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Pretreatment, Saccharification, and Bioethanol Production from Cotton ginning and Paper wastes by Saccharomyces Cerevisiae

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ABSTRACT

Cotton wastes comprise lignin, hemicelluloses and cellulose, which can be utilized as sustainable for the production of bioethanol. Cotton based lignocellulosic biomass requires specific pretreatment strategy to efficiently remove lignin and to solubilize hemicelluloses, which increases the accessibility of hydrolytic enzymes during saccharification. Waste paper, a major source of cellulosic biomass, could be utilized as a potential substrate for cellulase production. Different pretreated waste papers were used as substrates for cellulase production. *Trichoderma spp. i*s used for Bioethanol production because of high metabolic activity in production of cellulase enzyme and hydrolysis of cellulose to glucose. Bioethanol has been identified as the mostly used biofuel worldwide since it significantly contributes to the reduction of crude oil consumption and environmental pollutions. Compared to other types of microorganisms, yeasts especially *saccharomyces cerevisiae* is the common microbes employed in ethanol production due to its high ethanol productivity, high ethanol tolerance and ability of fermenting wide range of sugar. The production of bio-ethanol by microbial saccharification and fermentation process. The common process involves in ethanol production are pretreatment, hydrolysis and fermentation. Productions of bio-ethanol during fermentation depend on several factors such as temperature, pH, inoculum size, and sugar concentration.

KEYWORD

Cotton waste, Paper waste, Yeasts, Fermentation, Bioethanol

1. INTRODUCTION

Fuel ethanol production from lignocellulosic biomass is emerging as one of the most important technologies for sustainable production of renewable transportation fuels. Most of the fuel ethanol produced in the world is currently sourced from starchy biomass or sucrose (molasses or cane juice), but the technology for ethanol production from non-food plant sources is being developed rapidly so that large-scale production will be a reality in the coming years [1]. Lignocellulose is the most abundant renewable biological resource, constitute a major portion of agricultural wastes and forest wastes [2]. Lignocellulosic materials are cheap renewable resources available in large quantities [3].

The world population is estimated to increases from 6.7 billion to 8 billion by 2030 [4]. On the other hand, global oil production is expected to decline from 25 billion barrels to 5 billion barrels by 2050 [5]. Thus the energy demand of the future is likely to play a key role in geo-political economics. Given this reality, nations around the world are investing in alternative sources of energy, including bioethanol. The leading nations in bioethanol production are Brazil and the USA, and USA is the world's largest producer of bioethanol. Asian countries altogether account for about 14% of world's bioethanol production [6]. Bioethanol is known as the most widely used biofuel in transportation sector and have a long history as alternative fuels [7]. The steps involved in the production of bioethanol and chemicals from lignocellulosic biomass consist of feedstock preparation, pretreatment, fractionation, enzymatic hydrolysis (saccharification), fermentation, product recovery, and waste treatment [8].

Most of the previous studies have focused on utilizing office paper and newspaper as substrates [9]. Cardboard is widely used as a packing material and is found in large quantities all over the globe. [10]. and the physical properties of cardboard make it as a favorable candidate for biodegradation and bioproduction [11]. Huge quantity of cotton gin waste is generated in cotton mills. The disposal of this waste environmental regulation is one of the biggest problems faced by the cotton ginning industry worldwide [12]. Raw cotton processing generates cotton gin residue (CGR), which is composed of immature bolls, cotton seed, hulls, burs, sticks, leaves, cotton lint, and dirt [13].

Microorganisms such as yeasts play an essential role in bioethanol production by fermenting a wide range of sugars to ethanol. They are used in industrial plants due to valuable properties in ethanol yield, ethanol tolerance, ethanol productivity, growth in simple, in expensive media and undiluted fermentation broth with resistance to inhibitors and retard contaminants from growth condition [14]. As the main component in fermentation, yeasts affect the amount of ethanol yield.

