Review Paper:

Effective use of cellulose degrading bacteria from vermicompost in converting carbohydrate biomass to alcohol and lactic acid

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Abstract

The current scenario depicting an increase in agricultural waste is a major threat to our environment as most of these wastes are burnt releasing a lot of toxic gases affecting both aquatic and terrestrial life forms alike. However, there is an effective solution to this problem as 90 % of these wastes are composed of lignocellulose, an effective substrate for the production of lactic acid which can be polymerized to bio plastics. Bio plastics composed of polylactic acid act as a green substitute for plastic derived from petrochemicals. Furthermore, it also helps in the production of bioethanol. The conversion of lignocellulose into lactic acid involves several steps including pretreatment of lignocellulose to break down the structure of lignocellulose, degradation of cellulose to fermentable sugars like glucose and its fermentation into lactic acid and finally, polymerization and condensation to polylactic acid i.e. PLA. Microorganisms isolated from environment friendly vermicompost or earthworms can be of immense help during crucial stages of the process.

In particular, cellulose degrading bacteria from earthworms are known to degrade cellulose into fermentable sugars. They can also ferment the glucose into lactic acid. The yield of the lactic acid thus depends on the efficiency of cellulose degrading bacteria and lactic acid bacteria. The yield can also be affected by the pre-treatment of lignocellulose waste, thus the ideal method for each type of lignocellulose waste has to be optimized. This review describes the entire process of conversion of lignocellulose waste into lactic acid with emphasis on the source of cellulose degrading bacteria and lactic acid bacteria, specifically heterolactic bacteria.

Keywords: Lignocellulose waste, Cellulose degrading bacteria, Polylactic acid, Bio plastic.

Introduction

A surplus of agricultural waste poses serious problems of disposal. Between 40 to 50 billion tons of agricultural waste are produced every year. Lignocellulose comprises of 90 % of the total dry matter of agricultural wastes and it is continuously produced. The accumulating waste poses serious problems of disposal, since a major proportion of agricultural waste is not used effectively and results in the accumulation of organic waste. Most of it is either disposed in the open environment unchecked and left to rot near factories or burnt, releasing a lot of CO_2 into the atmosphere posing a risk for pollution and generate considerable harm to the environment. Some of it is shredded, composed and used for improving the quality of soil. There are certain ways to reduce the impact of the accumulating agricultural wastes. One way is to increase the commercial value of the agricultural waste by streamlining it into production of biofuels and biodegradable plastics.

Second generation biofuels and bio plastics: Lignocellulose biomass is the most abundant biopolymer available on earth and is an alternative sustainable energy source for the production of second generation fuels[14]. The cost effective transformation of lignocellulose will be a platform for bio based economy while preserving the biodiversity. It also minimizes the agricultural environmental footprint and conserves fresh water. First generation biofuels and bio plastics are made from sugar feed stock like corn starch. However, using lignocellulose waste from food and other industries cannot just increase the economic value of waste, but also environmentally friendly way of utilization of waste.

Structural organization of Lignocellulose: Lignocellulose, consisting of lignin, cellulose and hemicellulose, is a good source of fermentable sugars, especially cellulose and hemicellulose. The two carbohydrate polymers are cellulose and hemicellulose while lignin is the phenolic polymer. Lignocellulose also consists of traces of pectin. Although lignocellulose predominantly consists of lignin, cellulose and hemicellulose, the composition varies among different plants. The structure of each component of lignocellulose is described in details as follows:

Cellulose, a nontoxic component of the lignocellulose consist of a homopolysaccharide of glucose comprises of 30-60 % of the total feedstock[14]. The linear cellulose polymers called elemental fibrils are grouped together to form cellulose fiber and are generally covered by lignin and hemicellulose. The monomeric glucose molecules are linked by ß 1-4 glycosidic bonds. Cellulose is a rigid structure due to the orientation of the linkages, thus making it difficult to break, unless degraded by strong cellulose degrading enzymes. Furthermore, the cellulose is tightly linked to

lignin and hemicellulose, further making it challenging to isolate the fermentable sugars.

Hemicellulose, comprising a minor portion of the lignocellulose (20 % of the total feed stock), is branched with short lateral chained heteropolymer of pentose sugars, hexose sugars and acid sugars. Hemicelluloses from agricultural residues are mainly composed of xylose (a pentose sugar). Due to its highly branched nature, it is easier to degrade and it forms a gel connecting the cellulose with lignin. Hemicelluloses are imbedded into the paint cell walls and bind the cellulose micro fibrils thereby strengthening the cell walls.

