Food and Nutrition Sciences, 2022, 13, 463-470
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Optimization Studies in Simultaneous Saccharification and Fermentation of Wheat Bran Flour into Ethanol

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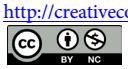
How to cite this paper: Manikandan, K., Rengadurai, S., Babu, E. and Sothivanan, S. (2022) Optimization Studies in Simultaneous Saccharification and Fermentation of Wheat Bran Flour into Ethanol. *Food and Nutrition Sciences*, **13**, 463-470.
https://doi.org/10.4236/fns.2022.135034

Received: March 14, 2022
Accepted: May 24, 2022
Published: May 27, 2022



Abstract

The effects of process variables in Simultaneous Saccharification and Fermentation (SSF) of wheat bran flour were studied in bulk fermentation using a coculture of *Aspergillus niger* - *Kluveromyces marxianus*. The effect of substrate density, pH, temperature, and enzyme concentration on wheat bran was predicted by designing experiments in which a single parameter is varied keeping other variables at a constant level. The above parameters were optimized for a batch culture in a fermentor. Optimal values for substrate concentration, pH, temperature, and enzyme concentration during processing were 200 g/l, 5.5, 65˚C, and 7.5 IU, respectively. In pre-treatment experiments, the concentration of enzymes and the pre-treatment temperature are highly correlated. The influence of pH, temperature, and substrate density on ethanol production was investigated. Temperature pH was determined as optimal, 32˚C and 5.5, respectively. After 48 hours of fermentation at optimum pH, a solution of wheat bran containing a maximum of 6% starch produces a maximum of 22.9 g/l ethanol.

Keywords

Simultaneous Saccharification and Fermentation (SSF), Co-Culture Fermentation, Single Factor Optimization, Ethanol

1. Introduction

Ethanol is gaining importance as a renewable fuel, beverage, and industrial solvent. With the rising prices of conventional fuels, it is a time to compromise on using starchy raw materials as a viable source to meet the increasing demand for ethanol. Industrial wheat makes up 14% - 19% of the outer layer of bran grain,

the aleurone layer, and the starch endosperm residue. Rich in starch, arabinoxilans, cellulose, beta-glucan, protein, and lignin, the wheat bran flour has the potential to serve as an economic feedstock to maximize ethanol production [1] [2]. Wheat bran is gelatinized and liquefied using amylase or amylase-producing microorganisms [3]. Simultaneous saccharification and fermentation of liquified starch is a promising viable technology to convert substrates into ethanol. The SSF process has certain limitations like different temperatures and pH favoring the growth of mold and yeast. It is essential to optimize the process variables which favor the growth of both cultures in a single fermentor. A thermotolerant yeast is used to favor a higher temperature in which saccharification is at a good rate and utilization of released sugars effectively by yeast *K. marxianus* without feedback inhibition of sugars. The effective synthesis of ethanol depends on the appropriate pre-treatment of wheat bran flour with fungal alpha-amylase. Conditions conducive to maximum enzymatic activity and microbial growth may vary [4] [5], so it is important to optimize the processing parameters of this SSF method. To solve this problem, it is important to use a single-component optimization strategy to optimize process variables. The aim of this study was to use *Aspergillus niger* (*MTCC* 1349) *and Kluyveromyces marxianus* (*MTCC*1389) in co-culture to simultaneously optimize the process factors influencing the decomposition of wheat bran starch into ethanol by fermentation.

2. Materials and Methods

2.1. Material

Wheat bran flour was procured from the local market and stored in airtight container.

2.2. Microorganisms

The heat-resistant *K. marxianus* (MTCC1389), and A. *niger* (MTCC 1349) were procured from IMTECH, Chandigarh. Initial pH 5.5 and at a temperature of 28˚C, *A. niger* was grown on a Potato Dextrose Agar (PDA) medium containing 200 g/l of potatoes and 40 g/l of dextrose, 20 g/l of agar.*K. marxianus* was grown in a YMP agar medium containing 3.0 g/l yeast extract, 3.0 g/l malt extract and 5.0 g/l peptone 20 g/l agar [6].

2.3. Pretreatment of Wheat Bran Flour

In an autoclave, a 10% (w/v) solution of wheat bran flour was gelatinized for one hour at 15 psi pressure. The solution was prepared by cooling with 10 IU of enzymatic fungal amylase enzyme purchased from Hemedia Laboratories [7]. The SSF procedure requires the preparation of liquid starch with specified concentrations.

