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Effective Bioconversion of Lignocellulosic Waste to Biodegradable Products for a Cleaner Earth

Nair P*, Kuriakose T and Das B

Department of Biotechnology, Mount Carmel College, India

***Corresponding author:** Preetha Nair, Department of Biotechnology, Mount Carmel College, Autonomous, #58, Palace Road, Bengaluru-560052, Karnataka, India, Email: preetram@gmail.com

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Abstract

Earth is constantly subjected to dramatic changes due to uncontrolled human activities and this has led to climatic variations associated with global warming resulting in unparalleled greenhouse gas emissions. One of the major causes is solid waste accumulation by human activities ranging from agricultural to industrial activities. The relation between solid waste and climatic change had forced scientists to propose a solid waste management plan to reuse, recycle and create energy from solid waste preventing its accumulation and subsequent pollution. When we consider human activities which are environmentally hazardous; plastic waste accumulation, agricultural wastes disposal and fossil fuel burning holds prominent positions. Plastic wastes. The accumulation of plastic wastes and agricultural wastes pose serious problems of disposal. Plastic wastes are often incinerated or left to be dumped in landfills. Similarly, agricultural wastes are also burnt, releasing a lot of toxic gases into the atmosphere. A common solution to curb both the problems is to streamline the production of bioplastic using agricultural waste (mostly lignocellulose) as a substrate. The third major environmental threat is the increased motor vehicle emission leading to air pollution associated with health threat. The extent of risk ranges from carcinogenic and noncarcinogenic health effects. Carbon dioxide emission by the increasing use of fossil fuels by ever increasing world population not only depletes the resource but also will result in anthropogenic climate change. Therefore, utilization of lignocellulosic waste material as raw material for monomers for bioplastic as well as for bioethanol production can be considered as a productive approach to address all the three problems mentioned above. In addition utilization of fermentation residue after bio products extraction can be used as a soil enriching agent. The intention is converting lignocellulosic waste to zero waste.

Keywords: Phyto-hormones; Lignocelluolosic; *Saccharomyces Cerevisiae*

Abbreviations: IPCC: Intergovernmental Panel on Climate Change; FBC: Fermented Bokashi Compost; LCW: Lignocellulosic Waste; CS: Cotton Stalks; SSF: Simultaneous Saccharification (hydrolytic) and Fermentation.

Introduction

Earth and its environment have a delicate balance which gets complex if it is perturbed. A greater negative impact on earth's environment is due to the growing amount of waste produced especially by the modernization in our human society. Lack of sustainable management of generated waste is detrimental to the environment. It affects the terrestrial and aquatic environment equally. Accumulation of waste affects earth and its environment in multiple ways; the natural flora and fauna will be threatened will have a negative effect on human health and it can even lead to climatic variation. Tons of solid waste produced by human activities ranges from domestic, industrial to municipal and agricultural wastes [1]. The first and most drastic impact of modernization is plastic waste accumulation around the world, irrespective of any region. Post -use of chemically synthesized polymerized plastic waste creates a deleterious environmental impact [2], which emphasizes on its proper management. Wide spread recognition of this problem had revealed that residual plastic will be persisting even for centuries on earth. Its long lasting implications are harmful [3] as plastic accumulation was neglected for a very long time and it had affected the terrestrial and aquatic ecosystem tremendously. As of now the advice is to reduce, reuse, and recycle plastic waste. But reuse and recycle had to be carried out carefully as it can create secondary problems [4]. The second serious issues to be addressed is road transport and related vehicle emissions leading air pollution. This has various detrimental effects on animal health as well as environmental sustainability [5]. The present era is suffering from diverse climatic changes which impose a huge threat on the human development [6]. Fifth assessment report of the Intergovernmental panel on climate change (IPCC) in 2013 (https://www.ipcc.ch/site/assets/uploads/2018/03/WG1AR5_SummaryVolume_FINAL.pdf) had emphasized that, enhanced fossil fuel usage and subsequent Carbon di oxide emissions are the dominant cause of global climatic change. Since this particular sector is becoming a serious issue due to increased usage of motor vehicles, it should be an area in need of serious research attention. The need for alternate fuels has become an ever increasing demand on human society.

