwivedi and group (2009) It can be seen that there were some details of existing conversion technologies for production of cellulosic ethanol. Both hydrolysis and thermochemical methods have been discussed with their adoption status. Furthermore, economies of ethanol production by using various conversion technologies has been investigated. Emerging conversion technologies and other development which might affect the production of cellulosic ethanol are also characterized. Based on the current estimates, it was found that about 400 million gallons of cellulosic ethanol will be produced in the country in years to come using different kinds of conversion technologies. It was noticed that out of several available conversion technologies, thermochemical-based technologies are gaining high popularity and it is projected that the use of conversion technologies will reduce the production of cellulosic ethanol cost significantly. Similarly, recent advancements in hydrolysis-based technologies have also assisted in reducing the production cost of cellulosic ethanol. Moreover, more resources will be required in the coming years to meet the policy goal of producing 21 billion gallons of cellulosic ethanol by the year 2022.

M. Teresa and group (2011) In this research they studied the chemical pretreatment of ozone rye and wheat straw was conducted in a fixed bed reactor. The effect of ozone pretreatment time on lignin removal was already determined. Glucose and xylose concentrations in the hydrolysates were also measured after the subsequent enzymatic

hydrolysis stage. Acid insoluble lignin reacts with ozone within 90 mins reaction. Insoluble lignin reduction was about 50%. The higher hydrolysis yields were obtained after 120 mins of ozonization process: the glucose yields ranged from 40% to 50% for rye straw and 34% to 39% for wheat straw, while xylose yields were about 30% of the cereal straw. The glucose yields corresponding to the raw straws were considerably lower at about 10%. Longer ozonization time sharply reduced the production of monosaccharides, probably due to the formation of the side products. The kinetic model, with the reaction parameters, estimated predicted reasonably well for the experimental data.

Ujjal Kaur and group (2011) In the present study, milled cotton stalks were subjected to alkali pretreatment stage with NaOH at 1–4% (w/v) concentrations at 121°C for time ranging from 30 to 90 mins. Ozone pretreatment was performed by passing 45 mg/l of ozone gas over 2 mm cotton stalks for 150 mins at a flow rate of 0.37 l/min. The residual 4% alkali pretreatment for 60 mins showed 46.6% lignin degradation accompanied by 83.2% increase in glucan content, compared with the untreated biomass. Hydrolysis of 4% alkali-treated and ozone-treated cotton stalks was conducted using enzyme combination of 20% filter paper cellulase units/gram dried substrate (FPU/g-ds), 45 IU/g-ds β-glucosidace and 15 IU/g-ds pectinase. Enzymatic hydrolysis of alkali-treated and ozone-treated biomass after 48 hrs resulted in 42.29 g/l glucose, 6.82 g/l xylose and 24.13 g/l glucose, 8.3 g/l xylose, respectively. About 99% of glucose was consumed in 24 hrs by Pichia kudriavzevii HOP-1 cells resulting in19.82 g/l of ethanol from alkali-treated cotton stalks and 10.96 g/l of ethanol from ozone-treated cotton stalks. Simultaneous saccharification and fermentation of the alkali-treated cotton stalks after 12 hours pre-hydrolysis resulted in ethanol concentration, ethanol yield on dry biomass basis and ethanol productivity of 19.48 g/l, 0.21 g/g, and 0.41 g/l/h, respectively which hold promise for further scale-up studies. To the best of our knowledge, this is the first study employing SSF for ethanol production from cotton stalks.

Yeoh Shen Yean (2013) The main objectives of the research are to study and optimize the process variables of pineapple waste and palm oil shell. In this study, there are four process variables such as ozonolysis time, moisture content, particle size and ozone concentrations. In addition, SRM technique using Statistica software was employed to predict the optimum condition for pineapple waste and palm oil shell, respectively. Based on the statistical result obtained, moisture content had the most significant effect in affecting lignin degradation of pineapple waste while ozonolysis time and particle size had the most influential effect in affecting lignin degradation of palm oil shell. Pineapple waste obtained experimental lignin degradation of 64.24% with percentage error of 3.65% at the optimum condition of ozonolysis time of 0.75 hr, moisture content of 42%, ozone concentration of 4 mg/L and particle size of 0.63 mm. Meanwhile, palm oil shell obtained lignin degradation of 44.84% with percentage error of 2.3 at condition same as pineapple waste and of 28.81% with percentage error of 56.19%at the optimum condition of ozonolysis time of 2 hours, moisture content of 50%, ozone concentration of 1 mg/l and particle size of 0.5 mm respectively. The huge percentage error at optimum condition of palm oil shell increase because the model prediction is only valid within the experimental range value. In addition, palm oil shell with higher lignin content obtained lower lignin degradation compared to pineapple waste at specific time since palm oil shell requires longer duration of ozonolysis time to obtain high lignin degradation.

