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Role of Aerobic and Anaerobic Bacteria in the Bioconversion of Lignocellulose waste material

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Abstract

The rise in accumulation of agricultural wastes has demanded ways of effectively utilizing this waste and converting them into useful products. A large portion of the agricultural waste comprises of lignocellulose. Lignocellulose, being the most abundant carbon source available, can be used as a substrate to convert into useful products like lactic acid and ethanol. However, lignocellulose bio-conversion comes with its own challenges, mainly due to its complex structure. There are several parameters affecting the efficiency of bioconversion of lignocellulose into lactic acid. This review will focus on one of the parameters involved i.e., the effect of oxygen in facilitating the efficient conversion of lignocellulose into lactic acid. Studies have shown that the yield of lactic acid is high-er when the fermentation is carried out under anaerobic conditions. The review also touches upon the different methods and compounds used for switching between aerobic and anaerobic conditions.

Keywords: *lignocellulose waste; biofuels; anaerobic; facultative anaerobes*

Bioconversion of lignocellulose waste

Over one-third of the food generated for human consumption is wasted globally, amounting to 1.3 billion tons of agricultural waste per year [5]. Agricultural wastes are the non-product outputs of production and processing of agricultural products [8]. Although they contain materials which can benefit man, their economic value is very low when compared to the costs of collection, transporta-tion and processing. The accumulation of these agricultural wastes or agro-wastes pose serious prob-lems of disposal, as an improper handling of the waste will result in various environmental problems ranging from air pollution to contamination of surface and ground water. Most of the agricultural wastes are either incinerated releasing toxic gases into the atmosphere or are just left to dump in landfills, resulting in environmental pollution. A large portion of agro-wastes i.e., 90 % of agricul-tural wastes comprises of lignocellulose [31]. It also contains of small portions of proteins, lipids, organic acids and some inorganic remainders. Lignocellulose typically constitutes 10-25 % lignin, 20-30 % hemicellulose and 40-50 % cellulose [5]. The environmental impact caused by their improp-er disposal led researchers to look for ways of disposing them in an environmentally friendly and cost-effective manner. Most waste management systems aren't very effective either due to high cost or due to its environmental impact [40]. One solution to solving this problem was by coming up with the agricultural waste management functions (AWM). AWM categorised the waste disposal into 6 options, including- prevention, minimisation, reuse, recycling, energy recovery and disposal (in the order of most favourable to least favourable methods) [8]. Despite the initiatives, the last 20 years has witnessed a shift in the focus of researchers from finding ways to dispose the lignocellulose waste to effectively utilising the lignocellulose waste into the production of useful products like single cell proteins, lactic acid and bioethanol [33]. In fact, studies suggest that lignocellulose is the most abundant carbon source available on earth. It is the only carbon source that could potentially provide sufficient substrate to satisfy the needs of our environment in a sustainable and renewable manner [37]. Apart from the lignocellulose waste, other naturally occurring sugar crops and starch crops like sugar canes and sugar beets can also be utilised to produce a large

number of commercial-ly important products, especially biofuels [5]. However, the use of lignocellulose waste has an add-ed advantage because as it not only helps in curbing the problems associated with the accumulating agricultural wastes, but also helps in increasing the economic value of the waste. According to a study, the most abundant lignocellulose waste available, is Rice Straw. About 731 million tons of rice straw is produced annually [48]. This agricultural waste generated is higher in Africa, Asia, Eu-rope, and America; with Asia being the leading generator of rice straw (667.6 million tons) [48]. This study, thus, potentially indicates the importance of effectively utilising lignocellulose waste to produce commercially important products.

The reason for lignocellulose waste having an added advantage over other crops can be attributed to the composition of lignocellulose itself. Lignocellulose, as the name suggests comprises of lignin, cellulose and hemicellulose (which connects lignin and cellulose). Lignin, a recalcitrant and non-de-gradable component forms the backbone of lignocellulose. It also constitutes of cellulose and hemi-cellulose, which upon hydrolysis can yield fermentable sugars like glucose. A fermentable sugar, as the name suggests can be fermented into useful products like lactic acid, single cell proteins, glycol-ic acid, phenolic compounds and ethanol. Of particular interest is the bioconversion of lignocellulose into lactic acid and ethanol. Lactic acid especially, is a product with high commercial importance, due to its use in several industries, ranging from packaging to pharmaceutical. Furthermore, it can be purified and polymerised to Polylactic acid (PLA), which can be used a greener alternative to petro-leum derived plastics. Also, the lignin from the lignocellulose is not wasted and can be used as a stabilising agent of PLA [49]. However, complete utilisation of the lignocellulose waste still remains a challenge. Recent research has thus focussed on optimising the parameters for improving the yield of these useful products, including changing the culture conditions, micro-organisms used and also metabolic engineering [22]. Among all the parameters, the most interesting one is the effect of oxy-gen on the biodegradation of lignocellulose to lactic acid and alcohol. The yield of lactic acid and alcohol is reported to be significantly higher under anaerobic conditions when compared to aerobic conditions.

