ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

REVIEW ARTICLE

Action of Thermophiles on Plant biomass to Bioenergy- A Review

Rajakumari*, Thiruchelvi. R, Venkataraghavan R, Abhishek Biswal. R
Department of Bio-Engineering, School of Engineering, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-117, Tamil Nadu, India.
*Corresponding Author E-mail: **rajikumari91@gmail.com**

ABSTRACT:
Lignocellulosic biomass is the only suitable raw material as a sustainable renewable resource in the production of biofuels. Everyday there are tons and tons of second generation feed stock disposed of. These biodegradable wastes are a high rich source of carbohydrates, lignin, cellulose and hemicellulose. These wastes can be transformed to bioenergy by the action of microbial and enzymatic processes. Thermophiles are extremophiles that are robust, efficient and can withstand very high range of temperatures during bioprocessing conditions which can be used for this transformation technology from waste to wheels. Thermophiles have high rates of conversion of biomass/ lignocellulose to biofuel due to the influence of its thermostable activity at high temperatures. Native species of thermophiles does not possess high rates of conversion of biomass, yield etc. In order for successful accomplishment of biofuels, the thermophiles have to be metabolically engineered and expressed in heterologous host to augment the conversion rates and yield of biofuel and reduces the amount of by-product yield. Therefore, this review scintillates on the current existing technologies for the effective conversion biomass conversion, simultaneous saccharification and fermentation (SSF) and separate hydrolysis fermentation (SHF) through consolidated bioprocessing (CBP) techniques.

KEYWORDS: Thermophiles, lignocellulose, hemicellulose, consolidated bioprocessing, simultaneous saccharification and fermentation, separate hydrolysis fermentation.

INTRODUCTION:
Commercially the first generation feed stocks such as food crops like corn, sugar cane molasses, beet molasses and wheat are widely being practiced throughout the world leading to the demand and the increase in their prices (Havlik *et al.*, 2011)[1]. The second generation feed stocks are the food/non-food agricultural wastes which includes the lignocellulosic components which includes lignin, cellulose and hemi cellulosic rich biological components are its major constituents (Hamelinck *et al.*, 2005)[2]. Lignocellulosic components including cellulose and hemicellulose are a rich source of carbon which is essential for the survival of any microorganisms for the conversion of biomass to biofuel.

Received on 22.03.2018 Modified on 16.05.2018
Accepted on 19.06.2018
Research J. Pharm. and Tech 2018; 11(9): 4191-4199.
DOI: 10.5958/0974-360X.2018.00769.2

In order to effectively access the lignocellulosic components, the pretreatment of the biomass feed stock has to be carried out to break down the complex lignocellulosic structure to simpler molecules (Sun and Cheng, 2002, (Alvira *et al.*, 2010)[3]. Then, lignocellulose-degrading enzymes, hemicellulases, cellulases and xylan degrading enzymes, xylanases are acted upon for the process of saccharification of the feed stock. Commercially, the existing saccharification of biomass polymers to monomeric sugars are carried out at less than 50 degree celcius, thereby exhibiting low enzymatic rates of conversion, often incomplete hydrolysis and low yield of fermentable sugars from lignocellulose biomass through the action of thermophiles (Viikari *et al.*, 2007, Yeoman *et al.*, 2010).

MATERIALS AND METHODS:
Thermophiles and their enzymes:
Thermophilic bacteria are a diverse group of bacteria that are robust to 50 to 85 degree celcius and possess a high thermostability of their enzymes (Rakshit, 2003, Rastogi *et al.*, 2009, Barnard *et al.*, 2010)[4-6].

Thermophiles are isolated from hot springs, volcano prone regions and near incinerators. Thermostability is defined as the ability of the thermostable enzymes to retain their activity and their structural conformations at a very high temperature over a long period of time. Thermostable enzymes have many potential commercial applications in many ways (Demirjian *et al.,* 2001)[7]. Thermostable enzymes possesses i) High rates of hydrolysis of lignocellulose biomass and yield fermentable sugars. ii) Augmented activity of enzymes at high temperatures. iii) Shorter hydrolysis time. iv) Low cost of pre-treatment, since at the complex lignocellulose is broken down to simpler components. v) Ease during product recovery. vi) Low risk of contamination during fermentation. Thermostable enzymes such as thermostable cellulases, hemicellulases and xylanases which are active at very high temperature play a vital role in the biofuel production (Shrinivas *et al.,* 2010, Liang *et al.,* 2011)[8, 9].

Thermostable enzymes:

Thermostable enzyme cellulases:

Cellulases are the enzymes that are actively reported to catalyze the cellulose hydrolysis (depolymerization) (Lynd *et al.,* 2002)[10]. Cellulases are produced by diverse group of microorganisms including bacteria, protozoans and bacteria. Cellulases hydrolyze the cellulose into simpler sugar monosaccharides such as D-glucose and oligosaccharides. The cellulose hydrolysis to sugars is necessary for the action of microorganisms to ferment the saccharides to convert them into useful product. Cellulases hydrolyze the 1, 4-β-D-glycosidic linkages in polymers like cellulose and hemicellulose. The three main classes of cellulases are i) 1, 4-β-D glucan glucohydrolase (EC 3.2.1.4) is an enzyme that hydrolyses cellulose to oligosaccharides and monosaccharides like glucose ii) 1, 4-β-D glucan cellulobiohydrolase (EC 3.2.1.91) hydrolyses the 1, 4-β-D glucosidic linkages and iii) β-D-glucoside glucohydrolase (EC 3.2.1.21) does the hydrolysis of cellulobiose and convert to glucose. The enzymes 1, 4-β-D glucan glucohydrolase are endoglucanase and 1, 4-β-D glucan cellulobiohydrolase are exoglucanase. The enzymes endoglucanase and exoglucanase are collectively referred to as "cellulases" (Blumer-Schuette *et al.,* 2008)[11]. The endoglucanase is called the primary cellulases and contains the carbohydrate binding molecule making the microorganisms to efficiently utilize the cellulose.

