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Journal of Hazardous Materials B137 (2006) 1643–1648

Journal of Hazardous Materials

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Hydrothermal decomposition of yeast cells for production of proteins and amino acids

Wiwat Lamoolphak [a], Motonobu Goto [c], Mitsuru Sasaki [c], Manop Suphantharika [b], Chirakarn Muangnapoh [a], Chattip Prommuag [a], Artiwan Shotipruk [a,*]

[a] *Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Patumwan, Payathai Road, Bangkok 10330, Thailand*
[b] *Department of Biotechnology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand*
[c] *Department of Applied Chemistry and Biochemistry, Kumamoto University, Kumamoto 850–8555, Japan*

Received 23 November 2005; received in revised form 1 May 2006; accepted 2 May 2006
Available online 16 May 2006

Abstract

This study examines hydrothermal decomposition of Baker's yeast cells, used as a model for spent Brewer's yeast waste, into protein and amino acids. The reaction was carried out in a closed batch reactor at various temperatures between 100 and 250 °C. The reaction products were separated into water-soluble and solid residue. The results demonstrated that the amount of yeast residue decreased with increasing hydrolysis temperature. After 20 min reaction in water at 250 °C, 78% of yeast was decomposed. The highest amount of protein produced was also obtained at this condition and was found to be 0.16 mg/mg dry yeast. The highest amount of amino acids (0.063 mg/mg dry yeast) was found at the lowest temperature tested after 15 min. The hydrolysis product obtained at 200 °C was tested as a nutrient source for yeast growth. The growth of yeast cells in the culture medium containing 2 w/v% of this product was comparable to that of the cells grown in the medium containing commercial yeast extract at the same concentration. These results demonstrated the feasibility of using subcritical water to potentially decompose proteinaceous waste such as spent Brewer's yeast while recovering more useful products.

Keywords: Subcritical water; Proteins; Amino acids; Hydrolysis; Baker's yeast; Spent Brewer's yeast

1. Introduction

Spent Brewer's yeast, the by product from the brewing industry, is being produced in large amount annually from main beer manufacturers due to increased volume of beer production. It is generally sold primarily as inexpensive animal feed after inactivation by heat, and much of this by product is considered industrial organic waste that causes a great deal of concerns. Such wastes are generally incinerated or put into landfill, in which case, the remaining proteins and amino acids, and other useful substances were not recovered. In addition, incineration of organic waste often gives toxic emission whose distribution degree is even higher than that of organic solid waste. Attempts have been made to recover higher value protein and amino acid products from spent Brewer's yeast [1] by employing various processes such as autolysis, plasmolysis [2,3] in organic salt solution or non-polar organic solvent, acid or alkali catalyzed hydrolysis, or enzymatic hydrolysis [1,4]. Plasmolysis and alkali or acid hydrolysis involves use of harmful chemicals and washing off these chemicals leads to generation of wastewater. Autolysis and enzymatic hydrolysis is therefore preferred, however autolysis requires long process time and the enzymes are usually costly to be practical in large scale.

Recently, hydrothermal conversion of organic wastes into more valuable substances using sub- or supercritical water without oxidants has been investigated. In this process, the ion product and dielectric constant of water play an important role. At the temperature below the critical point, the ion product of subcritical water, K_w, increases, for example, to approximately 10^{-11} at 250.3 °C and 25 MPa. The value then decreases to 10^{-16} at the critical point (374.2 °C) [5–7]. Two major types of reaction take place in sub-or supercritical water: oxidation and hydrolysis. Supercritical water oxidation is a process that converts organic materials completely into carbon dioxide, water,

* Corresponding author.
E-mail address: artiwan.s@chula.ac.th (A. Shotipruk).

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doi:10.1016/j.jhazmat.2006.05.029

Table 1
Approximate gross composition of dried yeast cells

Composition	Chemical composition (% as dry matter)	
	Brewer's yeast (*S. uvarum*)	Baker's yeast (*S. cerevisiae*)
Protein	48	42–46
Carbohydrates	36	30–37
Minerals	N/A	7–8
Nucleic acids	N/A	6–8
Lipids	1	4–7
Moisture	7	2–5
Ash	8	N/A

and nitrogen, and has been extensively studied [8,9]. The milder reaction, hydrolysis, is typically carried out in subcritical water. Hydrothermal conversions of cellulose [10–12] and disaccharides [13] in sub- and supercritical water have been studied. These materials were found to convert very rapidly into glucose and low-molecular-weight carboxylic acids [10–12,14]. Subcritical water hydrolysis of proteinaceous materials such as fish meat [15] and silk fibroin [16] into organic acids and amino acids was also examined.

