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

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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.

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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.

Carbohydrate assay: The carbohydrate concentration of the hydrolysate was determined according to a modified phenol-sulphuric acid assay[1]. The hydrolysate was diluted before mixing it with 1 mL of 5% (v/v) phenol. Then, the mixture was mixed evenly before the addition of 5 mL of 96% (v/v) concentrated sulphuric acid. The samples were left to cool in a water bath for 15 minutes at 25-30 °C. Following that, the absorbance of the suspension was measured at 490 nm using a spectrophotometer. The total carbohydrate concentration of the sample was determined based on an established glucose standard curve.

Scanning electron microscopy: The morphological changes of the hydrolysed yeast cells were observed using a Scanning electron microscope (SEM) (JSM-IT500HR, JEOL, Japan) referring to a modified protocol[17]. The hydrolysed cells were fixed using 2.5% glutaraldehyde solution for 30 minutes at 4 °C. Then the samples were rinsed using sterile distilled water for 5 minutes. The rinsing was repeated 3 times. The fixed samples were gradually dehydrated with a series of ethanol gradients (25%, 50%, 70%, 95%, 100%) for 15 minutes for every concentration. Then, the samples were dried in a desiccator before attaching them on carbon tape on the cover slips. The cover slips were mounted on aluminum sample stubs and the samples were observed under the microscope with a magnification of 5000x.

Statistical analysis: The results were analysed using Student's t-test using Microsoft excel. The results were regarded as significantly different when the p-value was less than 0.05.

Results and Discussion

Effect of different enzymes and their concentrations on protein concentration of yeast hydrolysate: The effect of using different enzymes namely alcalase and cellulase and their concentrations on the hydrolysis of spent *S. cerevisiae* generated from sago bioethanol fermentation was evaluated in terms of the protein released in the hydrolysate. Figures 1 and 2 show the profiles of protein concentration of the yeast hydrolysates obtained from the hydrolysis using alcalase and cellulase at different time intervals. The concentration of both enzymes during the hydrolysis was varied from 0.1 to 0.5% (v/v).

The results showed that in general, the hydrolysis using both enzymes have shown positive results where the protein concentrations in the hydrolysates were found to increase over time. This suggests that the hydrolysis of spent *S. cerevisiae* due to the action of alcalase and cellulase took place resulting in the release of protein in the hydrolysate. The highest protein profile for alcalase was shown by the hydrolysates derived from the hydrolysis using 0.4% (v/v) alcalase with the maximum peak of 12092.5 µg/mL after 72 hours of hydrolysis. The values represent an increment of 4-fold as compared to the control samples. Increasing the alcalase concentration to 0.5% (v/v) however, was found to result in a negligible difference of the maximum peak of protein concentration.

In the hydrolysis of spent yeast by cellulase, as shown in figure 2, there was a gradual increase of the protein profiles when the enzyme concentration was increased from 0.1 to 0.5% (v/v). The maximum profile was shown by the hydrolysis using 0.5% (v/v) cellulase with the maximum peak of 19760 µg/mL representing an increment of 1.6-fold over the maximum peak achieved in the hydrolysis using alcalase. This suggests the superiority of cellulase over alcalase in releasing the protein content from the lysed yeast cells.

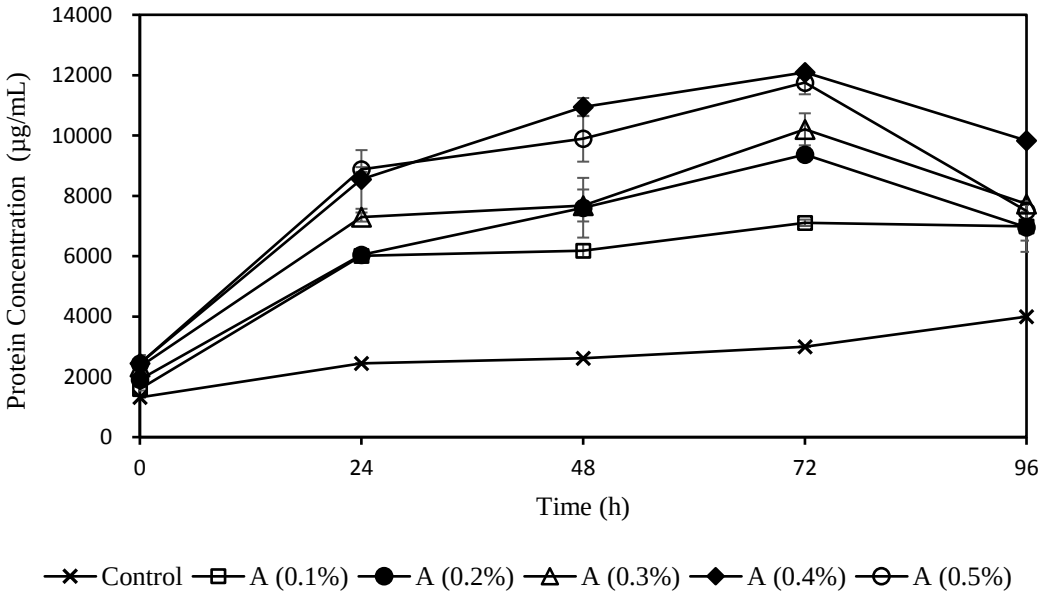

Figure 1: Change of protein concentration of hydrolysates over time. The hydrolysate was derived from the hydrolysis using alcalase at different concentrations [0.1%-0.5% (v/v)]