Biodegradation of lignocellulose

The complex structure of lignocellulose requires it to be hydrolysed and processed further in order to efficiently convert it into useful products. Lignocellulose consists of 3 components, namely-cellulose (the non-soluble fibre made of beta 1-4 glucan), hemicellulose (a non-cellulosic polysaccha-ride made of xylan and mannans) and lignin (a polyphenolic structure) [5]. As mentioned in the pre-vious section, the complete utilisation of lignocellulose waste is still a challenge due to its complex and rigid structure.

In the natural environment, composting of the agricultural waste/ plant waste occurs mostly in the dark. Due to this, the energy needed to support life for the bacteria and fungi involved in compost-ing cannot come from photosynthesis. They utilise the energy from the hexose and other fermenta-ble sugars formed after the breakdown of cellulose and hemicellulose [33]. The starting composition of the composting mixture is very important, depending on the C/N ratio, pH and abundance of mi-croorganisms. The C/N ratio is very crucial for the composting of lignocellulose. However, most lig-nocellulose materials like wood and dry grasses are rich in carbon, but contain insufficient amounts of nitrogen [33]. Furthermore, the recalcitrant structure of lignin makes it difficult to release the cel-lulose and hemicellulose from the lignin structure [32]. The lignocellulose waste has to be pretreated and hydrolysed in order to be effectively used for production of commercially important products. In most cases, dilute acid pretreatment is able to efficiently hydrolyse the hemicellulose to xylose, arabinose and glucose. This form of pre-treatment also enables the digestion of cellulose to glucose.

The heterogenous composition of microorganisms (including bacteria and fungi) present in the com-post plays a major role in the biodegradation process. All the different microbes present in the com-post work as a community to bring about the biodegradation of the lignocellulose. For instance, the cellulase enzyme of the cellulose degrading bacteria help breakdown the cellulose, while the ligno-lytic enzyme from yet another organism, which can help break down the lignin structure helps in releasing the cellulose and hemicellulose from the lignin. Once the cellulose is released and broken down into fermentable sugars, certain other organisms having enzymes which can ferment these sugars will act on them and the process continues. Thus, at each step, every organism will be in-volved in the compositing process and coexist as a community [33].

The same process can be reciprocated in the laboratory setup using various physical and chemical pretreatment methods for the breakdown of lignin to release cellulose and eventually use the cellu-lose

degrading bacteria and lactic acid bacteria to convert the cellulose to lactic acid. These experi-mentations are done to study and improve the bioconversion of Lignocellulosic waste with maxi-mum product yield, irrespective of ethanol, glycolic acid, Single Cell Protein or Lactic acid.

Lignocellulose waste to Lactic acid

Out of the several useful products of the bioconversion of lignocellulose waste, lactic acid is a promising source. Food waste, in particular are potential sources of nutrients for the growth of lactic acid bacteria [34]. The lactic acid obtained after the bioconversion of lignocellulose can be polymer-ised and fabricated into Poly Lactic Acid (PLA), which is a well-known bioplastic. PLA will thus replace the petroleum derived plastics which are non-biodegradable and cause serious problems of disposal. Apart from polymerising to PLA, lactic acid also has applications in a wide range of indus-tries including food, medicines and bioenergy [34]. The ethanol, formed as a byproduct, can be used as a biofuel.

Lignocellulose, as such cannot be used to convert into lactic acid. It requires pretreatment due to its complex structure. The importance of pretreatment lies in the fact that it increases the accessibility of cellulose to cellulase enzymes (present in certain bacteria) and disrupts the lignocellulose-hemicellulose-cellulose complex (LHC) [32]. Different pretreatment methods are available [5]. They mainly fall under 4 categories- Physical, Chemical, Physico chemical or biological.

Milling, microwave treatment, and ultrasonication are the most common physical techniques of pre-treatment. Ultrasonication as a pretreatment technique has the potential of disrupting the lignocellu-losic materials. It can facilitate the hydrolysis and the 80% of biomass but experimental benefit is not given to bio-fuel production by this pretreatment, as ultrasonication is energetically inefficient [17]

The use of microwaves for the pretreatment of lignocellulose has been carried out by Zongyuan Zhu et.al [47]. Microwaves are known to play a notable role in the hydrolysis of lignocellulose. Despite the increased solubility and decreased recalcitrance of the lignocellulose, the pretreatment process also produced byproducts which potentially affect the further processing of the lignocellulose.

Milling as a pretreatment has the advantage of no toxic or inhibitory compound production. There-fore, it is preferred either as a preliminary treatment along with chemical pretreatment method or in some cases, the only pretreatment method. A variety of lignocellulosic feedstocks can be subjected to this milling pretreatment [5].

