RAMA UNIVERSITY

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FACULTY OF ENGINEERING & TECHNOLOGY

Processing and pretreatment of raw materials used for industrial fermentation:

Physical pretreatments
Mechanical comminuting

▪The objective of the mechanical pretreatment is to reduce the particle size and crystallinity to increase the specific surface area.

▪The size of feedstock materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding.

▪Different milling processes (two-roll milling, hammer milling, colloid milling and vibratory milling) can be used to improve the digestibility of the lignocellulosic materials compared to ordinary ball milling. This process is generally not economically feasible because of high energy consumption for obtaining desired particle size.

Extrusion
Extrusion process has been used to produce gaseous products and residual char. In this process, materials are treated at a temperature higher than 300 °C followed by mixing and shearing which results in physical and chemical modifications of cellulose.

▪The screw speed and barrel temperature are believed to disrupt the lignocellulosic structure causing defibrillation, fibrillation and shortening of the fibers, followed by increased arability of carbohydrates to enzymatic attack.

▪The various parameters in bioreactor must be highly efficient in this process. In recent study, the application of enzymes during extrusion process is considered as a novel technology for ethanol production.

Physico-chemical pretreatments

Steam explosion (autohydrolysis)

▪Steam explosion is the most commonly used method for pretreatment of lignocellulosic biomass. In this method, the chipped biomass is treated to high pressure saturated steam for few seconds (30 s) to several minutes (20 min), and then pressure is suddenly reduced.

▪Steam explosion is typically a combination of mechanical forces and chemical effects due to the hydrolysis (autohydrolysis) of acetyl groups of hemicellulose. Autohydrolysis is initiated at high temperatures (160–260 °C) which promote the formation of acetic acid from acetyl groups.

- The advantage of steam explosion pretreatment also includes the possibility of using larger chip size, avoiding unnecessary addition of acid catalyst (except for softwoods), and its feasibility at industrial scale.

- Steam explosion is recognized as one of the most effective processes for cost reduction in hardwoods and agricultural residues.

- The main drawbacks of steam explosion pretreatment are the partial degradation of hemicelluloses and the formation of toxic components that could affect the enzymatic hydrolysis and fermentation process.

- Another drawback is the energy consumption for obtaining final chip size before pretreatment which can make up one-third of the power requirement of entire process.

Liquid hot water

▪Liquid hot water is one of the hydrothermal pretreatment which does not require rapid decompression and the addition of any catalyst or chemicals. Water pretreatment under high pressure is used to maintain the water in the liquid state at elevated temperatures.

•Temperature range between 170 and 230 °C and pressure (>5 MPa) are commonly used. Liquid hot water removes hemicellulose from lignocellulosic materials which makes the cellulose more accessible.

•The obtained slurry after pretreatment can be filtered to obtain two fractions: one solid cellulose-enriched fraction and a liquid fraction rich in hemicellulose derived sugars.

•This pretreatment allows better pH control (4–7) which minimizes the non-specific degradation of polysaccharides and also avoids formation of inhibitors. Liquid hot water has shown the potential to release high fraction of hemicellulosic sugars mostly in the form of oligomers contributing to reduce the undesired degrading products.

- In general, liquid hot water pretreatments are attractive from a cost savings poiIt was reported that temperature and time showed the most significant effect on the recovery of hemicellulosic sugars and the yield of subsequent enzymatic hydrolysis of pretreated corn stover, sugarcane bagasse and wheat straw.

- Three methods have also been developed to promote an effective contact between the biomass and the liquid water: co-current, countercurrent, and flow-through. In co-current pretreatments, slurry of biomass and water is heated to the desired temperature and held at the pretreatment conditions for controlled residence time before being cooled.

- Countercurrent pretreatment is designed to move water opposite to lignocellulose through the pretreatment system.

- Flow-through system allows hot water passage over a stationary bed of lignocelluloses; which hydrolyzes and dissolves lignocellulose components and carries them out of the system of view because no chemicals and corrosion-resistant materials are required for hydrolysis reactors.

•Another major advantage is that the solubilized hemicellulose and lignin products are present in lower concentration due to high water input.

