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Recent Insight into Production of Cellulase by Fungi and Its Industrial Applications

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ABSTRACT:
Cellulase has been extensively studied in the past years due to its potential in the industrial processes. It is mainly exploited due to its ability to hydrolyze cellulose into simpler sugars which can be used to make variety of products. This function also helps in making existing processes more efficient. Lignocellulose is used in many different forms to produce cellulase and is the most well-known bioenergy source. It acts as a substrate and is pretreated with number of techniques including physical, chemical, physiochemical and biological; to make fermentation less time consuming, effective and systematic. Production of cellulase is commercially carried out by a number of organisms but fungi are extensively used; as they give a higher yield, lower cost of production and show higher stability. Industrially, submerged fermentation has been the center of attention for decades where knowledge about solid state fermentation was not precisely distinguished but now it is being studied too, due to lower production cost, being a simpler process, low energy requirement and efficient product recovery. SSF fermentation supports microbial growth on a solid bed in the absence of free water and is a fool proof way of producing cellulase. This study focuses on the existing knowledge of cellulase and its industrial potential, overview of different lignocellulosic substrate and their pretreatment. The readers will be able to grasp comprehensive knowledge on different fungal genera and species involved in cellulase production in solid state fermentation and strategies to measure enzyme activity.

INTRODUCTION

Cellulose is the most abundant organic polymer in nature due to its presence as a vital and considerably present in high amounts as a structural component in plant cell wall; making it the highest constituent of plant biomass followed by hemicelluloses (Chaitanya et al., 2017)and also being secreted as a biofilm in some bacteria (Serra et al., 2013).

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Cellulose obtained from plants is usually present within a mixture of pectin, lignin, hemicelluloses and other substances, all present in different percentages, where as bacterial cellulose is pure and has higher chain length making it higher in tensile strength (Klemm et al., 2005). Structurally, it comprises of straight unbrached chains of D-glucose units that are linked together beta glucosidic bonds (Haider, 1982).

Cellulose has a partially crystalline and firm construction, it is intractable to enzymatic degradation (Nishiyama et al., 2002) but decomposition of cellulose is necessary for its biotechnological applications. This includes industries such as textile, paper industry, bioethanol production, laundry and detergent industry, production of food, agricultural industry, waste management (Amer and Aasia, 2018; Imran et al., 2018; Sun and Cheng, 2002). For this purpose, cellulase is used.

Cellulase (Fig. 1.1) is a synergistic enzymatic system (corporation of 3 structures) that degrades unbranched cellulose chains into glucose monomers and other oligosaccharide molecules by hydrolyzing glucosidal linkages, breaking bonds (Ahmed and Bibi, 2018; Chellapandi and Jani, 2008). It is widely used in industries due to this reason that is, breakdown of complex structures to simple and low molecular weight compounds.

Their broad and versatile use in the industry has increased its demand over the years. It has been commercially available for 30 years now (Kuhad et al., 2011). Since the demand has increased over the years, collecting valuable knowledge about cellulase may be beneficial in the future. This includes disciplines of biotechnology, molecular biology and genetics.

Complex organization of Cellulase

The action of cellulose is dependent on complex of 3 enzymes that makeup the structure: endoglucanase/CMCases, exoglucanases/FPases including cellobiohydrolases (CBH) and β-glucosidases (Yu et al., 1998). There is still more to be explored when it comes to sequential catalyses of cellulase but there is hypothesis that is accepted (Juturu and Wu, 2014; Ray, 2011). According to this (Fig 1.2): Endoglucanases degrades the amorphous regions of cellulose creating new chain ends and they can also hydrolyze carboxymethyl cellulose (CMC) and hydroxyethyl cellulose internal bonds.

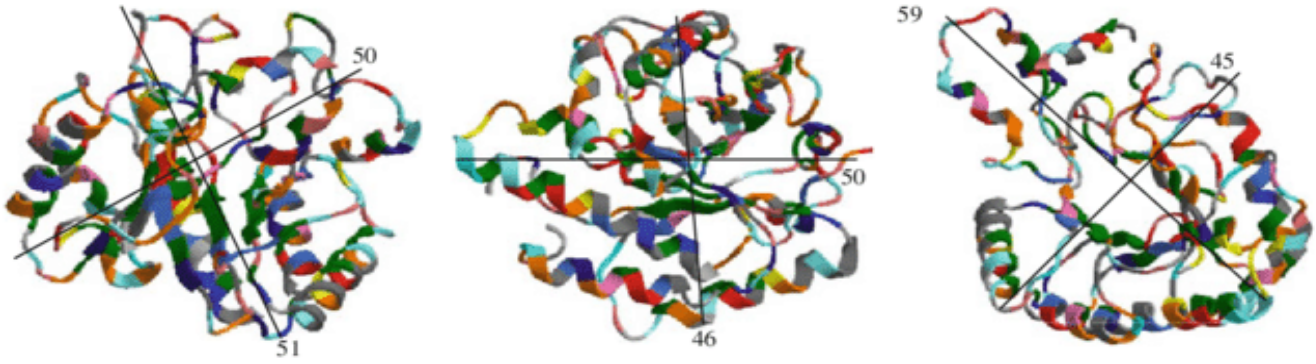

FIGURE 1.Cellulase structure from RCSB protein data base (Kannan and Jasra, 2011)

- Exoglucanases hydrolyzes the crystalline parts of cellulose producing tetrasaccharide (Moraïs et al., 2012) and disaccharide such as cellobiose, units either from non-reducing or reducing ends which are;
- Further acted upon by of β-glucosidase. These enzymes hydrolyze cellbioses into D-glucose (van den Brink and de Vries, 2011).