Lignin, an aromatic phenolic polymer comprises of about 10-15 % of the total feed stock and found in all vascular plants. Lignin binds to the cellulose fibers and strengthens the plant cell wall, thus it is highly resistant to chemical and biological degradation. Apart from providing strength and rigidity to the cell walls of plants, it also protects the plant from microbial degradation.[11] Lignin can be made available for its value added products rather than for use as a fuel. It can be used as a stabilizer during the polymerization of lactic acid into bio plastic.

The 3 components are tightly interlinked as illustrated in fig. 1, through strong covalent and non-covalent bonds like ether, ester and hydrogen bonds forming an intricately linked recalcitrant structure[11]. The degradation of lignocellulose is the greatest challenge due to its complex structure. Thus, obtaining the fermentable sugars from the lignocellulose requires efficient pretreatment to separate the lignin from the fermentable sugars like cellulose and hemicellulose. Lignin degradation is inevitable in order to obtain cellulose.

This is followed by enzymatic hydrolysis of the pentose or hexose sugars (glucose)[11]. These sugars can then be fermented to lactic acid, ethanol and several other valuable products. Ethanol can be used as a biofuel, while lactic acid can be polymerized to poly lactic acid (PLA), which can be used as a biodegradable plastic. The non-hydrolysable lignin can be added to the bio plastic as a stabilizer. This ensures maximum value for the agricultural waste.

Bioconversion of lignocellulose waste into lactic acid and alcohol: The conversion of lignocellulose into lactic acid happens in several steps as illustrated in fig. 2. It includes pretreatment of lignocellulose waste, breakdown of cellulose into fermentable pentose or hexose sugars like glucose and fermentation of the glucose obtained into lactic acid and alcohol.

Commercial importance of Lactic acid: Plastics are polymeric materials widely used globally and form an inevitable part of every household. However, they pose serious problems of disposal as they are non-biodegradable and recalcitrant in nature. This resulted in the shift from petrochemical plastics to a more environment friendly biodegradable plastic or bio plastic. The commercial importance of lactic acid has recently been demonstrated due to its possible use as a biodegradable plastic. The economic and environmental challenges have provoked the society to shift from petrochemicals to biodegradable plastics or bio plastics.

Poly lactic acid (PLA), a renewable polymer is known to act as a green substitute for petrochemical plastics. PLA is an aliphatic polyester manufactured chemically by ring opening polymerization of lactose or by polycondensation of lactic acid monomers derived from fermentation.However, this conventional method of PLA synthesis requires a catalyst and thus cannot be suitable for biological systems. Furthermore, since the optical purity of lactic acid is very important, the microbial production of lactic acid is favored.[21]

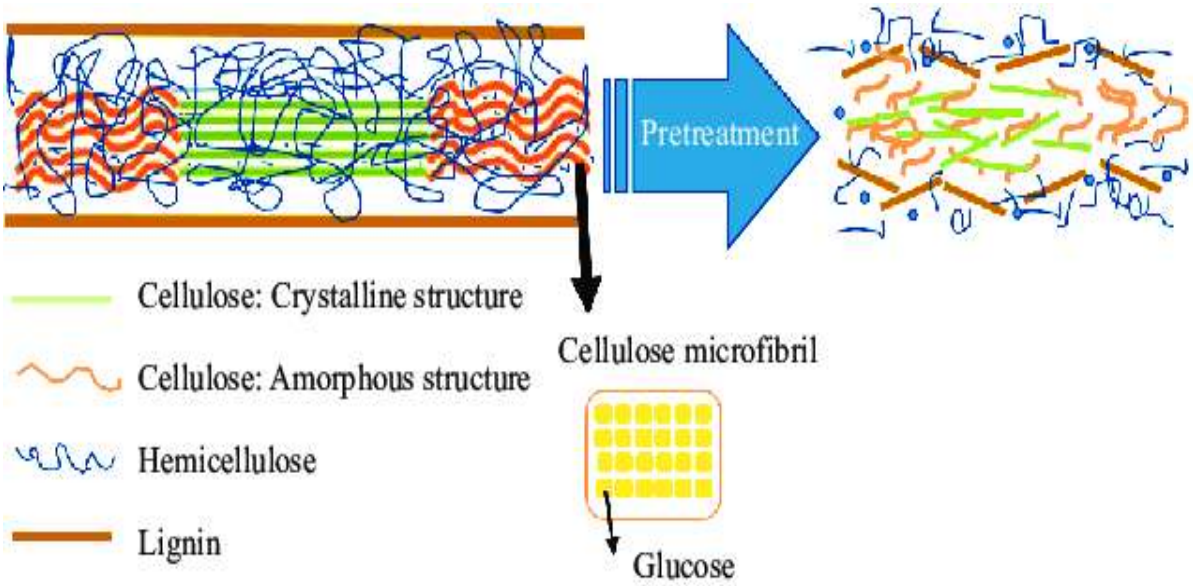

Fig. 1: Lignocellulose structure and the role of pretreatment in lignocellulosic structure.