• Higher pentose recovery and lower formation of inhibitory components are obtained in this pretreatment compared to steam explosion. However, this process is yet not developed at commercial scale because of higher water demand and high energy requirement.

Ammonia-based pretreatments

•Ammonia fiber explosion (*AFEX*) *process* is another type of physico-chemical pretreatment in which lignocellulosic biomass is treated with liquid ammonia at relatively moderate temperature (90–100 °C) for a period of 30–60 min. followed by a rapid pressure release.

• It results in a rapid expansion of the liquid ammonia that causes swelling and physical disruption of biomass fibers and partial decrystallization of cellulose. AFEX produces only a pretreated solid material. AFEX process can either modify or effectively reduce cellulose crystallinity and lignin fraction of the lignocellulosic materials. ..

•AFEX increases the digestibility of lignocellulosic biomass by removing the least acetyl groups by deacetylation process

•The main advantage of the ammonia pretreatment is that it does not produce inhibitors for the downstream biological processes, so water wash is not necessary. The herbaceous and agricultural residues are more effective for AFEX pretreatment, with limited effectiveness on woody biomass and other high lignin feedstocks demonstrated that AFEX pretreated corn grain yielded 1.5–3.0 folds higher enzymatic hydrolysis compared to untreated substrates.

•Sequential addition of cellulases after hydrolysis of starch resulted in 15–20 % higher hydrolysis yield compared to simultaneous addition of hydrolytic enzymes. AFEX pretreated corn stover resulted in 70 % glucan conversion after 72 h of hydrolysis. Ethanol fermentation of AFEX treated (at 6 % w/w glucan loading) corn stover resulted in 93 % ethanol yield optimized the conditions such as ammonia loading, temperature, blowdown pressure, moisture content of biomass and residence time in the AFEX process.

• It has been observed that at optimal conditions, AFEX can achieve over 90 % conversion of cellulose and hemicellulose to fermentable sugars for a broad variety of lignocellulosic materials. The high volatility of ammonia allows it to be recovered and recycled, leaving the dried biomass ready for enzymatic hydrolysis.

•The main disadvantage of AFEX process is that it is more effective on the biomass that contains less lignin. Furthermore, ammonia must be recycled after the pretreatment to reduce the cost and protect the environment. The cost of ammonia recovery may be significant regarding the commercial potential of the AFEX pretreatment.

CO_2 explosion

This method is based on the utilization of CO_2 as a supercritical fluid in which fluid displays gas like mass transfer properties besides a liquid-like solvating power. Supercritical pretreatment conditions can effectively remove lignin by increasing enzymatic digestibility of aspen (hardwood) and southern yellow pine (softwood).

- The delignification with CO_2 (SC-CO_2) at high pressure can be improved by the addition of co-solvents such as ethanol. Supercritical CO_2 has been mostly used as an extraction solvent for non-extractive purposes due to its several advantages such as availability at relatively low cost, non-toxicity, non-flammability, easy recovery after extraction, and the environmental acceptability.
- In aqueous solution, CO_2 forms carbonic acid and increases hydrolysis rate. The size of CO_2 molecules should be comparable to water and ammonia because CO_2molecules can penetrate small pores accessible to water and ammonia molecules.

- In this pretreatment, disruption of cellulose and hemicellulose structure occurs and consequently accessible surface area of the substrate to enzymatic attack increases.
- The comparison of CO_2 explosion with steam and ammonia expansion pretreatment methods on several substrates showed that CO_2 explosion was more cost-effective than ammonia expansion and the formation of inhibitors was lower compared to steam explosion.

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Oxidative pretreatment

- Oxidative pretreatment involves the addition of an oxidizing agent such as H_2O_2 or peracetic acid ($C_2H_4O_3$) to the water-suspended biomass.

- H_2O_2 is the most commonly used oxidizing agent. Studies have shown that dissolution of about 50 % of lignin and most of the hemicellulose has been achieved in a solution of 1–2 % H_2O_2 at 25–30 °C .

- This solubilization is generally five folds higher than those of sodium hydroxide (NaOH) treatment without H_2O_2 addition. This pretreatment method removes hemicellulose and lignin from biomass to increase accessibility to the cellulose.

