OPTIMIZATION FOR PRODUCTION OF BIOETHANOL USING SORGHUM STOVAR BY Saccharomyces cerevisiae

P. Udhayaraja* and J. Sriman Narayanan
Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram – 608 002.
E.mail:rise.raja@rediffmail.com

Received 12 October 2012; Accepted 17 October 2012

Abstract
Ethanol is a clear, colourless, flammable, oxygenated hydrocarbon with the chemical formula C\textsubscript{2}H\textsubscript{5}OH. Ethanol has been made since ancient times by fermenting sugars. All the ethanol used for fuel and alcoholic drinks including most of the industrial ethanol, is made by this process. In the present study, the bioethanol production was optimized by Saccharomyces cerevisiae using sorghum stovar as a substrate. Ethanol fermentation from crude enzymes hydrolysed sorghum stovar was analyzed in the present research and the reducing sugar content was recorded maximum after 60 hours. The effect of pH (3.5, 4.5, 5.5 and 6.5), temperatures (25°C, 30°C, 35°C and 40°C) and inoculum level (4%, 6%, 8% and 10%) on ethanol yield from sorghum stovar using the yeast Saccharomyces cerevisiae was estimated. It was concluded that the ethanol yield was maximum at pH 6.5, 35°C and 10% inoculum level.

© 2012 Universal Research Publications. All rights reserved

Key words: Bioethanol, Saccharomyces cerevisiae, Sorghum stovar and Fermentation.

1. INTRODUCTION
Agriculture wastes contain a high proportion of cellulosic matter which is easily decomposed by a combination of physical, chemical and biological processes. The bunch consists of 70 moisture and 30% solid; of which holocellulose accounts for 65.5, lignin 21.2, ash 3.5, hot water-soluble substances 5.6 and alcohol-benzene soluble 4%. Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation [2]. The recognition that environmental pollution is a worldwide threat to public health has given rise to a new massive industry for environmental restoration. Biological degradation, for both economic and ecological reasons, has become an increasingly popular alternative for the treatment of agricultural, industrial, organic as well as toxic waste.

Plant lignocellulosics as organic substances are subject to attacks by biological agents such as fungi, bacteria and insects. Acids can breakdown the long chains in cellulose to release the sugars through hydrolysis reaction, but because of their high specificity, cellulase can achieve higher yield of glucose from cellulose. A portion of pretreated biomass can be used to feed a fungus or other organism that produces cellulase that can then be added to pretreated solids to release glucose from cellulose. Filamentous fungi which use cellulose as carbon source possess the unique ability to degrade cellulose molecules in plant lignocellulose. Although, a large number of microorganisms are capable of degrading cellulose, only a few of these produce significant quantities of cell-free enzymes capable of completely hydrolyzing crystalline cellulose \textit{in vitro} [3].

Energy requirement is increasing day by day due to tremendous growth in population along with its modernization. Energy has been always in demand not only in the past but is a continuing crisis due to the advance technologies and increase in population. The fuel crisis has been precipitated because of fast depletion of the naturally occurring conventional fossil fuel reserves such as petrol, diesel, kerosene, coal etc., [4]. The biomass conversion for energy production is popular nowadays among many countries in order to fulfill their energy needs. The energy production through Agricultural raw material and their wastes play a major role to meet the energy need. Sweet sorghum stem is one of the important biomass used to produce bioethanol.