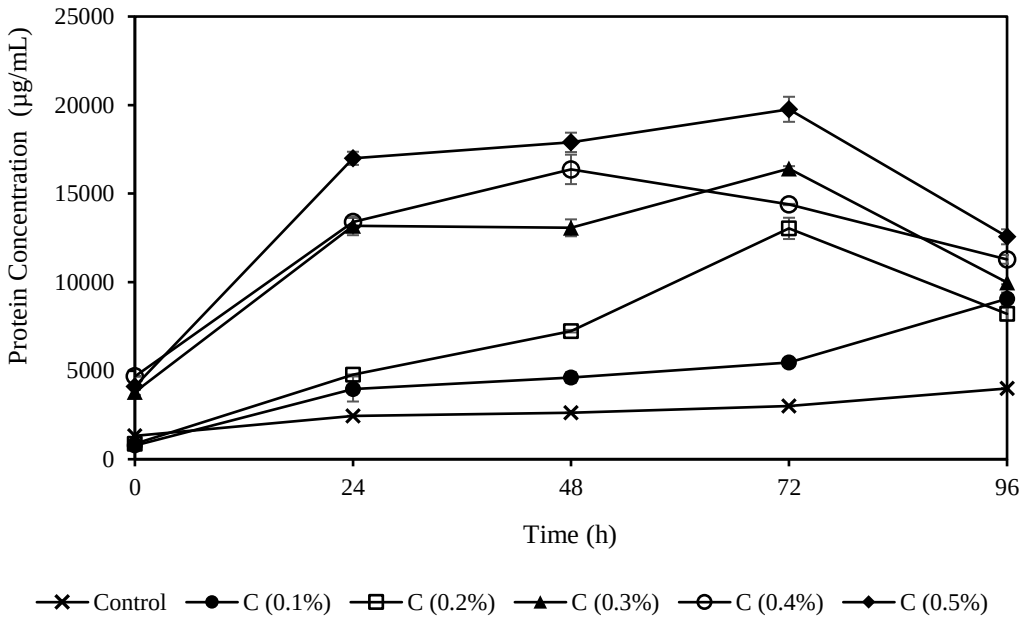

Figure 2: Change of protein concentration of hydrolysates over time. The hydrolysate was derived from the hydrolysis using cellulase at different concentrations [0.1%-0.5% (v/v)]

The degradation of the yeast cell in general is achieved by the synergistic activity of yeast endoenzymes and exoenzymes added[33]. Consequently, the recovery of the intracellular components from the lysed yeast cells strongly depends on this action. The choice of enzymes and their concentrations plays important roles in the enzymatic hydrolysis. Alcalase is a serine protease produced by *Bacillus licheniformis*[27]. The enzyme acts as an endopeptidase, in which it selectively cleaves peptide bond between amino acids within proteins in a particular position resulting in the rupture of the cytoplasmic membrane and the leakage of intracellular substances[29,33].

As a result, the protein is released into the hydrolysates as the enzymatic hydrolysis progresses. The hydrolysed proteins are rich in non-glycosylated soluble proteins and free amino acids originating from cytoplasm[10]. Meanwhile, cellulase is a carbohydrate enzyme derived from *Trichoderma reesei* which contains (1→3)-β-glucanase activity. β-1,3-glucanases hydrolyse the cell walls by breaking down glycosidic bonds in β-1,3-glucans which is the main constituents of the yeast cell wall[12,28].

The concentration of the enzyme influences the degree of the hydrolysis as well as the solubilisation of protein[11]. This can be seen in the present work where there was a consistent increase of the protein concentration when the enzyme concentration was increased from 0.1 to 0.5% (v/v). As the enzyme concentration increases, greater number of substrates bind to the active sites of the enzyme, forming enzyme-substrate complex which further increases the reaction rate until the maximum point where all the active sites of the enzyme have been bound to the substrate[5]. Further increase in the enzyme concentration beyond the saturation point will have no significant influence on the enzymatic hydrolysis.

The efficiency of alcalase in hydrolysing spent yeast cells as reported in this work is in parallel with the several findings reported in the literature. Alcalase was reported to be more superior than papain in hydrolysing spent Baker's yeast[35]. Moreover, the efficiency of alcalase in producing yeast hydrolysates with an antioxidative properties was also reported[30].