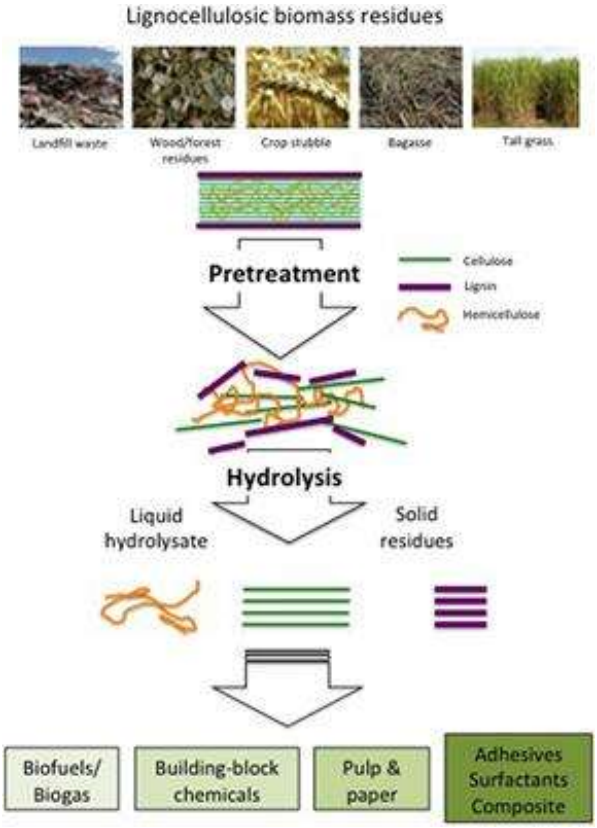

Fig. 2: Conversion process schematics and valorization of lignocellulose biomass residues, such as tall grass, bagasse, rice husks, wood chips and organic landfill waste. Pretreatment of this biomass depolymerizes the complex lignin and cellulosic structures and separate lignin from cellulose and hemicellulose via hydrolysis. Subsequent fermentation and/or chemical treatment converts them into valuable energy and chemical products.

Some strains of microbes can be specifically used for the production of lactic acid. Several reports have claimed the potential of both wild type and engineered bacteria in the production of lactic acid[21]. The lactic acid obtained after fermentation usually has a very high molecular weight and the impurities obtained in the process (mainly cell biomass) can be easily separated from the pure lactic acid. Among these groups of microbes, bacteria belonging to the bacillus species were found to be very effective in attaining high yields of lactic acid without compromising on the optical purity.

Although both bacteria and fungi are capable of producing lactic acid, fungal cultures require aerial conditions and also show slow reaction rate. The bacteria involved in lactic acid fermentation are classified into two groups- Homo fermentative and Hetero fermentative. Most industries use homo fermentative bacteria for the production of lactic acid. The most commonly studied homolactic bacteria is Bacillus *subtilis*[22].

PLA is known to have potential uses in a wide range of applications. They include packaging, medical, agricultural, transportation, building and agricultural. The main advantage of PLA which has encouraged its use in packaging industries is its high strength and bio degradability. PLA can be rapidly degraded into less toxic byproducts which cause less harm to the environment.

Although the production of pure lactic acid was initially done using 1st generation biomass, due to advancements in the optimization conditions for lactic acid fermentation using lignocellulose waste as a substrate, the use of 2nd generation biomass like agricultural wastes for the production of lactic acid is picking up[23].

Works from several laboratories have demonstrated the potential of agricultural wastes or food industrial wastes as a substrate for producing lactic acid. The production of lactic acid from second generation biomass also provides us with an added advantage of increasing the economic value of agricultural waste as demonstrated by a leading technology innovation centre in the UK.

Pretreatment of lignocellulose: The pretreatment of lignocellulose waste is necessary in order to hydrolyze cellulose. This ensures that the hemicellulose and lignin are partially broken down or degraded and also break up the cellulose to ensure maximum surface area for attachment of enzymes. The pretreatment reduces the crystallinity and increases the fraction of amorphous cellulose which is the most suitable form of enzymatic attack.