- Several reactions like electrophilic substitution, displacement of side chains, cleavage of alkyl/aryl ether linkages or the oxidative cleavage of aromatic nuclei can occur during this pretreatment. It has been observed that diluted alkaline peroxide treatment is an effective method for pretreatment of rice hulls, resulting in almost complete conversion (96 %) of rice hulls to sugars after enzymatic hydrolysis.

Wet oxidation

- Wet oxidation is considered as a suitable process for pretreatment of biomass having high lignin content. In this process, materials are treated with water and air/oxygen at temperatures higher than 120 °C for 30 min.

- The temperature, reaction time and oxygen pressure are the most effective parameters in wet oxidation.

- The addition of oxygen at temperatures higher than 170 °C makes the process exothermic, and it becomes self-supporting system with respect to heat. The wet oxidation pretreatment catalyzes the formation of acids from hydrolytic processes and oxidative reactions.

- All three fractions of lignocellulosic materials are affected in this process. The hemicelluloses are extensively cleaved to low molecular weight sugars that become soluble in water.

- Lignin undergoes cleavage and oxidation, and cellulose is partly degraded. The cellulose becomes highly susceptible to enzymatic hydrolysis. However, addition of some alkaline agent such as sodium carbonate may help to solubilize hemicellulose fraction and also minimizes the formation of furan-based degradation products that could inhibit enzymes.

Microwave pretreatment

- Microwave irradiation is a process which has been widely used because of its high heating efficiency and easy operation. The residence time in microwave irradiation ranges from 5 to 20 min.

- It could change the ultra structure of cellulose by degrading lignin and hemicelluloses and by increasing the enzymatic susceptibility of lignocellulosic materials. Preliminary experiments identified alkali-treated rice straw as suitable biomass for microwave-based pretreatment.

- NaOH is the most effective alkali reagent for microwave-based pretreatment. One of the studies on microwave-based alkali pretreatment of switchgrass observed the low energy requirement for extended pretreatment time and obtained 70–90 % sugar yields.

- The main advantage of this process is the short reaction times and homogeneous heating of the reaction mixture. Microwave-assisted pretreatment of biomass could be a useful process to save time, energy and minimum generation of inhibitors. It could be considered as one of the most promising pretreatment methods to change the native structure of cellulose with lignin and hemicelluloses degradation, and thus increasing the enzymatic susceptibility. Microwave approach could be further combined with the addition of chemicals to improve the sugar yield from the substrate.

Chemical pretreatments

Acid pretreatment

▪The main objective of the acid pretreatment is chemical hydrolysis which can cause solubilization of hemicelluloses and lignin, and to make the cellulose more accessible to enzymes.

▪Acid pretreatment technologies can be performed with concentrated or diluted acid but use of concentrated acid is less attractive due to the formation of inhibiting compounds (furfural, 5-hydroxymethylfurfural, phenolic acids and aldehydes). Concentrated acids are toxic, corrosive, hazardous, and require equipment that is resistant to corrosion.

▪Diluted acid pretreatment method is the most feasible for industrial scale. Different types of reactors such as percolation, plug flow, shrinking-bed, batch, flow-through reactor and countercurrent reactors have been developed for this approach.

▪There are two types of dilute acid pretreatment processes: high temperature (e.g., 180 °C) during a short period of time and lower temperature (e.g., 120 °C) for longer retention time (30–90 min).

- High hydrolysis yields have been reported with dilute H_2SO_4 which is also the most widely used acid. However, use of hydrochloric acid (HCl), phosphoric acid, nitric acid, $C_2H_4O_3$, oxalic acid, formic acid, acetic acid and maleic acid has also been tested.

- Oxalic acid treatment in corn cobs produced low level of inhibitors with a total sugar yield of 13.1 %; while it was 10 % in maleic acid treatment with the generation of higher levels of furfural and hydroxymethylfurfural. Acid pretreatment of biomass could be inexpensive because H_2SO_4 and HCl are cheap

- The process is carried out at high temperatures, and therefore, it requires high energy input, which is costly.

- The presence of acids at high temperatures can be corrosive, thus, the process requires specific reaction vessels which must be resistant to these conditions. In addition, acid treatment generates inhibitors which need to be removed.