Thermophilic fungal species:

There are diverse group of thermophilic fungal and bacterial species that are capable of producing thermostable enzymes. For example, the fungal species includes *Trichoderma, Aspergillus, Sclerotium, Thermoascus thermophile and Rhizopus*(Barnard *et al.,* 2010)[6]. These fungal species secrete cellulases, but the efficiency of cellulose hydrolysis was not complete, the enzymes are only capable of partial hydrolysis of cellulose. The prime reason for this incomplete hydrolysis is due to the low activity of the β-D-glucoside glucohydrolase enzyme (Gusakov *et al.,* 2007)[12].

Thermophilic bacterial species:

Diverse group of thermophilic bacteria have reported to produce cellulases enzymes which includes bacterial species like *Geobacillus, Bacillus, Caldibacillus* and *Clostridium* (Bok *et al.,* 1998)[13]. These organisms are often called thermoanaerobic bacteria since they are capable of withstanding very high temperature zones with high enzyme activity. Hyperthermophilic bacteria produces thermostable lignolytic enzymes have enhanced activity at 80 degree celcius. These enzymes are isolated from hyperthermophiles such as *Thermotoga* wrapped with unique protective sheath like outer membrane called "toga" which protects the bacteria from extreme heat, *Anaerocellum* is an anaerobic hyperthermophile, *Sulfolobus* and *Rhodothermus.* Many research reports reveal that hyperthermophiles cannot completely degrade the crystalline cellulose molecule at temperature greater than 75 degree celcius due to the lack of the carbohydrate binding molecule (Maki *et al.,* 2009, Graham *et al.,* 2011)[14]. Nevertheless, the multi domain thermostable enzymes like cellulases in archaeal environment degrade the cellulosic polymers efficiently at above 90 degree celcius. The impressive adaptability of thermophiles and hyperthermophiles to a wide range of pH known to be thermoaciodophiles and thermoalkalophiles during bioprocessing makes them effective for the conversion of lignocellulosic feed stock to biofuel. For an example, thermophilic cellulases endoglucanases isolated from the strain *Acidothermus cellulolyticus* which is a thermoacidophilic bacterium have temperature resistance up to 80 degree celcius and the activity of the enzyme is efficient at acidic pH of 5 (Lindenmuth and McDonald, 2011). Bacterial strain *Bacillus* KSM-S237is a thermoalkalophilic bacterium whose thermostability of enzymes is active even at 100 degree celcius and at a pH of 8.5 to 9.1. Many other thermoaciodophiles and thermoalkalophiles have tolerance to pH even up to 1 to 2 and pH of 10 at 100 degree celcius. Many thermophiles have very high enzyme activity of 80% at 90 to 95 degree celcius(Hakamada *et al.,* 1997)[15].

Thermostable enzyme xylanases:

Xylanases (EC 3.2.1.8) are xylan degrading hydrolytic enzymes. This enzyme cleaves the β-1, 4-xylan to xylose thereby breaking down the cellulose polymer which is the predominant components of plant cell wall. The xylanases are classified as i) endo-(1->4)-β-xylan 4-

xylanohydrolase, ii) endo-1,4-xylanase, iii) endo-1,4-β-xylanase iv) β-1,4-xylanase v) β-1,4-xylanase vi) 1,4-β-xylan xylanohydrolase vii) β-xylanase viii) β-1,4-xylan xylanohydrolase and ix) β-D-xylanase. Xylanases is produced by diverse group of microorganisms including bacteria, fungi, protozoans, photosynthetic algae and yeasts (Sunna and Antranikian, 1997)[16]. Mammals do not produce xylanases. Often, predominantly xylanases are produced by filamentous fungi. Xylanases are commercially used in paper or pulp industries, wood industries etc. In this review, the xylanases are used in the lignolytic cleavage of lignocellulose to obtain biofuels.

Thermophilic fungal species:

Nonomuraea (Zhang *et al.,* 2011), *Thermomyces, Talaromyces* (Maalej *et al.,* 2009), *Rhizomucor* and *Laetiporus* are some fungal species that produces thermostable xylanases[17,18]. Generally, fungal thermostable enzymes are not preferred for the lignocellulosic cleavage for biofuel production. This may be due to lack of tolerance to ethanol for the microbe and also difficult during in-situ product recovery in purification.

Thermophilic bacterial species:

Bacterial thermostable enzymes are often used for lignolytic cleavage of cellulose to biofuel than the fungal thermostable xylanases because of the high thermostability of bacterial thermostable xylanases activity over fungi. These enzymes are isolated from thermophilic bacteria. This covers a wide diverse species of bacteria which includes *Actinomadura, Geobacillus, Bacillus, Cellulomonas, Acidothermus, Enterobacter* and *Thermotoga* and so on. Hyperthermophilic bacteria produce xylanases that possesses a very high thermostability and retains its activity even at an elevated temperature up to 90 degree celcius. Apart from thermophiles and hyperthermophiles, thermoalkalophiles, thermoaciodophiles and thermohalophiles have reported to produce xylanases. For an example, a marine thermohalophile, *Thermoanaerobacterium saccharolyticum* NTOU1, strain has been recorded to possess tolerance even at very high salt concentrations. The bacterium was tested for the tolerance and was reported to survive at 2 Molar sodium chloride solutions and had enzyme activity retention up to 71%. And also the xylanases isolated from this strain of bacteria retained enzyme activity up to 50% when subjected to 60 to 65 degree celcius at 0.91 hours of incubation (Hung *et al.,* 2011)[19].