2.4. Simultaneous Saccharification and Fermentation

Figure 1 shows the flow sheet of simultaneous saccharification and fermentation

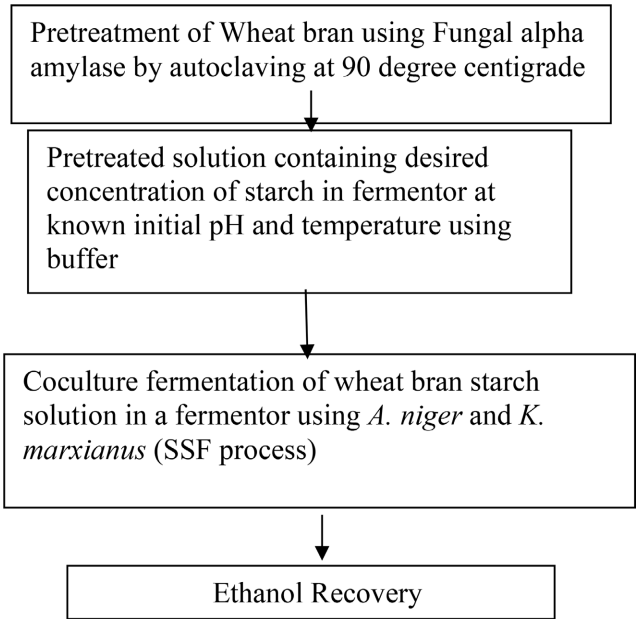


Figure 1. Flowsheet for SSF process used in ethanol production from wheat bran starch.

process [8]. The wheat bran solution was pretreated with fungal alpha amylase enzyme at 90˚C. The wheat bran starch solution of known initial concentration was taken in a fermentor and inoculated with cells of *A. niger* and *K. marxianus* The Simultaneous saccharification and fermentation was carried out at 150 rpm for 2 days at 30˚C. For every six hours, samples were taken and centrifuged cat 1200 and 5000 rpm and the supernatants were analyzed for glucose and ethanol concentration.

Fermentation was conducted at 26˚C, 28˚C, 30˚C, 32, ˚C and 34˚C at baseline concentrations of 2˚C and 5.5 initial pH. The experiments were performed at optimized temperature using 2% starch with different pH values of 4.5, 5.0, 5.5, 6.0 and 6.5. Different substrate concentrations of 2% and 4% 6%, 8%, and 10% (w/v), starch respectively, was fermented at optimized temperature and pH to maximize the ethanol production.

2.5. Cell Mass and Analysis

Cells were harvested at 5000 rpm, dried under vacuum at 70˚C to constant weight and used to calculate cell biomass by reporting dry weight in grams per liter of culture medium. The starch content of wheat bran flour was determined using a biospectrophotometer, the phenol-sulfuric acid technique.

2.6. Ethanol Estimation

The amount of ethanol in the fermented broth was measured using a NUCON 5765 gas chromatography (GC) system with a flame ionization detector and a Carbowax column (2 m × 0.3 cm) with nitrogen at 2 kg/cm^2. The oven was kept at 80˚C all the time. Temperatures of 200˚C were maintained in the injector and detector.

3. Results and Discussion

3.1. Effect of Temperature on Ethanol Production

Fermentation tests were conducted at temperatures of 26˚C, 28˚C, 30˚C, 32˚C and 34˚C, with a substrate concentration of 2% starch, 150 rpm and an initial pH of 5.5. Figure 2 shows the ethanol concentrations at different temperatures in the SSF process.

Ethanol concentration was found to increase with increasing temperature from 26˚C to 32˚C, which here was due to the increased activity of saccharifying enzymes secreted by the fungal culture [9] [10] [11]. The authors reported a maximum optimum temperature of 37˚C for co-culture fermentation using *S. cerevisiae* and *S. diastus*,In our studies the temperature beyond 32˚C resulted in decline of ethanol production. The optimum temperature turned out to be 32˚C, to produce a maximum of 6.03 g/l of ethanol.

3.2. Effect of Initial pH on Ethanol Production Using SSF

Fermentation studies were performed at various initial pH levels of 4.5, 5, 5.5, 6.0 and 6.5, maintaining the temperature at 32˚C, 150 rpm and 2% starch substrate. Figure 3 illustrates the results. Figure 3 shows the concentration of ethanol at an initial pH of 4.5, 5.0, 5.5, 6.0 and 6.5, maintaining 2% starch content at 32˚C. After 48 hours of fermentation, maximum ethanol concentration 7.53 g/l. l, was obtained.

The ethanol output increased as the starting pH was raised from 4.5 to 5.5 during the fermentation process. The best initial pH was discovered to be 5.5, which produced a maximum ethanol concentration of 7.53 g/l. The ethanol output decreased dramatically as the initial pH was elevated from 5.5 to 6.5, which was due to unfavorable acidic pH of wheat bran starch solution [12] [13].