In the same work, alcalase is also revealed as one of the most effective proteases in recovering the intracellular content from the yeast cells. Meanwhile, the use of cellulase for hydrolysing spent yeast cells has also been reported in the literature. In a study by Garcia[12], high release of free glucose was observed when cellulase was used in hydrolysing the yeast cell wall.

Previously, we reported the feasibility of autolysis for recovering protein and carbohydrate from spent *S. cerevisiae* obtained from sago bioethanol[19]. The maximum recovery of protein from spent *S. cerevisiae* via enzymatic hydrolysis using alcalase and cellulase as reported in the current work was found to be 1.1 to 1.8-fold higher than that obtained via autolysis.

This can be associated with the fact that autolysis only depends on the action of endogenous enzymes whilst in the enzymatic hydrolysis by either alcalase or cellulase, both exogenous and endogenous enzymes play roles in the yeast cell degradation resulting in higher recovery of the yeast intracellular components.

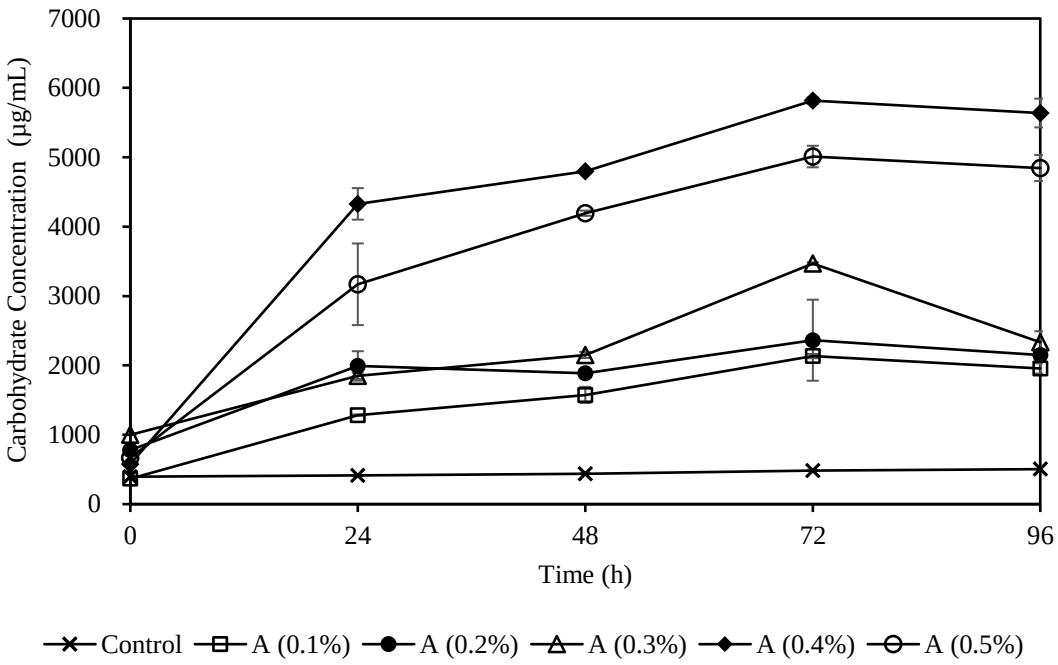

Figure 3: Change of total carbohydrate concentration of hydrolysate over time. The hydrolysates was derived from hydrolysis using alcalase at different concentrations [0.1%-0.5% (v/v)]