Overexpression of thermostable in the hydrolysis of lignocellulose:

Most thermophilic bacteria produce lower quantities of enzymes of interest even at optimized conditions. For example, the bacterial species *Geobacillus* at 60 degree celcius produced just 0.0113 U/mL and 0.058 U/mL of endoglucanase which is negligible (Rastogi *et al.,* 2009)[5]. Under optimal conditions of 55 degree celcius, the *Geobacillus* species produced only 0.064 U/mL of endoglucanase. In order to efficiently utilize the thermostable enzymes for industrial applications, one must overexpress these thermostable enzymes in a suitable selective heterologous host (Abdel-Fattah *et al.,* 2007)[20]. An example of such over expressed recombinant enzyme is the endoglucanase from *Geobacillus* species 70PC53 was been expressed into *Escherichia coli* host (Ng *et al.,* 2009)[21]. Another example is the expression of xylanases gene from *Geobacillus* strain MT-1 was been isolated, cloned and overexpressed into the Escherichia coli host. Both the wild native type xylanases and recombinant xylanases possessed optimal activity at 70 degree celcius with similar enzyme activity between 20 and 90 degree celcius. Endoglucanases from *Bacillus subtilis, Fervidobacterium nodosum,* and *Thermoanaerobacter tengcongensis* MB4 strain have reported to express their thermostable enzyme genes into the host organism and exhibited very high enzyme activity (Liang *et al.,* 2011)[9]. Heterologous host such as *Bacillus megaterium* and *Pichia pastoris* have also reported to produce recombinant thermostable cellulases and thermostable xylanases.

It has been reported in the recent research that the thermostable xylanase enzyme produced by the species *Actinomadura* S14 was more thermostable when overexpressed in Pichia pastoris as host when compared to the *Escherichia coli* as a heterologous host (Zhang *et al.,* 2010)[22]. These research studies of expressing the thermostable responsible genes in heterologous host can be utilized in the lignocellulosic deconstruction of biomasses in the commercial production of biofuels like bio ethanol and bio butanol. These overexpressed thermostable enzymes onto a heterologous host can be isolated form the host and can be purified by using affinity chromatography using affinity tags like His-6-tag and later can be eluted by using imidazole salts from the column (Huang *et al.,* 2005)[23].

Applications of thermostable enzymes and thermophiles in lignocellulosic biomass conversion:

Many varieties of microorganisms are able to ferment the saccharides to biofuels such as bioethanol, biodiesel and Biobutanol. Several microorganisms including thermophilic bacteria and thermophilic fungal species have recorded to produce ethanol very efficient manner (Wu *et al.,* 1986, Cripps *et al.,* 2009)[24].

Table 1: Thermostable endoglucanases and their properties

Organism	Optimum pH	Optimum Temperature (ºC)	Molecular Weight (KDa)	Stability of enzymes	References
Endoglucanase isolated from *Thermotoga neapolitana* cel B	6.0-6.5	106	30	Half-life of 0.43 hours at 110 ºC	Bok et al. (1998)
Endoglucanase isolated from *Thermotoga neapolitana* cel A	6.0	95	29	half-life 2.16 hours at 106 ºC	Bok et al. (1998)
Endogloucanase isolated from *Rhodothermus marinus*	7.0	95	49	Retained 50% of enzyme activity after 3 and half hours at 100 ºC and retained 80% of its activity after 16 hours at 90ºC	Hreggvidsson et al.(1996)
The expression of *E. coli* endoglucanase gene isolated from *Aquifex aeolicus*	7	80	36.75	Possess a half-life of 2 hours at 100 ºC and 4 hours at 90 ºC	Kim et al. (1998)
Endoglucanase isolated from *Caldibacillus cellulovorans*	6.5 – 7.0	80	85.1	Possess half-life of 0.53 hours at 80 ºC, 0.03 hours at 85 ºC half-life and retained 83% of enzyme of activity at 70 ºC after 3 hours	Huang and Monk (2004)
Endoglucanase isolated from *Geobacillus sp.* Strain B39	6.5	60	148	Retained 80% of enzyme activity at 70 ºC after 30 minutes.	Wang et al. (2008)
The expression of *E. coli* endoglucanase gene isolated from Bacillus licheniformis strain B-41361	6.0	65	42	Retained 10% of its enzyme activity at 65 ºC incubation after 1 hour.	Bischoff at al. (2007)
Endogloucanase isolated from *Clostridium sp* TCW1	6.0	65	45,53,70	NA	Lo et al. (2011)
The expression of *E. coli* endoglucanase gene isolated from *clostridium thermocellum*	5.4	60	66	Retained 50% of enzyme activity at an incubation of 70 ºC for 1 hour.	Peng et al. (2011)
The expression of *E. coli* endoglucanase gene isolated from *Thermobifida halotolerans YIM 90462*	8.0	55	98.9	Retained 74% of enzyme activity at 65 ºC after half an hour.	Zhang et al. (2011)

Initially, yeast species such as *Saccharomyces cerevisiae* was used for the fermentation of saccharides and other carbon sources. Several researches reveal that genetically engineered species of *Saccharomyces cerevisiae*, *Zymomonas mobilis* and *Escherichia coli* strains were identified and tend to possess the capabilities to ferment pentose and hexose saccharides to fuel (Taylor *et al.*, 2009)[25]. These engineered species of microorganism uses cellulose, lignocellulose as carbon sources to transform into biofuel. The process of conversion of lignocellulose biomass to biofuel consists of several stages which includes i) Pretreatment of the raw material feedstock, ii) Release of thermostable enzymes by the microbes iii) Hydrolysis of the lignocellulose biomass leading to the fermentation of sugars.

Most of the ongoing technology of lignocellulose conversion is not that very effective, since due to end product inhibition, involvement of various difficult steps in its conversion, different hydrolysis and the varying high temperatures involved in the process. This technology does not seem to be cost-effective. For instance, in the process of Simultaneous Saccharification and Fermentation, abbreviated as (SSF) the saccharification and the fermentation process take place at the same time, which involves the hydrolysis of the lignocellulose biomass to sugars. But unfortunately, the hydrolysis occurs at non-optimal temperature of the enzymes, which ultimately leads to longer time for hydrolysis and the process is also prone to several contaminations since operated at low temperature. In order to make the lignocellulose conversion a cost-effective process, several ideas have come upon to combine or to eliminate some steps involved in the reaction. The idea is to implement the fermentative thermophilic bacteria and their thermostable lignocellulose degrading enzymes in the conversion of the lignocellulosic feedstock. Many diverse commercial lignocellulose degrading enzymes like xylanases and cellulases are active and potential at temperatures at 50 degree celcius. Thus the xylanases and cellulases obtained from the thermophilic organisms would be a very good solution in the production of the cost-efficient conversion of lignocellulosic biomass to biofuels.