Alkali/ acid pretreatment are widely studied chemical pretreatment methods. However, in the pre-sent days, ionic liquids and organo solvents are used alongside alkali/ acid pretreatment methods [27, 32]. Studies have shown that alkali pretreatment is an effective technique in removing lignin and makes carbohydrates more exposed to use for the downstream processes, although the removal of alkali is a disadvantage associated with this technique. Organic acids in conjunction with inor-ganic acids such as Hydrochloric acid, Sulphuric acid and Nitric acid for lignocellulosic pretreat-ment is an area that needs to be explored yet.

Some researchers have also demonstrated the use of Response Surface Methodology (RSM), which combines the effect of three parameters including organic acid concentration, treatment time and reaction temperature to evaluate its effect on the pre-treatment of lignocellulose. A significant im-provement was demonstrated with regard to the breakdown of lignin as well as the yield of the end products [1].

Irrespective of the method used, the objective of pretreatment is to release the cellulose and hemi-cellulose (in some cases) from the lignin and make it accessible by the different microbes present in the compost. In this case, i.e., conversion of lignocellulose to lactic acid and ethanol, there are main-ly 2 groups of microbes involved in this bioconversion, namely-

- Cellulose degrading bacteria- to break down the cellulose into fermentable sugars [50]
- Lactic acid bacteria- to ferment the hexose monomers into lactic acid and ethanol [51]

Groups of bacteria involved in the bioconversion of lignocellulose to lactic acid

Cellulose degrading bacteria

They are an interesting class of microorganisms which carry out a process called cellulolysis. En-zymes belonging to the cellulase system of cellulose degrading bacteria help process the cellulolysis. The cellulase enzyme systems comprises of 3 extracellular enzymes- 1, 4-β-endoglucanase, 1, 4-β-exoglucanase, and β-glucosidase (β-D-glucoside glucohydrolase or cellobiose) [23]. These enzymes are commonly produced by some bacterial genera such as Cellulomonas, Pseudomonas, Bacillus and

Micrococcus, that are now widely used several in industrial applications [3]. The cellulose de-grading bacteria can be isolated from different sources including gut of termites, earthworms or even vermicompost.

Lactic Acid Bacteria

Lactic acid bacteria are the most important and versatile group of microorganisms. They are a vast and diverse microbial group with potential uses in several industries including dairy, animal feed silage, fish and meat processing and are even found to be a part of the intestinal flora of humans. But they are mainly used in food fermentation. Fermentation is an anaerobic enzymatic conversion process. This series of chemical reactions involves the breakdown of glucose or other fermentable sugars to commercially useful products. The distinct feature of lactic acid bacteria is lactic acid pro-duction. Most lactic acid bacteria are free living or live in mutualistic associations with humans and other animals as intestinal gut bacteria, although some are also opportunistic bacteria [42]. Chen et al had reported around 32 isolated bacteria from 68 soil samples collected, of which are acid produc-ing bacterial species. Both physiological and getic tests were done and identified as lactic acid bac-teria belonging to: Lactobacillus, Lactococcus, Enterococcus, Leuconostoc and Weissella [52].

Lactic acid bacteria play a major role in the fermentation of hexose monomers like glucose into lac-tic acid. The first pure culture of lactic acid fermenting bacteria is Lactococcus lactis isolated by Joseph lister in the year 1873 [34]. Although the most commonly studied fermentable sugar is glu-cose, studies have also reported the fermentation of fructose into lactic acid along with other prod-ucts like mannitol, acetic acid, alcohol and CO2. This particular pathway was studied in Leuconos-toc mesenteroids [20]. The pathway of lactic acid formation is same in both the sugars, however, the main difference lies in the fact that fructose is a hydrogen acceptor, while glucose is not.

Characteristics of Lactic Acid Bacteria

Conventionally, lactic acid bacteria belong to the following genera- Lactobacillus; Leuconostoc; Pediococcus; Lactococcus; and Streptococcus in addition to Carnobacterium, Enterococcus, Oenococcus, Tetragenococcus, Vagococcus, and Weisella [42]. The first pure culture of lactic acid bacteria was as already mentioned is that of Lactococcus lactis in the year 1873 by Joseph Lister [34]

Lactic acid bacteria [LAB's] are known to possess unique features. They are gram positive cocci or rods which show optimal growth at 35 to 40 degrees C and at a pH of 5 to 6 [35]. They have a tol-erance to low pH and are found to inhabit a broad range of diversity. They are chemotrophs and grow in conditions with high sugar. They lack catalase enzyme in addition to lacking some compo-nents of the respiratory chain like cytochromes, so the only way they generate ATP is through fer-mentation of glucose. Since lactic acid bacteria also do not possess cytochromes, they do not require oxygen, thus, they can grow without them. Hence, most of the lactic acid bacteria are facultative anaerobes. They are protected from oxygen due to the presence of peroxidase enzymes [35]. It can be observed that a common feature among all the LAB's is the enzymes of the glycolytic cycle. This is the universal feature of LAB's due to the fact that they recover their primary source of energy via glycolysis. A recent study on L. acidophilus examined the transcriptional analysis upon its growth on 8 different carbohydrates. It was observed that the highest expression was shown by the en-zymes of the glycolytic pathway [30]. However, this is one of the very few genetic traits that ap-pears to be conserved among all LAB's. They ferment glucose primarily to lactic acid, ethanol and CO2. All LABs grow anaerobically, however, unlike other anaerobes, they possess the ability to grow in the presence of oxygen as aero tolerant anaerobes/ facultative anaerobes. Another interest-ing feature of LAB's is their small genome size (1.7 to 3.3 Mb), thereby harbours a limited set of genes (from 1600 to 3000 genes) [4]. This corresponds to their lack of certain enzymes, like catalase and other key genes of the respiratory chain, making them different from the other groups of bacte-ria. In fact, these small genomes only code for transporters to assimilate carbon and nitrogen sources from their adaptive environments and lack the other genes, thereby leading to their small genome sizes.