2. Cotton ginning wastes

About 218 kg of cotton fiber generates 68–91 kg of cotton gin trash (CGT) [15]. Worldwide production of this waste is approximately 3.23 million tones per year [16]. The USA produces about 1.8 million tones of CGT, whereas an average of 800 tones of cotton gin trash is produced by Texas in its 30 principal counties. With this large quantity of wastes, the final disposal becomes a major problem to the cotton industry which becomes more critical during winter and rainy seasons when insects use these residues as survival sites [17]. Availability is one of the most important factors in feasibility of using any product for bioenergy production [18]. In this context, though abundance of cotton gin waste throughout the world is a major problem of disposal, it's a simultaneous solution for bioenergy production. Alternative fuels produced from renewable resources, such as fuel ethanol, provide numerous benefits in terms of environmental protection, economic development, and national energy security [19].

3. Cardboard Wastes

The organic fraction of municipal solid waste contains lignocellulose in the form of waste paper products which could be an adequate raw material produce value-added products [20]. In municipal solid waste, the major cellulosic wastes are Paper, cardboard, wood, and agricultural residues and discarding these wastes in the landfills pollute the environment and cause the emission of greenhouse gases [21]. Utilizing these paper wastes as a biomass resource for microbial fermentations would be a better alternative way to solving this challenge. Waste paper could be utilized as a potential carbon source for cellulase enzyme production as it consists of 40–80% cellulose [22]. Very few studies have been carried out using cardboard as a resource [23]. Cardboard waste contains at least 50% of cellulose [10].

4. Yeasts

The production of bioethanol is founded on the ability of yeasts to catabolize six-carbon molecules such as glucose into two carbon components, such as ethanol, without proceeding to the final oxidation product which is CO_2. Crabtree positive yeasts such as *S. cerevisiae* accumulate ethanol in the presence of oxygen; however *Candia albicans* which is crabtree-negative yeast catabolizes sugars into CO_2 in the presence of oxygen [24].

4.1 yeasts in bioethanol production

Yeast such as *S. cerevisiae* have been used in alcohol production especially in the brewery and wine industries. It keeps the distillation cost low as it gives a high ethanol yield, a high productivity and high ethanol concentration [25]. Nowadays, yeasts are used to generate fuel ethanol from renewable energy sources [26].

S. cerevisiae is the most commonly employed yeast in industrial ethanol production as it tolerates a wide range of pH [27]. Thus making the process less susceptible to infection. Yeast was traditionally used as a starter culture in ethanol production due to its low cost and easy availability [28]. Flocculent yeasts were also used during biological fermentation for ethanol production as it facilities downstream processing, allows operation at high cell density and gives higher overall productivity [29, 30].

There are common challenges to yeasts during sugar fermentation which are rise in temperature (35-45°C) and ethanol concentration (over 20%) [31]. Inability of *S. cerevisiae* to grow in media containing high level of alcohols lead to the inhibition of ethanol production [32]. The other problems in bioethanol fermentation by yeast are the ability to ferment hexoses but not pentoses [33]. The efficiency of ethanol production on an industrial scale will be increased by using yeasts that are tolerant to inhibitors [31]. The common challenges of yeasts can be overcome by using ethanol tolerant and thermotolerant yeasts. Ethanol-tolerant and thermotolerant strain which can resist stresses can be isolated from natural resources such as soil, water, plants and animals. These are because cells adapt to their environment over time by natural selection [34].

Genetically engineered *S. cerevisiae* and co-culture of two strains have been developed to produce bioethanol from xylose with high yield [35]. Co-culture shows better ethanol production as compared to its pure culture [36]. Yeast strains that have been used in bioethanol production are *S. cerevisiae* was most widely studied yeasts [37]. At the optimum condition for sugar release, the levels of toxic degradation products exceed the critical level and made the condition unsuitable for yeast fermentation.

5. Process in bioethanol production

The process of ethanol production depends on the type of feed-stocks used. Generally, there are three major steps in ethanol production: (1) obtaining solution that contains fermentable sugars, (2) converting sugars to ethanol by fermentation and (3) ethanol separation and purification [38]. Feedstocks are usually pretreated in order to reduce its size and facilitate subsequent processes. Yeast are given the responsibility to ferment these sugars into ethanol [39].