Consolidated Bioprocessing (CBP):

The lignocellulosic biomass is widely used as renewable source of feed stock as it involves low-cost technologies to efficiently degrade the complex structure of cellulose. Four major steps occur during the conversion of lignocellulose to biofuels by the action of thermostable enzyme hydrolysis, they include: i) Production of saccharide deconstructing enzymes like xylanases, hemi-cellulases and cellulases, ii) Saccharification: Hydrolysis of saccharides in pretreated biomass, iii) Fermentation of the hexose and pentose sugars by the microbes.

Recent researches proposed the idea of the hydrolysis of sugars and its fermentation, both the processes combining together in a single bioreactor simultaneously known as the Simultaneous Saccharification and Fermentation of hexose sugars (SSF) and hydrolysis of hexose and pentose sugars together and their fermentation is referred to as Simultaneous Saccharification and Co-Fermentation (SSCF).

The consolidated bioprocessing reduces the cost and separate efforts involved in hydrolysis of biomass and fermentation of sugars.

Simultaneous Saccharification and fermentation:

Biofuels (Bioethanol) produced from natural plant biomass (second generation feedstock) possess several advantages over the biofuels produced from starch or sugar derived (first generation feedstock) carbon source in terms of both environmental and energy utilizing point of view. The most important environmental consideration is that the release of greenhouse gases during fermentation is very much lower when compared to saccharide or starch dependent fermentation due to the fact of reduced overall oil input required in the entire process. The process starts with the physical or chemical pretreatment of lignocellulose followed by the production and hydrolysis of the complex lignocellulosic feedstock to simple sugars using thermophiles and their enzymes and finally fermentation of sugars to obtain desired products.

The concept of SSF:

The first idea of Simultaneous Saccharification and Fermentation was given by Gauss *et al*. in the year 1976 and filed patent for the same[26]. They discovered that glucose yield after enzyme hydrolysis was lower due to end-product inhibition of cellulobiose and glucose after hydrolysis.

Gauss stated that the glucose need not be separated from lignin which is followed by the hydrolysis using enzymes and fermentation, so that the loss of sugars during the process can be avoided. Moreover, this process reduces the overall investment cost and as well as the number of reactors used to carry out several reactions.

The use of thermophiles is more advantageous when compared with simultaneous hydrolysis fermentation; since the optimum temperature required for the hydrolysis of the cellulose biomass with the action of enzymes are higher that than the fermentation. As thermophiles can withstand very high temperature, there is no requirement for temperature adjustment and obviously the cost of the process reduces.

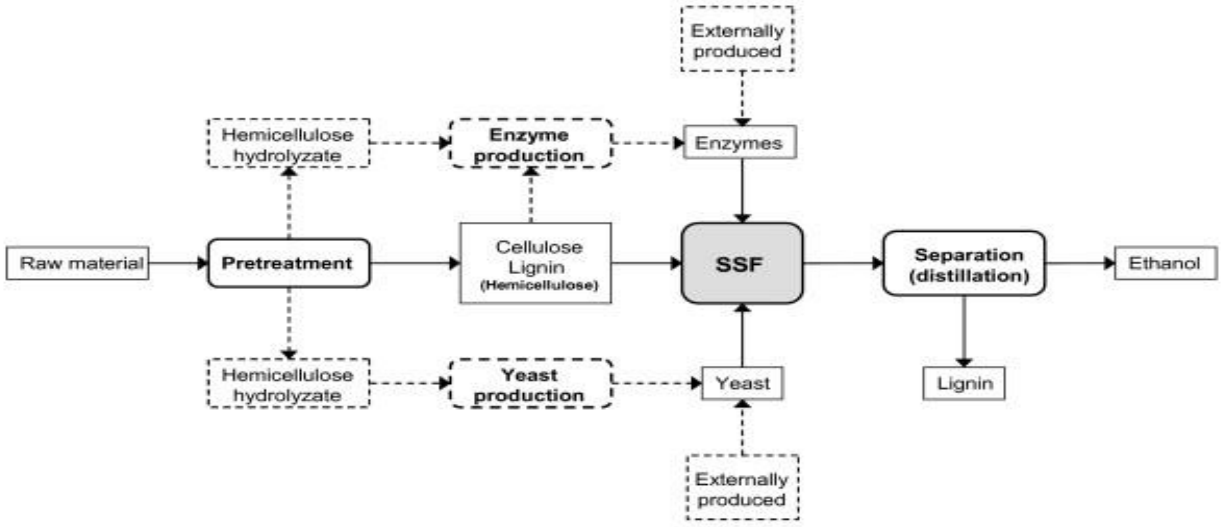

Figure 1 Commercial Simultaneous Saccharification ad Fermentation (SSF)

The above image gives an overall representation of the simultaneous saccharification and fermentation using yeast. This figure gives the commercial production of alcohol form the lignocellulosic biomass. Instead using organisms like thermophiles reduces the number of steps and reactions involved in the process in several ways. The pre-treatment can be skipped, since the thermophiles are resistant to high temperatures, the high temperature could acts as one of the excellent pre-treatment factors and there is no need for any alterations in the temperature during the fermentation process as well. The enzymes used in the hydrolysis, the microbial strains utilized and the pretreatment conditions depend upon the heterogeneity of the feedstock as the lignocellulose biomasses are heterogeneous in terms of both the chemical composition and as well as the complex structure of the compound.