Culturing of lactic acid bacteria requires the use of complex media to fulfil all their nutritional re-quirements as they have limited biosynthetic ability. A lot of factors have contributed to the present structure and shape of the genomes of LAB's. The most important factor is their adaptation to dif-ferent environments [30]. They have evolved in environments rich in vitamins, amino acids and pu-rines and pyrimidines. Most LAB's are highly deficient in key biosynthetic pathways corresponding to their adaptation to different nutritionally rich environments. In fact, this adaptation to nutritional-ly rich environments has resulted in gene simplification and

even degradation in some lactic acid bacteria, and this decay was seen in genes involved in carbohydrate metabolism. However, interest-ing evidence was found in S. thermophilus, which showed the presence of a lactic acid symporter which was absent from the other pathogenic streptococci. A yet another evidence also suggests the horizontal gene transfer from Staphylococcus thermophilus to other organisms inhabiting the diary environment. Similarly, Lactococcus lactis obtains genes for critical functions for growth and com-petence in the milk environment, like genes encoding lactose metabolism, resistance to bacteriocins and bacteriophages. The introduction of these genes which promote its competition is attained due to horizontal gene transfer.

Genes present in lactic acid bacteria

The genome analysis of LABs has revolutionalized the understanding of metabolic pathways of LAB's and also their potential roles in overall health and well-being. Among the LAB's that were completely sequenced are Lactobacillus brevis, L. casei, L. gasseri, Lc. cremoris, Leuconostoc mesenteroides, Oenococcus oeni, Pediococcus pentosaceus, and S. thermophilus. Their genomes are completely annotated and published. Genome analysis of other LAB's like lactobacilli, bifidobacteria, streptococci, and lactococci possess a broad range of saccharolytic potentials, which explains the broad range of diversity in their habitat. The organism's broad capacity to metabolise a range of carbohydrates from a varied range of environments is what makes LAB's different and interesting from the other groups of bacteria. For instance, the genome analysis of L. plantarum has shown the presence of a transport system called Phosphotransferase system (PTS) involved the or-ganism's capacity to metabolise a broad range of carbohydrates. Interestingly, a specific region in the genome of LAB's is a 213 kb region called the "lifestyle adaptation island" which is dedicated to the broad range of metabolism of carbohydrates. They are involved in sugar transport and metab-olism [4]. Apart from their unique genomic sequences, the proteolytic system of lactic acid bacteria is very extensive and unique, thus provides an added advantage over other groups of bacteria when applied for the manufacturing of many different compounds [34]. The proteolytic system of lactic acid bacteria converts proteins to the main amino acids which are essential for flavour compounds like aldehydes, alcohol and esters. Components of the proteolytic system is seen in a diverse range of lactic acid bacteria and a few enzymes, like the cell wall bound proteinase PrtP are very unique to lactic acid bacteria, and that gives them an advantage over other groups of bacteria.

Generally, the predominant end product of carbohydrate fermentation of lactic acid bacteria is lactic acid. However, upon adaptation to various conditions and their change in metabolism, it could po-tentially lead to significant end product patterns. Biochemically, lactic acid bacteria are classified into 2 groups, namely- Homolactic Bacteria and Heterolactic Bacteria

Homo Lactic Bacteria - They involve the fermentation of hexoses using the Embden-Meyerhof (E-M) pathway. The major end product is 2 moles of lactic acid. This pathway is generally exhibited by enterococci, lactococci, pediococci, streptococci, tetragenococci, and vagococci [42]. This type of fermentations occurs under excess glucose and limited oxygen. The EM pathway yields 2 moles of pyruvate from 1 mole of glucose. The pyruvate is then reduced to lactic acid. The process involves the production of 2 moles of ATP per molecule of glucose.