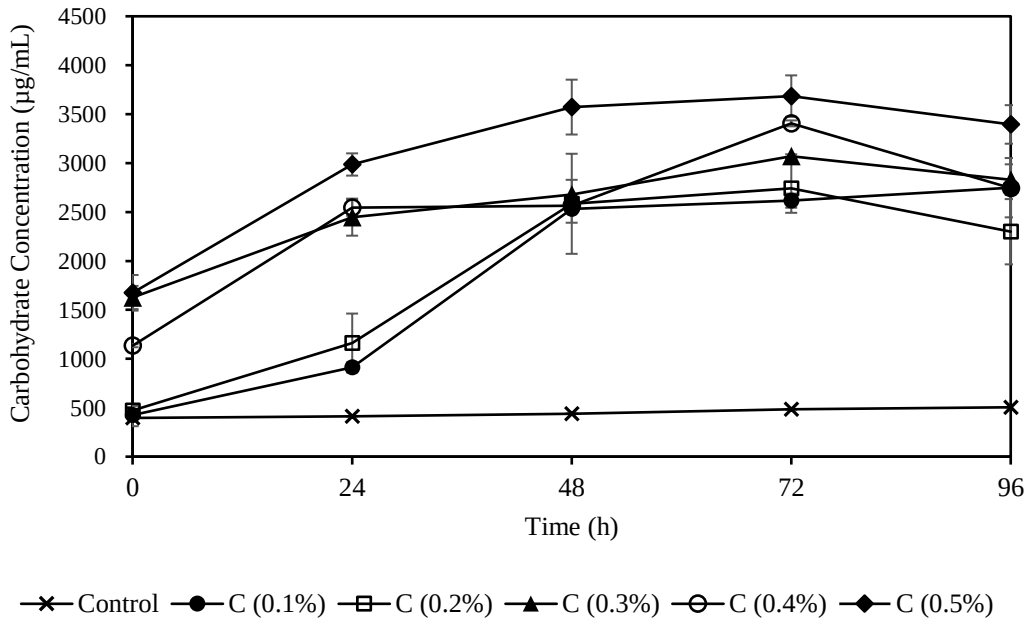

Figure 4: Change of total carbohydrate concentration of hydrolysate over time. The hydrolysate was derived from hydrolysis using cellulase at different concentrations [0.1%-0.5% (v/v)]

Effect of different enzymes and their concentrations on carbohydrate concentration of yeast hydrolysate: Similar to protein, the amount of carbohydrate released in the yeast hydrolysate can be associated with the degree of the enzymatic hydrolysis and hence it is referred to as one of the important indicators. Figures 3 and 4 illustrate the change of total carbohydrate of hydrolysate derived from the hydrolysis using alcalase and cellulase conducted at different enzyme concentrations throughout 96 hours of hydrolysis.

The results showed that in general, there was a notable increase of total carbohydrate concentration during the first 72 hours of hydrolysis conducted using both alcalase and cellulase. Prolonging the hydrolysis to 96 hours, however, resulted in a minimal change of the total carbohydrate concentration. Moreover, increasing the enzyme concentration from 0.1 to 0.5% (v/v) also results in an increase of the total carbohydrate released in the hydrolysate. The variation of the performance of the two aforementioned enzymes in releasing protein and

carbohydrate can be associated with their different efficiencies in degrading the yeast cells and different recovery levels of the intracellular components.

Furthermore, different type of exogenous enzyme added to the hydrolysis may result in the variation of the synergistic activity with the yeast endoenzymes, which consequently may influence the protein and carbohydrate content in the hydrolysate. The maximum profiles of total carbohydrate concentration were achieved in the hydrolysis conducted using 0.4% (v/v) alcalase and 0.5% (v/v) cellulase where the highest peaks were 5817 µg/mL and 3684 µg/mL respectively. The values were found to be 5.6 and 3.5-fold over that achieved via autolysis as reported in our earlier work[19].

Saccharomyces cerevisiae has cell wall consisting of 80 to 90% polysaccharides with the main components of glucans and mannoproteins which make up 15 to 30% of the dry mass of the cell[34]. The increasing amount of carbohydrates released into the hydrolysates can be associated with the decomposition of components in cell walls by the enzymes which led to the rupture of the intact cell wall structure followed by the collapse of cytoplasmic membrane and leakage of intracellular substance[33].

Therefore, an increased total carbohydrate content in the yeast, hydrolysate is to be expected. Unlike autolysis where the release of carbohydrates occurs in two phases, degradation of structural carbohydrates during the enzymatic hydrolysis starts from the beginning of the process[33]. The results from the present work are in agreement with some of previous works that reported the release of carbohydrate in the hydrolysate upon the hydrolysis of spent yeast using proteases[8,12,33].

It can be concluded here that alcalase and cellulase are suitable to be employed for hydrolysing spent *S. cerevisiae* derived from sago bioethanol fermentation with their suggested concentrations of 0.4% (v/v) and 0.5% (v/v) respectively. Further increase of the enzyme concentrations may not just increase the cost of the process but also may result in minimal benefits of the recovery of protein and carbohydrate. Our results also showed that 72 hours are the optimal duration of the hydrolysis of spent *S. cerevisiae* by both alcalase and cellulase with regards to the release of protein and carbohydrate in the hydrolysate.