Cardona Eliana and group (2013) In this study, elephant grass is a cellulosic material with high potential for ethanol production in tropical nations because of their high rate of availability and adaptability. For this research, chemical and physicochemical pretreatments like lignin removal using alkaline, diluted acid hydrolysis, steam explosion, alkaline peroxide, and aqueous ammonia soaking were implemented in order to determine their effect on the hydrolysis and the fermentability of the cellulosic fraction of this material. In an initial screening of the methods, the alkaline pretreatment with NaOH yields the highest concentrations of reducing sugars (34.4 g/l) and ethanol (15.1 g/l). A more detailed study of the effect of the alkaline pretreatment conditions (temperature, solid to liquid ratio, NaOH concentrations and residence time) on the fermentability of elephants grass was conducted. Results showed that under pretreatment conditions of 120 ^{o}C for 1 hr with 2 wt% NaOH and a solid to liquid ratio of 1:20 (weight) the highest yield of ethanol was obtained such as 26.1 g/l (141.5 mg ethanol/g dry biomass, 95 % of

theoretical yield). Furthermore, this pretreatment allowed the removal of most of the existing lignin in this material. That is, 88% lignin removal. Besides, this pretreatment allowed a high recovery of the cellulosic fraction in the solid.

Weakley and Owens (1975) experimented with the application of ozone as a pretreatment method for low quality roughages. Wood hemicellulose, alfalfa hay, and a low quality range forage were treated with 4.5% ozone for 2-4 hrs. The in vitro dry matter digestibility of the low quality range forage was significantly improved with the ozone treatment, however the alfalfa hay was only slightly improved, and the digestibility of the wood hemicellulose was depressed with ozone treatment. It was particularly noted that no toxic compounds were produced by ozonation of roughages.

Ben-Ghedalia and group (1980) treated cotton stalks with ozone, NH_4OH and a combination of the two treatments. Cotton stalks, which were classified as "woody" in structure, responded well to ozonation with a 50% reduction in lignin content note. In vitro dry matter disappearance was increased 100% with ozone treatment, and 120% with the hydroxide-ozone combination. Cotton stalks were only improved 20% with NH_4OH treatment alone. Ben-Ghedalia and Miron (1981b) treated wheat straw with 5% NaOH, ozone and 5% sulfur dioxide (70º C for 72 hours). The straw was ozonated until it had a bleached appearance. In vitro organic matter disappearance was increased from 44% to 80% with sulfur dioxide treatment, and from 44% to 66% for ozone and NaOH treatments. Large increases in the percent of reducing sugars were also noted with sulfur dioxide and ozone treatments. Reducing sugar was increased from 2.2% to 15.6% and 24.3% by ozone and sulfur dioxide, respectively.

Tock and group (1982) found that ozone could be successfully used to improve the nutritious value of ground mesquite, increasing the in vitro dry matter disappearance of ground mesquite nearly 100% with ozone treatment. It was also noted that ozone treatment produced no toxic chemical residues, giving it a feeding advantage over other chemical treatment methods. Runte, et al. (1981) included ozone treated mesquite as 10% of a lamb ration, with no significant depression in performance relative to controls.

Jiangning Wu et al. (2013) tested Ozonation as a pretreatment method for enhanced biohydrogen production from wheat straw. The more increase in the amount of ozone dose applied, the more delignification increases. This clearly shows that ozone pretreatment method degraded lignin from wheat straw significantly. Then we conducted both enzyme hydrolysis and dark fermentation experiment simultaneously using a mixed anaerobic consortium. It is shown that ozone pretreatment considerably increased biohydrogen production.