Types of pretreatment

Several physical, physicochemical and biological methods of pretreatments have been demonstrated by several teams.

Mechanical pretreatment: In most cases, pretreatment of the lignocellulose waste material involves mechanical treatment by grinding, chipping, sheering or milling. This simple technique makes it easier to handle the material and also increases the surface to volume ratio of cellulose. Yet another form of mechanical treatment is ultra sonication. Other useful methods include pyrolysis, irradiation with gamma, microwave and infrared radiation. However, several times, it is necessary to combine mechanical treatment with chemical treatment as well. In fact, mechanical pretreatment is the prerequisite for the other forms of pretreatment. However, one disadvantage of mechanical pretreatment is its high energy consumption, although it also depends on the type of wood/ lignocellulose waste you are using. Some of the most common forms of physical pretreatment are described in detail as follows:

- **Milling**: It reduces the crystallinity and the particle size. It is the simplest form of physical pretreatment. There are different types of milling namely two-roll milling, ball milling, rod milling, hammer milling, vibratory milling, colloid milling and wet disk milling. However, its biggest disadvantages are that the energy consumption is very high. Furthermore, the cost of the equipment used for the milling is very high. Wet disk milling has a comparatively lower energy consumption rate when compared to the other forms of milling[2].

- **Microwave assisted size reduction**: The work on microwave irradiation was first done in the year 1984 and ever since emerged as a convenient method of pretreatment due to its low energy consumption, easy handling and minimum inhibitor formations. The dielectric polarizations causes molecular collisions and generates thermal energy resulting in the disruption of the complex lignocellulose structure. Some teams have used irradiation with UV radiation as an upgrading attempt along with other forms of treatment[2].

- **Ultra sonication**: it is based on the principle of formation of pores or cavities in the sample by employing ultrasonic radiation. It is known to increase the crystallinity of the lignocellulose waste. Although it is a viable pretreatment technique, the process is energy intensive and parameters are yet to be optimized for large scale production.

Chemical pretreatment: The use of several chemicals including strong acids and alkalis to hydrolyze the hemicellulose helps in effective pretreatment of the lignocellulose resulting in the release of fermentable sugars. Several companies have demonstrated the use of dilute acids like H_2SO_4 as a pretreatment for lignocellulose, however, it also generates toxic byproducts and thus, they have to be removed before proceeding. Apart from strong acids, the use of supercritical fluids helps in lignocellulose decomposition[11]. However, it is not cost effective due to the expensive cost of the high pressure equipment.

Despite the release of hemicellulose and other fermentable sugars, it poses the release of inhibitory compounds as a major drawback. The inhibitors affected the fermentation medium and thus the growth of the bacteria. Some works also indicated the simultaneous saccharification and fermentation (SSF) of the lignocellulose waste. SSF is especially useful as it removes the inhibitors released after the degradation of lignocellulose. Some forms of chemical pretreatment are described in detail below:

- **Alkali pretreatment**: It is the most widely used form of chemical pretreatment and is based on the solubility of lignin in the alkali solution. Several alkaline reagents are used like hydroxides of sodium, potassium, calcium and magnesium and ammonium. Alkali involves a saponification reaction which cleaves the intermolecular ester linkages between lignin and hemicellulose. This results in the solubilisation of lignin and hemicellulose in the alkali solution thereby exposing the cellulose to the cellulose degrading enzymes. Alkali treatment also increases the internal surface area of lignocellulose by reducing the crystallinity and degree of polarization.

  In addition, it also removes the acetyl groups and ironic acid substitutions from the hemicellulose thereby enabling the access of the carbohydrates by the enzymes. Although alkali pretreatment is used extensively and has proved to be very efficient in separating lignin from the fermentable sugars, they have their own disadvantages. The recovery of the added alkali needs to be further researched[3]. Moreover, the alkali pretreatment works best only for lignocellulose with low lignin content like herbaceous crops and agricultural woods[2].

- **Acid pretreatment:** This method exploits the susceptibility of glycosidic bonds to certain acids. Glycosidic bonds connect the hemicellulose and cellulose. Hydronium ions from the acid catalyst break down the long cellulose chains into glucose monomers. They are used as concentrated acids at low temperature or dilute acids at high temperatures for efficient degradation/ breakdown of lignocellulose. Although the use of concentrated acids can highly accelerate the rate of the reaction, the maintenance and operational costs for these chemicals are high due to its toxic and corrosive nature[3]. This also generates inhibitory compounds and additional detoxification methods need to be coupled with acid pretreatment. Activated charcoal is the best form of chemical detoxification to remove the inhibitory compounds.