Alkali pretreatments

- In the alkaline treatment, biomass is treated with alkali such as sodium, potassium, calcium and ammonium hydroxides at normal temperature and pressure. The main advantage of the process is efficient removal of lignin from the biomass .

- This process removes acetyl and uronic acid groups present on hemicelluloses, thus enhances the accessibility of enzyme that degrades hemicellulose. Ester linkages between xylan and hemicelluloses residues are also hydrolyzed.

- This process can largely improve the cellulose digestibility and it is also more effective for lignin solubilization, exhibiting minor cellulose and hemicellulose solubilization compared to acid pretreatment. Alkali pretreatment can also be operational at lower temperature, pressure and time ranging from hours to days.

- NaOH is more effective than others. It was found to be more effective on increasing the internal surface area of cellulose, decreasing the degree of polymerization and crystallinity, and disrupting the lignin structure.

Lime [$Ca(OH)_2$] is another widely used alkali. It also removes acetyl groups and lignin-carbohydrate ester and enhances cellulose digestibility.

It has been proven successful for pretreatment of wheat straw, poplar wood, switchgrass and corn stover. This pretreatment has the additional benefits of low reagent cost and less safety requirements compared to NaOH or KOH pretreatments and can be easily recovered from hydrolysate by reaction with CO_2 . The addition of air/oxygen to alkaline pretreatment [$NaOH/Ca(OH)_2$] can improve the treatment efficiency by increasing lignin removal. Some researchers have also tried combination of two pretreatment processes for significant recovery of reducing sugars: combination of alkaline treatment (lime) with oxidative delignification process. Although, lime and other hydroxides are inexpensive but downstream processing costs are high, thus making it a costly process. The process also utilizes a huge amount of water for washing salts of calcium and sodium. Moreover, it is difficult to remove them.
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(c)Ozonolysis

•The biomass is treated with ozone (O3) which is a powerful oxidizing agent. It degrades lignin by attacking aromatic rings structures, and does not affect hemicellulose and cellulose.

•It can be used to disrupt the structure of many lignocellulosic materials such as wheat straw, bagasse, pine, peanut, cotton straw, rye straw and poplar sawdust. Ozonolysis is usually performed at room temperature and pressure, and it does not produce toxic residues that can affect the subsequent hydrolysis and fermentation.

•The O3 gas is passed through a reaction vessel containing the substrate. The vessel could be packed beds, fixed beds or stirred semi-batch reactors.

•Moisture content and type of biomass significantly affect ozonolysis. A major drawback of ozonolysis is the requirement of large amounts of O3, making the process expensive.

(d)Organosolv

•Organosolv process uses organic or aqueous organic solvent mixtures with inorganic acid catalysts to extract lignin from lignocellulosic biomass. Numerous organic solvent mixtures including methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol have been used.

•Some organic or aqueous organic solvents like oxalic, acetylsalicylic and salicylic acid can also be used as catalysts at higher temperatures with or without addition of some organic acids. Pretreatment of wheat straw by glycerol-based autocatalytic organosolv pretreatment resulted in removal of 70 % hemicelluloses and 65 % lignin.

•It also resulted in 98 % cellulose retention. A modified organosolv method using ethanol under mild conditions followed by H_2O_2post-treatment in horticultural waste resulted in a hydrolysate containing 26.9 g/L reducing sugar.

•Fermentation of this hydrolysate medium produced 11.69 g/L ethanol using *Saccharomyces cerevisiae* have reported that the application of alcohol-based organosolv treatment in combination with Ball Milling (BM) for pretreatment of Japanese cypress (*Chamaecyparis obtusa*) significantly improved the enzymatic digestibility and decreased the required severity of organosolv treatment.

•It was also observed that the combination of alcohol-based organosolv treatment in mild conditions and short time BM had a synergistic effect on the enzymatic digestibility of Japanese cypress.

•Organosolv process has been extensively used for extraction of high quality lignin which is a value added product. This process has shown high amounts of enzymatic hydrolysis of treated biomass (around 90 %) due to efficient removal of lignin.

•The main drawback of the process is the cost of solvent and the catalysts. Removal and recovery of the solvent can considerably reduce the operational cost.

•Another important aspect is safety measures which have to be implemented because organic solvents are inflammable and uncontrolled use can cause fires and explosions.