There is a fourth class of enzyme that has been classified as: the lytic polysaccharide mono-oxygenases (Johansen, 2016). These enzymes work systematically in order to completely hydrolyze cellulose. Apart from this, there is very little known about how certain mode of action of cellulase are carried out in different organisms, especially between Eukaryotes and Prokaryotes (Ghose and Collaborator, 1987).

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However, according to another hypothesis, the opposite is proposed. In this hypothesis, not 2 but 3 enzymes are necessary for proper and efficient hydrolysis of cellulosic biomass (Reese, 1956).

Solid state fermentation VS Submerged fermentation

SSF, a cultivation process, is a technique different from conventional submerged fermentation in many ways and has arisen as a promising process for formation and large-scale production of industrial enzymes (Coradi et al., 2013). It has its own physiochemical drawbacks and advantages.

Unlike SmF, SSF is carried out on a solid substrate bed in aerobic or anaerobic conditions (Doriya et al., 2016). Microbial growth occurs with no "free" water available rather, the water is embedded onto the solid substrate for the cells to consume (Hashemi et al., 2010; Pirota et al., 2014). The presence of less water content is a favorable condition for growth of filamentous fungus especially since they grow on solid surfaces (Pandey, 1992) but, will not support any organism that require higher water content such as bacteria (Satyanarayana, 1994).

It can reduce the production cost of enzymes as well as improve it (Lin et al., 2010). The microbial growth and metabolism is supported by substrates rich in carbon, nitrogen and other nutrients (Maroušek et al., 2014). These namely include bran, bagasse, and paper pulp (Satyanarayana, 1994) as well as; wheat straw, wheat bran and rice straw for production of cellulase (Alegre et al., 2009; Biswas et al., 1990; Singhania et al., 2010). These are renewable sources and are easily recycled as substrates.

The utilization of the source is done very slowly and steadily accounting for a longer duration for the fermentation to be carried out. This ensures the control release of nutrients and product, very little contamination and identified on a very early stage (Doriya et al., 2016). Another benefit of SSF is that there is no foam build up (Szendefy et al., 2006).

There are some problems one might face in this process as well (Manan and Webb, 2017). These issues include: media may lose moisture and optimization is an issue including optimization of pH and temperature. These are require to produce enzymes as they are very sensitive proteins: scaling-up as an industrial application is a question mark (Kumar Ramamoorthy et al., 2019). Uniform mass transfer, fungus may start to grow in aerial direction on the side surfaces due to presence of mycelia (Grajek, 1987). However, a very few techniques and methods to ensure the presence of exact biomass concentration in the fermentor such as; infrared spectroscopy, pressure drop measurements and image analysis. (Viniegra-Gonzàlez, 1997).

Submerged fermentation (SmF)

Submerged fermentation is quite different from solid state fermentation in many aspects. Traditionally, enzymes have been obtained from SmF. Microorganisms are present in a liquid medium like molasses or broths and utilize nutrients from this. The product is released in the broth as well (Mrudula and Murugammal, 2011a). It has free water available unlike SSF. This makes it ideal for organisms that require more moisture content to survive such as bacteria.

The substrates are replaced with nutrients after specific time intervals to keep the fermentation going as it can cause depletion in nutrients this is so, because nutrients are utilized exceptional fast (Hansen et al., 2015). So in order to keep the microorganism alive and obtaining the enzymes, this is important. Since the substrate is liquid, purification of the final enzyme is easy.

A primary culture is necessary for submerged fermentation that is later shifted to a pilot fermentor in the industries. Certain parameters are to be monitored after inoculation such as temperature, pH, agitation (Rangaswamy, 2012) and antifoam (Yu et al., 1998). However, in SmF filamentous fungi do not show major growth due to the fact that they grow on solid surfaces. Since mycelia are not able to attach to the surface because of constant movement of the impeller, not enough is formed and the product formation is not satisfactory.

Following problems are faced by scientists with SMF:

- Cellulase release may make the broth more viscose which increases agitation and results in considerably more foaming (Etoc et al., 2006)

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- Since cellulase is released in a culture broth it may act on the lignocellulosic biomass causing accumulation of glucose and xylose (Casey et al., 2010)
- Unregulated agitation causes foaming

Comparative Study

- Microbial strains are exceptionally different in both the fermentation techniques. The strains involved SmF are usually wild type strains that is, their natural form but those used in submerged fermentation are usually genetically modified. Almost 90% of the enzymes are produced by genetically modified microbes when it comes to SmF. (Adsul et al., 2011; Singhania et al., 2015).
- In SmF, there can be larger by-product accumulation than in SSF. SmF may also lead to more secondary metabolite production.
- SSF is considered to be more environmental friendly. SmF causes more environmental pollution due to presence of liquid broth which may be harder to treat and be dumped. Especially since most organism are genetically modified so may cause disruption in the natural environment (Kumar et al., 2011).
- SSF acts as a good way for recycling and utilizing agricultural waste. SSF utilized cardboard and waste surgical cotton to produce cellulase in a study conducted (Kumar Ramamoorthy et al., 2019).
- SSF is more suitable for microbes that need a surface to get attached to like filamentous fungus (Binod et al., 2010).
- SSF is more preferred for large scale production of cellulase due to being a simpler technique, lower capital investment, superior production, less water waste, less water requirement and lower energy consumption. (Aidoo et al., 1982; Behera and Ray, 2016; Cen and Xia, 1999; de Souza and de Oliveira Magalhães, 2010)
- In previous studies (Cunha et al., 2012) it was demonstrated that SSF yields more enzymes than SmF. Production of cellulase by using waste lignocellulose in both SSF and SmF was done. Estimation of enzyme activity by using enzyme assays showed that SSF produced more cellulase than SmF. (Kumar Ramamoorthy et al., 2019).
- SmF requires a few additional steps in the down streaming process than in SSF (Fig 1.3).