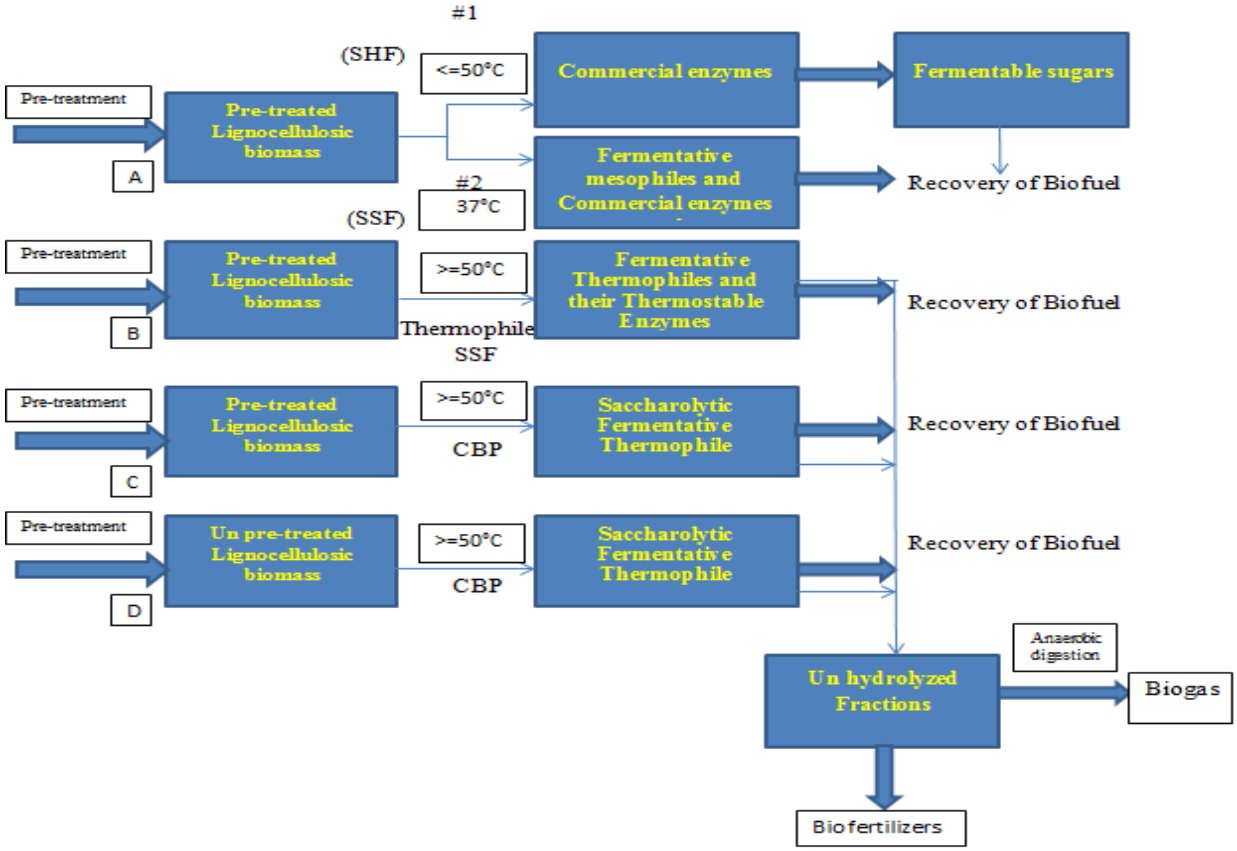

Figure 2 Schematic representation of lignocellulose to biofuel production through various bioprocess technologies

Table 2: Thermophilic strains and their optimum fermentation temperature, substrates used, yield (g/g) and side products obtained

Organism and strains	Fermentation (°C)	Substrates used	Yield of Ethanol (g/g)[a]	Side products	Citations
Thermoanaerobacterium aotearoense	45–60	Xylose	0.16	Lactate	Cai *et al.*, 2011
Thermoanaerobacterium aotearoense Δ*ldh mutant*	50-65	Xylose	0.35	Acetate	Cai *et al.*, 2011
Bacillus coagulans	50	Crystalline cellulose (mixed saccharides)	0.02	Acetate and Lactate	Ou *et al.*, 2009
Thermoanaerobacter mathranii BG1G1	70	Xylose	0.47	Acetate	Yao and Mikkelsen, 2010
Geobacillus thermoglucosidasius TM242	60	Glucose	0.42	Lactate	Cripps *etal., 2009*

a=Yield of ethanol in grams produced per gram cellulose or saccharide used

The Fig.2 represents the following processes: A) current bioprocess, B) simultaneous saccharification and fermentation (SSF), C) consolidated bioprocessing using saccharolytic thermophiles and D) consolidated bioprocessing using saccharolytic thermophiles.

In the Fig.2, (A) the route #1 represents the typical industrial bioprocess involved in lignocellulosic biomass to biofuel production using the mesophilic microorganisms like Saccharomyces cerevisiae; and is performed in several steps which include physical and chemical pre-treatment of the feedstock, followed by the enzymatic hydrolysis of the pre-treated biomass at temperature less than 50°C and fermentation at 37°C. All these steps take place at different temperatures and thus making the entire process insufficient.

Fig.2, (A) route #2 denotes that the enzymatic hydrolysis of biomass followed by the fermentation in the bioreactor operate simultaneously at a lower temperature of about 37°C compared to the optimum active temperature of the enzymes at 50°C. This process is no cost-effective as there is a large amount of enzyme input (Podkaminer *et al.*, 2011)[27]. In order to develop a cost-efficient bioprocessing technology, thermostable enzymes combined with a fermentative thermophilic microorganism would be a better option, since there is a great potential in the hydrolysis and fermentation as they

occur at an elevated temperature. (Shaw *et al*., 2008) revealed that there is a 2.5 fold decrease in the enzyme input required to get hydrolysis for simultaneous saccharification and fermentation at 50°C using the strain *Thermoanaerobacterium saccharolyticum* ALK2 in contrast to mesophilic simultaneous saccharification and fermentation with Saccharomyces cerevisiae at 37°C[28]. Diverse thermophiles have shown to possess the unique property to ferment saccharides from the lignocellulosic biomasses like corn stover and wheat straw (Shaw *et al.,* 2008;Yao and Mikkelsen, 2010; Cripps *et al.,* 2009)[24,29]. Many proven researches of the fermentation of saccharides from biomass using thermophiles and their thermostable enzymes to ethanol can be found in the literature. (Georgieva *et al.,* 2008) revealed that the commercial enzymes like xylanases and cellulases have a significant hydrolysis effect on pre-treated lignocellulosic biomass into fermentable saccharides and fermentation of the saccharides into biofuel (ethanol) using the thermophilic strains of *Thermoanaerobacter*BG1L[30]. The overall yield in the conversion of saccharides to ethanol is 68 to 78%, making the process commercialize.