  Several microbes can bring about changes to the inhibitors and they can be used for biological detoxification. The use of dilute H_2SO_4 is known to be very effective being both economic and environmental friendly. It is the most feasible acid pretreatment for lignocellulose wastes[2].

- **H_2O_2 Pretreatment**: The use of H_2O_2 for the pretreatment of lignocellulose is known to be an effective form of pretreatment. The basic principle behind this form of pretreatment is Fenton reaction. Although the detailed molecular mechanisms underlying the Fenton reaction are yet to be described, it is a catalytic reaction which converts hydrogen peroxide into hydronium ions known to damage the lignocellulose structure. Unlike most other forms of pretreatment, the use of H_2O_2, it completely separates the lignin from the cellulose and over a few weeks also releases the hemicellulose and other components of the lignocellulose. The maximal recovery of fermentable sugars at the same time is minimizing the inhibitors by using a modification to Fenton reaction by a combination of solvents i.e. water and DMSO. 94% of the carbohydrates were recovered as soluble monosaccharides like glucose and xylose[10].

Figure 3 summarizes all the different forms of pretreatment available for the breakdown of lignocellulose waste.

Importance of Cellulose Degrading Bacteria and cellulase enzyme: As described previously, the complexity of lignocellulose makes the pretreatment of the agricultural wastes difficult. Furthermore, the rigid structure of cellulose makes it challenging to release the fermentable sugars like glucose. The breakdown of cellulose is exhibited by an interesting class of bacteria called Cellulose Degrading Bacteria (CDB) characterized by the presence of cellulolytic enzymes or cellulases like endoglucanases and exoglucanases.

Endoglucanases, also known as carboxymethyl cellulases or CMCase are responsible for random cleavage of B 1-4 glycosidic bonds along a cellulose chains. While exoglucanases is necessary for cleavage of the non-reducing end of a cellulose chain, yet another enzyme ß 1-4 glucosidase converts cellobiose and cello dextrin to glucose. Cellulose degrading bacteria from an important community and carry out critical functions in the host by enhancing the metabolism, synthesis or catabolism. The cellulolytic systems present in these bacteria cellulose can be used for converting to glucose which is a multi-utility product. Enzymatic saccharification (with the help of exoglucanases and endoglucanases) is inexpensive and less harmful due to its microbial source.

They cause depolymerisation of cellulose thereby liberating cellobiose and glucose, thus making it available for fermentation into lactic acid. Cellulases play a critical role in the saccharification of of feedstocks. Due to its industrial and commercial importance, much research has been aimed at obtaining new microorganisms producing cellulase enzymes with higher specific activities and efficiency. Cellulases offer an opportunity for tremendous benefits of biomass utilization. A wide range of bacteria including aerobic and anaerobic synthesize cellulolytic enzymes. Although cellulolytic enzymes are produced by both bacteria and fungi, there is an increasing interest in celluloses produced by bacteria as they have a higher growth rate[12]. It is economically feasible to isolate celluloses from bacteria with high efficiency and can tolerate a wide range of pH, temperatures and salt concentrations[12].