Hetero Lactic Bacteria- it involves the fermentation of pentoses and gluconate. This is not seen in homofermentative LABs as they lack an enzyme phosphoketolase. Furthermore, the heterolactic bacteria also lacks aldolase and isomerase enzymes, suggesting that they do not follow the Embden-Mayerhof pathway [7]. Instead, the main pathway used by these organisms is the phosphoketolase pathway/ hexose monophosphate shunt pathway, which shares many similarities with pentose phos-phate pathway. The end products of this pathway is, in most cases, equimolar amounts of lactic ac-id, alcohol and CO2. This group includes leuconostoc, some lactobacilli, oenococci, and weisella species [36]. Within the group of heterolactic bacteria, two fermentation patterns were observed and was described by Nelson et.al in 1935 [18]. The final products of the two fermentations were found to be as follows- 1. Equimolar quantities of lactate, ethanol and carbon dioxide along with traces of acetate. The production of glycerol along with lactate, ethanol and carbon dioxide [18].

An interesting example of heterofermentative lactic acid bacteria is Leuconostoc mesenteroids. In fact, the earliest works on heterolactic bacteria was done using this model organism in the year 1961 [20]. In a yet another interesting study, the bacteria Streptococcus lactis, which is a homofermenta-tive bacteria, has shown the capacity to switch between homolactic and heterolactic fermentation under

anaerobic conditions [29]. According to the study, when glucose was available as a fermenta-ble sugar (hexose sugar), almost 90-95 % of the glucose was fermented to lactic acid. However, un-der anaerobic conditions, when glucose was depleted, the cells could shift to heterolactic pathway and ferment galactose (pentose sugar) into several products including lactic acid, ethanol, acetate, fumarate and CO2. The cells obtained energy under anaerobic conditions by a mechanism involving pyruvate formate lyase. In addition to this pathway, they also induced arginine catabolism to release more ATP [29]. Many Lactic acid bacterias can also ferment pentoses to lactate and acetate without CO2 production through the bifidum pathway [34].

Based on their metabolism, lactic acid bacteria can be classified into 3 groups- Aerobic, Facultative Anaerobic and Obligate Anaerobe.

Respiration is more efficient in the presence of oxygen, while fermentation is more efficient in the absence of oxygen. The response of bacteria to oxygen is not determined simply by their metabolic needs. Oxygen, being a highly reactive molecule, forms several toxic by products like superoxide and peroxide. Aerobic microbes have the enzymes necessary to detoxify these toxic products, the most common enzyme being catalase. These toxic by products, if not detoxified properly by cata-lase, have the potential to cause severe damage to the cell, especially upon reaction with iron. Since many anaerobes do not possess catalase, they are hypersensitive to oxygen. Some aero tolerant mi-crobes are more tolerant towards oxygen [35]. However, those who rely on oxygen for some of the biosynthetic reactions, like aerobes, they do not survive under anaerobic conditions [45].

Obligate Anaerobe Vs Facultative Anaerobe

Obligatory anaerobe- They typically live in oxygen free places like gut of organisms or the mud. They can tolerate oxygen concentrations ranging from 0.5 to 8 %. Their mechanism of metabolising energy does not require oxygen. If they are exposed to oxygen, these microbes become dormant and start forming endospores until they are exposed to an anaerobic environment [44]. Based on their relationship to oxygen, they can further be differentiated into two groups.

They are-

1. Aero- tolerant anaerobes, which are slightly inhibited by oxygen, however, they can grow on the surface of agar plates incubated at atmospheric pressure, and

2. Strict Anaerobes, which represent the other extreme, which die immediately upon exposure to low levels of oxygen. Most strict anaerobes also require redox potential below 300 mV [44].

Facultative anaerobe- Although they can grow well in oxygen, they can also continue to grow in its absence. Therefore, they can switch between aerobic and anaerobic conditions with ease. They can change their metabolism based on the presence of oxygen.

The most interesting class of bacteria is the facultative anaerobe. The detailed chemistry of lactic acid fermentation by facultative anaerobes will be described in section 10.

Apart from bacteria, some studies have shown the potential of other groups of microorganisms like yeasts in lactic acid fermentation. For instance, a study led by Christopher. D. Skory, demonstrates the potential of Rhizopus oryzae in lactic acid fermentation. It is a filamentous hetero- thallic micro-fungus [26]. However, lactic acid production using fungi requires an aerial condition as it slows the reaction rate. But, the heterologous gene expression of Lactate Dehydrogenase (a key gene in lactic acid bacteria) in Saccharomyces cerevisiae has proven to enhance the yield of lactic acid [19].

Simultaneous Saccharification and Fermentation (SSF)

Simultaneous saccharification and fermentation is a process of carrying out both hydrolysis of cellu-lose and fermentation of glucose in the same step [24]. The main advantage of SSF is reduced end product inhibition of the enzymatic hydrolysis. This also dramatically reduces the cost of the entire process as less equipments and vessels [16]. In a study led by Keikhosro karimi, simultaneous sac-charification and fermentation was carried out through dilute acid pretreatment, and it was ob-served that lactic acid was the significantly increased under anaerobic conditions [15]. Furthermore, glucose does not need to be separated from the lignin, so, this potentially avoids the loss of sugar. However, SSF also has some disadvantage that the optimum temperature for hydrolysis and fer-mentation is different, especially in yeast. Thus, a compromise has to be made with regard to the temperature.