Fig.2, (C) represent the consolidated bioprocessing (CBP) which denotes the combination of enzyme production, hydrolysis of biomass to sugars and fermentation of sugars to biofuel happens in the same bioreactor at the same temperature. The consolidated bioprocessing technologies have proven yields which could be commercialized because of its economical cost-effective lignocellulosic biomass to biofuel. There is no requirement of separate process for commercial degrading enzyme production. Therefore, the use of saccharolytic thermophilic microorganisms that perform both the process of lignolytic/deconstructing enzyme production and fermentation of sugars in a combined bioreactor for effective conversion of biomass to biofuel would definitely lead to a cost-efficient process when compared to (Fig.2 (B) route 2 simultaneous saccharification and fermentation. CBP has higher conversion rates of lignin and cellulase degradation. (Lynd et al., 2005) stated that CBP showed four fold decreases in terms of cost when compared to other technologies like simultaneous saccharification and co-fermentation process (SSCF)[31]. Several genetically modified strains of thermophiles are developed for the construction of bacterial cellulases in a single step process for the consolidated bioprocessing in lignocellulose deconstruction (Shaw *et al.,* 2008, Cai *et al.,* 2011, Liao *et al.,* 2011)[32, 33].

Clostridium thermocellum, an anaerobic thermophilic microbe is widely used in the commercialization of biofuel through effective degradation of biomass to biofuel through consolidated bioprocessing technologies. The *Clostridium thermocellum* produces a multi-protein complex called cellulosome that helps in complete degradation of crystalline cellulose at an elevated temperature of 60°C (Olson *et al.,* 2011). (Shao et al., 2011) stated that metabolic engineering of microbial strain and their yielding pathway could be an effective tool for this process[34]. By then used of metabolically engineered organisms, we can able to produce high ethanol tolerant species of thermophiles. (Willquist et al., 2010) stated that *Caldicellulosiruptor saccharolyticus* is a thermophile which is the principle organism for the Biohydrogen production[35]. *Caldicellulosiruptor saccharolyticus* grow on various plant biomasses like sugarcane bagasse, sweet sorghum, crystalline cellulose, maize leaves (Blumer-Schuette *et al.,* 2010) and hemicellulose optimally at 70°C and produces thermostable lignolytic, xylanolytic and cellulolytic enzymes. Moreover, by the use of metabolic engineering technologies, by-products such as acetate, lactate and ethanol have been considerably reduced in case of Biohydrogen production (Hallenbeck, 2005)[36]. *Caldicellulosiruptor bescii*DSM 6725 is capable of growing efficiently on plant biomass such as crystalline cellulose at an optimum temperature of 80°C (Dam et al., 2011). Additionally, as discussed earlier thermophilic microbes and their thermostable deconstructing enzymes reduces the cost for pre-treatment of complex plant biomasses like lignin, cellulose and hemicellulose, which is an added advantage over the consolidated bioprocessing. Moreover, the high temperature involved in the bioreactor due to thermophiles that is used in the CBP is an added advantage as there is low risk of possible contaminations due to other mesophilic microorganisms during the fermentation process[36].

If the un-treated lignocellulosic biomass hydrolysis, saccharification (conversion of complex polysaccharides to fermentable sugars), fermentation of sugars and the separation of products obtained can be processed at (>=50°C) temperature, then the consolidation of diverse unit operations can be performed in one single step (Fig.1) (D), which reduces the cost considerably and as well as is more efficient. For instance, un-processed biomasses like un-treated switchgrass, yellow Indian grass, napier grass, poplar and panic grass are the commonly used as more efficient feed for Biohydrogen production in one single bioreactor along with CBP technology with the help of Clostridium bescii DSM 6725, which is one of the principle candidate for Biohydrogen production (Yang et al., 2009)[37]. In the Fig.2, there is a fraction of biomass left unused after the CBP fermentation as a waste; which could be either used as a substrate for anaerobic digestion to produce Biohydrogen and Biogas or the remaining unused slurry could be used as soil amendments such as bio fertilizers

and manure to the crops as a source of rich nutrition. The spent slurry discarded after anaerobic digestion is a wonderful source of liquid bio fertilizer (Rabelo et al., 2011)[38].

Pitfalls in the process:

Even though as mentioned in the above contents, the thermophiles and their thermostable enzymes are useful to us in many ways for simultaneous saccharification fermentation and consolidated bioprocessing, misfortunately there are also some practical pitfalls during the commercialization. There are some factors responsible for these pitfalls, they include the following; 1) The cost involved in the production of biofuel, 2) The efficiency of enzyme catalysis, 3) The net overall energy consumed during the production and 4) The environmental impacts on the conversion of lignocellulose plant biomass. The commercial level culturing and maintenance of thermophilic microorganisms are its negligible cell yield and are scrupulous. If in the case of biomass conversion to biofuel using aerobic thermophile, then the challenge lies in the continuous sparging of sterile oxygen to the microorganism in the bioreactor, making this process non cost effective and due to low solubility of gases at very an elevated high temperatures tends to cause product yield inhibition and as well as the high temperature optimum for the survival of thermophiles may damage the raw materials in case of complex media. These characteristics of thermophiles make the entire process, non-cost efficient.