5.1 Pretreatment

Pretreatment has a significant effect on the overall process which makes the hydrolysis easier and produces higher amount of fermentable sugars. And produce higher amount of ethanol yield and production cost [40]. Methods that are currently used for pretreatments are physical, chemical, biological and physicochemical. Physical pretreatment uses mechanical milling to ground the substrate. The common chemical pretreatment includes ozonolysis, acid hydrolysis, and alkaline hydrolysis [41]. And organosoly based process [42]. Different fungal species are involved in biological pretreatment while physicochemical pretreatment includes ammonia fiber explosion [43] and steam [44]. Yeast fermentation is inhibited by the week acid stress induced from lignocellulosic materials. However, the low concentration of week acid stress induced from lignocellulosic materials. However the low concentration of week acid can increase ethanol production by cellular division [45].

5.2 Hydrolysis

Hydrolysis process takes place after pretreatment to break down the feedstocks into fermentable sugar for bioethanol production. The two most commonly used hydrolysis methods are acidic and enzymatic [46]. Acidic hydrolysis can be divided into two types namely dilute and concentrated. Dilute acid hydrolysis is performed at higher temperature using low acid concentration while concentrated acid hydrolysis is carried out at lower temperature using high acid concentration. Concentrated acid process generates high sugar recovery (90%) in shorter period of time [47].

Enzymatic hydrolysis requires enzymes to hydrolyse the feedstocks into fermentable sugars. The activity of cellulase enzyme is influenced by the concentration and source of enzyme. The efficiency of enzymatic hydrolysis is influenced by optimized conditions such temperature, time, pH, enzyme loading and substrate concentration [48]. The amount of fermentable sugar obtained increase as the enzyme load increases while cellulose load decreases. Enzymatic saccharification of cellulose can be enhanced by using surfactants which function to block lignin. The efficiency of cellulose hydrolysis can be improved by adding polyethylene glycol (PEG) or Tween 20 to increase enzymatic saccharification and reduce the adsorption of cellulase on lignin [47].

5.3 SACCHARIFICATION AND FERMENTATION

The most successful method for ethanol production from lignocellulosic and cellulosic material is combination of the enzymatic hydrolysis of pretreated sample and fermentation in one step, called simultaneous saccharification and fermentation (SSF).

In this process, the glucose produced by the hydrolyzing enzyme which is consumed immediately by the fermenting microorganism present in the culture. SSF gives higher reported ethanol yield from cellulose then separate Enzymatic Hydrolysis and Fermentation (SHF) and requires lower amount of enzyme [49]. Hydrolysis of lignocellulosic

materials is separated from ethanol fermentation. The separation of enzymatic hydrolysis and fermentation allows enzyme to be operated at high temperature for better performance while fermentation organisms can be operated at moderate temperature for optimizing sugar utilization [48].

Fermentation of bioethanol can be carried out in batch, fed-batch, repeated batch or continuous mode. In batch process, substrate is provided at the beginning of the process without addition or removal of the medium [50]. Is known as the simplest system of bioreactor with multi-vessel, flexible or easy control process. The fermentation process was carried out in a closed-loop system with high sugars and inhibitors concentration at the beginning and ends with high product concentration [51]. There are several benefits of batch system including complete sterilization, does not require labour skills, easy to manage the feedstocks, can be control easily and flexible to various product specifications [52, 53]. However, the productivity is low and need intensive and high labour costs. The presence of high sugar concentration in the fermentation medium may lead to substrate inhibition and result in inhibition of cell growth and ethanol production [54].