The reason for high yield of the products and reduced end product inhibition can be attributed to the fact that yeasts convert the inhibitors of enzymatic hydrolysis like Hydroxymethyl furfuralde-hyde (HMF) to produce weak acids like acetic acid and formic acid. These weak acids are otherwise produced by the de-

acetylation of hemicellulose [16]. Recent research by a team led by Asiminia et.al in 2019, has also demonstrated a one pot chemocatalytic process which can also synthesise lac-tic acid from cellulose directly [2].

Factors affecting the yield of lactic acid

1. C/N ratio- the composition of the medium used has a significant impact on the yield of lactic ac-id. Specifically, the C/N ratio is known to play an important role in the yield of lactic acid. Most lignocellulosic materials like woods and dry grasses have more carbon and very little nitrogen. A study conducted by Zhang et.al studied the role of nitrogen sources on the lactic acid production using Rhizopus arrhizus. A low C:N ratio is known to enhance the yield of lactic acid and alcohol, while a higher C:N ratio is known to favour the formation of fumaric acid [32]. Among the nitrogen sources studied, Ammonium nitrate was the most useful nitrogen source for lactic acid production, while the least useful was urea. In general, the yield of lactic acid is reduced upon nitrogen defi-ciency. This is because they cannot synthesise amino acids from inorganic nitrogen sources. Fur-thermore, the carbon content is known to decrease as the composting proceeds and that can be at-tributed to the fact that cellulose and glucose are being efficiently converted to other products [33].

2. Microorganism used for fermentation- In general, the type of microorganism used depends on the type of fermentation product. In case of lactic acid fermentation, the main organisms used in the study of lactic acid fermentation belong to the lactic acid bacteria. The different species of lactic acid bacteria include species of Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Weisella. Different microorganisms have demonstrated varying levels of lactic acids upon fermentation of glucose.

3.Cellulose degradation - cellulose degradation is affected by the action of cellulase enzymes pre-sent in cellulose degrading bacteria. Cellulase enzymes fall into three major categories, namely- en-doglucanases, exoglucanases and glucosidase. A specific thermophilic strain Geobacillus stea-rothermophilus has gained importance in the recent past due to its characteristic cellulase enzyme [25]. The biggest advantage of the cellulase enzyme isolated from this particular strain is its stability at high temperatures and a broad substrate range. This directly affects their breakdown into fer-mentable sugars and other monomers. This in turn affects the yield of lactic acid and ethanol. Fur-thermore, certain lignolytic chemicals like hydrogen peroxide can breakdown lignin more effectively when compared to dilute acids, thus resulting in an increased release of cellulose from the lignin. This in turn affects the overall yield of lactic acid [33].

4. Effect of immobilisation- the yield of lactic acid can be enhanced by carrying out the fermenta-tion in an enclosed environment [22]. Immobilisation can also reduce the fermentation time. Fur-thermore, immobilised cells have an excellent mechanical strength to prevent them from breakage during agitation. All these advantages help have an effect on the yield of lactic acid. Such an envi-ronment can be attained by encapsulating the substrate and the organism in a hydrogel containing sodium alginate and calcium chloride or polyvinyl chloride [13].

5. pH and temperature- External pH and temperature can also affect the yield of lactic acid [22]. The yield of lactic acid over a few days of the week can decrease also due to the non-availability of substrate and product inhibition.

6. Effect of oxygen - As mentioned previously, there are 3 groups of lactic acid bacteria based on their metabolism. In particular, 1 group of bacteria- facultative heterofermentative/ facultative an-aerobes [22]. It is observed that the yield of lactic acid is significantly higher under anaerobic condi-tions when compared to the aerobic conditions, as demonstrated by several studies. They will be discussed in detail in the following section.

Evidence for higher yield of lactic acid under anaerobic conditions

Experiments carried out using Streptococcus, by a team led by J C White in New York, revealed that the percentage of lactic acid under aerobic conditions was significantly lower than under anaer-obic conditions [11].

A similar study done using another model organism, Lactobacillus plantarum, a facultative het-erofermentive bacteria, where they studied lactic acid fermentation under 2 conditions- Aerobic and Anaerobic conditions. It was observed that the yield of lactic acid was about 2.3 times than that of aerobic fermentation under optimal pH conditions (pH: 5 to 6) [12].

Not just bacteria, but such experiments have also been reported in yeasts/ Fungi. For instance, lactic acid production was studied under oxygen limiting conditions using Rhizopus oryzae as a model organism [26]. According to the study, under aerobic conditions, Rhizopus oryzae is known to pro-duce

lactic acid by the reduction of pyruvate using lactate dehydrogenase, while the oxygen limit-ing/ anaerobic conditions yielded primarily alcohol. However, a mutant of this fungus was identi-fied, which expressed only 5 % of the wild type of alcohol and nearly 40 g/ l of lactic acid over a period of 70 hours. This is nearly 10 times than that of the parent strain [26]. This indicates that some strains can be engineered (genetically or metabolically) to enhance the yield of lactic acid un-der anaerobic conditions.