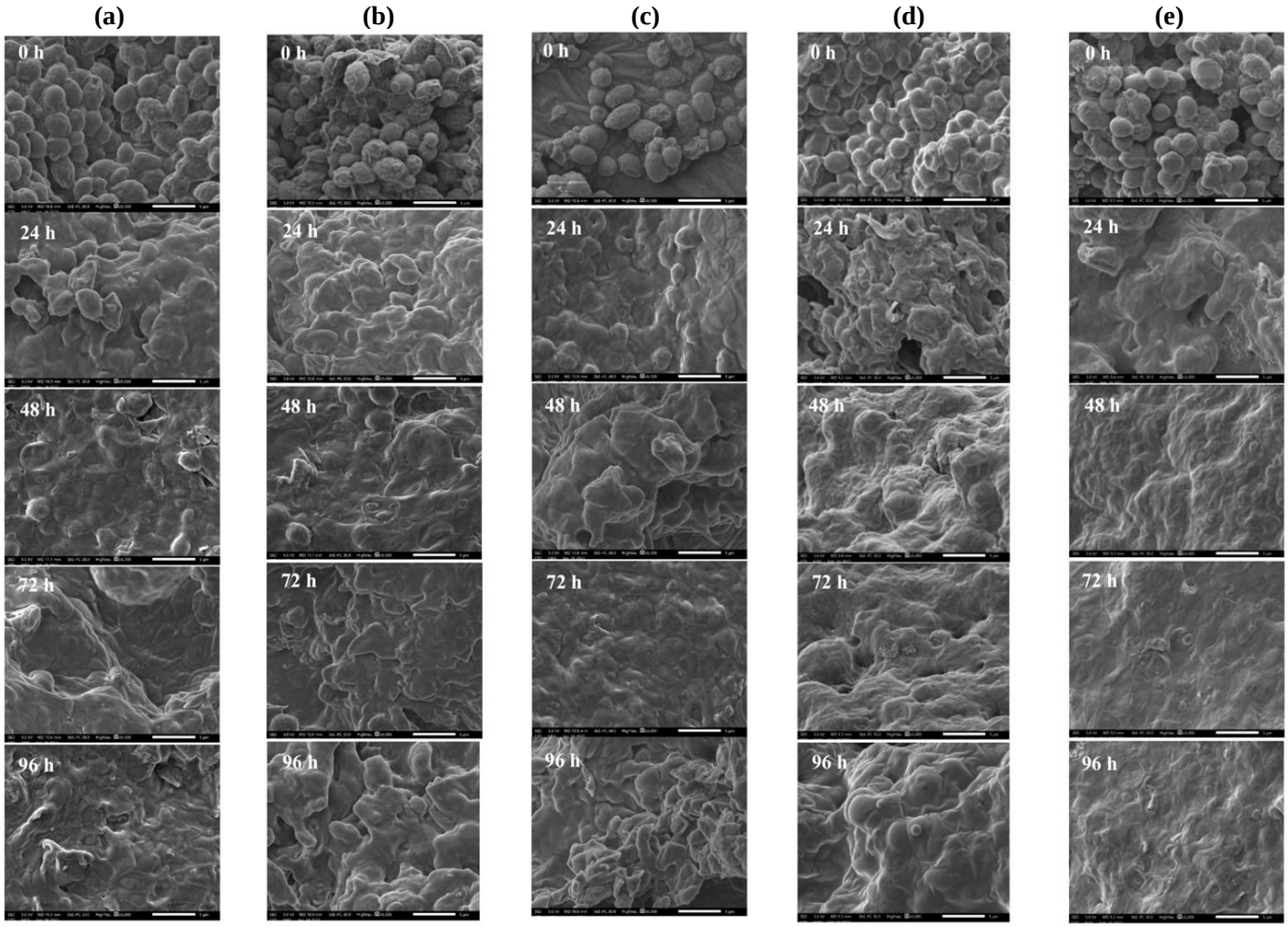

Figure 5: Changes of surface morphology of yeast cells treated with alcalase at (a) 0.1% (v/v); (b) 0.2% (v/v); (c) 0.3% (v/v); (d) 0.4% (v/v) and (e) 0.5% (v/v). Magnification is 5000 times