Christopher J. Chuck et al. (2013) applied ozonolysis with ethanol to depolymerise lignin. This results in a low conversion of oxygenated aromatics over short reaction times, or a range of saturated esters over 24 hours. To improve the fuel properties, short chain oxygenates can be added in the fuel to lower the percentage of hydrocarbon.

Le Duy Khuong et al. (2014) studied the method for optimizing alkaline pretreatment process of sugarcane bagasse using a fungus called Phlebia sp. MG-60. From the study, the amount of cellulase and xylanase productions from this fungus rose rapidly over 120 hours. When Phlebia sp. MG-60 was cultured with 20 grams of L-1 of sugarcane bagasse pretreated with NaOH in the amount of 0.8 wt% at the temperature of 121 degrees Celsius for 60 minutes, 4.5 grams L-1 ethanol was produced. This is equivalent to 210 milligrams of ethanol and 1 gram of untreated bagasse after 240 hours of fermentation. This clearly shows that Phlebia sp. MG-60 is a suitable for ethanol production.

Nur Izyan Wan Azelee et al. (2014) focus on getting rid of a major part of the lignin layer from kenaf , at the same time, preserving the majority of the hemicellulose. They adopted a two-stage pretreatment process using calcium hydroxide, Ca(OH)2, and peracetic acid, PPA to break the recalcitrant lignin layer from other structural polysaccharides. To enable an optimally designed pretreatment process for kenaf, an experimental screening of several pretreatment chemicals, concentrations, temperatures and solid–liquid ratios is required.

Sujala Bhattarai et al. (2015) studied the change in lignin concentration with a range of ozonolysis time from 0 – 60 minutes. It is suitable for two types of kinetic models. The first one is a model developed by Garcia-Cubero et al. (2012) and the second one includes an outer mass transfer barrier or ‘‘cuticle’’ region where ozone mass transport decreased in proportion to the mass of unreacted insoluble lignin in the cuticle. They determined The kinetic parameters of two mathematical models for predicting the soluble and insoluble lignin at different pretreatment time from the result, it can be seen that the parameters derived from the cuticle-based model provided a better suitability to experimental results compared to a model without a cuticle layer.

Mehdi Jonoobi et al. (2016) aimed to investigate the mechanical and physical properties of particleboard panels made of bagasse, which were treated or untreated with ozone. For mechanical properties, modulus of elasticity (MOE), modulus of rupture (MOR), and internal bonding strength (IBS) were tested. For physical properties, water absorption (WA) and thickness swelling (TS) were tested. All tests are conducted according to EN standards. From the overall results, it is shown that all panels made of treated bagasse surpassed the EN standards for MOE, MOR, and IBS but WA and TS values were reduced after ozone pretreatment compared with the untreated panels. Duncan’s Multiple Range Test was also applied. Hence, the effects of both types of variables, excluding their interactions, on the mechanical and physical properties were very noteworthy.

Rodolfo Travaini et al. (2016) applied orthogonal array to study the four most important parameters in the ozonolysis pretreatment (moisture content, ozone concentration, ozone/oxygen flow and particle size) on production of ethanol from sugarcane bagasse. From statistical analysis, ozone concentration has the highest influence parameter on reaction time and sugars release after the process of enzymatic hydrolysis. There is an increase on reaction time and a decrease on ozone/oxygen flow. This could result in slight differences of ozone consumption.

Olavo Micali Perrone et al. (2016) treated Sugarcane bagasse in three following stages; ozone oxidation (O), washing in an alkaline medium (B) and ultrasonic irradiation (U). It was then evaluated by its chemical composition using an infrared technique (FTIR-ATR) and thermogravimetric analysis (TGA/DTG). From the result, the pretreatment sequence O, B and U significantly resulted in a reduction of lignin and hemicellulose.

Iliana Barrera-Martínez et al. (2016) aimed to study effects on solubilization of lignin using ozonification process pretreatment of Alkaline lignin (AkL) and sugarcane bagasse (SCB) as well as acid and enzymatic saccharification. Clearly, AkL suffered oxidation after 60 minutes and soluble lignin from SCB was observed within 30 minutes of the ozonification reaction. The total (TS) and reducing sugars (RS) produced from ozonized SCB in acid hydrolysis were 30.83 and 27.17%; while enzymatic hydrolysis resulted in 18.41 and 13.43% after 120 minutes of ozonation.