The third and more or less neglected area is disposal of agricultural solid wastes. More than a waste disposal nuisance, in many countries burning of indiscriminately deposited agricultural waste leads to air pollution as it releases toxic gases along with smoke and dust. In addition it leads soil contamination and a threat to terrestrial and aquatic microbial life [7]. If agricultural solid waste can be recycled effectively it can be a major promising step in the field of bio economy. The present century faces the greatest challenge of growing demand on the rapidly depleting fossil fuels for not only transportation but also for industrial processes. Even when we are aware of its impact on global warming apart from the ever increasing price factor, no safety measures are taken. If biofuels/bioethanol can solve this negative impact to a certain extent, it can be considered as a unique available alternate energy source [8]. As already stated, solid waste accumulation irrespective of plastic wastes [9] or agricultural wastes [10,11] imposes the urgent need to find out a sustainable management as well production/consumption mechanisms. It should be either to replace the non-biodegradable plastic or to channelize the agricultural waste to usable alternative bio product such as bioplastics [12,13]. This stimulates a thought provoking idea that whether lignocellulosic waste - a byproduct of agriculture can be used as the raw material for ethanol and lactic acid production? Ethanol can be modified as bioethanol and can replace or supplement the fuel usage. Lactic acid can be polymerized to poly lactic acid the biopolymer which can replace synthetic plastic from almost all areas of human activity.

Another area which can be addressed in this context is usage of bio fertilizers in sustainable agriculture sector. It's increasing popularity because it promotes the plant beneficial microorganisms in soil [14]. Most of these microbial systems possess multifunctional properties apart from soil enrichment, such as it provides plant stimulating metabolites and phyto-hormones, and even bring about plant pathogen suppression [15]. Biotechnology always tries to find new bio formulations and one such attempt to increase soil fertility is usage of Fermented Bokashi Compost (FBC), which was proved to be a best organic wastes. It changes the net nitrogen mineralization and chemical properties of soil [16]. This creates an inquisitiveness to find out whether lignocellulosic agricultural waste fermentation will result in a fermentation residue which may be effective in enhancing soil fertility.

Common Remedy to All Problems

Our suggestion is to find out a common remedy to three hazardous problems; a replacement for depleting hazardous fossil fuels, an alternative to non-degradable synthetic plastic and recycling of accumulating agricultural solid wastes. Conversion of lignocellulosic agricultural wastes to either bioethanol or monomer of bioplastic PLA called lactic acid by fermentation. In addition the fermentation residue can be utilized as a soil amendment agent to increase soil fertility. This leads to zero waste with beneficial bio products. Agricultural wastes consist of lignocellulosic biomass and main consists of polysaccharides like cellulose and hemicellulose; and lignin which are a polymer of aromatic nature. This lignocellulosic waste (LCW) material can be obtained from various sources including agricultural and forestry residues, pulp and paper industry wastes and plant based wastes generated in day to day life. Recycling of LCW irrespective of the source to various beneficial chemical compounds is an attractive area of research. This attempt not only helps in managing the LCW based solid waste accumulation but also helps in generation of eco-friendly bio products of commercial value. This review article focuses on three such products from LCW by microbial conversion. Alcohol such as ethanol and lactic acid the monomer for PLA a reliable bioplastic can be generated from LCW by the saccharification and consequent fermentation process mediated by specific microbial strains.