CONCLUSION:
AND FUTURE PROSPECTUS:

Thermophiles are extremophilic microorganisms that are capable of living at very high temperature of more than 50°C such as hot springs, volcanic regions, etc. They are capable of producing thermostable enzymes which are involved in the active hydrolysis of plant biomasses such as lignin, cellulose, hemicellulose using enzymes like xylanases and cellulases. These enzymes are active even at elevated temperatures. Using thermophiles is one such idea which performs the process of deconstruction of lignolytic cleavage of complex plant biomasses into simpler compounds which is not be by the mesophilic organisms like *Saccharomyces cerevisiae.* The thermophiles have several advantages such as 1) Faster growth rates of thermophiles is an important characteristic of thermophiles towards rapid biofuel production. 2) Higher metabolic rates of conversion of biomass to biofuel. 3) Lignolytic enzymes produced by thermophiles are flexible at high temperatures and are heat stable. 4) Thermophiles are resistant to pH and organic solvents that are produced during the fermentation process. 5) The viscosity of fermentation broth decreases with an increase in the temperature. 6) Facilitates faster rates of in-situ product recovery. 7) Minimum risk of contamination, since the process is operated at high temperature so that no other microbes can survive at that temperature, 8) Low cost of pre-treatment since the operating temperature of thermophiles are high which brakes the complex plant biomass and satisfy the effect of pre-treatment[39, 40].

Another interesting fact is that, the raw material cost for fermentation is very low as food waste and other plant wastes are its major components. Moreover, the fermentation can be practically performed in laboratory and the resultant product Biohydrogen or biogas can be instantly used to light up the flame of Bunsen burner in the laboratory so that the cost investment in commercial LPG cylinders can be minimized. Through metabolic engineering, the side-products yielding metabolic pathway of the thermophilic conversion of lignocellulose to biofuel such as lactate and acetate can be altered and minimized. With the help of genetic engineering, recombinant strains of thermophiles can be used in order to produce very high temperature and pH tolerant thermophiles.

ACKNOWLEDGEMENT:

We, the authors sincerely thank Vels Institute of Science, Technology and Advanced Studies management for their kind support and facilities provided to carry out our review work.

CONFLICTS OF INTERESTS:

The authors declare that they do not have any Conflicts of Interests.

REFERENCES:

1. Havlík P, Schneider UA, Schmid E, Böttcher H, Fritz S, Skalský R, Aoki K, De Cara S, Kindermann G, Kraxner F, Leduc S. Global land-use implications of first and second generation biofuel targets. Energy policy. 2011; 39(10):5690-5702.
2. Hamelinck CN, Van Hooijdonk G, Faaij AP. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle-and long-term. Biomass and bioenergy. 2005; 28(4):384-410.
3. Alvira P, Tomás-Pejó E, Ballesteros MJ, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresource technology. 2010; 101(13):4851-4861.
4. Haki GD, Rakshit SK. Developments in industrially important thermostable enzymes: a review. Bioresource technology. 2003; 89(1):17-34.
5. Rastogi G, Muppidi GL, Gurram RN, Adhikari A, Bischoff KM, Hughes SR, Apel WA, Bang SS, Dixon DJ, Sani RK. Isolation and characterization of cellulose-degrading bacteria from the deep subsurface of the Homestake gold mine, Lead, South Dakota, USA. Journal of industrial microbiology & biotechnology. 2009; 36(4):585.
6. Barnard D, Casanueva A, Tuffin M, Cowan D. Extremophiles in biofuel synthesis. Environmental technology. 2010; 31(8-9):871-888.
7. Demirjian DC, Morís-Varas F, Cassidy CS. Enzymes from extremophiles. Current opinion in chemical biology. 2001; 5(2):144-151.