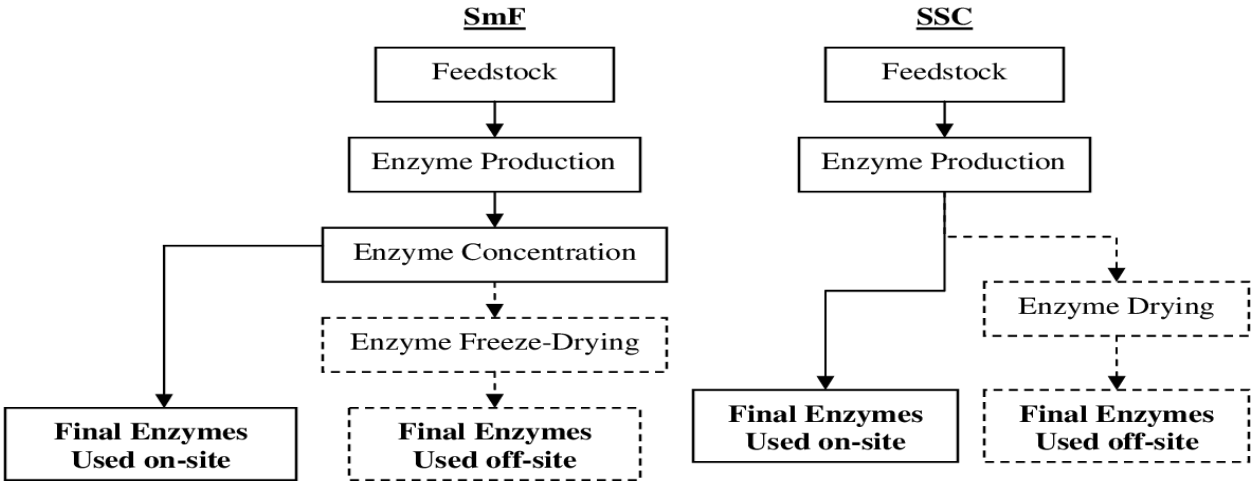

FIGURE 3. Flowcharts showing comparison between SmF and SSF processes (Zhuang et al., 2007)

Lignocellulosic material for SSF

Substrate in solid state fermentation does more than just provide nutrients; it also acts as an anchor for the microorganisms, especially fungi. Many different kinds of materials have been employed in SSF like rice straws, wheat straws, waste cotton, waste cardboard, sugarcane bagasse, wheat barn and many more. Some of the materias used along with the microorganisms are shown in table 1.

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TABLE 1. Variety of lignocellulosic materials, mostly waste, used in cellulase production

Lignocellolusic material used in Cellulase production	References
Rice Husk	(Kuhad and Singh, 1993)
Cassava Bagasse	(Falkoski et al., 2013; John et al., 2006)
Rice Bran	(Kodali and Ravindra, 2006; Liang et al., 2014)
Sugarcane Bagasse	(Wahono et al., 2014)
Orange peel	(Karmakar and Ray, 2010)
Dried flower	(Karmakar and Ray, 2010)
Wheat straw	(Pensupa et al., 2013)
Wheat bran	(Bharti et al., 2018)
Sea weed	(Trivedi et al., 2015a)
Banana Peel	(Dabhi et al., 2014)
Saw dust	(Acharya et al., 2008)
Cardboard and surgical waste cotton	(Kumar Ramamoorthy et al., 2019)
Corncobs	(Ojumu et al., 2003)
Coconut Coir pith	(Muniswaran et al., 1994)
Reed	(Philippoussis et al., 2011)
Bean stalks	(Philippoussis et al., 2011)
Soybean hulls	(Brijwani et al., 2010)
Wood Chips	(Shin et al., 2000)

Measurement of Cellulase Enzymatic Activity in SSF

Cellulase enzyme activity can be measured in SSF by using enzyme essays (Adney and Baker, 1996). These empirical procedures have been developed in order to understand the practical nature and aspect of most cellulase work since it is not well known. The following systems have been accepted for evaluation of cellulase produced by Trichoderma which is known for releasing large amounts of cellulase enzyme. (Kubicek, 1992).

2.1.1 ***Extraction of Cellulase in SSF***

There several ways to extract cellulase and were reported by many studies (Adney and Baker, 1996; Brijwani and Vadlani, 2011; Cunha et al., 2014; Lee et al., 2011; Pensupa et al., 2013). To extract the enzyme, substrate on which fermentation took place is put and submerged into sodium acetate buffer. It is gently shaken in to and fro motion. The mixture is then filtered through a muslin cloth (double layer)/cheese cloth taking out bigger perticles. It is then subjected to centrifugation. This is done at 7000rpm at for 15min. The clear supernantant obtained is the enzyme mix used for enzyme assays.