For ease of storage and handling, here, dried Baker's yeast was used as a model for spent Brewer's yeast for the hydrothermal decomposition study. This is due to the fact that Baker's yeast and Brewer's yeast have close resemblance in their physical compositions (Table 1). The purpose of this investigation was to determine the effect of temperature and hydrolysis time on the amount of residual yeast, TOC, and the amount of protein and amino acids in the soluble products. The hydrothermal reaction was carried out in a batch reactor, and the results were compared with those obtained by autolysis of yeast cells. Moreover, the usefulness of the hydrolysis product as a nutrient source for the yeast growth itself was demonstrated and the result was compared with that obtained with commercial yeast extract. In addition, a kinetic model for the hydrothermal decomposition of yeast cells was proposed and corresponding parameters were determined.

2. Experimental

2.1. Subcritical water hydrolysis

Baker's yeast (Oriental Yeast Co., Ltd., Japan) was suspended in distilled water to about 10 w/v%. Then, 5 ml of this yeast suspension was charged into a 5 ml stainless steel (SUS-316) closed batch reactor (AKICO Co., Japan). The reactor was then heated with an electric heater to the desired temperature (100–250 °C). The schematic diagram of the apparatus is shown in Fig. 1. The pressure in the reactor was estimated based on saturated steam to be between 101.35 kPa and 3.97 MPa for the temperature range studied. After a desire reaction time (5–30 min), the reactor was immediately cooled to room temperature by immersion in a cool water bath. The liquid and solid contents in the reactor were collected and the remaining solid in the reactor was washed out with water. The residual yeast was separated from the soluble product

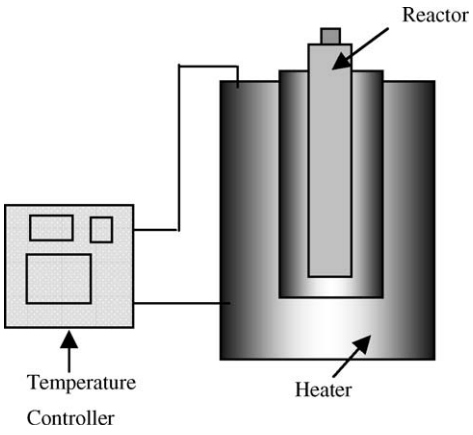

Fig. 1. Schematic diagram of subcritical water hydrolysis apparatus.

with a filter paper (Whatman no. 1) and weighed after drying in a vacuum oven at 50 °C. The soluble portion was assayed for the amount of protein, amino acids, and total organic carbon (TOC).

2.2. Autolysis

Yeast suspension was adjusted to obtain the pH of 5.2 using 0.5 N hydrochloric acid solution. Then autolysis was allowed to take place in a stirred vessel (Julabo HC-2/8, Labortechnik GMBH, Germany) at 50 °C. After autolysis for 19 h, the autolysate was heated to 85 °C for 20 min to terminate the enzyme activity. After autolysis, the weight of the yeast residue was measured and TOC and the protein and amino acid contents of the soluble product were measured.

2.3. Analytical methods

The protein content of the soluble portion was assayed using Lowry, 1951 method [17] using bovine serum albumin (BSA) as a standard. Amino acids content was analyzed by Ninhydrin assays [18] using L-glutamic acid as a standard. Briefly, Ninhydrin reagent, containing 1 ml of 1%w/w ninhydrin solution, 2.4 ml of 55% v/v glycerol solution, 0.2 ml of 0.5 M citrate buffer and 100 mg/ml manganese chloride, was added to 0.2 ml of the sample solution. The mixture was then shaken and boiled for 12 min, after which it was cooled in a water bath. Spectroscopic absorbance of the sample mixture was then measured at 570 nm in triplicates.

The TOC of the soluble reaction products was measured with a TOC analyzer (Shimadzu TOC-5050A), into which 7 μl of aqueous phase was injected. TOC was calculated by subtracting inorganic carbon (IC) from total carbon (TC). The IC values were less than 10% of the TC values for all samples examined.

The TOC yield of the aqueous phase was defined by the following equation:

$$\text{TOC yield} = \frac{\text{TOC} \times V}{w}$$

where TOC, V, and w were TOC of the aqueous phase [mg/l], volume of the aqueous phase [l], Baker's yeast weight [mg], respectively.