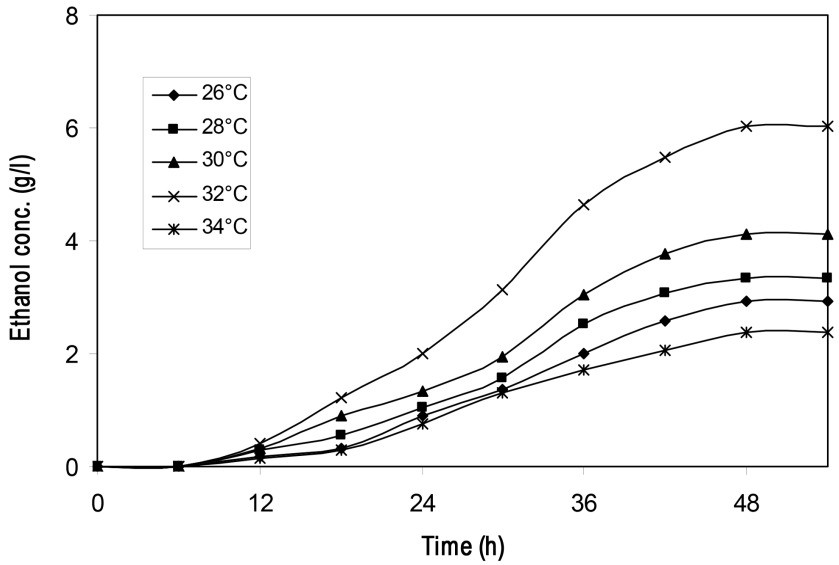


Figure 2. Ethanol concentration at different temperatures in SSF process carried out with an initial pH of 5.5, starch concentration 2% and 150 rpm.

The limitations of coculture fermentation process were low production of ethanol. The concentration of ethanol can be improved using glucoamylase enzyme and a high ethanol tolerant strain of yeast *S. cerevisiae*.

3.3. Effect of Substrate Concentration

The studies were conducted out at varying substrate concentrations of 2%, 4%, 6%, 8%, and 10% starch while holding all other parameters constant at an optimal beginning pH of 5.5, a temperature of 32˚C, and a rotational speed of 150 rpm. Figure 4 shows the ethanol production from wheat bran starch solution for

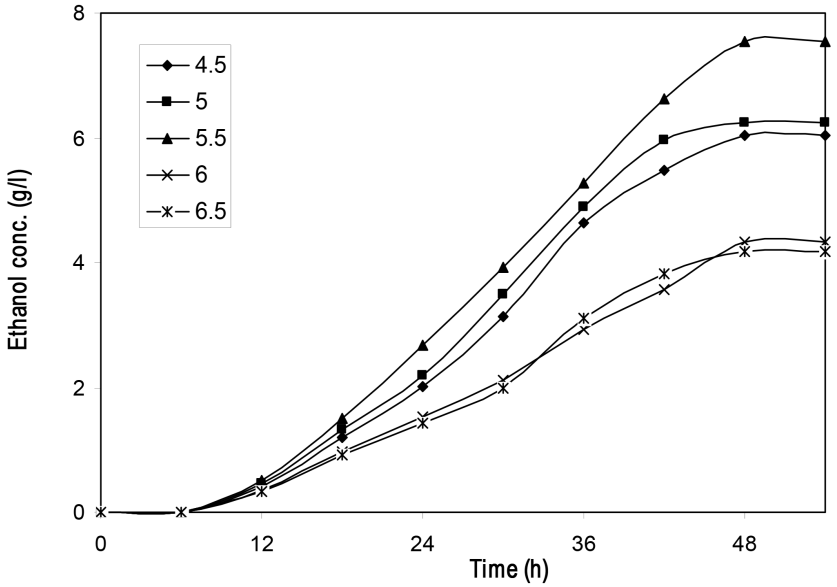


Figure 3. Effect of initial pH on ethanol concentration by co-culture of *A. niger* and *K. marxianus* for the experiment carried out with a starch concentration 2%, temperature 32˚C.