2.1.2 ***Filter Paper Activity Assay***

FPase measures the overall enzymatic capacity for hydrolyzing cellulose of the obtained microbial cellulase. Conditions of FPase need to be standardized in order to acquire comparable data (Delabona et al., 2012; Thomas et al., 2013). It is dependent on dilution of enzyme in the assay and extraction volumes but other cellulosic enzyme assays such as endoglucanase and the rest, are much less sensitive to extraction volumes (Urbánszki et al., 2000).

In this enzyme assay, 1ml of 0.05M of Na-citrate pH 4.8 is mixed with 0.5ml enzyme diluted with citrate buffer. Each enzyme sample should have two dilutions. Each above and below 2.0mg of glucose, by minute quanitiy. Whatman No. 1 filter paper strip is added to this mixture and incubated at 50°C for 60 min.

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Then after addition of DNS, it is heated in a water bath for 5mins (Delabona, Pirota et al. 2012). Deionized water is added followed by mixing so the solution would separate to the bottom of the tube. When the pulp settles, the color formed is measured against the spectro zero at 540 nm (Ghose and Collaborator, 1987). The activity is measured in filter paper units that is defined as the amount of enzyme that releases 1µmol of reducing sugar per min.

2.1.3 *Carboxymethyl Cellulase Activity (CMCase) for endo-13-1, 4-glucanase*

Endo-13-1, 4-glucanase activity is measured through CMCase. In this assay, 0.5 ml of diluted enzyme is taken. This dilution is done in Citrate buffer. 2 dilutions for each sample are taken, one slightly above and one slightly less than 0.5mg of glucose.

To this, 0.5 ml substrate solution i.e. 2% Carboxymethyl cellulose in 0.05 M sodium citrate buffer with pH 4.8 is added after the temperature is brought to 50°C. After mixing, it is incubated at. DNS is added then mixture is boiled. After this, it is moved to a cold water bath where 20 ml of distilled water is added. Tube is then inverted several times to separate solution settled at the bottom. The color formed against the spectro zero at 540 nm (Ghose and Collaborator, 1987).

2.1.4 *Cellobiase assay*

1 ml of diluted β-glucosidase is taken in a test tube. 2 Dilutions need to be taken with a minute difference. This is heated to 50°C and then 1 ml of substrate solution is added. Here, the substrate is 15.0 mM cellobiose in 0.05 M citrate buffer pH 4.8.

Incubation is done for 30 min at 50°C. After that is completed, the tube is transferred to a cold water bath. Then glucose is measured using a kit based on the glucose oxidase reaction. The unit used express enzyme activity in SSF is activity units per mass of initial dry solid substrate (IU/gds or FPU/gds). These techniques are all based on estation of fixed amount of glucose from the relevant substrate.

2.1.5 *Xylanase assay*

0.1 ml of diluted enzyme is mixed with xylan solution as substrate is used. The mxture is then incubated at 50°C for 15 min. This is used to measure xylanase activity where I unit of xylanase activity = 1µmol of xylose released per min (da Silva Delabona et al., 2012).

In another study, xylanase assay was conducted by using 1% birchwood xylan as substrate with 20mM Tris−HCl buffer wuth the pH of 8. It was then incubated for 40°C for 60 min. DNS method is used to calculated the amount of reduced sugar released after incubation (Kumar Ramamoorthy et al., 2019).

2.2 Microorganism for production of Cellulase

Cellulase, this naturally occurring enzyme is synthesized by various bacteria (Immanuel et al., 2006), fungi (Liu et al., 2012), plants and some animals; however, microorganisms are the major contributor to cellulose degradation (Lynd et al., 2002). This makes them ideal for industrial production of cellulase. Microorganisms also happen to have number of features that make them the most capable among the rest of the organisms.They have the ability to adapt to various environmental conditions; many strains can be improved for different biotechnological applications such as in yeast through adaptive laboratory evolution (Bachmann et al., 2015; Kutyna et al., 2010). Apart from this they require less space for higher amount of cells and are easier to supervise. However, Fungi are the most preferred microorganism among the rest for cellulase production.

Fungi are prominent organisms when it comes to producing cellulase for industrial applications. This is because they secrete larger quantities of enzymes as compared to bacteria as well as the attribute of producing a complete cellulase system (Yoon et al., 2014). Among these, filamentous fungi are in the spotlight. The hyphae, being branching filaments, get attaches to the solid nutritive surface, completely covering it (Mienda and Idi, 2011). It also exerts mechanical pressure on the cellulosic complex and hyphae elongating over the surface causing more amount of enzyme to be produced by increasing the surface area.

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Bacterial species produce low amount of cellulase as compared to fungal species (Thurston et al., 1993). When it comes to aerobic or anaerobic species, aerobic species are more efficient in cellulase production than anaerobic due to restriction of activity to cellulose and hydrolytic products (Ng and Zeikus, 1982).

The growth of these filamentous fungi depends on two things: high content of polymerized sugars in the media (substrate) and the high capacity of enzyme synthesis that are hydrolytic (Sachslehner et al., 1997). These two factors are also responsible for inducing gene expression in the enzymes (Sternberg, 1976). There is a remarkable number of fungi species that produce and optimize cellulase enzyme but in them, some species have been extensively studied due to better performers as producers(Lynd et al., 2002). Many fungal genera have been exploited for the extraction of cellulase such as Trichoderma, Aspergillus, Humicola & Penicillium.

