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Research Progress on Bioconversion of Wooden Biomass to Single Cell Protein

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Abstract

Wooden biomass resources are in a situation of being seriously lavished at present in China; while at the same time, protein resources are seriously short for feed. Study on bioconversion of wooden biomass to single cell protein is becoming a task that scientific researchers are facing. This article summarizes the research situation, facing problems and developing tendency of bioconversion of agricultural and forestry residues, sugar cane bagasse and wood residues to single cell proteins (SCP), in order to offer the theoretical foundation for application.

Keywords: wooden biomass; bioconversion; single cell protein.

1. Introduction

Wooden biomass resources are very precious natural resources, but they are in a situation of being seriously lavished at present. Along with the exhausting of non-regeneration resources on the earth, it is important to convert the wooden biomass to useful products by biotechnology. Cellulose and hemicellulose can be hydrolyzed to single sugars by enzymes, the single sugars can be fermented to single cell protein (SCP), organic acid and alcohol these high cost products[1,2], the application foreground is very wide.

At the same time, with the economy development and the living standard improving in China, the increasing demand for protein food has promoted the breeding industry development, which made the lacking of protein resources for feed. Single cell proteins are the microbial proteins obtained by cultivating single cells or wirelike organisms, they are rich in protein and essential amino acids, abundant vitamins and minerals, manifold enzymes and hormones, which can promote animals to grow [3].

Bioconverting the deserted wooden biomass to single cell protein is important to solve both the agricultural and forestry residues and the lacking of feed protein, and the bioconversion process do not pollute the environment, it can make people realize continuable development, the foreground is very wide.

In order to make clear the flow sheet of bioconversion, the proposed process was initially identified in Figure 1.

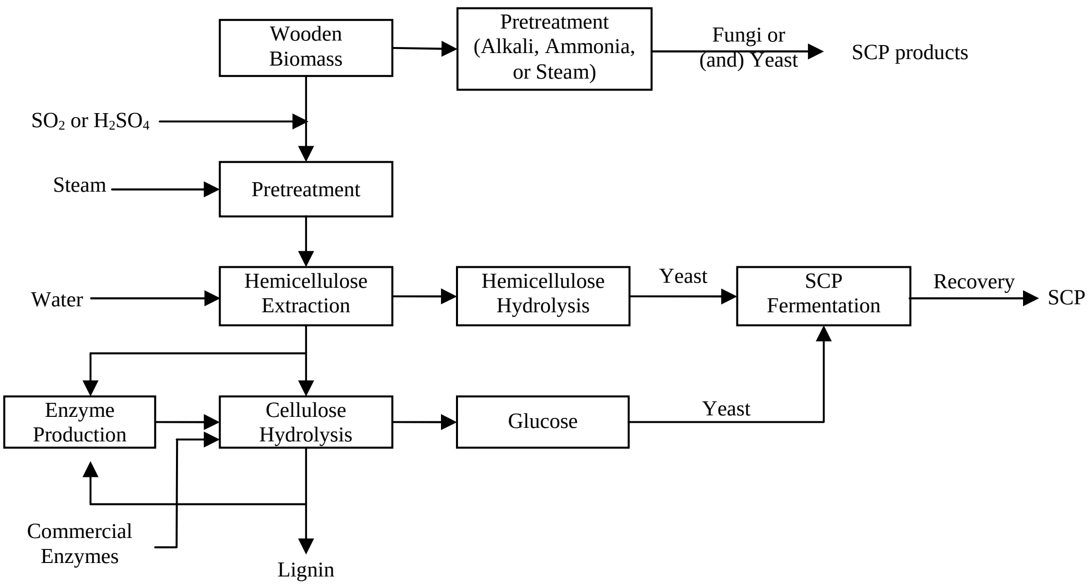

Figure 1. Schematic diagram of a bioconversion to SCP process.