8. Shrinivas D, Savitha G, Raviranjan K, Naik GR. A highly thermostable alkaline cellulase-free xylanase from thermoalkalophilic Bacillus sp. JB 99 suitable for paper and pulp industry: purification and characterization. Applied biochemistry and biotechnology. 2010; 162(7):2049-2057.
9. Liang C, Xue Y, Fioroni M, Rodríguez-Ropero F, Zhou C, Schwaneberg U, Ma Y. Cloning and characterization of a thermostable and halo-tolerant endoglucanase from Thermoanaerobacter tengcongensis MB4. Applied microbiology and biotechnology. 2011; 89(2):315-326.
10. Lynd LR, Weimer PJ, Van Zyl WH, Pretorius IS. Microbial cellulose utilization: fundamentals and biotechnology. Microbiology and molecular biology reviews. 2002; 66(3):506-577.
11. Blumer-Schuette SE, Kataeva I, Westpheling J, Adams MW, Kelly RM. Extremely thermophilic microorganisms for biomass conversion: status and prospects. Current Opinion in Biotechnology. 2008; 19(3):210-217.
12. Gusakov AV, Salanovich TN, Antonov AI, Ustinov BB, Okunev ON, Burlingame R, Emalfarb M, Baez M, Sinitsyn AP. Design of highly efficient cellulase mixtures for enzymatic hydrolysis of cellulose. Biotechnology and bioengineering. 2007; 97(5):1028-1038.
13. Bok JD, Yernool DA, Eveleigh DE. Purification, characterization, and molecular analysis of thermostable cellulases CelA and CelB fromThermotoga neapolitana. Applied and Environmental Microbiology. 1998; 64(12):4774-4781.
14. Graham JE, Clark ME, Nadler DC, Huffer S, Chokhawala HA, Rowland SE, Blanch HW, Clark DS, Robb FT. Identification and characterization of a multidomain hyperthermophilic cellulase from an archaeal enrichment. Nature communications. 2011; 2:375.
15. Hakamada Y, Koike K, Yoshimatsu T, Mori H, Kobayashi T, Ito S. Thermostable alkaline cellulase from an alkaliphilic isolate, Bacillus sp. KSM-S237. Extremophiles. 1997; 1(3):151-156.
16. Sunna A, Antranikian G. Xylanolytic enzymes from fungi and bacteria. Critical reviews in biotechnology. 1997; 17(1):39-67.
17. Zhang J, Siika-aho M, Puranen T, Tang M, Tenkanen M, Viikari L. Thermostable recombinant xylanases from Nonomuraea flexuosa and Thermoascus aurantiacus show distinct properties in the hydrolysis of xylans and pretreated wheat straw. Biotechnology for biofuels. 2011; 4(1):12.
18. Maalej I, Belhaj I, Masmoudi NF, Belghith H. Highly thermostable xylanase of the thermophilic fungus Talaromyces thermophilus: purification and characterization. Applied biochemistry and biotechnology. 2009; 158(1):200-212.
19. Hung KS, Liu SM, Tzou WS, Lin FP, Pan CL, Fang TY, Sun KH, Tang SJ. Characterization of a novel GH10 thermostable, halophilic xylanase from the marine bacterium Thermoanaerobacterium saccharolyticum NTOU1. Process biochemistry. 2011; 46(6):1257-1263.
20. Abdel-Fattah YR, El-Helow ER, Ghanem KM, Lotfy WA. Application of factorial designs for optimization of avicelase production by a thermophilic Geobacillus isolate. Research Journal of Microbiology. 2007; 2(1):13-23.
21. Ng IS, Li CW, Yeh YF, Chen PT, Chir JL, Ma CH, Yu SM, Ho TH, Tong CG. A novel endo-glucanase from the thermophilic bacterium Geobacillus sp. 70PC53 with high activity and stability over a broad range of temperatures. Extremophiles. 2009; 13(3):425-435.
22. Zhang G, Mao L, Zhao Y, Xue Y, Ma Y. Characterization of a thermostable xylanase from an alkaliphilic Bacillus sp. Biotechnology letters. 2010; 32(12):1915-1920.
23. Huang Y, Krauss G, Cottaz S, Driguez H, Lipps G. A highly acid-stable and thermostable endo-β-glucanase from the thermoacidophilic archaeon Sulfolobus solfataricus. Biochemical Journal. 2005; 385(2):581-588.
24. Cripps RE, Eley K, Leak DJ, Rudd B, Taylor M, Todd M, Boakes S, Martin S, Atkinson T. Metabolic engineering of Geobacillus thermoglucosidasius for high yield ethanol production. Metabolic engineering. 2009; 11(6):398-408.
25. Taylor MP, Eley KL, Martin S, Tuffin MI, Burton SG, Cowan DA. Thermophilic ethanologenesis: future prospects for second-generation bioethanol production. Trends in biotechnology. 2009; 27(7):398-405.
26. Gauss WF, Suzuki S, Takagi M, inventors; Bio Research Center Co Ltd, assignee. Manufacture of alcohol from cellulosic materials using plural ferments. United States patent US 3,990,944. 1976.
27. Podkaminer KK, Shao X, Hogsett DA, Lynd LR. Enzyme inactivation by ethanol and development of a kinetic model for thermophilic simultaneous saccharification and fermentation at 50 C with Thermoanaerobacterium saccharolyticum ALK2. Biotechnology and bioengineering. 2011; 108(6):1268-1278.
28. Shaw AJ, Podkaminer KK, Desai SG, Bardsley JS, Rogers SR, Thorne PG, Hogsett DA, Lynd LR. Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. Proceedings of the National Academy of Sciences. 2008; 105(37):13769-13774.
29. Yao S, Mikkelsen MJ. Metabolic engineering to improve ethanol production in Thermoanaerobacter mathranii. Applied microbiology and biotechnology. 2010; 88(1):199-208.
30. Georgieva TI, Ahring BK. Evaluation of continuous ethanol fermentation of dilute-acid corn stover hydrolysate using thermophilic anaerobic bacterium Thermoanaerobacter BG1L1. Applied Microbiology and Biotechnology. 2007; 77(1):61-68.
31. Lynd LR, Van Zyl WH, McBride JE, Laser M. Consolidated bioprocessing of cellulosic biomass: an update. Current opinion in biotechnology. 2005; 16(5):577-583.
32. Cai Y, Lai C, Li S, Liang Z, Zhu M, Liang S, Wang J. Disruption of lactate dehydrogenase through homologous recombination to improve bioethanol production in Thermoanaerobacterium aotearoense. Enzyme and microbial technology. 2011; 48(2):155-161.
33. Liao H, Zhang XZ, Rollin JA, Zhang YH. A minimal set of bacterial cellulases for consolidated bioprocessing of lignocellulose. Biotechnology journal. 2011; 6(11):1409-1418.
34. Shao X, Raman B, Zhu M, Mielenz JR, Brown SD, Guss AM, Lynd LR. Mutant selection and phenotypic and genetic characterization of ethanol-tolerant strains of Clostridium thermocellum. Applied microbiology and biotechnology. 2011; 92(3):641-652.
35. Willquist K, Zeidan AA, van Niel EW. Physiological characteristics of the extreme thermophile Caldicellulosiruptor saccharolyticus: an efficient hydrogen cell factory. Microbial Cell Factories. 2010; 9(1):89.
36. Hallenbeck PC. Fundamentals of the fermentative production of hydrogen. Water Science and Technology. 2005; 52(1-2):21-29.
37. Yang SJ, Kataeva I, Hamilton-Brehm SD, Engle NL, Tschaplinski TJ, Doeppke C, Davis M, Westpheling J, Adams MW. Efficient degradation of lignocellulosic plant biomass, without pretreatment, by the thermophilic anaerobe "Anaerocellum thermophilum" DSM 6725. Applied and environmental microbiology. 2009; 75(14):4762-4769.
38. Rabelo SC, Carrere H, Maciel Filho R, Costa AC. Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresource technology. 2011; 102(17):7887-7895.
39. Bhalla A, Bansal N, Kumar S, Bischoff KM, Sani RK. Improved lignocellulose conversion to biofuels with thermophilic bacteria and thermostable enzymes. Bioresource technology. 2013; 128:751-759.
40. Yang B, Wyman CE. Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels, Bioproducts and Biorefining. 2008; 2(1):26-40.