Lignocellulosic Waste (LCW) Materials and Processing

Various sources of agricultural residues are generated as LCW. Rice and wheat straw, sugar industry waste such as sugar cane waste and bagasse, corn husk, vegetable and fruit wastes like potato and orange peel are some of them. Their efficient use in biofuel production was already reviewed [17]. Methodologies were developed based on chemical reactions and biochemical pathways to convert LCW to chemically important building blocks such as ethanol or butanol or such organic compounds. Similarly lactic acid, the bio-based monomer for polylactide (poly lactic acid - PLA) synthesis can also be generated from LCW by microbial saccharification and bio fermentation [18]. In places like India the important food crops are Wheat and rice which generate enormous biomass residues of lignocellulosic nature. These two high yielding feed stocks are also economically efficient energy yielding crop [19]. Even though wheat and rice, the major food crops which provides enormous biomass residues, due to lignocellulosic recalcitrance, the biomass conversion is a difficult task [20]. The recalcitrance of LCW is due to its structure, composition and cell wall network consisting of various polymers like cellulose, pectic polymers and lignin [21,22]. The complicated cell wall structure consisting of above mentioned major components makes LCW less accessible to biomass-digestibility [23]. This demands specific pre-treatment steps to make lignocellulosic material more accessible to microbial or direct enzymatic conversion of polysaccharides into fermentable sugars. This is achieved by changing the physical as well as the chemical structure of lignocellulosic plant content by various methods [24]. Elaborate research has to be done to devise various pretreatment techniques depending on the type of LCW material. These techniques can be either of the available physical, chemical, physicochemical, biological methods or a combination of such methods [25].

Depending upon the source of LCW and also on the desired bio product to be produced; the pretreatment method has to be selected. Complexity of cell wall components and their diverse biological factors should be considered, for selecting the process. For bioethanol production the general scheme is appropriate pre-treatment, hydrolysis of complex polysaccharides and bio-fermentation of saccharified sugars. The pre-treatment process suggested are ionic liquid, microwave, dilute acid, or steam explosion [17]. Dimos, et al. [26] explained on their study on high carbohydrate containing cotton stalks (CS) as potential candidate for fuel-ethanol production and found out that sequential combination of organosolv and hydrothermal pretreatment brings improvement in ethanol production. Conde-Mejia, et al. [27] suggested that the key factor to be considered in bioethanol production is consumption of energy during the process. Therefore selection of an energy efficient pretreatment method contributes the success of the overall bioethanol production process. It is applicable to lactic acid production from LCW raw material also. In both bioconversions the goal of the pretreatment is to subject the LCW in such a way to improve the saccharification to produce fermentable sugar which is accessible to further microbial fermentation. Marzo, et al. [28] compared the effect of pretreatment on lactic acid production using sugar beet pulp as raw material. According to their study, thermochemical pretreatment with H_2SO_4 resulted in enhanced lactic acid production compared to enzymatic/biological or alkaline H_2O_2 based chemical methods. These studies suggest that irrespective of ethanol or lactic acid production a suitable pretreatment process for rice and wheat straw LCW has to be standardized.

According to Conde-Mejia, et al. [27], even though the preliminary screening metric is energy consumption and later it should be complemented with other parameters. They are inhibition of process due to change in environmental factors, water consumption, as well as chemical cost. The primary goal is conversion of lignocellulose to fermentable sugar which can be further subjected to bio fermentation with a suitable microbial system to produce specifically ethanol or lactic acid. The specific bioconversion of LCW to desired product depends on the type of microbial system used. Hence selection of such a system is of utmost importance. Isolation, screening and selection of suitable microorganism for specific saccharification and bio fermentation have to be critically analyzed for an efficient economically feasible process.

Isolation of Microbial System to Degrade Lignocellulosic Waste

Identifying and separating a specific strain of a microorganism for a predetermined purpose from a consortium of mixed population from an exclusively natural habitat is very important. Some microbial species are well known for the process of biodegradation of solid waste. Eco friendly management of biodegradable waste with microorganisms is an area needs exploration. Many of the soil bacterial strains use organic solid waste as nutrients. The waste products will be converted to products which are safe and some times during this conversion of complex waste, several metabolites produced found to industrially important [29,30]. Most of these microorganisms are obscure in their role in maintaining a sustainable environment. The ability of bacterial species which can survive in diverse ecological habitats like waste dumps, are due to their adaptive capacity in utilizing the organic waste. This is achieved by producing specific enzyme systems for metabolizing it [30]. The need to select effective bacterial strains is very important in biodegradation of waste. Identification, selection and isolation of microorganisms especially bacterial species from a suitable source is a great challenge to environmentalists. Always there is a demand to isolate a microbial strain from a diverse environment which can employ unique enzymes/ molecules for waste degradation. A very good example of organic waste accumulation is agricultural residues, which are generated in large quantities. It can be considered as an underutilized source of renewable by product of agricultural practice. More than an ecological necessity, recycling these wastes has become an economic compulsion for a country like India [31].

