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Bioethanol Production Using *Saccharomyces cerevisiae* with Different Perspectives: Substrates, Growth Variables, Inhibitor Reduction and Immobilization

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Abstract

In the transportation sector, the most commonly used biofuel is 'bioethanol' to reduce greenhouse gases. Ethanol production at the industrial level is employed by many yeast, bacteria, and fungi. But *Saccharomyces Cerevisiae* is most employed yeast. Wide range of substrates has been used for ethanol production such as lignocellulose, molasses, sweat sorghum cane extract, starch based substrate and other wastes. Lignocellulosic hydrolysates contain many inhibitors that can be reduced by treatment with activated charcoal and reducing agents, repeated sequential fermentation, over-liming, evaporation, anion exchanger, enzymatic treatment using peroxidase and laccase, and in-situ detoxification with fermenting microbes. Co-culturing of *S. Cerevisiae* with other microbes is targeted for optimization of ethanol production, short fermentation time, and for reduced process cost. Yeast cell immobilization has been considered as a potential alternative to enhance ethanol productivity. This paper also reviews the effects of various factors on yeast fermentation for ethanol optimization.

Keywords: Bioethanol; *S. cerevisiae*; Lignocellulose; Fermentation; immobilization

Introduction

As the world population and industrialization are increasing, the demand for energy is also increasing. Therefore, the cost of coal, natural gas, and crude oil is increasing. Thus the uncertainty of the fossil fuel and global climate changes have led to renewable energy development. Among biofuels biodiesel, biogas and bioethanol are dominant renewable energy. In the transportation sector, bioethanol is the most commonly used biofuel. Several substrates have been used for ethanol production such as lignocelluloses, starch, and different wastes [1]. Lignocellulosic biomass (LCB) is more preferred for ethanol production because of the two major reasons: a) it does not compete with food, b) it takes care of plant and agricultural residues in environmentally sustainable process [2,3]. Due to high processing cost, cellulosic ethanol production at industrial level is still a challenge. The major reason for the high cost is the consumption of high steam energy for distillation of fermentation broth with the low ethanol titer when LCB is used as feedstock [4]. Higher feedstock price is the second reason for the high cost of ethanol production [5,6]. Ethanol titer can be upgraded by different pretreatment methods that increase cellulosic content in fermentation system [3,7] and hence reduces the cost. Various microorganisms carry out fermentation such as yeast, fungi, and bacteria. But *S. cerevisiae* is widely studied and used at both household and industrial levels. Ethanol is generated as the main fermentation product of *S. cerevisiae*. *S. cerevisiae* is superior to filamentous fungi, bacteria and other yeasts in its various physiological characteristics for ethanol production at industrial level. It can tolerate wide range of pH [8] with acidic pH as optimum [9], which protects contamination. It can also tolerate ethanol better than other ethanol producers [10]. It is also GRAS (generally regarded as safe) for human consumption. This paper reviews trends for ethanol production using *S. cerevisiae* with different perspectives like substrates, growth variables, inhibitors reduction from hydrolysate and different immobilization techniques.

Substrate for Yeast

Nonfood source acts as the substrate for ethanol production. Various substrates have been used for ethanol production (Table 1).

S. cerevisiae strains	Substrate	Pretreatment	Enzymatic hydrolysis (g/l)	Ethanol Produced
TISTR 5596	starch cassava pulp		Amaylase and glucoamylase	9.9
ATCC 26602	Wheat straw	H_2O_2	cellulase	10
SOL/M5	Leaf and stem of *Dendratherma Grandiflora*		Crude extract from *Pleurotus ostreatus*	10.64
Baker yeast	Sticky coffee husks			13.6

MTCC 174	Rice husks	Na OH	Crude unprocessed enzyme	14
ATCC 96581	Waste newspaper	sodium dodecyl sulphate	Cellulase and glucosidase	14.29
RCK-1	newspaper cellulosics		exoglucanase, glucosidase and xylanases with tween 80 and $CoCl_2$	5.64
Y5	Corn stover	Steam explosion	cellulase	40
TJ14	Microcrystalline Cellulose		Commercial cellulase	45
DQ1	Corn stover	H_2SO_4 supplemented with	Cellulase	48
Y5	Corn stover	Steam explosion	Cellulase and glucodiase	50
DQ1	Corn stover	steam explosion	Cellulase	55
L2524a	Empty palm fruit bunch fibers	Alkali (NaOH)	Cellulase	64.2
var. ellipsoideus	Corn meal		Heat stable –amylase and glucoamylase	79.6
ATCC 6508	Sweet potato chips		Amylase and glucomylase	104.3

Table 1: Different Substrates for *S. cerevisiae* for Ethanol Production at varying Treatment Conditions.

Ethanol production from nonfood sources provide two advantages: a) cost of waste disposal is reduced, b) since wastes are cheap, thus the cost of ethanol production is also reduced.