•This additional requirement increases the cost of the process. Organic solvents are also the inhibitors of enzymatic hydrolysis, so their removal is necessary for proper enzymatic hydrolysis. Removal of organic solvents also burdens an additional cost.

e) Ionic liquids (ILs)

•This pretreatment process uses ILs in a ratio of biomass and ionic liquid (1:10 w/w) and temperatures ranging from 100 to 150 °C. The antisolvent such as water, methanol and ethanol use the regeneration of soluble biomass and then subject to enzymatic hydrolysis to produce fermentable sugars.

•ILs behaves like salt which is typically a combined effect of large organic cations and small inorganic anions and it exist as liquids at relatively low temperatures (room temperature). ILs have the capability to form hydrogen bonds with cellulose at high temperatures because of the presence of anions like chloride, formate, acetate or alkyl phosphonate.

•ILs have tremendous potential for pretreating lignocellulosic biomass and producing a substrate that can achieve more than 90 % cellulose digestibility.

•Residual ILs remaining in the biomass could interfere with hydrolytic enzyme activities and downstream fermentation steps. It may affect the final sugar and biofuel yields. After regeneration, ILs may be recovered from antisolvents by flash distillation and it could be reused.

•Development of energy efficient recycling methods for ILs is a prerequisite for large-scale application.

•Toxicity to enzymes and fermentative microorganisms must also be considered before their application in biomass pretreatment. Significant negative effect on cellulase activity may also occur in ILs treatment.

•Further research is needed to improve the economics of ILs pretreatment before they can be applied at industrial scale. In addition, techniques need to be developed to recover hemicellulose and lignin from solutions after extraction of cellulose.

•Despite these current limitations, development of ILs pretreatment offers a great potential for future biorefinering processes of lignocelluloses.

Biological pretreatments

Conventional physico-chemical methods for lignin degradation require large inputs of energy and also cause pollution. Therefore, biological pretreatment of lignocellulosic biomass is considered as an efficient, ecofriendly and cheap alternative. The biological pretreatment of lignocellulosic biomass is usually performed using cellulolytic and hemicellulolytic microorganisms.

•The commonly used microorganisms are filamentous fungi which are ubiquitous and can be isolated from the soil, living plants or lignocellulosic waste material.

•Studies have shown that white-rot fungi are the most effective microorganisms for the pretreatment of most of the lignocellulosic materials. Several white-rot fungi such as *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus stercolerus*, *Ceriporiopsis subvermispora*, *Pycnoporuscinnarbarinus*, *Pleurotusostreaus* and *P. chrysosporium*produce lignin peroxidases which is lignin-degrading enzymes and manganese-dependent peroxidases.

•These have shown high delignification efficiency on various lignocellulosic biomasses. An effective delignification of various feedstocks was reported by fungus *Ceriporiopsis subvermispora* in the combined action of manganese peroxidase and laccase .

• A glucose yield of 24.2–56.5 % was reported during enzymatic hydrolysis which was 2–3 folds higher than those of the raw materials.

•Biological pretreatment of rice husks by fungus *Phanerochaete chrysosporium* resulted in 44.7 % reducing sugars.

•Biological treatments of wheat straw by solid state and submerged fermentations in the presence of white-rot basidiomycetes such as *Bjerkandera adusta*, *Fomes fomentarius*, *Ganoderma resinaceum*, *Irpex lacteus*, *Phanerochaete chrysosporium*, *Trametes versicolor*, *Euc*-1 and *Lepista nuda* were evaluated and *T. versicolor* for enzymatic hydrolysis of holocellulose proved better strain compared to others.

•The treatment of hardwood and softwood was also found to be effective with *Streptomyces griseus*.It has been observed that performing saccharification and fermentation processes at high-substrate concentration may increase the concentration of inhibitors (furan derivatives and phenolic compounds).

•Treatment with enzymes such as laccases has been suggested to prevent production of such inhibitors. Some other advantages of biological pretreatments are: low-capital cost, low energy requirement, no chemicals requirement, and mild environmental conditions.

•However, the main drawback to develop biological methods is that the rate of hydrolysis is very low. There is need to keep on testing more and more isolates such as basidiomycetes fungi for their ability to delignify the plant material quickly and efficiently.