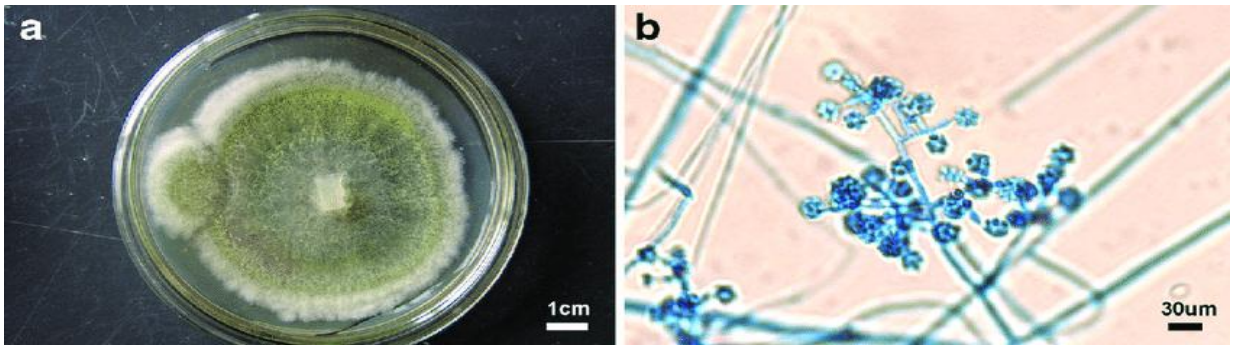
2.2.1 *Trichoderma*

Trichoderma (Fig 2.1) and Aspergillus are especially known commercially in the market for the production of many enzymes in the industry (van Peij et al., 1998). Trichoderma is extensively studied for the production of cellulase preparations as it produces low levels of β-glucosidases which represses the production of the enzyme as it is the cause of accumulation of glucose (Tiwari et al., 2013) but high levels of endoglucanases and exoglucanases which is required for full hydrolysis of cellulose (Singhania et al., 2012).

The especially known specie from this genra is *Trichoderma reesei which is more popular from an industrial point of view. Trichoderma is responsible for the production of two enzymes that are:* cellulase and chitinase but is dominantly known for cellulase. Many strains are known to do so but best in these have to do with better resistance to product inhibition.

For this, strains need to be extensively studied and researches on their activity and productivity need to be made. *T. viride,* though producing higher amount of cellulase as compared to *T. reesei* strains, not much is known about it, so using it is a risk.

FIGURE 2. Culture of Trichoderma sp. a) colonies in Potato dextrose Agar. b) Conodia (Li et al., 2014)



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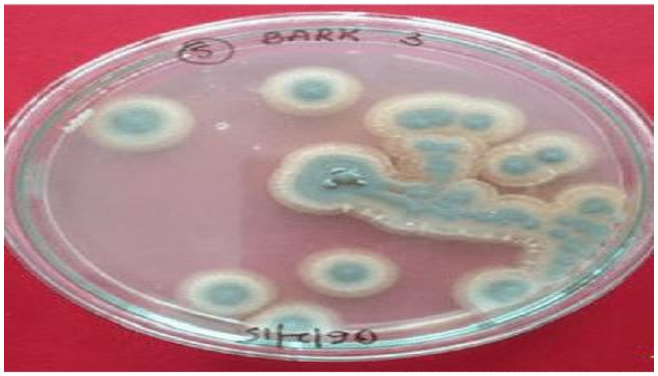

FIGURE 2. purified Penicillium species in PDA(Raghavendra, 2016)

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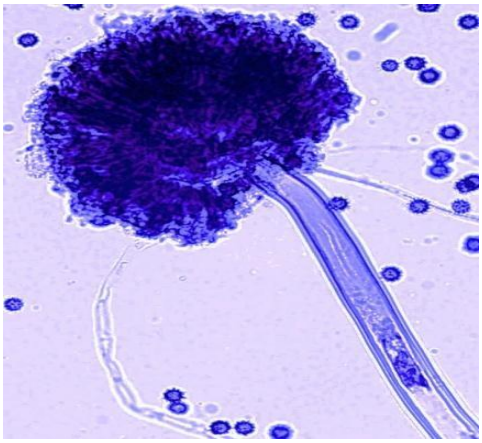

FIGURE 3.Aspergillus fumigatus(Raksha et al., 2015)

2.2.2 *Aspergillus*

In contrast to Trichoderma, Aspergillus (Fig 2.3) species produces greater amount of β-glucosidase (Vaithanomsat et al., 2011)but low levels of low CBH and EGL (Hanif et al., 2004). All the species of Aspergillus have the ability to produce this enzyme so have the tendency to dominate the enzyme industry. *A. niger* is in the spotlight when it comes to industries. It has all the cellulytic genes and proficiently degrades plant cell wall. However, Its growth on cellulose is unsatisfactory (Coutinho et al., 2009).

Other species include Aspergillus ornatus, Aspergillus terreus (Sohail et al., 2016), Aspergillus terreus M1 (Gao et al., 2008), Aspergillus niger (Mrudula and Murugammal, 2011b) among many others.

2.2.3 *Penicillium*

Penicillium (Fig 2.3) produces higher amounts of β-glucosidase making it more recognizable than Trichoderma (Vaishnav et al., 2018). It shows higher activity of cellulytic enzymes during saccharification (Picart et al., 2007). This results in lower enzyme requirement making cellulase more economical. Penicellia, however, is still in its initial stages of research and extensive understanding for their full enzymatic potential but studies are being done on a rapid pace in cellulase production (Prasanna et al., 2016).