2.4. Use of hydrolysis product on yeast growth

The hydrolysis product was tested as a nutrient source for yeast growth. Because the 5 ml reactor employed in this study was too small to prepare a large amount of yeast product required for yeast growth, the sample was thus prepared in a 120 ml autoclave reactor. The product was obtained after 5 min at 200 °C hydrolysis temperature. The yeast (*Saccharomyces cerevisiae*) suspension was precultured overnight in a medium comprised of 2 w/v% of glucose (Wako Chemical Industries, Ltd., Japan), 2 w/v% of commercial yeast extract (Merck KgaA, Darmstadt, Germany), and 2 w/v% peptone (Wako Chemical Industries, Ltd., Japan) in 60 ml distilled water. Under sterile condition, 5 ml of this culture was added to each of the culture flasks containing 100 ml of different culture medium that was autoclaved at 110 °C for 10 min and cooled prior to use. The different culture media tested were (1) control medium which was a 2 w/v% glucose solution in distilled water, (2) the test medium containing 2 w/v% of glucose and 2 w/v% of subcritical water hydrolysis yeast product (3) a culture medium containing 2 w/v% of glucose and 2 w/v% of commercial yeast extract granulated for microbiology (Merck KgaA, Darmstadt, Germany). The test cultures were incubated in a Controlled Environment Incubator Shaker (New Brunswick Scientific Co., Inc., Edison, NJ, USA) at 30 °C and 200 rpm. The yeast growth was examined by sampling the suspension every 2 h for the measurement of the optical density with UV–vis spectrophotometer (UV-1201, Shimadzu Corporation, Japan) at 660 nm. All experiments were carried out in triplicate.

3. Results and discussion

3.1. Amount of residual yeast and TOC of soluble product

The reaction products consisted of two parts: solid yeast residue and aqueous solution. Fig. 2 shows the photographs of the soluble reaction products at 125, 200, and 250 °C after the reaction time of 10 min. The color of the soluble product became darker as more organic content are dissolved. Also, cells started to burn at high temperature.

Fig. 2. Soluble reaction product obtained at 125, 200, and 250 °C after 10 min.

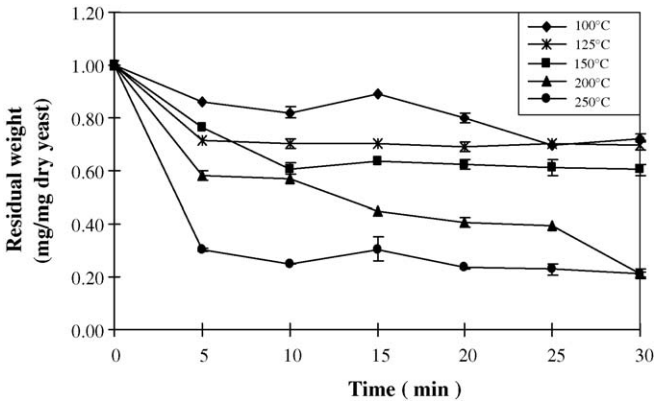

Fig. 3. Effect of reaction time and reaction temperature on residual weight of dry yeast.

The weight of residual yeast decreased with an increase in temperature because at high temperature, hydrolysis proceeded to the larger extent than at low temperature (Fig. 3). When the reaction temperature was increased to 250 °C (3.97 MPa), the residual weight of solid was about 0.22 mg/mg of dry yeast. This is a 78% reduction of the total solid waste, which is significantly higher than that obtained after 19 h of autolysis in which only 32% was hydrolyzed. The conversion of yeast cells into soluble products was supported by the TOC of the soluble products, which was generally found to increase as the temperature increases (Fig. 4).

From Fig. 4, it can be seen that TOC yields increased and became stable after 10 min under temperature conditions lower than 200 °C. TOC yields were found to increase from 0.04 mg C/mg of dry yeast at the start of the reaction and reached the highest yield of about 0.42 mg C/mg of dry yeast after 20 min at the reaction temperature of 200 °C (1.55 MPa), indicating that about 42% of the organic carbon in dry yeast cells was recovered into soluble solution. The TOC yield of the soluble product

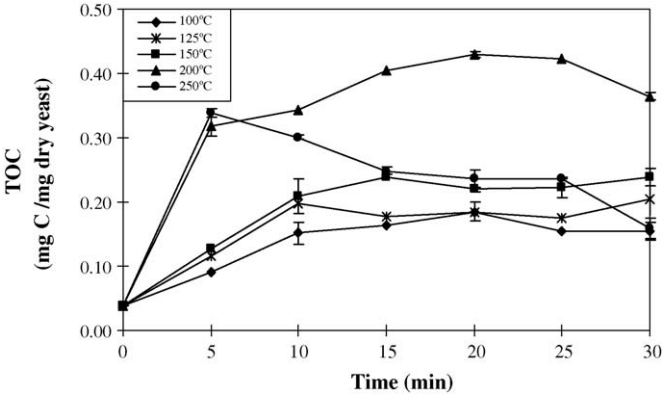

Fig. 4. Effect of reaction time and reaction temperature on TOC of supernatant.