2. Study on pre-treatment of wooden biomass materials for bioconversion

The most important step of wooden biomass bioconversion is cellulase hydrolysis, but due to the complex structure of natural wooden materials, the difficulty of bioconversion is how to decompose it to fermentable sugars high efficiently. Therefore, removing the lignin block and making the cellulase contact with cellulose are the key to improve the hydrolysis. The enzymatic hydrolysis rate of untreated natural biomass materials was less than 20%, so the materials must be pretreated, during which the cellulose, hemicellulose, and lignin be separated, the cellulase could be easily penetrated to cellulose, and be hydrolyzed effectively [4].

There are many kinds of methods for pretreatment, including physical, chemical, physical and chemical, and biological methods, among them, the steam explosion pretreatment was studied excessively, and was regarded as the most economical and efficient method for hardwood [5]. Because in the process of steam explosion, the physical and chemical actions made the hemicelluloses to be hydrolyzed to mono-saccharides and oligosaccharides, part of lignin dissolved, and the cellulose solubility increase so as to be fit for being acted by cellulase enzymes. The conventional mechanical methods require roughly 70 % more energy to achieve the same size reduction than explosive decompression [6].

Steam explosion of wood chips with SO_2 addition, or impregnating SO_2 or H_2SO_4 before explosion, could greatly improve the hydrolyzing process, and was also effective for softwood [7,8]. Clark et al. [7] even described by empirical models the effects of the variables time, temperature, and sulphur dioxide level to total sugar yields, and obtained the optimal conditions: 3minutes; 215°C; 2.55 % SO_2. Under these conditions, the steam-exploded fibre was 82% digestable by the cellulase enzymes used, and the total sugar yield was 57g /100g. Mackie et al. [8] examined the action mode and found that approximately 50% of the input sulphur had been binded to the substrate, forming, most likely, lignosulphonates. Although the steam explosion made the content of water-soluble lignin decrease but the content of alkali-soluble lignin increase, which was in favor of the lignin dissolving for next treatment using alkali.

3. Studies on bioconversion of wooden biomass to single cell protein

The crude protein concentration of various materials and pretreatments fermented with different strains was listed in Table 1.

Table 1. Crude protein of various materials and strains by fermentation.

Fermentation means	Materials and pretreatments	Strains	Crude Protein	References
Solid Fermentation	Steam exploded corn stalks	*Trichoderma reesei* *Candida tropicalis*	31.82%	[9]
	Milled poplar leaves	*Saccharomy cescerevisiae* *Candida tropicalis*	16.25%	[10]
Liquid Fermentation	Ammonia pretreated corn stalks	*Trichoderma reesei* *Aspergillus niger* *Candida tropicalis* 2.21	18.13%	[11]
	Ammonia pretreated Corn stalks	*Trichoderma reesei* TB101 *Saccharomy cescerevisiae* 2.339	21.0%	[12]
	NaOH treated rice straw(＜1mm)	*Candida tropicalis* 321,1817	＞ 50%	[13]
	Defatted rice polishing	*Neurospora sitophila*	27.8%	[14]
	Milled sugar canebagasse	*Candida utilis* *Rhizopus nigricans*	26.02%	[15]
	Alkali pretreated bagasse	*Trichoderma sp.* *Aspergillus terreus*	21-28%	[16]
	$NaClO_2$ pretreated aspen wood	*Chaetomium cellulolyticum*	37.9%	[17]
	Steam exploded aspen wood	*Chaetomium cellulolyticum*	21.4%	[17]

3.1 Agricultural residues

With ammonia pretreated corn stalks as materials, and with high yield cellulase of *Trichoderma reesei* and the feed-yeast, Qingsen Chen et al. [11,12] developed thoroughly for systhesis of SCP by liquid co-fermentation. Two optimized systems of muti-strains co-fermentation systems were set up, the crude protein achieved 18.13% and 21% (Table 1) after 5 days co-fermentation, and the cellulose conversion rate were 66.55% and 72%, respectively. Mixed solid fermentation of *Trichoderma reesei* and *Candida tropicalis* was studied by Chenghua Wang [9] for production of high enzymatic activity SCP from steam explosion maize stalk. Under the optimal conditions, the crude protein obtained by mixed fermentation reached 31.82 %, 56.88 % of the content of raw cellulose was reduced, and the cellulase activity reached 105 µ /g.