2.2.4 *Humicola*

Humicola genus resides the capability of producing higher amounts of gIucosidases than Trichoderma (Takashima et al., 1999). *H. Insolens*, an aerobic fungi typically found on mushroom composts of soil, was recently discovered to produce β-glucosidase by mycelial glucose- and xylose (Souza et al., 2010). Further studies are to be conducted on Hemicola though to grasp a better understanding.

2.3 Application of cellulase in industries

In the past few decades, enzymes have been tackled by many research groups due to their growing demand in many different industries. Many have focused on understanding their structure, production, characteristics and properties, factors effecting their efficiency and applications in the past (Kuhad et al., 2011). As annual sale of cellulase is expected to exceed the protease market in the future, contributing to 10% of the worldwide industrial enzyme demand (Singh et al., 2016) and has already reached up to 8% of the total enzyme market, better and efficient ways of producing these enzymes is now the center of attention of many (Horn et al., 2012).

Alternative sources have been explored and invested in by many governments and researchers, the result of which led to biomass utilization for producing energy. Concerns like environmental issues made the biomass utilization resurface (Ramos and Wilhelm, 2005; Zaldivar et al., 2001). For this to work, obtaining monosacharrides by cellulase from biomass for liquid fuel production and other chemicals was the main focus (Bozell and Petersen, 2010). Now cellulase is used to aid the production of biofuels especially after the oil crises in 1970’s.

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2.3.1 *Textile Industry:*

Reported data has shown, that there was 13.77% demand in the textile industry of cellulase in 2016 (Jayasekara and Ratnayake, 2019b). In the textile industry, It helps create a competitive platform as it is used for finishing, manufacturing of cellulose containing/based fabric and creating new fabric by improving basic processing steps, this does not require any extra machinery or equipment.

It roots back to the 1980's, where biostoning (Arja, 2007b; Mondal and Khan, 2014) came into existence. Before that, amylase along with pumice stone wash (Menon and Rao, 2012) was used but it had several draw back such as, it had to be used on bulk fabric, tear effects could be caused, stone was to be removed manually. Later, Cellulase was used to create a stonewashed appearance along with biofinishing of cellulase based fabric was created making the fabric more wearable and superior quality wise. The yarn surface has small fibers sticking out; these are what cellulase acts on loosening the dye forming water soluble sugars, making it easy to be removed while washing (GALANTE et al., 1998; Singh et al., 2007; Sukumaran et al., 2005a). *Trichoderma reesei* is considered to be the best candidate in the textile industry (Miettinen-Oinonen, 2004).

Cellulose based textile such as cotton or linen often times form fuzz on their surfaces. This fuzz is actually loosely attached fibers that form small balls called pilling. This gives the fabric an unattractive look and feel to it. In order to avoid this, fabric is treated with cellulose in the wet processing stages. This acidic cellulase removes the microfibers and improving fabric softness as well as giving a shiny appearance to the surface (Sreenath et al., 1996). This not only betters the hand feel but the water absorption properties of the fabric as well (Bhat, 2000). Cellulase especially rich in endogulcanases, restore and brightens the color, softens the fabric; improves stability of cellulosic fabrics and removes excess dye (Ibrahim et al., 2011).

Cellulase in particular are used in the textile industry due to their easy use and no need of extra equipment, rather are run on already present equipment. They do not harm the environment either as they are biodegradable, economical by saving energy and chemicals, cutting the prices on the final product.

2.3.2 *Laundry and detergent:*

Laundry industries have adopted the use of enzyme in biological detergents that times as far back as 1960s, however, use of cellulase along with other enzymes such as proteases and lipases is a fairly new practice (Singh et al., 2007). According to a report, in 2014, the detergent industry stood at the top of market for enzyme due to its total sales of 25-30% (Jayasekara and Ratnayake, 2019a).

Cellulase trend in the laundry industry is the use of alkaline cellulase. This is being actively perused (Singh et al., 2007). Alkaline cellulase contacts the cellulose within the fabric and removes soil from the fibrils. But all this is done in the presence of other detergent constituents (Sukumaran et al., 2005b).

Using cellulase comes with certain perks, mainly including cleaning and textile care benefits. It is used to remove fuzz and stray strands from fabric that helps enhance and brighten the color in faded garment giving it a better appearance (Maurer et al., 1997). Trochoderma species, *Aspergillus niger* and a few Humicola species have been studied for applications in the detergent industry.

2.3.3 Paper and Pulp industry:

Paper industry contributes to the largest industrial sector in the world and has continued to expand (Nagar et al., 2011). The demand of cellulase has increased from 320 to 395 million tons in just the span of the last decade (Mai et al., 2004; Przybysz Buzała et al., 2018). Many enzymes have been employed throughout the years for many different processes in this industry including deinking for recycling purposes, pulping, and strength properties. Some of the enzymes used are xylanase, hemicellulase, laccase, lipase (Demuner et al., 2011b) and cellulase (Garg et al., 2011; Subramaniyan and Prema, 2002).

Cellulase is mainly used in the pulping process to extract cellulose from raw material and removing impurities before paper making. This process can either be carried out mechanically or biochemically (Bajpai, 1999). However, mechanically doing this has certain drawbacks. It involves grinding woody raw materials even though, end product has high amounts of bulk, fine and stiffness. It also consumes high amounts of energy in order to carry this out.

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On the other hand, utilizing enzymes such as cellulase for biomechanical pulping not only saves energy from 20-40% (Bhat, 2000; Demuner et al., 2011a) but also improves the quality of paper. This makes it more profitable for the industries as it decreases energy consumption so lowers the overall cost as well as improves pulp quality (Demuner et al., 2011b).