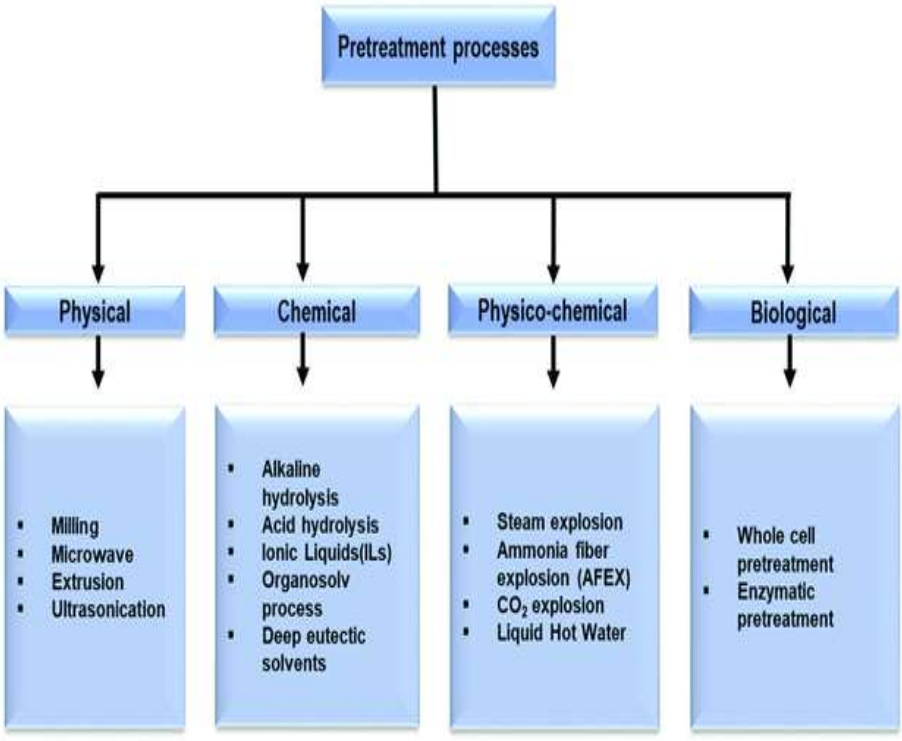

Fig. 3: Flow chart diagram of pretreatment processes.

In other words, a more robust strain with high efficiency would be economically preferred. Since the final product of cellulose degradation is soluble, fermentable sugar- glucose, it forms an important aspect of biofuel and bio plastic research. Although cellulose degrading enzymes were demonstrated to be efficient in both aerobic and anaerobic organisms, anaerobic organisms with cellulolytic enzymes, specifically facultative anaerobes are preferred as they can breakdown cellulose to glucose and ferment the glucose into lactic acid and alcohol i.e. heterolactic fermentation as opposed to homolactic fermentation from aerobic microorganisms.

Heterolactic fermentation is known to produce more lactic acid as it is known that some facultative anaerobes switch between the 2 modes of fermentation thereby producing more lactic acid.

Vermicompost and intestinal fluid of earthworms- a promising source of cellulose degrading bacteria: Although there are several sources available for the isolation of cellulose degrading bacteria like industrial wastes, sewage wastes and different types of soil like garden soil where cellulose degradation takes place as demonstrated by Rawway et al[20]. However, the easiest and environment friendly source for the isolation of cellulose degrading bacteria is earthworms.

Earthworms are hermaphrodites which thrive in rotting vegetation, compost and manure. They are the most important soil invertebrates due to their beneficial effects on soil environment including decomposition of organic matter. Infact, earthworms are often termed the "keystone species" or "ecosystem engineers" of the soil[5]. Earthworms convert organic waste constituents into a more constructive form by grinding and digesting with the help of aerobic and anaerobic microbes[16]. The interdependency between the earthworms and its intestinal microorganisms directly influences the carbon dynamics, nutrient cycling and soil physical conditions. Many species of earthworm have demonstrated the ability to decompose this waste by enhancing the dynamics of the gut microbes.

Earthworms are crucial drivers of the decomposition process as they provide an aerobic condition and fragment the substrate thereby enhancing the biodegradability of the waste. Earthworms mainly consume lignocellulose waste or soil organic matter from which they derive their energy. Several enzymes, intestinal mucous and antibiotics in the intestinal tract of earthworm play an important role in the breakdown of organic macromolecules including cellulose. Different groups of earthworm have been used to analyze the presence of cellulose degrading bacteria which can breakdown cellulose.

Although, cellulose degrading properties of both bacteria and fungi have been studied, the yield of lactic acid in fungi was very low. Several bacteria among them are obligate or facultative anaerobes which ferment glucose into lactic acid and alcohol. These bacteria are especially useful from the environmental perspective as they can degrade lignocellulose waste into lactic acid which can be polymerized for its use as a bio plastic. Several earthworms have been studied for the presence of cellulose degrading bacteria in their gut[7].

Some studies focused on vermicompost while the other groups isolated and characterized bacteria from the mid gut and intestinal fluid. Most studies have worked on the isolation of the cellulose degrading bacteria identifying the cellulolytic enzymes using Congo red staining and endoglucanase activity. They were then biochemically characterized using several tests including starch utilization, citrate utilization, catalase test and MR-VP test, after morphological identification through gram staining[6]. Few works have further characterized the strains using 16S rRNA studies and identified the bacteria at a species level. Most bacteria belonged to the genus Bacillus[18].