The most effective method suggested is composting by lignocellulolytic microorganisms. This specific microbial system brings about organic matter assimilation due to their capacity to employ degradative enzymes. Based on this they can be selected [32]. Varied microbial species ranging from bacteria and actinomycetes to fungi and even invertebrates like earthworm play crucial role in composting. Though these systems independently capable of carrying out composting, it is observed that mixed cultures of microorganisms employs synergistic action. It is by utilizing intermediary metabolites produced and thereby enhancing the rate of lignocellulose degradation [33]. Brémond, et al. [34] emphasized biological pretreatment methods with the usage of suitable microbial consortium producing potent ligninolytic and cellulolytic enzymes. Microorganisms evolve a natural mechanism to change their metabolism to access lignocellulosic biomass. Natural modification and degradation provides a natural, low-input method of preparing LCW for beneficial by-products such as ethanol or lactic acid.

Vermicompost technology is one of the eco-friendly methods for transforming waste into a nutrient-rich fertilizer, through the joint action of earthworms and microorganisms. Cellulolytic bacterial isolation, screening and optimization of enzyme production was studied by Karthika, et al. [35] in vermicomposting of used paper cups. Vermicomposting can be described as a bio oxidative process involving earthworms and associated microbes. Soil biodiversity will be enriched with beneficial microbes during vermicomposting [36]. During this process earthworms influences soil chemical processes indirectly, by affecting the activity of the soil micro-flora [37,38]. Edwards and Lofty as early as in 1977 Petersen H, et al. [39] reported that there will be a five time increase in soil bacterial population in association with earthworms. Therefore vermicomposting done with agricultural waste like rice and paddy straw is expected to enrich lignocellulolytic microbial population in the compost and they adapt to this composting by developing suitable cellulolytic enzyme systems. Isolation, selection, screening and identifying the best cellulolytic microbes more precisely bacterial strains from straw based vermicompost is easier.

Isolation and Selection of Cellulolytic Bacteria

The vermicompost is subjected to serial dilution technique for bacterial isolation. The isolated bacteria were grown on CMC (carboxy methyl cellulose) agar plates for assessing their ability to degrade cellulosic materials. Bacterial strains producing clear zones in CMC agar plate were isolated and cultured [40]. Alternate sources of bacterial species can also be isolated for their cellulolytic activity. They can be isolated from earthworm or termite intestinal micro flora [41,42]. The saccharification and bio fermentation activity of selected bacterial species can be analyzed in a pilot scale study in the laboratory with the pretreated rice and wheat straw based LCW. Efficiency of bacterial species in fermentable sugar production is indicative of saccharification. It is not necessary that a good saccharifying bacterial species is capable of converting fermentable sugar to either alcohol or lactic acid. In such cases a mixed population of saccharifying bacterial species can be co-cultured with a lactic acid or alcohol producing microbial species, for example Yeast cells (*Saccharomyces cerevisiae)* [43,44]. Yeast is selected for bioethanol production due to its high ethanol productivity along with high ethanol tolerance and ability of fermenting wide range of sugars [45]. Yeast cells can be easily manipulated due to their adaptability to simple nutritional requirements. Moreover Lactic acid production can lower the pH of culture medium and yeast is capable of withstanding low pH than bacterial species. Heterologous expression of lactate dehydrogenases is one way of metabolic manipulation. Lactic acid accumulation can suppress LDH activity which can be circumvented by periodic product (lactic acid) removal [46]. An approach to improvise lactic acid production is suppressing the activity of pyruvate decarboxylase and alcohol dehydrogenase the key enzymes in alcohol synthesis. It is to promote conversion of the pyruvate produced in glycolysis to the synthesis of lactate [47].