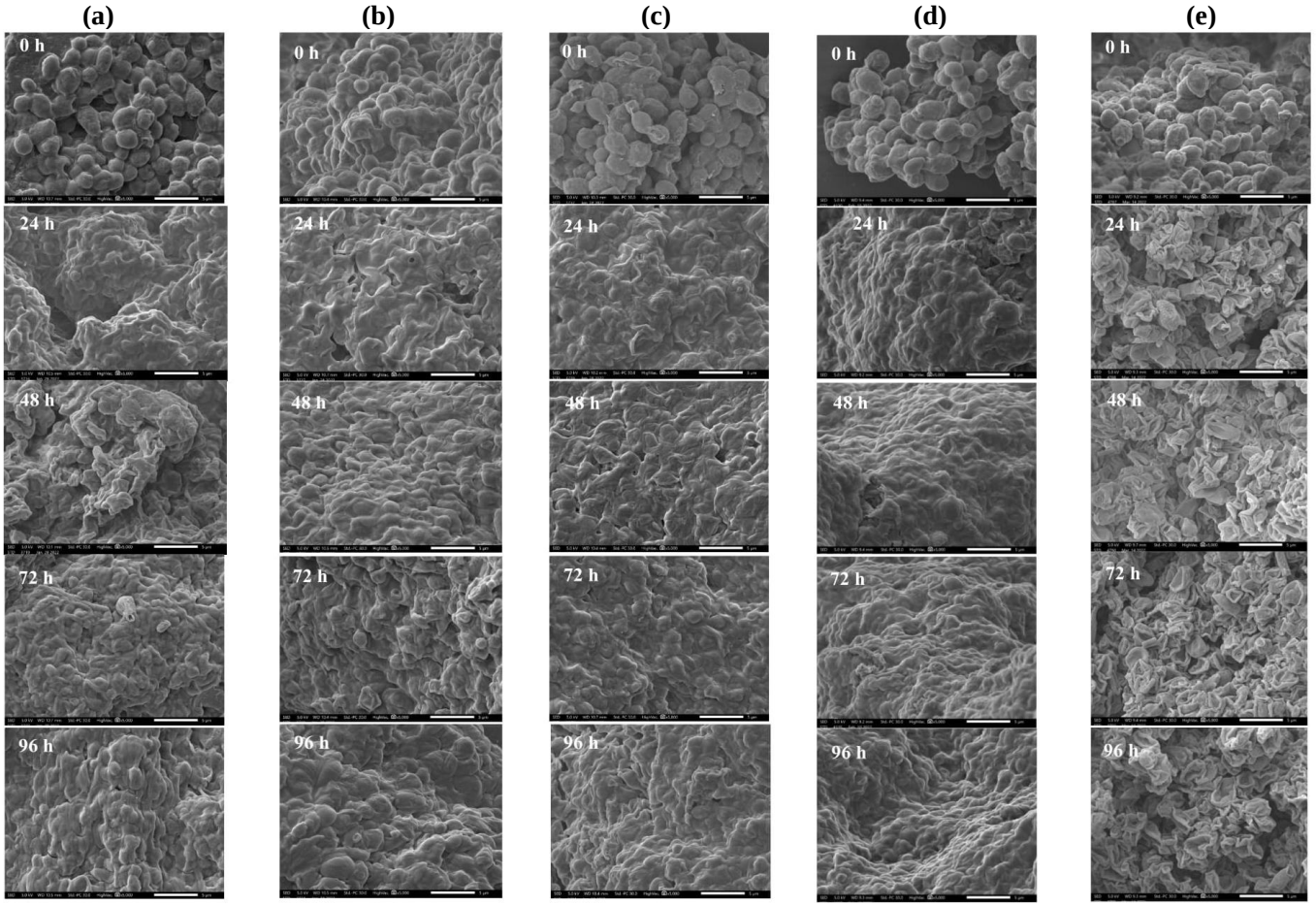

Figure 6: Changes of surface morphology of yeast cells treated with cellulase at (a) 0.1% (v/v); (b) 0.2% (v/v); (c) 0.3% (v/v); (d) 0.4% (v/v) and (e) 0.5% (v/v). Magnification is 5000 times

Prolonging the incubation period during hydrolysis may be disadvantageous since it may increase the cost and risk of contamination owing to the development of pathogen such as *Clostridium* sp. which prefers protein-rich and low oxygen condition that is found during the yeast hydrolysis[3]. Similar to our prior work[19], it was also shown that upon the enzymatic hydrolysis of spent *S. cerevisiae*, the concentration of protein in the hydrolysate was found to be higher than carbohydrate. This is consistent with the fact that protein is the primary component of yeast extract[30].

Morphological analysis of hydrolysed yeast cells: In addition to the protein and carbohydrate analysis of the hydrolysates, which serve as the important indicators of the hydrolysis, morphological analysis of the hydrolysed yeast cells was also conducted. Figures 5 and 6 show the morphological changes of the yeast cells during the enzymatic hydrolysis using alcalase and cellulase.

It is clearly shown that the yeast cells had an ellipsoidal shape with a plump and smooth surface at 0 hour in all cases. The yeast cells that were subjected to alcalase and cellulase hydrolysis, on the other hand, changed drastically in their appearance and cell volume over the course of the 96-hour incubation period. The cells became flattened, shriveled up and became severely deformed, losing their plump and mellow appearance. In figure 5, some of the yeast cells when treated with alcalase at 0.1% and 0.2% (v/v) still appeared in spherical shape during the 24 hour of hydrolysis. However, the yeast cells from the hydrolysis using other concentrations of alcalase began to flatten and distort at this hour.

In contrary to the yeast cells from the hydrolysis using cellulase (Figure 6), the yeast cells started to flatten after 24 hours of hydrolysis in all cases. These results suggest that the enzymatic hydrolysis facilitated by alcalase and cellulase caused the morphological change of the surface structure of the yeast cells which can be linked to the gradual degradation of cell wall during the process. It is observed that in general, the yeast cells treated with cellulase showed more prominent damage as compared to those treated by alcalase. This is in line with the high release of protein content than that catalysed by alcalase as discussed earlier.

The yeast cell wall, which has a thickness of 100-150 nm, drives the main function in establishing and maintaining the cell morphology and structural integrity against cell lysis[16,34]. The loss of cell wall components predominantly comprised of β-glucans and mannoproteins will alter the wall porosity, hence allowing intracellular macromolecules should be released[13]. During the enzymatic hydrolysis, the cell wall comes into a direct contact with the external culture

media, triggering a sequence of biological activities in the cell to adapt to the changing environment.