Characterization of isolated bacteria for potential biodegradation: Cellulose degrading bacteria can be identified for their potential to degrade cellulose by growing them on CMC agar plates followed by Congo red staining. CMC stands for carboxymethyl cellulose and it acts as a screening medium for cellulolytic bacteria. The composition of CMC agar plates also matters since too much percentage of CMC can be difficult for the bacteria to degrade while too little percentage of the CMC medium will not result in gel formation. The ideal composition of CMC agar plates is 0.5 % CMC along with 1 % agar. 24 hour cultures of the bacteria grown on CMC medium plates can be subjected to Congo red overlay method[13]. Congo red staining involves the addition of 1% Congo red and detaining it with 1 M NaCl after incubating for 20 minutes. The zones of lysis of cellulose can be seen as yellow after destaining.

The destaining can be enhanced by adding 2 % acetic acid. One disadvantage to this method of characterization is that Congo red is toxic to the bacterial cell, therefore, an excessive use of Congo red should be avoided. In some cases, the toxicity of Congo red might also dislodge the bacteria from the agar plate. Furthermore, the characterization of cellulose degrading bacteria can be confirmed by its ability to degrade filter paper[12]. Once the identification of cellulose degrading bacteria is done, the cellulase enzymes can be quantified using several assays for the estimation of endoglucanase activity and exoglucanase activity.

Importance of 16 S rRNA sequencing of cellulose degrading bacteria: Although most studies focused on the isolation and characterization at a genus level, it is important to know the species of the organisms. Each specific strain of bacteria differs in their ability to ferment glucose into lactic acid. Literature study has revealed the presence of different strains of bacillus isolated from vermicompost or gut isolate

of earthworms which have demonstrated the ability to ferment glucose to lactic acid using lignocellulose waste as a substrate[18].

A few organisms which are isolated, characterized and sequenced using 16 S rRNA are Bacillus *pumilis*, Bacillus *subtilis* and Bacillus *cereus*[10]. They were isolated and characterized (including 16 S rRNA) from the mid gut of Eudrilis *eugenia* by different teams.[9,10] Eudrilis *eugenia* is the most commonly used earthworm used for these studies[26].

Heterolactic fermentation of bacteria in converting cellulose to lactic acid: Some examples of bacteria that carry out heterolactic fermentation are *Leuconostoc mesenteroides*, *Lactobacillus bifermentous* and *Leconostoc lactis*. Figure 4 illustrates the differences between homolactic and heterolactic fermentation.

The flowchart on the left depicts the homolactic fermentation, with only 2 molecules of lactate as its end product, while the flowchart on the right depicts heterolactic fermentation with lactate and ethanol as its end products[4].

Apart from the above mentioned organisms, *Bacillus licheniformis*, a facultative anaerobe well known for its ability to heterolactic fermentation, was studied in the gut isolate of Etroplus *suratensis*, a fish[24]. In this work, the bacteria was isolated and characterized for its cellulose degrading properties. Although the study was done on fish, literature has also shown the presence of this particular bacterium in the mid gut of earthworm. Furthermore, its ability to effectively ferment glucose from lignocellulose waste into lactic acid is also demonstrated[15]. *Bacillus coagulans* is yet another facultative anaerobe known to effectively ferment glucose into lactic acid using lignocellulose as. a substrate[1].

Similarly, there are several other bacteria which were identified in the mid gut of earthworms and these bacteria can also ferment glucose to lactic acid. Although most of them are aerobic bacteria like *bacillus subtilis* (the most common homo fermentative bacteria[19] and they can ferment glucose to lactic acid, the use of facultative anaerobes is preferred since they are known to produce more lactic acid in addition to other byproducts like alcohol and carbon dioxide. This is because some facultative anaerobes tend to switch to heterolactic fermentation under anaerobic conditions.

Studies on the presence of these bacteria (*Bacillus licheniformis* and *Bacillus coagulans*) in vermicompost and gut isolate of earthworms would help in building a strong platform for the use of earthworms for the isolation of cellulose degrading bacteria which can utilize lignocellulose waste as a substrate for heterolactic fermentation.