Zhang et al. [18] investigated the factors of cellulase and SCP by means of mixed fermentation of *Trichoderma viride* and *Candida utilis* yeast, and determined the parameters of mixed solid fermentation. Hongzhang Chen et al. [19] extracted the hemicellulose hydrolysate from steam-exploded wheat straw to produce SCP by *Trichosporon cutaneum* 851. In a 2 liter auto-fermentor, the biomass concentration of 45g / L and the productivity of 4.4g / (L·h) were obtained in batch-fed fermentation.

In abroad, Banerjee et al. [13] studied the effects of the size of straw and different pretreatment to protein yield in their research of bioconversion rice straw to SCP by *Neurospora sitophila*. The results showed that cellulose utilization and crude protein yield increase with the size of straw reducing and the concentration of pretreated solution enhancing. The cellulose utilization was about 90% and the crude protein content in product exceeded 50 % if the rice straw of less than 1mm was pretreated with 0.15 kg NaOH / kg rice straw. Ibrahim Rajoka [14] studied microbial protein production from defatted rice polishings using *Candida utilis* in shake-flasks and a 14-L fermentor to optimize fermentation conditions before producing biomass in a 50-L fermentor, and calculated the kinetics of crude protein production, cell mass formation and solid substrate present in the medium. All values of fermentation variables were significantly higher than that reported by Nigam [20] and Paul [21], biomass yield was 0.62 g cells / g substrate and the crude protein content was 27.8%.

3.2 Forestry residues

Wang [10] from Nanjing Forestry University cultivated yeast by solid fermentation on substrate of poplar leaves, the results showed that forestry wastes as poplar leaves could be regarded as materials to produce feed yeast. He analyzed the ingredient of yeast cultures and discovered that the total amino acids content increased 92.5%, the L-cystine which was advantageous to poultry breeding, increased 2-3 times,. The yeast cultures were used to cultivate fish, the mix-cultured *Parabramis pekinbnsis* and *Calossoma* grow quickly and the feed coefficient decrease. Further more, due to the price of the yeast cultures was lower than that of the fishing feed, the feed cost reduced and the gross profit increased greatly [22].

3.3 Sugar cane bagasse

Sugar cane bagasse, the left residue of sugar cane after being extracted of sucrose, in which, cellulose, hemicellulose and lignin contents are ca. 38%, 33% and 22% respectively, so it is applicable to produce single cell protein [20]. Wu [15] used mix-culture technique to produce single cell protein utilizing bagasse as the sole carbon source on laboratory, the results indicated that the mixed-strains of *Rhizopus* and *Trichoderma* had better ability to degrade bagasse. The liquid fermentation was carried in rotating inculator for 108h at 32°C, pH 6.0, the crude protein of the fermentation dry product was 260.2 g / kg.

The effect of chemical solutions sprayed on sugar cane bagasse pith to produce single cell protein was investigated by Rodriguez-Vazquez [23], he discovered that the pore size of vessels in pith pretreated with $Ca(OH)_2$ and NaOH increase and hemicellulose-phenolic compound linkage breakdown, which made the carbohydrate be easily attacked by microbial organisms, it was favorable for fermentation. Uniformly, Zayed [24] also reported that delignification to bagasse promote the saccharification of *Aspergillus niger* to bagasse.