Conversion of Lignocellulosic Wastes into Biofuels and Bioplastics

Lignocellulosic biomass is processed for biofuel and bioplastic preparation in four major phases: pre-treatment, hydrolysis (saccharification), fermentation and product purification. When organic solid wastes are subjected to bioconversion to useful bio products, the chemical composition of biomass has to analysed. In agro wastes, depending upon the type of wastes, there will be significant variation in the relative content of lignin, cellulose and hemicellulose. This relative proportion of major polymers determines type of pretreatment and fermentation methods employed. Even type of microorganisms for such bioconversion is decided based on this polymer proportion in fermentable material. Anwar, et al. [48] had compared the percentage composition of lignin (%), hemicelluloses (%) and cellulose (%) in various lignocellulosic materials. Sugar cane bagasse has 20%, 25% and 42% of lignin, hemicellulose and cellulose respectively and in Rice straw and Wheat straw their percentage composition is 18%, 24% 32.1% and 16- 21% 26- 32% and 29-35% respectively. This shows that irrespective of the origin of LCW it has lesser percentage of lignin. Pretreatment methods should be selected in such a way to release the cellulose from lignin based biomass. Mechanical pretreatment will be done initially by grinding or milling to reduce the particle size. It is accompanied by changes in surface area and porosity of LCW material [49]. Preliminary acid and alkali based treatments are conventional approaches. Dilute acid based methods can be applied to agricultural LCW. Solubilisation of hemicellulose can be achieved by this method [50]. On the other hand, depletion of lignin barrier, cellulose swelling, and partial decrystallization and solvation of cellulose and hemicelluloses, respectively can be achieved by alkaline hydrolysis [51]. But it is accepted that compared to physical or chemical pretreatments, biological pretreatments are more effective, economical, eco-friendly and less health hazardous [52]. Irrespective of the methods employed, the primary goal is depolymerisation of cellulose reducing cellulose crystallinity achieved by removing lignin. Therefore a combination of physical, physicochemical and biological methods can be used as pretreatment of LCW.

Saccharification is the process by which the newly freed carbohydrate polymers hydrolyzed to simple monosaccharides. Even though the physical and chemical saccharification brings about lignocellulose hydrolysis, it results in toxic byproducts like furfural formation resulting in fermentation inhibition [53]. Since biological saccharification can also results in lignocellulosic hydrolysis under milder conditions without toxic product formation. Hence it is considered as ideal method of saccharification [54]. Variety of microorganisms like fungi and bacteria are used for biological saccharification, of which thermophilic anaerobic bacterial cellulase is found to be superior. These bacteria use multienzyme complexes for saccharification [55,56]. Maki, et al. [57] has reported that *Paenibacillus* and specific *Bacillus* strains produces cellulases which are stable at high temperature and at wide pH range, and certain other bacterial species like *Cellulomonas flavigena* and *Terendinibacter turnerae* were identified with multifunctional and broader substrate utilization enzymes Singh S, et al. [57,58] reported identification of 6 isolates of anaerobic soil bacteria identified as *Thermoanaerobacterium* sp. with the ability to utilize a wide range lignocellulosic substrates. At this point our suggestion is to isolate thermophilic anaerobic bacterial species from lignocellulosic rice/wheat straw based vermicompost. Further characterization can be done by standard biochemical tests and 16Sr RNA ribotyping.