Cell recycle batch fermentation is a strategic method for effective ethanol production as it reduces time and cost for inoculum preparation. The other advantages of repeated-batch process are easy cell collection, stable operation and long-term productivity [55, 56]. Sugar materials and immobilized yeast cells are used to facilitate cell separation for cell recycling [57, 58]. However, its application in SSF process of lignocellulosic materials is extremely difficult because lignocellulosic residue remain in the fermentation medium together with yeast cells [59]. Fed-batch fermentation is a combination of batch and continuous mode which involves the addition of substrate into the fermentor without removing the medium. It has been used to overcome the problem of substrate inhibition in batch operation. Productivity of fed-batch fermentation can be increased by maintain substrate at low concentration which allows the conversion of sufficient amount of fermentable sugars to ethanol [53]. This process has higher productivity, higher dissolved oxygen in medium, shorter fermentation time and lower toxic effect of the medium components compared to other types of fermentation [54]. However, ethanol productivity in fed-batch is limited by feed rate and cell mass concentration [60]. Fed-batch operation has been applied successfully in non-uniform SSF system by continuously adding a pretreated substrate in order to achieve relatively high sugar and ethanol concentration [61]. Culture volume in continuous operation must be constant and the fermentation products are taken continuously from the media. Various types of products can be obtained from the top of the bioreactor volumes and less investment and operational costs [53]. At high dilution rate, ethanol productivity is increased while ethanol yield is decreased due to incompletely substrate consumption by yeasts. [62]. However, the possibility for contamination to occur is higher that other types of fermentation [63].

Enzymatic hydrolysis is the preferred saccharification method because of its higher yields, higher selectivity, lower energy cost and milder operating condition than chemical processes [64]. The most commonly used pretreatment method is steam explosion. This is contributed by the attractive features of steam explosion which has less environmental impact, low capital investment, high energy efficiency, less hazardous process chemicals and conditions and complete sugar recovery [65].

5.4 Factors affecting bioethanol production

There are several factors which influence the production of bioethanol including temperature, sugar concentration, pH, fermentation time, agitation rate, and inoculums size [66]. The growth rate of the microorganisms is directly affected by the temperature [67]. High temperature which is unfavorable for cells growth becomes a stress factor for microorganisms [68]. The ideal temperature range for fermentation is between 20 and 35°C [69]. Moreover, enzymes which regulate microbial activity and fermentation process are sensitive to high temperature which can denature its tertiary structure and inactivates the enzymes [70].

The increase in sugar concentration up to a certain level caused fermentation rate is increase. The initial sugar concentration also has been considered as an important factor in ethanol production. High ethanol productivity and yield in batch fermentation can obtained by using higher initial sugar concentration the maximum rate of ethanol production is achieved when using sugar at the concentration of 150 g/L. [66].

Ethanol production in influenced by pH of the broth as it affects bacterial contamination, yeast growth, fermentation rate and by-product formation. The permeability of some essential nutrients into the cells is influenced by the concentration in the fermentation broth [66]. Moreover, the survival and growth of yeasts is influenced by the pH in the range of 2.75-4.25 [71]. In fermentation for ethanol production, the optimum pH range of *S. cerevisiae* is 4.0-5.0 [27].

Inoculum concentration does not give significant effects on the final ethanol concentration but it affects the consumption rate of sugar and ethanol productivity [72]. The production of ethanol was seen to be increased with the increase in cell numbers from 1×10^4 to 1×10^7 cells per ml but there was no significant ethanol production found between 10^7 and 10^8 cells per ml [66].

Agitation rate controls the permeability of nutrients from the fermentation broth to inside the cells and removal of ethanol from the cell to the fermentation broth. It increases the amount of sugar consumption or reduces the inhibition of ethanol on cells. The common agitation rate for fermentation by yeast cells is 150-200 rpm [66].

Fermentation time affect the growth of microorganisms. Shorter fermentation time causes inefficient fermentation due to inadequate growth of microorganisms. Complete fermentation can be achieved at lower temperature by using longer fermentation time which results in lowest ethanol yield [66].

6. Conclusion

In this present study cotton waste and paper waste used a substrate for the production of bioethanol. Yeast which is the most common microorganisms in bioethanol production plays important function in fermenting sugar to ethanol. The enzyme cellulase obtained enzymatic hydrolysis of the substrate cotton by simultaneous saccharification and fermentation. Fermentation process exihibited significant effect on ethanol production. Continuous SSF method has shown its ability in producing high ethanol concentration with high productivity.

7. References

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