Ethanol is one of the good sources of liquid energy for automobiles and industries. Ethanol is used as universal solvent. It is also used as fuel. In future, ethanol is going to blend with petrol in high proportion. Among the liquid fuels, ethanol is used as an alternative to petroleum (gasohol) by blending with petrol at the rate of 20% [4]. To reach the future demand of ethanol, it should be produced in high quantity from the agricultural raw material. The production of ethanol was basically by chemical methods but now, it can be effectively produced using microbial
process. The sweet sorghum juice is taken for the bioconversion of ethanol using yeast *Saccharomyces cerevisiae*. There is a high sugar content in the juice of sweet sorghum stem. The sugar is composed mainly of saccharose (60-65%), fructose and glucose. Sorghum is an indigenous African plant belonging to the family *Sorghum bicolor* (L) Moench [5]. Sweet sorghum produces grain, which is harvested for human consumption and contains sweet juice in the stalk. After harvest, the stalks are squeezed for the sweet juice, which can be turned into sugar or fermented to ethanol. The stalk material remaining after the sugar juice has been squeezed out is called the bagasse and can be used as animal feed or pretreated, hydrolyzed and fermented to ethanol. Sweet sorghum is considered as one of the most promising crops for the production of ethanol at low cost [6, 7, 8]. There are approximately four thousand varieties of sweet sorghum throughout the world [9]. Fermentation of sweet sorghum using yeast has an advantage of rapid fermentation [10]. Sufficient nutrients like carbon and nitrogen are required for the yeast to grow and reproduce but inorganic salts present in sweet sorghum juice is not enough to meet the need of fermentation [11]. *Saccharomyces cerevisiae* shows high ethanol productivity, high tolerance to ethanol and tolerance to inhibitory compounds present in hydrolysate of LB. However, wild type *Saccharomyces cerevisiae* has limitation being unable to ferment pentoses and hard efforts have been made to design a suitable engineered *Saccharomyces cerevisiae* [12]. Main strategies have been the construction of recombinant strains by introduction of genes *XYL1* and *XYL2* encoding for xylose reductase (XR) and xylitol dehydrogenase (XDH) respectively or by introduction of gene encoding for xylose isomerase (XI) due to its ability to ferment xylulose to ethanol [13]. Former strategy also need an over expression of endogenous xylulokinase (XK) for efficient xylose metabolism. Another hurdle to overcome when using xylose fermenting *Saccharomyces cerevisiae* is that xylose uptake competes with glucose uptake, because they are sharing membrane transporters [14]. *Saccharomyces cerevisiae* takes up xylose by both low and high affinity glucose transport systems, however, xylose uptake through these transporters is significantly less efficient compared to glucose. Therefore, various metabolic engineering efforts involving recombinant *Saccharomyces cerevisiae* have led to improvements in the initial rate of xylose consumption, being improvement of xylose transport in *Saccharomyces cerevisiae*, a great challenge to optimize xylose metabolic pathway [15].

2. MATERIALS AND METHODS

2.1. Pretreatment by enzymatic hydrolysis

Pretreatment of the substrate was one of the most important methods in order to process the material to release the fermentable sugars. Here different pretreated methods were employed with the aim of comparing the efficiency of each method to release maximum reducing sugars. For the study of enzymatic hydrolysis, the substrate was taken in 50gm Erlenmeyer flask, pH adjusted and inoculated with organism. The culture filtrate which was obtained after incubation of 7days contained enzyme source. The crude enzyme extract were take in different quantities (2ml and 4ml) were added in a 250ml Erlenmeyer flask containing substrates, acetate buffer 0.1M pH of 4.8, kept on a rotary shaker at a temperature of 50ºC for 72 hours. The clear supernatant of the hydrolysate from different time intervals *viz.*, 2, 4 upto 12 hours was taken for the estimation of reducing sugars.

2.2. Fermentation medium

The cellulosic hydrolysate obtained after the saccharification with enzyme was filtered and centrifuged to remove unhydrolysate, the pH of the supernatant was adjusted to 5.0 with 10% ammonium hydroxide solution before inoculation. In the case of alkali hydrolysate, the pH of the supernatant was adjusted to 5.0 with 10% sulphuric acid solution before inoculation. The 100ml clear supernatant was then enriched with 0.2 % urea as nitrogen source and the fermentation was carried out at 28-30ºC using *Saccharomyces cerevisiae* and their combination (4%w/v).

2.3. Optimization of fermentation process

Parameter optima are important in any type of fermentation process carried out by *Saccharomyces cerevisiae* and *Zymomonas mobilies* are known to vary with respect to pH, temperature, substrates, its concentrations and inoculums size etc. It is therefore imperative to optimize the fermentation conditions for Cellulosic hydrolysate of 100ml was used as substrate and yeast and bacterial cells as fermenting microorganisms so that the production efficiency increased.

2.3.1. Effect of pH on ethanol production

The pH of the hydrolysate was adjusted to 3.5, 4.5 5.5 and 6.5 with potassium hydroxide solution. The yeast culture of *Saccharomyces cerevisiae*, *Zymomonas mobilies* was inoculated and fermentation was carried up 7 days at 40ºC. The sample was analyzed for ethanol yield.

2.3.2. Effect of temperature on ethanol production

The yeast and bacterial culture at 1% level was inoculated to fermentation medium, pH was adjusted to 5.0 and incubated at different temperature *viz.*, 40, 44, 48°C for 7 days. After fermentation, the samples were analyzed for ethanol yield and unfermented residual sugar present.

