Enzymatic hydrolysis of spent *Saccharomyces cerevisiae* derived from sago bioethanol fermentation

Nik Nur Aziati Mahmod[1], Nurashikin Suhaili[1*], Awang Ahmad Sallehin Awang Husaini[1], Dayang Salwani Awang Adeni[1] and Siti Efliza Ashari[2]
1. Faculty of Resource Science and Technology, UNIMAS, 94300 Kota Samarahan, Sarawak, MALAYSIA
2. Centre of Foundation Studies for Agricultural Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, MALAYSIA
*snurashikin@unimas.my

Abstract

Spent Saccharomyces cerevisiae is a by-product of bioethanol fermentation. The spent yeast is abundant in valuable components which can be used for many applications. One of the ways to prepare yeast extract is through enzymatic hydrolysis which is by rupturing the yeast cell walls using exogenous enzymes under certain conditions that promote the leakage of intracellular compounds. To date, enzymatic hydrolysis of spent S. cerevisiae derived from the production of sago bioethanol is yet to be thoroughly explored. In the present study, we examine the feasibility of enzymatic hydrolysis of spent S. cerevisiae generated from sago bioethanol fermentation. The effect of two enzymes namely alcalase and cellulase and their concentrations (0.1-0.5% (v/v) on the release of protein and carbohydrate in the hydrolysate was also investigated.

Additionally, the surface morphology of the hydrolysed yeast cells was observed using a Scanning electron microscope (SEM). Our results showed that the optimal concentration of alcalase and cellulase for enzymatic hydrolysis of spent S. cerevisiae was 0.4% (v/v) and 0.5% (v/v) respectively. In addition, cellulase was found to be more superior than alcalase with respect to the protein content in the hydrolysate. The enzymatic hydrolysis of spent yeast by alcalase and cellulase yielded improvements of 1.1 to 1.8-fold and 3.5 to 5.6-fold of protein and total carbohydrate concentration respectively in comparison to that achieved via autolysis. It was evident from the SEM analysis that there was a notable change in the surface morphology of the lysed yeast cells indicating the lysis of the yeast cells throughout the enzymatic hydrolysis. In summary, the current work provides useful insights into the strategies of valorising spent S. cerevisiae generated from sago bioethanol production. This will further help the development of value-added products from the waste, hence promoting a sustainable economy besides reducing the environmental impacts associated with the disposal of spent S. cerevisiae.

Keywords: Enzymatic hydrolysis, Bioethanol fermentation, *Saccharomyces cerevisiae*, Spent Baker's yeast, Yeast extract.

**** Author for Correspondence***

Introduction

Spent *Saccharomyces cerevisiae* is a major by-product of the brewing industry. The spent yeast is rich in protein, essential amino acids, RNA, vitamin B and minerals, making it as a promising source for the production of yeast extract[14,32]. Yeast extract refers to the soluble portion of yeast cells after the separation of the insoluble components[33]. It has been reported to possess biological properties such as antioxidant properties. Besides that, the yeast extract is also used as a source of peptides for value-added functional foods and as a component for microbiological growth media[21]. In addition, yeast extract has also been widely used as a flavouring agent and flavour enhancer in food industry[6]. To date, spent *S. cerevisiae* is typically used either as a low-cost protein source in animal feed formulations or is discarded to the environment causing severe ecological impacts[15,26].

There is a growing interest in valorising the spent *S. cerevisiae* for various applications. One of the ways to derive the yeast components is by enzymatic hydrolysis. Enzymatic hydrolysis is an efficient method in yeast extract production since it offers higher process specificity compared to conventional processes[9]. Enzymatic hydrolysis is performed by adding exogenous enzymes that accelerate the rupture of yeast cell wall[22]. Furthermore, these enzymes also increase the activity of endogenous enzymes in releasing intracellular compounds, resulting in hydrolysates of superior sensorial quality and improved functional and biological functions with minimal salt content[30,31].

An alternative to increase the bioactivity of ingredients at a reduced cost would be through the use of commercial enzyme pools for enzymatic production of bioactive peptides from complex feedstock mixtures[25]. Several studies have reported the use of commercial lytic enzymes such as pancreatin, flavourzyme, brauzyn, papain, lyticase and alcalase for lysing yeast cells[4,21,33]. These enzymes have one or more of the following activities: proteolytic activity (proteases or peptidases), RNA degrading activity (nucleases), or deaminase activity (deaminases)[8,9,33].

Most of previous reports on enzymatic hydrolysis in the literature focused on the use of either fresh yeast or spent yeast from brewing fermentation. In general, there is still scarce information on the valorisation of spent *S. cerevisiae* derived from bioethanol fermentation using agricultural

waste such as sago fibre. The production of bioethanol from sago fibre has been reported in the literature[2]. Looking at the potential of sago bioethanol in the future, it is also crucial to investigate the possible direction of the waste generated from the sago bioethanol production in order to ensure the sustainability of the whole process.