The external hydrolysing enzymes breakdown the cell membrane components, causing holes to appear in the cell membrane. As a result, the cell becomes porous and large amount of the intracellular components are released into the surrounding media[34]. This in turn, causes wrinkling of the cell wall and subsequently reduction of the cell diameter throughout the lysis. The results obtained in our work are in accordance with those reported in the literature[8,23,30]. In summary, the morphological analysis here confirms the lysis of spent *S. cerevisiae* due to the use of alcalase and cellulase in the hydrolysis as described earlier.

Conclusion

In summary, this work has demonstrated the feasibility of enzymatic hydrolysis of spent *S. cerevisiae* derived from sago bioethanol fermentation using alcalase and cellulase. It has been shown that the optimal concentrations of alcalase and cellulase in this work were 0.4% (v/v) and 0.5% (v/v) respectively. Cellulase was found to be more superior than alcalase with respect to protein concentration of the hydrolysate.

The use of alcalase and cellulase in the enzymatic hydrolysis of spent *S. cerevisiae* 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 as reported in our prior work[19]. Future work should consider the effect of coupling alcalase and cellulase on the hydrolysis of spent *S. cerevisiae* derived from sago bioethanol fermentation.

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References

1. Agrawal N., Minj D.K. and Rani K., Estimation of total carbohydrate present in dry fruits, *IOSR J. Environ. Sci. Toxicol. Food Technol*., **1(6)**, 2319-2399 **(2015)**

2. Awang-Adeni D.S., Bujang K.B., Hassan M.A. and Abd-Aziz S., Recovery of glucose from residual starch of sago hampas for bioethanol production, *BioMed. Res. Int.*, **2013**, 1-8 **(2013)**

3. Bataeva D.S., Makhova A.A., Krylova V.B., Gustova T.V. and Minaev M.Y., *Clostridium* spp. detection in food samples using 16S rDNA-based PCR method, In IOP Conf. Ser.: Environ. Earth Sci, IOP Publishing, **421(5)**, 052025 **(2020)**

4. Bayarjargal M., Munkhbat E., Ariunsaikhan T., Odonchimeg M., Uurzaikh T., Gan-Erdene T. and Regdel D., Utilization of spent brewer's yeast *Saccharomyces cerevisiae* for the production of yeast enzymatic hydrolysate, *Mong. J. Chem.*, **12(38)**, 88-91 **(2011)**

5. Blanco A. and Blanco G., Enzymes, Medical biochemistry, Academic Press, 153-175 **(2017)**

6. Boonraeng S., Foo-Trakul P., Kanlayakrit W. and Chetanachitra C., Effects of chemical, biochemical and physical treatments on the kinetics and on the role of some endogenous enzymes action of baker's yeast lysis for food-grade yeast extract production, *Agric. Nat. Resour*., **34(2)**, 270-278 **(2000)**

7. Boonyeun P., Shotipruk A., Prommuak C., Suphantharika M. and Muangnapoh C., Enhancement of amino acid production by two-step autolysis of spent brewer's yeast, *Chem. Eng. Commun.*, **198(12)**, 1594-1602 **(2011)**

8. Cao R., Yang X., Shang W., Zhou Z., Strappe P. and Blanchard C., Functional enrichment of mannanase-treated spent brewer yeast, *Prep. Biochem. Biotechnol.*, **47(8)**, 789-794 **(2017)**

9. Chae H.J., Joo H. and In M.J., Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part 1: Effects of different enzymatic treatments on solid and protein recovery and flavor characteristics, *Bioresour. Technol.*, **76(3)**, 253-258 **(2001)**

10. Comuzzo P., Tat L., Liessi A., Brotto L., Battistutta F. and Zironi R., Effect of different lysis treatments on the characteristics of yeast derivatives for winemaking, *J. Agric. Food Chem.*, **60(12)**, 3211-3222 **(2012)**

11. Demirhan E., Apar D.K. and Özbek B., Sesame cake protein hydrolysis by alcalase: Effects of process parameters on hydrolysis, solubilisation and enzyme inactivation, *Korean J. Chem. Eng*., **28(1)**, 195-202 **(2011)**

12. Garcia R.B. Yeast cell wall hydrolysis by enzymes for the production of value-added products, Master's Thesis, Universidade NOVA de Lisboa **(2017)**

13. Hernawan T. and Fleet G., Chemical and cytological changes during the autolysis of yeasts, *J. Ind. Microbiol.*, **14(6)**, 440-450 **(1995)**

14. Jacob F.F., Striegel L., Rychlik M., Hutzler M. and Methner F.J., Spent yeast from brewing processes: A biodiverse starting material for yeast extract production, *Ferment*, **5(2)**, 51 **(2019)**

15. Jaeger A., Arendt E.K., Zannini E. and Sahin A.W., Brewer's spent yeast (BSY), an underutilized brewing by-product, *Ferment*, **6(4)**, 123 **(2020)**

16. Levin D.E., Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: The cell wall integrity signaling pathway, *Genetics*, **189(4)**, 1145-1175 **(2011)**