2.3.3. Effect of inoculum size on ethanol production

The cellulosic hydrolysate extract (100ml of pH 5.0) was inoculated with 0.4, 0.6 , 8.0 and 10.0 % of inoculums levels of yeast and bacterial cultures and kept for fermentation at 40ºC for 7 days and thereafter samples were analyzed for unfermented residual sugar and ethanol yield.

3. RESULTS AND DISCUSSION

Due to rapid increase in population along with industrialization require large quantities of food and energy to serve the demand. The energy need was mainly for the automobile industry where petrol, diesel, gasoline and other energy sources are used. But they are non-renewable sources of energy, due to their large use it becomes depletion in energy source especially in transportation. So, alcohol fermentation is one of the major area of interest nowadays to meet out the present scenario of global energy. In the present study, sorghum stover was selected to find out its suitability for alcohol production to get maximum yield of ethanol, a viable technology has to be developed.
Ethanol fermentation from crude enzymes hydrolysed sorghum stover was analyzed in the present research and results were given in Fig. 1. The reducing sugar content was recorded maximum after 60 hours. Problems associated with the hydrolysis process need more improvements in order to reduce the operating cost for an ethanol-producing plant that uses wood waste as feedstock. Using a mixture of α-amylase and glucoamylase as the catalyst at 70°C and the dewatered potato enzyme/substrate ratio of 6U/g, the starch hydrolysis yield of 82g/g was obtained. Under these reaction conditions, the enzymes catalyzed hydrolysis of the potato starch was found to be irreversible first order reaction. These kinetic data were used in this study for the CSTR sizing in the enzyme catalyzed hydrolysis process [16]. Srichu Wong et al. [17] achieved the ethanol yield of 16.61% (v/v) in the bench scale SSF process carried out under the optimal conditions in 61.5 hours. Furthermore, large amounts of exogenous enzymes are required. Currently the Solid State Fermentation process is not used in continuous system on the industrial scale [18]. The effect of pH (3.5, 4.5, 5.5 and 6.5) on ethanol yield from sorghum stover using the yeast Saccharomyces cerevisiae was estimated and the results were furnished in Fig. 2. The ethanol yield was maximum at pH 6.5 followed by pH 4.5 and 5.5. Minimum ethanol yield was recorded in pH 3.5. Sreenath and Jeffries [19] reported maximum alcohol concentration of 5.8% (v/v) at pH 4.5 from aqueous carob extract after 120 hours of fermentation. Ethanol production was maximum at pH 6 and it was 30% less in pH 7.7.

Sreenath and Jeffries [19] reported maximum alcohol concentration of 5.8% (v/v) at pH 4.5 from aqueous carob extract after 120 hours of fermentation. Ethanol production was maximum at pH 6 and it was 30% less in pH 7.7.

Sreenath and Jeffries [19] reported maximum alcohol concentration of 5.8% (v/v) at pH 4.5 from aqueous carob extract after 120 hours of fermentation. Ethanol production was maximum at pH 6 and it was 30% less in pH 7.7.

The increase in ethanol yield at different temperatures (25°C, 30°C, 35°C and 40°C) was estimated and the results were showed in Fig-3. Highest ethanol yield was observed at 35°C followed by 30°C and 40°C. Low ethanol yield was noted at 25°C. The temperature and its role was also taken in consideration in order to assess the optimum temperature for the effective ethanol production. Verma et al. [20] tested the ethanol production at different temperatures viz., 25, 30 and 40°C from starch and reported maximum ethanol concentration 21.8 g L⁻¹ at optimum temperature of 30°C in 48 hours of fermentation period. The effect of inoculum level (4%, 6%, 8% and 10%) for the maximization of ethanol yield was studied in the present study and the results were showed in Fig-4. The ethanol yield was maximum at 10% inoculum level followed by 8% and 6%. Minimum ethanol yield was observed in 4% inoculum level. Nimbkar et al. [21] studied the effect of different inoculum level viz., 2,4,6,8 and 10% on the ethanol production from unspecialized juice of sweet sorghum and obtained maximum alcohol concentration of 12.45 and 12.23% (v/v) at 6% and 2% respectively. The findings of their research coincide with the present study by giving maximum ethanol yield at maximum inoculum level.

4. REFERENCES


Source of support: Nil; Conflict of interest: None declared