Cellulase is used for another process known as deinking. In traditional method, many chemicals were used to remove the ink from the paper; it caused yellowing, dullness in paper, increased process cost and contributed to environment pollution. Small amount of enzyme is used to digest surface cellulose in order to loosen the ink. In biobleaching cellulase and xylanase mixture maybe used (Kumar and Satyanarayana, 2012). It enhances paper appearance, makes it brighter, whiter and clean looking in addition to lessening environmental pollution.

2.3.4 *Bioethanol and Biofeul production*

As the world is developing, energy requirement is increasing. To fulfill this requirement alternatives methods of producing sufficient energy have been explored by researchers and scientists. Recently, many new studies have been conducted on utilizing lignocellulosic waste for production of biofuel (Binod et al., 2010; Liang et al., 2014; Zhu et al., 2015).

The pretreated lignocellulosic waste is broken-down by cellulase into sugars that are further converted into bioethanol (Amer and Aasia, 2018). It holds enough potential to produce energy to meet the requirements of the fast, energy driven world (Anwar et al., 2014). According to some studies conducted, it is revealed that cellulase can successfully convert lignocellulose substrates into ethanol. If produced from SSF, it enhances and improves the quality of ethanol being produced (Shrestha et al., 2010).

Cellulase helps in producing ethanol so it also contributes to minimizing environmental pollution that is extensively caused by burning of fossil fuels otherwise (Horn et al., 2012). A worldwide energy crisis started in 1970s that compelled not only researchers but governments to look for alternative sources. Forces combined and attention was given to biofuels. So cellulase not only helps with the environment but with the energy crises as well; since biofuels are renewable and sustainable. (Srivastava et al., 2018)

2.3.5 Food Industry

Food industry has a prominent place for cellulase. The orange juice present on the tables in the morning to bottle of olive oil in the kitchen, these are there due the refinement of food by cellulase. Cellulase is used to enhance flavor, texture and aroma (Baker and Wicker, 1996) of many fruits and plants. It is used to clarify and stabilize juices, this also helps in increasing the production amount of certain juices (de Carvalho et al., 2008).

Many nectars and purees of fruits like peaches, mango, plum, and papaya; are stabilized and improved texture is obtained with the right thickness by using cellulase (Bhat, 2000; de Carvalho et al., 2008). It betters the taste of baked goods and nutritive quality of food for the cattle and other animals as well (Brøkner et al., 2012).

2.3.6 Agriculture

Agricultural industries employ cellulase for a number of reasons. Cellulase is used to control certain diseases in plants and pathogens that may cause further complications. This promotes healthier growth of crops when used with other enzymes (Bhat, 2000).

it is known for improving soil quality where it breaks down straw that is incorporated into the soil as an alternative to synthetic fertilizers. Exogenouse cellulase is able to hydrolyze the cellulose present in the straw, decomposing and release sugars which further increases soil fertility (Han and He, 2010).

2.4 Lignocellulose pretreatment

Cellulose and hemicelluloses in lignocellulose digestibility, by cellulase is hindered due to a number of factors. Presence of sugars, lignin, oils, mineral; the partial crystalline structure of cellulose or particle size, for example, may contribute to limiting the hydrolysis (Hendriks and Zeeman, 2009b; Zoghlami and Paës, 2019). Due to this obstacle, pretreatment of lignocellulose is done. Pretreatment helps with making substrate more accessible to microorganisms and increases enzyme activity thus more enzymes are produced (Sarkar et al., 2012).

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The finer the substrate the better, however, there are different methods for treating substrate before use in fermentation, each method having a different impact on the constituents of lignocellulose (Baruah et al., 2018; Den et al., 2018; Gírio et al., 2010). So different techniques are chosen and employed according to the fermentation steps involved (FitzPatrick et al., 2010). Since it helps in decreasing time needed for fermentation to be carried out, it makes the process economically feasible due to less energy requirement (Brodeur et al., 2011).

2.4.1 *Physical Pretreatments*

Before moving towards other techniques for pretreatments, the raw material, be it rice straw, wheat straw or sugarcane bagasse, undergoes physically being reduced into smaller particles. This reduction size contributes to increased surface area, decreased crystalline structure making it better for further treatment, modification of lignin structure (Canilha et al., 2012; Margeot et al., 2009).

To mechanically breakdown lignocellulose, grinding, milling, rolling, hacking so on are used so fragmentation is successful (Hendriks and Zeeman, 2009a; Quéméneur et al., 2012; Zhu et al., 2010). Another benefit is that, breakdown of the structure may limit by-product formation during fermentation (Abbasi and Abbasi, 2010) this in return improves the downstream process favoring the enzymatic conversion. One thing to be kept in mind is that overdoing depolymerization and using very small, fine particles will lead to clumping (Sarkar et al., 2012).

2.4.2 *Chemical Pretreatments*

Chemical pretreatment is usually employed by industries as it is the most feasible method (Bensah and Mensah, 2013; Trivedi et al., 2015b) . It ensures better product formation and less degradation, release high rates of sugar, decreases the need of enzymes and solvent loads when used with other techniques (Bensah and Mensah, 2013).

Chemical methods usually employ chemicals like acids, alkalis, ammonia (Kumar Ramamoorthy et al., 2019) along with ethylamine, ozone and more (Trivedi et al., 2015b). These chemicals are used in variety of manner depending on the type of substrate that is needed for the solid state fermentation.