Previously, we reported the utilisation of sago bioethanol liquid waste for production of an industrial biocatalyst[20]. We have also explored the feasibility of autolysis of spent *S. cerevisiae* derived from the bioethanol fermentation[19]. The work serves as an important stepping stone for valorising the solid waste from sago bioethanol production. Nonetheless, there are still more strategies that need to be explored for valorising spent *S. cerevisiae* as autolysis may have some limitations due to low titre of yeast hydrolysate.

The aim of the current work is to investigate the feasibility of enzymatic hydrolysis of spent *S. cerevisiae* derived from sago bioethanol fermentation. The study focuses on the effect of two enzymes namely alcalase and cellulase and their concentrations on the hydrolysis of the spent *S. cerevisiae* on the basis of protein and carbohydrate concentration in the yeast hydrolysate. In general, this work provides useful insights into the recovery of spent *S. cerevisiae* that can be used for production of various value-added products.

Material and Methods

Microorganism: Commercial *Saccharomyces cerevisiae* was used in this work for bioethanol fermentation.

Enzymes: Two types of enzymes namely alcalase (EC 3.4.21.14) (Millipore, USA) from *Bacillus licheniformis* and cellulase (EC 3.2.1.4) (Sigma-Aldrich, Denmark) from *Trichoderma reesei* were used in the enzymatic hydrolysis of spent *S. cerevisiae* in this work.

Pre-treatment and hydrolysis of sago fibre: Sago fibre was supplied by Herdsen Sago Mill, Sarawak and it was used as the feedstock for bioethanol fermentation. The pre-treatment procedure was based on a published protocol.[2]. The fibre was initially ground into powder prior to drying until a constant weight was achieved. Approximately, 7% (w/v) of sago fibre suspension was gelatinised at 90-100 °C for 30 minutes. Subsequently, the resulting slurry underwent liquefaction stage for another 30 minutes by adding Liquozyme® (Novozymes, Denmark) at 2 µl/g.

The suspension was constantly stirred for 30 minutes to ensure homogeneity between enzyme and substrate. The suspension was allowed to cool down and 1 µl/g of Spirizyme® (Novozymes, Denmark) was added for the saccharification stage.

Following that, the suspension was incubated for overnight at 50 °C. Sago fibre hydrolysate (SFH) was recovered from the residual lignocellulosic fibre by filtrating the suspension through a fabric mesh filter.

Bioethanol fermentation: Bioethanol fermentation by *S. cerevisiae* was carried out in 500 mL Erlenmeyer flasks with a working volume of 250 mL. The fermentations were performed in triplicate. The fermentation media consisted of SFH supplemented with 5 g/L yeast extract (Oxoid, UK). The initial pH was adjusted to 5.5. The yeast cultures were incubated on an orbital shaker at 150 rpm shaking speed and at room temperature for 48 hours. An aliquot of the culture broth was taken every 8 h during the fermentation in order to quantify the residual glucose and bioethanol. Following the bioethanol fermentation, the culture broth was centrifuged at 4 °C for 10 minutes at 3000 rpm in order to recover the pellet which consisted of spent *S. cerevisiae*.

Enzymatic hydrolysis: The enzymatic hydrolysis of spent *S. cerevisiae* was carried out following the methods described by Takalloo et al[30] and Xie et al[35]. The yeast slurry from the bioethanol fermentation was subjected to 3 washing processes in order to remove any fermentation residues[7]. The yeast suspension with a final concentration of 14% (w/v) was placed in 100 mL Erlenmeyer flasks and the flasks were immersed in a shaking water bath (Digital Precise Shaking Water Bath, Daihan, Korea). The flasks were shaken at a shaking speed of 100 rpm for 96 h. The hydrolysis with alcalase was performed at 55 ºC and at an initial pH of 7.0[30] whilst hydrolysis using cellulase was performed at 55 ºC and at an initial pH of 5.5[24].

Two portions of the sampled suspension were withdrawn at different time intervals during the enzymatic hydrolysis for the analysis of protein, carbohydrate and surface morphology. The first portion was centrifuged immediately with the pellet retrieved proceeded for the morphological analysis. The second portion of the sampled suspension was used for the quantification of protein and carbohydrate concentration of the hydrolysate. The sample was initially heated at 100 ºC for 5 minutes to halt the enzymatic activity. Then, the sample was centrifuged at 3000 rpm for 10 minutes in order to obtain the supernatant, which was then used for the protein and carbohydrate analysis.

Protein assay: The protein content of the hydrolysate was assayed based on Lowry method by using bovine serum albumin as a standard[18]. The diluted hydrolysate was mixed with 100 µL of 2 N NaOH before the mixture was heated at 100 °C for 10 minutes. Then, 1000 µL of freshly mixed Lowry solution was added and left for 10 minutes. The mixture was then incubated for another 30 minutes at room temperature in a dark environment after being incubated with 100 µL of diluted Folin reagent. The absorbance of the samples was measured using a spectrophotometer (Shimadzu UV Mini 1240 UV-vis) at a wavelength of 750 nm. The absorbance values were then translated into equivalent protein concentration based on an established protein standard curve.