Both of Ferrell Miller [25] and El-Nawwi [16] took alkali pretreated bagasse as materials, investigated the cultivation conditions of SCP and cellulase production with *Aspergillus terreus*. The former analyzed the SCP production under conditions of batch, semicontinuous, and continuous cultivation, he found that the doubling time of continuous cultivation was short, the crude protein content was steady at different temperature. The latter achieved 21%~28% of SCP content and 11~14.5g / kg bagasse of SCP yield under 1.5% alkali concentration, pH 4.5, 35 ℃ fermentation temperature, 4% inoculum and 7d continuous cultivation in shake flask. Carboxymethyl Cellulase (CMCase) and Filter Paper Activity (FPA) were 0.85 ~1.2 U / ml and 0.08 ~ 0.11 U / ml respectively, the enzymeatic activity were proportionate with crude protein content in product.

Other than the whole sugar cane bagasse, hemicellulosic hydrolyzate of bagasse can also be used to ferment yeast SCP, for the bagasse contains ca.30 ~35% hemicellulose. Nigam [20] and Pessoa [26] both investigated the microbial protein production and its kinetics with sugar cane bagasse hemicellulosic hydrolyzate, the total protein in biomass product reached 48.2 % and 31.3 % separately, and contained essential amino acids for animal feed. Nigam [20] also compared the biomass production of *Candida langeronii* and *Candida utilis* from hemicellulosic hydrolyzate, and discovered that *C. langeronii* was superior to *Candida utilis* in that it utilized L-arabinose in except of D-xylose and was capable of growth at higher temperature.

3.4 Wood residues

Compared to agricultural residues, the lignification degree and cellulose crystallinity are higher, which cause more difficult for organisms to attack wood cellulose, so pretreatment to wood before bioconversion appeared to be more necessary. YingKai Tong [27] studied the fermented sawdust to be used as feed, and found that the sawdust was pretreated by heat spurt before fermentation, and then was inoculated cellulose degradation organisms, the crude protein content increased after 72 h fermentation. The results to feed egg-chicken using fermented sawdust showed that it could substitute part of corn in day feed, the laying quotiety and feed to eggs ratio were both unnotable compared to the contrast group.

Chahal [17] investigated the effects of different pretreatments to aspen wood for SCP production with *Chaetomium cellulolyticum*, and found that high pressure steam was superior to atmospheric pressure steam, because high pressure steam could made wood break to smaller pieces. More complete delignification of wood using $NaClO_2$ increased the protein composition in the final product to 37.9 %, at a specific growth rate of 0.19 h^{-1}, and the cellulose utilization was highest, reached 90%.

The hemicellulose fraction of eucalyptus wood can be easily removed by acid treatment and the hydrolyzate is rich in fermentable sugars, mainly xylose, it has been utilized as a substrate for different bioconversion products. Almeida [28,2] conducted studies of bioconversion to SCP with eucalyptus wood hydrolyzate, and used the response surface methology to select nutrient level for culturing *Paecilomyces variotii* IOC-3764 in eucalyptus hemicellulosic hydrolyzate. Cell biomass concentration achieved 12.06 g / L in medium of 10 g / L rice pollard, 2.0 g / L nitrogen and 1.1 g / L sodium phosphorus acid and after 89 h cultivating.

4. Problems and directions

In process of bioconverting wooden biomass to SCP, the hydrolyzate contains pentoses (mainly xylose) except hexose, for the pentosan is the important composition in hemicellulose, so the simultaneous saccharification and fermentation (SSF) of pentose and hexose is the research aspect in this field.

Because acetic acid, furfural, hydroxymethyl furfural and soluble lignin existed in hemicellulose hydrolyzate, they could bring restrain action to microbial growth. So the next task is how to take off the toxin from wooden biomass hydrolyzate economically and effectively, thereby the fermentation performance can be improved.

In addition, how to prevent the pollution of other foreign bacteria, how to control the ferment process are also the tasks we should work over. At present, to study the dynamics of fermentation process is the important and basic work to rein fermentation process.

In conclusion, bioconversion of wooden biomass to SCP is the important approach to resolve the food supply and environment problems in face of the world, is the important guarantee to realize continuable development of mankind. China is an agricultural country, and has huge population, studies on this area should be developed widely.

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