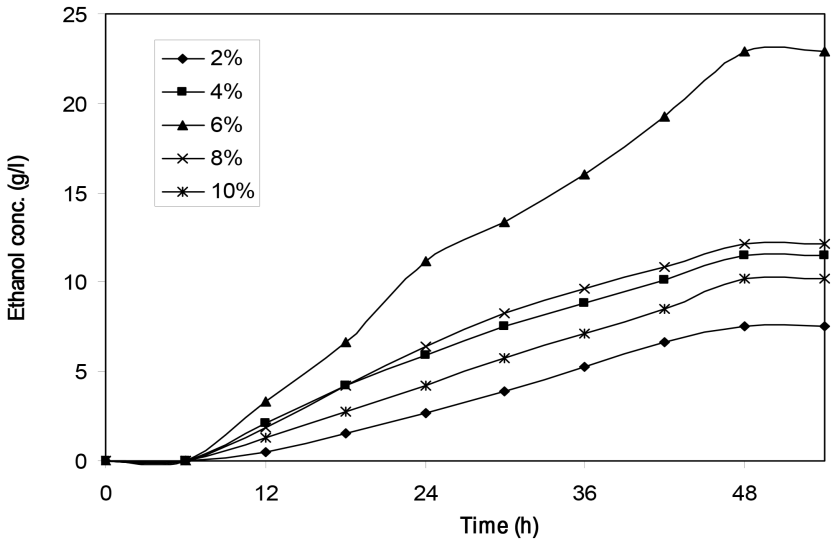


Figure 4. Ethanol concentration at different starch concentration in SSF process carried out with an initial pH-5.5, temperature 32˚C and 150 rpm.

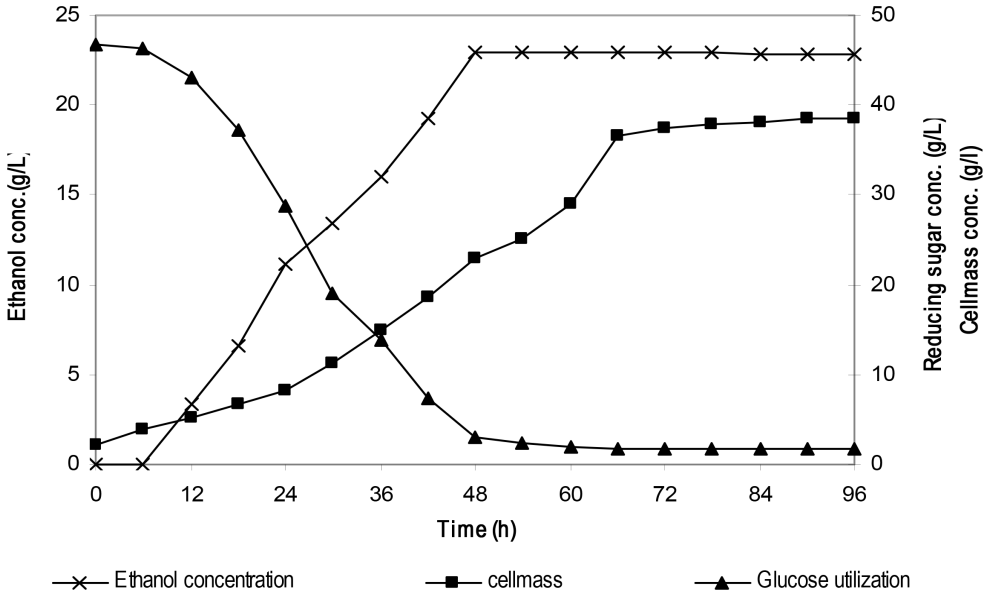


Figure 5. Time course of wheat bran starch fermentation by cocultures of *A. niger* and *K. marxianus* for the experiment carried out with an initial pH 5.5, temperature 32˚C, starch concentration 6 % (w/v) and 150 rpm.

different initial substrate concentration.

An increase in the starch content resulted in an increase in the concentration of ethanol to 6% with a maximum value of 22.9 g/l. A further increase in the starch content resulted in a decrease in the ethanol concentration, which was due to the substrate suppression of microbial growth [14] [15].

Figure 5 shows the decrease in sugar consumption, and increase in biomass and ethanol concentration during fermentation of pretreated wheat bran flour solution containing 6% starch at 32˚C, 150 rpm, and an initial pH of 5.5. The highest glucose consumption was recorded between 6 and 48 hours of fermentation. After 48 hours of fermentation, the ethanol concentration was at a maximum level of 22.9 g/L.

4. Conclusion

The starch content in wheat bran flour was used as the substrate for ethanol production by suitable pretreatment with fungal alpha-amylase solution. The liberated sugars in the solution were diluted to a known concentration and used as a medium for ethanol production. The simultaneous saccharification and fermentation were made in a single fermentor using an amylotic mold and sugar-consuming yeast. The process variables namely fermentation temperature, initial pH, and substrate concentration were optimized by using a single component optimization strategy. A maximum ethanol concentration of 22.9 g/l was obtained for the initial starch concentration of 6%, 5.5 initial pH, and 2˚C temperature.

Acknowledgments

The experiments were carried out in the Bioprocess Laboratory of Annamalai

University. We sincerely thank the Department of Chemical Engineering, Annamalai University to use the equipment for doing this research work in 2021.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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