Acid treatment causes reduction in hemicelluloses and lignin (Sun and Cheng, 2002; Zhang et al., 2011; Zhang and Lynd, 2004), Alkaline treatment converts lignocellulose into soap and alcohol along with altering the structure of lignin (Zhang and Lynd, 2004).

In an experiment conducted by Kumar, (Kumar et al., 2011) waste cardboard and surgical cotton was washed and a lignocellulosic mixture was prepared. Two variations of the pretreatment were done. One batch was treated with acid and the other with ammonia. In the acid pretreatment, 5% sulphuric acid was added to the lignocellulosic mixture which was then autoclaved at 121 °C for 60 min. It resulted in removal of hemicelluloses and a significant decrease in lignin. It also showed a vast difference when enzyme activity was measured between pretreated and untreated substrate. In lignocellulose treated with ammonia, 15% ammonia was used reducing hemicelluloses and more than half of lignin present. When enzyme activities were compared in table 2.2 in both Treated and untreated substrate, pretreated substrate showed high activity.

TABLE 2.Enzyme activities (IU/mL) of Cellulases and xylanase produced using the cotton-card board mixture by SSF.

Process	Incubati-on (days)	FPase (IU/mL)	CMCase (IU/mL)	Xylanase (IU/mL)	Beta – Glucosidase (IU/mL)
Solid state fermentation-Untreated lignocellulosic mixture	9	2.4 ± 0.12	16.78 ± 0.72	940 ± 0.66	3920 ± 0.8
Solid state					

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fermentation-Sulphuric acid pre-treated lignocellulosic mixture	9	3.14 ± 1.4	21.38 ± 1.8	1230 ± 1.0	5409 ± 1.2
Solid state fermentation-Ammonia pre-treated substrate mixture	9	3.23 ± 1.3	20.93 ± 0.45	1259 ± 0.7	5264 ± 1.5

2.4.3 *Physio-Chemcal Pretreatment*

Lignocellulose has an ability to resist deconstruction and overcoming this is needed for better performance of SSF. This resistance can be disrupted by using physiochemical techniques (Fig 2.4) such as steam explosion, liquid hot water and ammonia fiber explosion. (Zhao et al., 2012)

Steam explosion is widely known as enables lignin alteration and solubilization of hemicelluloses (Brodeur et al., 2011). The temperature ranges from 190 to 270 degrees with the time of exposure from 1 to 10 min. The time and temperature depends on the starting material (Duff and Murray, 1996). It enables high yield of cellulose.

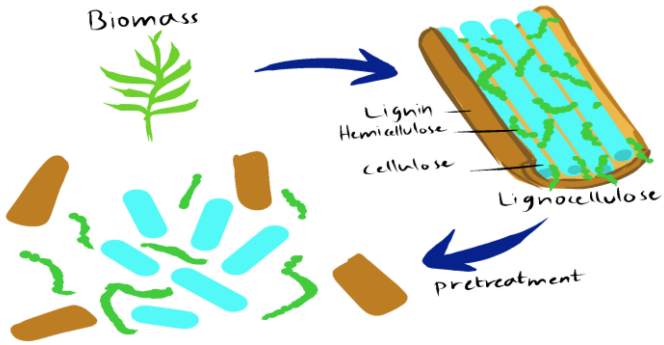

FIGURE 4. Pretreatment of lignocellulose

2.4.4 *Biological pretreatment*

In biological pretreatment other microbes are used such as brown-rot, white-rot and soft rot fungus. These are employed to delignify the material for fermentation, though, white rot is the most effective (Sindhu et al., 2016). These are mostly environmental friendly and do not produce inhibitors or toxic material.

Discussion

With the modern world moving fast and developing at a high rate, a demand for fast and improved production of products has increased along with it. Industries as well as scientists have been focusing on more efficient and less laborious ways to meet these demands. Cellulase production and solid state fermentation seems to be the future of this work. SSF is a fool proof method of recovering cellulase due to a number of reasons; 1)It is a cheaper technology as compared to SmF 2) It is easier to familiarize with as does not require too much maintenance 3)It makes product recovery easier, making downstream process less of a task 4)It can utilize pretreated lignocellolusic waste, not competing with animal fodder 5)making use of pretreated lignocellolusic substrate makes it easier for microorganisms to attack and produce higher quantities of cellulase 6) Decreases environmental pollution risks. It produces higher quantities than the much used submerged fermentation, in a study it showed that Aspergillus Niger produced 14.6 times more cellulase in SSF than it did in SmF (Mrudula and Murugammal, 2011a).

Cellulase has already shown its capabilities in producing biofuels, which maybe the fate of the world. Researches are being done on producing biofuels to replace the use on nonrenewable fossil fuels. The biggest obstacle in this matter is conversion of biomass to sugars. Here cellulase engineering is the edge cutting technology.

According to allied market research, the global enzyme market is expected to reach $10, 519 by 2024, growing at an astonishing rate. Cellulase having immense potential in many different industries, making many processes feasible and; introducing new and better products in the market, should be focused on more. It is the crux of many future industrial processes. It brought about revolution decades earlier like for biostoning in the textile industry in 1980s (Arja, 2007a); and will continue to do so as more research will be conducted.

Conclusion

Developing countries should take cellulase production and utilization under consideration as the enzyme market is growing is going to prove beneficial according to the future perspective. This will help them compete with developed countries in the industrial aspect and modify existing processes. Researches should be done as cellulose produced by solid state fermentation has promising advantages and should be applied to different techniques.

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