The above works illustrate that the yield of lactic acid is significantly higher under anaerobic condi-tions.

Chemistry of Heterolactic fermentation by facultative anaerobes

Although facultative anaerobes prefer an anaerobic condition, they can easily switch between the two states depending on the presence or absence of oxygen. In general, facultative anaerobes are capable of oxidative phosphorylation in the presence of oxygen. Upon fermentation of glucose to lactate or ethanol and CO2, the yield of ATP is less I.e., 2 mol/ mole of substrate [41]. This is be-cause pyruvate is used to revive the NADH produced during glycolysis. Thus, the end product of this fermentation is lactate or ethanol and CO2. This reaction is in general wasteful because ATP can otherwise be generated by further fermentation of pyruvate to acetate [41].

Due to its low energy yield, lactic acid fermentation is only important in places where easily de-gradable/ fermentable sugars are available in high concentrations. The nature of these environments is an added advantage for the acid tolerant lactobacilli over other groups of fermenting bacteria [41]. Lactic acid bacteria, in general, are an important class of microorganisms. However, 3 specific species of lactic acid bacteria namely- Lactobacillus case, Lactobacillus plantarum and Lactobacil-lus's sake are the most versatile among all other lactic acid bacteria, since they can deal with aerobic and anaerobic growth conditions [28]. They are facultative anaerobes.

Facultative anaerobes, in contrast to the obligatory anaerobes (where oxygen is toxic for their growth) can tolerate oxygen and can even use it for respiration. They basically thrive in aerobic con-ditions I.e.; they require oxygen for their growth. However, under anaerobic conditions, they shift their metabolism towards fermentation. The implications of this shift in metabolism under aerobic and anaerobic conditions is evident in Lactobacillus plantarum [9]. According to the study, the growth and kinetics of Lactobacillus plantarum in fermentation was studied. It was observed that although the growth of the bacteria was more under aerobic condition, the lactic acid production was more under anaerobic conditions. A similar study was done by a team in Slovakia, wherein they studied the influence of aerobic and anaerobic conditions on selected strains of Lactobacillus plantarum. It was observed that the yield of lactic acid under anaerobic conditions was about 2.3 times more than under aerobic conditions [12]. This indicates the significance of the shift between aerobic and anaerobic bacteria, and this is why facultative anaerobes are an interesting group of an-aerobic bacteria.

They have the ability to respire and ferment organic substances, under both aerobic and anaerobic conditions, however, the yield of the metabolic product remains high under anaerobic conditions. In addition to the high levels of lactic acid under anaerobic conditions, the shift from anaerobic to aer-obic conditions offer physiological advantages to the lactic acid bacteria [28]. They have increased tolerance to heat and oxidative stress. Although they require aerobic conditions for enhanced growth, aerobic conditions also have some disadvantages. In general, the aerobic growth conditions result in the formation of reactive oxygen species. But Lactic acid bacteria possess ROS removing enzymes like catalase that can completely remove the reactive oxygen species from the system. Cat-alase enzyme is a degradative enzyme which can breakdown hydrogen peroxide to water and mo-lecular oxygen. Lactic acid bacteria possess 2 families of catalase enzymes namely- Super oxide dismutase (SOD) and NADH oxidase/ NADH peroxidase system (NOX/NPR). The presence of this enzyme is responsible for the ability of lactic acid bacteria to shift between the two pathways based on the presence or absence of oxygen. Ideally, aerobic conditions enhance the growth, amp levels and stress tolerance of the lactic acid bacteria, while anaerobic conditions result enhances the levels of the metabolic products, in this case, Lactic acid and alcohol.

Although facultative anaerobes follow a different pathway for respiration and fermentation, the first step in metabolism common to both aerobes and anaerobes is glycolysis, wherein a simple sugar like glucose is converted to phosphoenolpyruvate and further converted to pyruvate.

Aerobic respiration in the presence of oxygen, goes through 2 more phases- TCA (Tricarboxylic ac-id) and ETC (Electron transport chain), resulting in the conversion of pyruvate into acetyl CoA with the help of pyruvate dehydrogenase. Acetyl CoA, is oxidised in 8 enzymatic steps to NADH, which is a reducing

agent. NADH thus acts as an electron donor to oxygen (the terminal electron accep-tor). This step converts oxygen to water. Thus, under aerobic conditions, the complete breakdown of glucose results in 36 molecules of ATP per molecule of glucose.

However, under anaerobic conditions, the organism takes another route to obtain energy. This pathway involves the transformation of pyruvate to regenerate NAD acceptors like sulphate, nitrate and fumarate [43]. Thus, NAD donates electrons to sulphate, nitrate and fumarate instead of oxy-gen. For instance, Bacillus subtilis, a well-known anaerobe, can utilise Nitrate as an electron accep-tor in place of oxygen, for the production of lactic acid [6]. However, since their affinity for elec-trons is low compared to oxygen, it yields less amount of energy.