17. Li X., Shi X., Zou M., Luo Y. Tan Y., Wu Y., Chen L. and Li P., Characteristics of morphological and physiological changes during the autolysis process of *Saccharomyces cerevisiae* FX-2, *Microbiol. Biotechnol. Lett.*, **43(3)**, 249-258 **(2015)**

18. Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, **193(1)**, 265-275 **(1951)**

19. Mahmod N.N.A., Suhaili N., Awang-Husaini A.A.S. and Awang-Adeni D.S., Autolysis of spent baker's yeast generated

from sago bioethanol fermentation: A preliminary study, *Res. J. Biotech.*, **17(7)**, 91-97 **(2022)**

20. Mamat F.W., Suhaili N., Ngieng N.S., Vincent M. and Awang-Adeni D.S., Feasibility of Sago bioethanol liquid waste as a feedstock for laccase production in recombinant *Pichia pastoris*, *Res. J. Biotech.*, **16(4)**, 172-179 **(2021)**

21. Marson G.V., da Costa Machado M.T., de Castro R.J.S. and Hubinger M.D., Sequential hydrolysis of spent brewer's yeast improved its physico-chemical characteristics and antioxidant properties: A strategy to transform waste into added-value biomolecules, *Process Biochem.*, **84**, 91-102 **(2019)**

22. Martınez-Rodrıguez A.J., Polo M.C. and Carrascosa A.V., Structural and ultrastructural changes in yeast cells during autolysis in a model wine system and in sparkling wines, *Int. J. Food Microbiol*., **71(1)**, 45-51 **(2001)**

23. Middelberg A.P., Process-scale disruption of microorganisms, *Biotechnol. Adv.*, **13(3)**, 491-551 **(1995)**

24. Nataraja S., Chetan D.M. and Krishnappa M., Effect of temperature on cellulose enzyme activity in crude extracts isolated from solid wastes microbes, *Int. J. Microbiol Res*., **2(2)**, 44-47 **(2010)**

25. Ortiz-Chao P.A. and Jauregi P., Enzymatic production of bioactive peptides from milk and whey proteins. In Novel enzyme technology for food applications, Woodhead Publishing, 160-182 **(2007)**

26. Podpora B., Swiderski F., Sadowska A., Piotrowska A. and Rakowska R., Spent brewer's yeast autolysates as a new and valuable component of functional food and dietary supplements, *J. Food Process Technol.*, **6(12)**, 1 **(2015)**

27. Razzaq A., Shamsi S., Ali A., Ali Q., Sajjad M., Malik A. and Ashraf M., Microbial proteases applications, *Front. Bioeng. Biotechnol*., **7(110)**, 1-20 **(2019)**

28. Souza R.S., Diaz-Albiter H.M., Dillon V.M., Dillon R.J. and Genta F.A., Digestion of yeasts and beta-1,3-glucanases in mosquito larvae: physiological and biochemical considerations, *PLoS One*, **11(3)**, e0151403 **(2016)**

29. Tacias-Pascacio V.G., Morellon-Sterling R., Siar E.H., Tavano O., Berenguer-Murcia Á. and Fernandez-Lafuente R., Use of Alcalase in the production of bioactive peptides: A review, *Int. J. Biol. Macromolecules*, **165**, 2143-2196 **(2020)**

30. Takalloo Z., Nikkhah M., Nemati R., Jalilian N. and Sajedi R.H., Autolysis, plasmolysis and enzymatic hydrolysis of baker's yeast (*Saccharomyces cerevisiae*): A comparative study, *World J. Microbiol. Biotechnol.*, **36(5)**, 1-14 **(2020)**

31. Torresi S., Frangipane M.T., Garzillo A.M., Massantini R. and Contini M., Effects of a β-glucanase enzymatic preparation on yeast lysis during aging of traditional sparkling wines, *Food Res. Int.*, **55**, 83-92 **(2014)**

32. Vieira E.F., Carvalho J., Pinto E., Cunha S., Almeida A.A. and Ferreira I.M., Nutritive value, antioxidant activity and phenolic compounds profile of brewer's spent yeast extract, *J. Food Compost. Anal.*, **52**, 44-51 **(2016)**

33. Vukašinović-Milić T., Rakin M. and Šiler-Marinković S., Utilization of baker's yeast (*Saccharomyces cerevisiae*) for the production of yeast extract: Effects of different enzymatic treatments on solid, protein and carbohydrate recovery, *J. Serb. Chem. Soc.*, **72(5)**, 451-457 **(2007)**

34. Wang J., Li M., Zheng F., Niu C., Liu C., Li Q. and Sun J., Cell wall polysaccharides: Before and after autolysis of brewer's yeast, *World J. Microbiol. Biotechnol.*, **34(9)**, 1-8 **(2018)**

35. Xie J., Cui C., Ren J., Zhao M., Zhao L. and Wang W., High solid concentrations facilitate enzymatic hydrolysis of yeast cells, *Food Bioprod. Process.*, **103**, 114-121 **(2017)**.

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