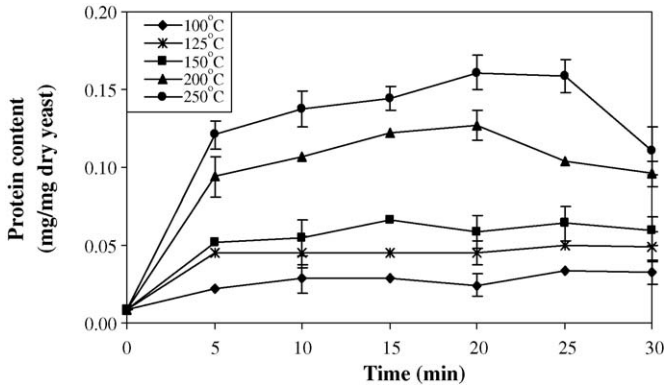

Fig. 5. Effect of reaction time and reaction temperature on protein production.

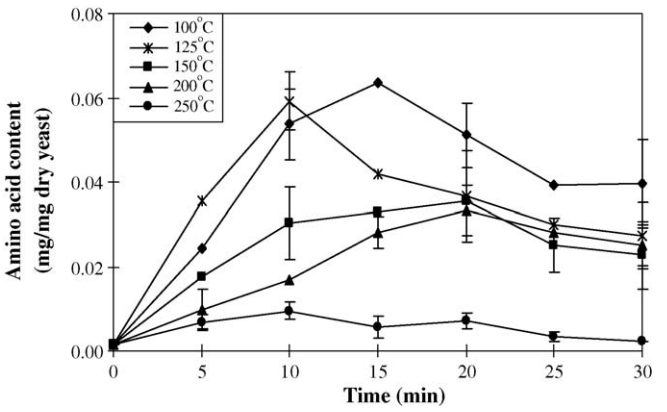

Fig. 6. Effect of reaction time and reaction temperature on amino acids production.

after a 19 h autolysis process was found to be only about 22.8%, indicating that the lower amount of organic carbon in dry yeast cells was recovered. This was about 50% lower than the TOC yield obtained by hydrolysis with subcritical water (at 200 °C, 20 min). However, at the temperature of 250 °C, the TOC values decreased at higher reaction times and were lower than those at 200 °C. This result suggested that formation of gaseous product might have occurred at these conditions and that some part of water-soluble may be converted to water-insoluble through carbonization.

3.2. Soluble protein product

The effect of the temperature and time of subcritical water on the hydrolysis yield was determined. As shown in Fig. 5, the yield of protein almost reached plateaus by 5 min for all reaction temperatures. The amount of protein produced increased with an increase in temperature and was the highest for the reaction that took place at 250 °C (3.97 MPa) for 20 min, which was 0.16 mg/mg of dry yeast. At higher temperature, the product was found to decrease when the reaction was extended beyond 20 min. When compared with the protein yield of Baker's yeast obtained by autolysis process (0.095 mg/mg of dry yeast after 19 h), the protein yield obtained by hydrolysis in subcritical water was higher at the above condition. This demonstrates that hydrothermal decomposition yields valuable protein products in favorable amount in a short time.

3.3. Total amino acids product

The results in Fig. 6 revealed that the amount of total amino acids produced decreased with an increase in temperature. The highest yield was obtained at 100 °C (101.35 kPa) for 15 min reaction time, and was found to be 0.063 mg/mg of dry yeast. Compared with the total amino acids yield obtained after 19 h of autolysis (about 0.45 mg/mg of dry yeast), the amino acids yields obtained by hydrolysis of Baker's yeast in subcritical water was much inferior, despite the higher TOC values. This strongly suggests that amino acids decomposed at high reaction temperature and longer reaction times into low-molecular-weight carboxylic acids and gaseous products [14,16].

3.4. Use of hydrolysis product on yeast growth

The product of subcritical water hydrolysis was tested as nutrient source for yeast growth. Fig. 7, indicated that the OD_{660} of the suspension of yeast cells grown in the product resulted from subcritical water hydrolysis of Baker's yeast did not significantly differ from that grown in commercial yeast extract. The resulted growth was much higher than that obtained in glucose solution, which confirmed that the yeast product from subcritical water could positively be applied for the culture of yeast cells. The potentials of application of the product with growth of other microorganisms are under investigation.