Bio fermentation by microbial system especially by bacterial species breaks complex organic molecules like cellulose to simpler fermentable sugars like glucose. LCW pretreated appropriately and hydrolytically saccharified prior to biofermentation. These saccharified simpler sugars can be subjected to microbial fermentation to produce either bioethanol or monomers of bioplastic (PLA) called lactic acid. Glucose molecules during glycolytic cycle produce pyruvate. Pyruvic acid can be reduced to ethanol and carbon dioxide [59] during fermentation. Under anaerobic conditions, pyruvate can be metabolized to acetaldehyde with the release of carbon dioxide. Subsequently, acetaldehyde can then be reduced to ethanol by alcohol dehydrogenase [60]. If we can inhibit the activity of alcohol dehydrogenase then lactate dehydrogenase activity will be favored anaerobically and Lactic acid production will be favored [61]. Microbial fermentation is best brought about by *Saccharomyces cerevisiae* compared to other types of microorganisms as it results in high ethanol production. In addition, sensitivity of organism to high ethanol concentration and temperature are less with yeast cells compared to bacterial strains. But its ability to ferment pentose sugars is less. Simple sugars are fermented to ethanol by yeasts so it is better to convert complex sugars to fermentable sugars before employing yeast to bring about ethanol fermentation. Temperature, pH, concentration of sugar, time duration of fermentation, agitation rate, and size of the inoculum determines the ethanol production rate [45]. Olofsson, et al. [62] has suggested simultaneous saccharification (hydrolytic) and fermentation (SSF) of LCW with yeast cells. Advantages of this method are reduced end-product inhibition and investment costs. According to them low yeast inoculum with high LCW substrate loading, results in high ethanol yield. Since metabolic engineering is needed for pentose utilization by yeast cells [63], our suggestion is to use thermophilic anaerobic bacteria for hydrolytic saccharification of LCW for maximum fermentable sugar production and yeast (*Saccharomyces cerevisiae)* for fermentation of sugar to alcohol. For successful conversion we can even employ simultaneous saccharification (hydrolytic) and fermentation (SSF) of LCW. We can try a co-culture of both to achieve this. It is also observed that the efficiency ethanol production can be enhanced by immobilizing the yeast cells.

The intention is to use LCW as a raw material for Lactic acid monomer production for bioplastic production also. *Lactobacillus* species can be used for sugar fermentation to lactic acid but the limitation for this fermentation is due to sensitivity of this bacterial sps to low pH when there is an accumulation of lactic acid. Therefore during commercial industrial production, large quantity of neutralizing agents may be needed [46]. The next suitable microbial system is yeast cells as they are widely used for carboxylic acid production. Channelizing pyruvate to lactate conversion can be achieved by suppressing key enzyme activities in the alcohol production. The ideal method is bio fermentable sugar production by thermophilic anaerobic bacterial saccharification and then suppressing alcohol pathway enzymes in yeast bio fermentation. By this method the fermentable sugar can be converted to Lactic acid. Immobilization methods can be employed for more controlled environment for this bioconversion. The ethanol produced or lactic acid produced can be extracted and purified and commercialized for bioethanol and bioplastic formation.

Amendment Studies

There are reports showing that the residue of fermentation called the anaerobic slurry after purifying the main product can be used as soil amendment agent to enhance soil fertility. The organic content and the digested residues found to have beneficial effects on soil enrichment safeguarding environmental protection [64]. It was reported by them that, anaerobic digestion can increase the nutrient availability without compromising soil carbon content. Simultaneously this approach decreases the soil pathogen load as well as the total greenhouse gas emission. Baryga, et al. [65] reported that dig estate, a by-product of the anaerobic digestion of sugar beet pulp can be supplemented as a soil amendment agent. Anaerobic digestion of plant materials increases the labile fraction of organic matter. Dig estate has nitrogen and phosphate containing organic fraction and on decomposition and digestion they amend soil with N2 and P. Readily available nutrients in it improves soil texture and increases its ability to retain water and nutrients. Our unpublished data also supporting this finding .The fermentation residue after extracting the ethanol or lactic acid is used for soil amendment studies and its beneficial effect was assessed by improved plant growth, increased soil respiration due to active soil microbial population.

This approach is a zero waste strategy utilizing lignocellulosic waste material for commercially important products like ethanol and lactic acid to avoid conventional disposal of agro wastes. And the effective application of fermentation residue as a soil enriching agent add on to its utilization. This is a hypothetical approach to address problems like fossil fuel depletion as well as the air pollution caused by it; to reduce plastic accumulation related environmental problems. In addition this has an indirect effect of increasing soil fertility with zero waste accumulation.

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