Reduction of Inhibitors in Hydrolysates

During hydrolysis, various inhibitors are generated that pose hindrances for ethanol production, such as inhibition of cell growth and sugar consumption during yeast cultivation. Such inhibitors are- furfural, hydroxymethylfurfural (HMF), acetic acid, formic acid, and phenolic compounds etc. Various approaches are being used to solve this problem, such as- a) repeated sequential fermentation so that yeast can adapt the inhibitory chemicals, b) over-liming [10-27], c) anion exchanger [28], d) activated charcoal addition [25], e) treatment with reducing agents [29], f) evaporation [10], g) in-situ detoxification by fermenting microbes [30,31], h) enzymatic treatment with peroxidase and laccase [32], i) membrane extraction [33] and, j) solvent extraction [32]. In biological methods, enzymes or the microorganisms are used to detoxify the inhibitors in co-culture. Sequential co-culturing of *S.cerevisiae* and *Thermoanaerobacter pentosaceus* was found to reduce inhibitory compounds and also enhance ethanol production [30]. According to results, *T. pentosaceus* was able to metabolize furfural and HMF up to 0.5 and 1 g/L, respectively. Phenolic compounds were also detoxified from *Trametes versicolor* using immobilized laccase [28]. Activated charcoal treatment, neutralization, solvent extraction, ion exchanger and over-liming are chemical treatment methods. Activated charcoal treatment reduces the inhibitors due to their high adsorption capacity and also shorten the fermentation time [26]. Evaporation helps in the reduction of volatile inhibiting compounds in LCB hydrolysates [10]. Over-liming detoxifies the inhibitors by precipitating them at high pH [27]. Precipitation reduces levulinic acid and acetic acid by neutralization chemistry principle.

Factors Affecting Rate of Yeast Fermentation

There are many factors that could affect the rate of yeast fermentation [8], like - a) type of carbohydrate, b) concentration of carbohydrate, c) concentration of salt, d) osmolarity, e) ethanol concentration, f) pH, g) temperature. Optimum temperature for *S. cerevisiae* is 30-40°C. Higher temperature shorten the exponential phase of yeast cell [8]. At 50°C, ethanol production is considerably reduced due to change in transport system that can increase toxin accumulation in the cell [8]. Optimum pH for *S. cerevisiae* was found to be 4.0-5.0 [8]. Below 4.0, the incubation period was prolonged and favored the formation of acetic acid and above 5.0, the concentration of ethanol diminished subsequently and it also favored butyric acid production [8]. Thus various parameters affect ethanol production that must be optimized to enhance ethanol productivity.

Immobilization to Improve Ethanol Productivity

Calcium or sodium alginate and agar-agar cubes are commonly used immobilizing agents [7]. Also, several studies have been done to investigate new immobilizing agents that are cheap and easy to use (Table 2). Yeast immobilization enhances ethanol productivity because- a) it reduces risk of contamination [33-35], b) it makes it easy to separate cell mass for the bulk liquid [36-43], c) it reduces production costs [18,36,42], d) biocatalyst can be recycled [43], e) fermentation time can be reduced [7,18], f) cells can be protected from inhibitors [44] g) more ethanol production compared to free cells [7,18,38,35].

S. cerevisiae Strain	Substrate	Initial sugar (g/l)	Residual Sugar	Immobilizing materials	Ethanol produced	Ethanol yield
MTCC 174	Sugar cane Bagasse	50	22	Agar-agar cubes	9.4	0.33
Baker yeast	Glucose	100	16	Lyophilized cellulose gel	36.12	0.43
MTCC 174	Sugar cane Bagasse	50	15	Sugar cane bagasse	15.4	0.44
CBS 8066	Glucose	30	0.3	Alginate-chitosan beads	13.37	0.45
CTCRI	Mahula flowers	89.75	7.99	Luffa sponge discs	37.2	0.455
Mutant baker	Glucose + Sucrose	280	7.21	Sweet sorghum pith	130.12	0.477
TISTR 5048	Sweet sorghum	240	26.69	Corncobs	102.39	0.48
NP 01	Sweet sorghum Juice	240	54.8	Corncobs	90.75	0.49
Baker yeast	Cashew apple juice	70.01	3.92	Cashew apple bagasse	36.91	0.49
DTN	Sugar beet Molases	130	6.3	Alginate-maize stem ground tissue	60.36	0.493
Saccharomyces cerevisiae var. ellipsoideus	Corn meal hydrolysates	176	8.02	Calcium alginate	89.68	0.52
Pakmaya Yeast Company	Glucose	120	6.03	Sodium alginate grafted with N-vinyl-2-pyrrolidone	69.68	0.697

Table 2: Enhancement of Ethanol Production using different immobilizing agents.

Conclusion and Future Perspectives

Starch and molasses have been used for ethanol production for long period of time, but they lead to competition for food with respect to land and price. Therefore, LCB is being used to solve such challenges. Ethanol production at industrial level is not successful due to two major reasons- a) low ethanol titer, b) different inhibitors in hydrolysates. Various optimization techniques are being used to enhance ethanol titer. Adsorption with activated charcoal, over liming, treatment with reducing agents, solvent and membrane extractions potentially reduce inhibitors to enhance ethanol titer. Immobilization of yeast cells is another strategy for optimization of production process in less cost manner. Thus lignocellulose pretreatment and fermentation are still an area of research interest. At present, transformation and over-expression of a gene for specific traits (eg cellulase) in yeast can be fruitful to solve challenges such as inability to use ribose and polysaccharide. Hence, an economic process analysis is required for the development of an industrially suitable production strategy to solve our energy crisis by producing more ethanol in a stable way.

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