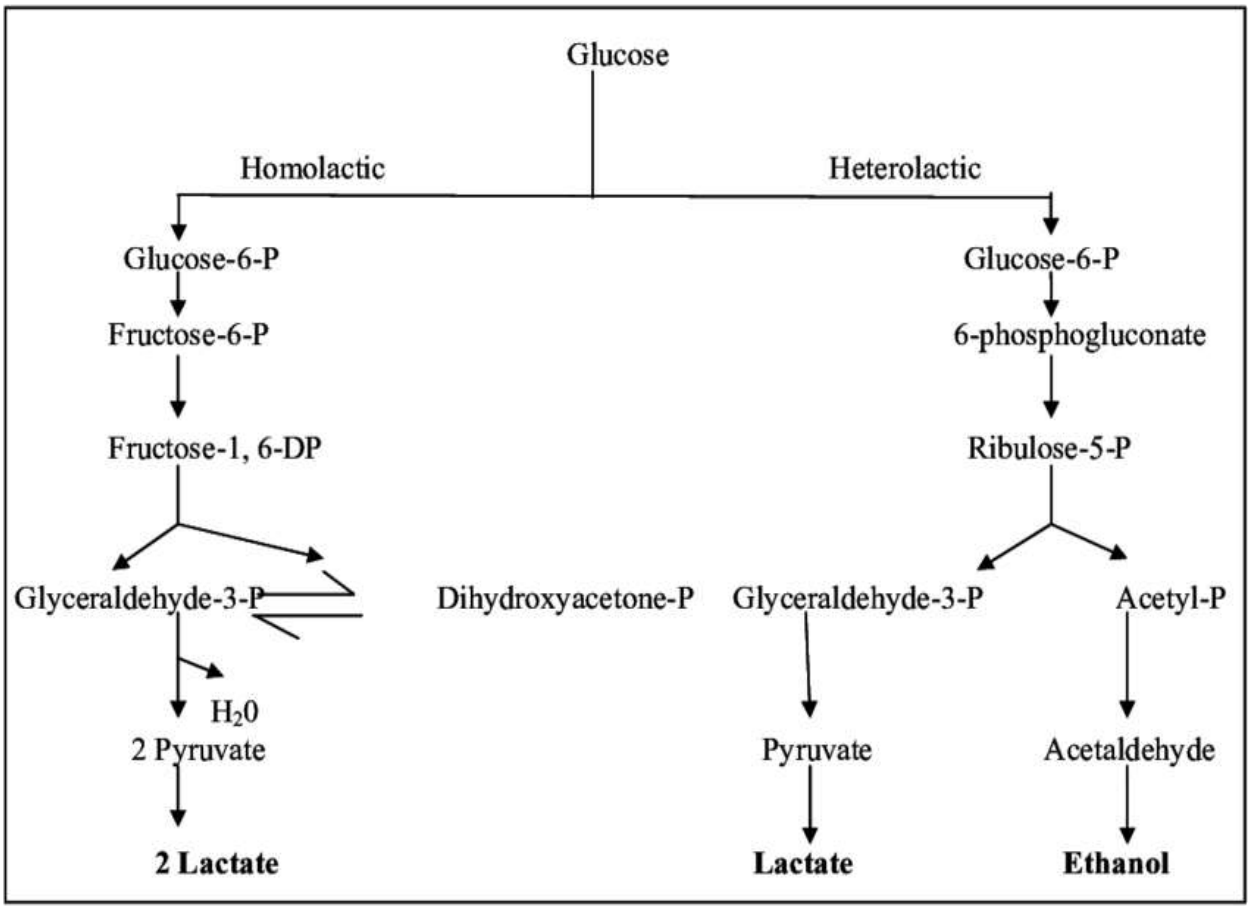

Fig. 4: Different Fermentation Pathway of Bacteria

In addition to these microbes belonging to the bacillus species, there are also several other facultative anaerobes which can exhibit heterolactic fermentation.

Conclusion

The most important factor in the production of lactic acid and bioethanol is the substrate. Lignocellulose waste is proven to be an efficient and environmentally friendly substrate. Furthermore, lignocellulose waste is continuously produced and easily available. The cellulose degrading bacteria, specifically, facultative anaerobes which can ferment glucose to lactic acid and ethanol can be isolated from earthworms. The isolation of these bacteria could be from the intestinal fluid, gut isolate or the vermicompost. Once the bacteria are isolated, they can be characterized by specific tests for CDB like Congo red staining or by testing their ability to metabolize filter paper.

Further characterizations with regard to morphology and biochemistry can be done by biochemical tests namely- Gram's staining, TSI test, catalase production. Once these bacteria are characterized, they can be inoculated along with the lignocellulose for submerged fermentation to produce lactic acid and alcohol. Before inoculation, an efficient method of pretreatment of lignocellulose should be designed. The pretreatment strategy depends on the source of lignocellulose. The cellulose, glucose, lactic acid and alcohol content can be estimated. The number of days of incubation after inoculation into lignocellulose needs to be optimized as it varies for every species of bacteria. The lactic acid production needs to be scaled up from the lab scale to a fermenter level. The resulting lactic acid can be polymerized and condensed to form a thin film of bio plastic and the biodegradable plastic can be stabilized by adding the lignin obtained from the lignocellulose waste.

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