3.5. Determination of kinetics parameter

For the process to be applied on a large scale, determination of degradation kinetics is important. Degradation of yeast cells was described by the following kinetic model.

$$\frac{-\mathrm{d}C}{\mathrm{d}t} = -r_{\text{yeast}} = \frac{kC}{1 - k'C} \tag{1}$$

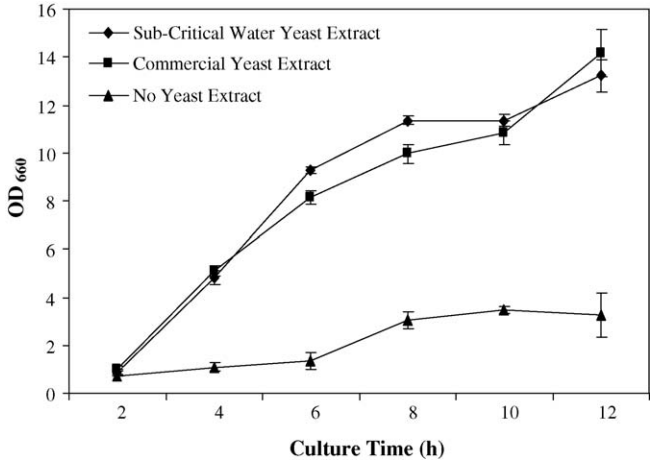

Fig. 7. Optical densities of yeast cultures incubated in control medium, and that containing product of sub-critical water hydrolysis and commercial yeast extract.

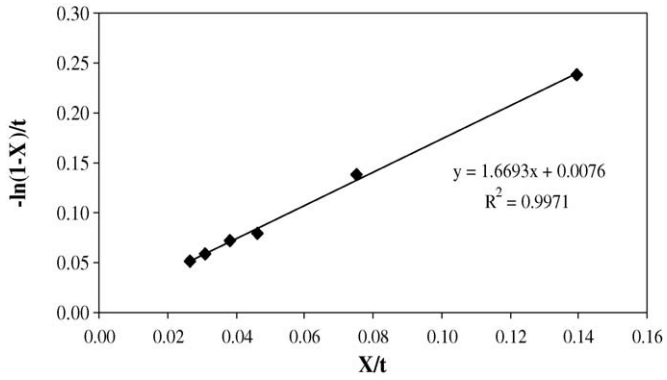

Fig. 8. Plot of $-\ln(1-X)/t$ and X/t at hydrolysis temperature of 250 °C.

Table 2
Kinetic parameter, k and k', of decomposition reaction rate at difference temperature

Temperature (°C)	k (min^{-1})	k' (ml/g)
100	0.0008	10.543
125	0.0020	11.750
150	0.0230	11.391
200	0.0135	11.131
250	0.0076	16.693

where C is the concentration of yeast cells (g cm^{-3}) and k and k' are the kinetic parameters of decomposition. This equation describes a reaction whose rate is first order at the beginning when the cell concentration is high and becomes zeroth order at lower cell concentration.

After an integration of Eq. (1), the following equation results

$$t = \frac{1}{k}\ln\frac{C_0}{C} - \frac{k'}{k}(C_0 - C) \quad (2)$$

where C_0 is an initial yeast cell concentration.

Eq. (2) can be written in terms of mass conversion, X, as:

$$t = \frac{1}{k}\ln\frac{1}{1-X} - \frac{k'C_0X}{k} \quad (3)$$

Multiply Eq. (3) with k/t and rearranging the equation, the following equation results:

$$-\frac{\ln(1-X)}{t} = k + \frac{k'C_0X}{t} \quad (4)$$

From this equation, k and k' can then be determine from the plot of $-\ln(1-X)/t$ and X/t as demonstrated in Fig. 8 for the decomposition of yeast cells at 250 °C. The kinetic parameters for decomposition of yeast powder at different temperatures are summarized in Table 2. The kinetics model proposed could further be used for the design of a large scale process.

4. Conclusions

This study demonstrates that subcritical water could be used to potentially hydrolyze spent Brewer's yeast cells, organic waste from brewing industries into more valuable proteins and amino acids. The amount of protein produced increases with an increase in temperature, while that of amino acids decreases with increasing temperature. The highest yield of protein and amino acids were 0.16 and 0.063 mg/mg of dry yeast, respectively. The amount of total organic carbon was found to increase with increasing temperature. However, the value was decreased at the temperature as high as 250 °C suggesting the gaseous products were formed.

Tests of the hydrolysis product obtained at 200 °C on yeast growth suggested a possible use of this product for cultivation of yeast and other microorganisms.

Acknowledgement

The authors thank National Research Council of Thailand for financial support.

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