It is only in cases where even these alternatives are not available, that facultative anaerobes resort to using fermentation as a means of energy production. Fermentation involves the conversion of py-ruvate to lactate and alcohol using lactate dehydrogenase and alcohol dehydrogenase, respectively. But, the amount of energy produced is significantly less than aerobic conditions, yielding just 2 molecules of ATP per unit of glucose. This is the reason why some lactic acid bacteria show a good growth under aerobic conditions and a higher yield of lactic acid yield under anaerobic conditions [9].

Switch between aerobic and anaerobic conditions

Since the yield of lactic acid and ethanol is clearly higher under anaerobic conditions and that facul-tative anaerobes are able to ferment glucose to lactic acid and ethanol better than the aerobes, it would be interesting to switch between aerobic and anaerobic conditions in order to yield a maximal production of lactic acid and alcohol.

Several studies have used different methods to bring about this switch from aerobic to anaerobic conditions. The initial studies done on aerobic and anaerobic conditions used a basic air pump or water pump for aerobic and using stoppered flasks or cotton plugs for growing the cultures under anaerobic conditions [12]. Some studies also grow the cells as a thin layer on the liquid culture fol-lowed by vigorous shaking or aeration to maintain aerobic conditions. Some studies also used a mix-ture of 95 % Nitrogen and 5 % CO2. One particular study, in an attempt to isolate anaerobes from spacecraft clean rooms, prepared an anaerobic storage buffer consisting of sodium sulphide after flushing the vial with nitrogen. Sodium resazurin was added as a redox indicator [21]. As a redox sensitive dye, it is nontoxic to the bacteria and is effective even under low concentrations. The in-active form of the indicator is dark blue in colour, and it has to undergo a reduction step to convert to its active form which is pink in colour [21]. In addition to these reagents, anaerobic mixtures con-taining cysteine HCL and sodium sulphide are also available [21].

However, although the above methods were useful at that time, several advancements came up. Over the years, several researchers have shifted to chemical reduction of oxygen tension. They add compounds like thioglycolic acid (TGA), which can be directly added to the culture medium and thereby maintain the anaerobic conditions [46]. Such reducing compounds act by utilising the oxy-gen and thereby removing the oxygen from the medium. Thioglycolic acid is a reducing agent. The main advantage of thioglycolate is its relative stability at room temperature. One can thus add it di-rectly to the media without degassing the medium. It inactivated only at 100 deg C and effectively removes the oxygen from the medium. The standard redox potential of thioglycolate is -100mV [44]. Some studies have even demonstrated the use of phosphorous to culture bacteria under strictly anaerobic conditions. A few other examples of reducing agents used for the culture of anaerobes include iron compounds, alkaline pyrogallol, cysteine [10].

Conclusion

The environmental problems we are currently dealing, like accumulation of agricultural as well as plastic wastes has led researchers to study how they can utilise agricultural wastes, which mainly comprised of lignocellulose to produce bioplastics. The research on the pretreatment of lignocellu-lose, microbes used for the conversion of lignocellulose to lactic acid and the parameters used to optimise the conditions for maximal production of lactic acid has been trending. Lignocellulose, due to its abundance has proven to be a promising substrate for certain groups of microorganisms like cellulose degrading bacteria and lactic acid bacteria for the production of lactic acid and ethanol. Apart from the production of lactic acid, it can also be polymerised to polylactic acid (PLA), for its use as bioplastic. Despite several parameters affect the yield of lactic acid, a major factor affecting the yield of lactic acid is the presence or absence of oxygen. There are several instances which demonstrate that the yield of lactic acid is higher under anaerobic conditions. Although the yield is higher under anaerobic conditions, aerobic conditions is required for the growth of lactic acid

bacte-ria. An interesting class of lactic acid bacteria- the facultative anaerobes, which can thrive under both aerobic and anaerobic conditions can be employed here. The aerobic to anaerobic switch can be efficiently carried out by adding reducing agents like thioglycolic acid (TGA) to the medium.

Declaration

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Conflicts of interest- Not Applicable

Ethics approval- Not Applicable since living animals are not used

Consent to participate- As the principal investigator of the project Dr. Preetha Nair and as a re-search assistant in the project Ms. Gayathri L N are extending the consent to participate to be au-thors in the manuscript "Role of Aerobic and Anaerobic bacteria in the bioconversion of lignocellu-lose waste material".

Consent for publication- As the principal investigator of the project Dr. Preetha Nair and as a re-search assistant in the project Ms. Gayathri L N are extending the consent for publication of the manuscript "Role of Aerobic and Anaerobic bacteria in the bioconversion of lignocellulose waste material".

Availability of data and material- the data and material will be submitted whenever required. It is not applicable since it is a review article.

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Authors contribution- Both authors equally contributed in the preparation of manuscript.

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