แก่นเกษตร ๓๔ (๓) : ๑๖๔-๑๗๒ (๒๕๔๕) KHON KAEN AGRIC. 34 (3) : 164-172 (2006)

Some Fermentation Parameters Influencing Single Cell Protein Production from Cassava Starch by *Schwanniomyces occidentalis* TISTR

Pornthap Thanonkeo[1,2*], Kanit Vichitphan[1,2] and Pattana Laopaiboon[1,2]

Abstract

The yeast *Schwanniomyces occidentalis* TISTR5555 was cultured in the modified YM medium containing cassava starch as a sole carbon source. The optimum cultural conditions for growth and protein production were investigated. In a shake-flask culture at 30° C, the maximum growth and protein concentration were obtained when yeast cells were grown in the culture medium consisting of (w/v) 4% cassava starch, 0.5 % NH_4NO_3 and 0.5 % yeast extract. The optimum initial pH of the growth medium was 5.5. In the experiment conducted in 5 and 10-l bioreactor, 2.43 and 2.84 g/l of protein were obtained, respectively, under optimum growth conditions. Chemical composition analysis of the yeast biomass produced in the bioreactor revealed 43.87% dry weight of total protein. The highest amino acid and fatty acid were glutamic acid (3.65 g/ 100 g protein) and oleic acid (1.44 g/ 100 g dry weight), respectively.

Keywords : Cassava starch, SCP, *Schwanniomyces occidentalis*

[1] Department of Biotechnology, Faculty of Technology, Khon Kaen University

[2] Fermentation Research Center for Valued Added Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand

* Corresponding author. Tel : 66-43-362121; Fax : 66-43-343182, E-mail : portha@kku.ac.th

Introduction

Cassava (*Manihot esculenta* Crantz), a major root crop of the tropics, is currently the subject of worldwide efforts to develop its potential as a food, a feed and the industrial commodities such as liquid glucose, dextrin, and citric acid (Ravindra, 2000). In general, cassava is extremely low in protein but gives the highest yield of starch per hectare per year of any crop known. The dry matter of the root may be 90% fermentable carbohydrates, whereas the crude protein content averages only 5.3% (Reade and Gregory, 1975; Azoulay et al. 1980; Grace, 1986). Because its protein content is too low, it must be supplemented with a protein concentrate when it is to be used for animal feeding. An alternative to the use of a protein concentrate would be to use cassava as the carbon and energy sources in a microbial fermentation in which non-protein nitrogen is converted into microbial protein or single-cell protein (SCP).

The SCP production in a commercial process the starch is first hydrolyzed to fermentable sugars by commercial amylolytic enzymes (Nojiri, 1979). This requires significant raw material, equipment, energy, and labor which could be saved if this enzyme hydrolysis step could be eliminated. Therefore, current research focuses on the microorganism which produces its own amylolytic enzyme for starch conversion and which is also capable of fermentation. Previous studies in our laboratory revealed that the *Schwanniomyces occidentalis*, an amylolytic yeast which posses both alpha-amylase and glucoamylase, can efficiently utilize starch and convert it to glucose with significant higher specific growth rate compared to other microorganisms tested. To optimize the SCP production from cassava starch by Sc. occidentalis, medium components and cultivation conditions were investigated in this work.

Materials and Methods

Microorganism and medium

The yeast, *Sc. occidentalis* TISTR5555 was obtained from the culture collection of the Thailand Institute of Scientific and Technological Research, Bangkok. Culture was maintained on YM agar slant (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% glucose, and 2.0% agar) and was stored at 4 ° C with subculturing every 2 months.

Inoculum preparation

Yeast cells of *Sc. occidentalis* from a stock culture on YM slant were transferred into 250-ml flasks containing 100 ml of sterilized YM medium. The flasks were ncubated for 48 h at 30 ° C on a rotary shaker at 200 rpm. In all experiments, a 2% (v/v) inoculum of active cells was used.

Cultural conditions

The yeast strain was cultivated in 250-ml flaks, each containing 100-ml of modified YM medium using cassava starch as a sole carbon source. The initial pH of the culture medium was adjusted to 5.5 using 0.1 N HCl/NaOH. The flasks were incubated in a rotary shaker incubator (New Brunswick Scientific, USA) at 30° C and culture broth was taken at specific time intervals.

Large-scale fermentation was carried out in a 5 and 10-1 bioreactor (Biostat (R) M, B. Braun Biotech, Germany) with a working volume of 3 and 6-1, respectively. Agitation was set at 100 rpm and aeration rate was 1.0 vvm during the whole culture period. Temperature was maintained at 30° C and pH was controlled at 5.5 with a 0.1 N HCl/NaOH. An antifoam probe was connected to the bioreactor to allow adding adequate amounts of antifoam when necessary. At the end of cultivation, cells were collected by centrifugation and analyzed.

Optimization of production parameters

The effect of growth temperature and initial pH on SCP production was determined by growing the organism in shake-flasks at different temperatures (25, 30 and 35° C) and pH values (4.5, 5.0, and 5.5) for 72 h. The media and cultural conditions were as described earlier.

The yeast strain was also cultivated in modified YM medium containing different starch concentrations (1 to 4%, w/v) and various nitrogen sources by substituting yeast extract with equimolar concentration of nitrogen. The effect of different levels of KH_2PO_4 (0.25 to 1.0% w/v) on SCP production were also investigated. All the experiments were carried out in triplicate, and the mean values are presented.

Sample analyses

The protein content of the biomass was estimated by the Micro-Kjeldahl method (calculated as N x 6.25) as proposed by Ma and Zuaga (1941). True protein was examined using the method described by Lowry et al. (1951) with bovine serum albumin as the standard. Total reducing sugars were determined using DNS method (Miller, 1959). Cell biomass was measured as cell dry weight. Yeast cells were harvested by centrifugation 5 ml of the sample at 6000 g for 10 min, washed twice with distilled water and weight after a 24 h period at 105° C. For amino acid and fatty acid analysis, samples were prepared according to the AOAC (1984) method and determined using Gas chromatography (Model 9A, Shimadsu, Japan).

Results and Discussion

Effect of temperature and initial pH

The optimum temperature and initial pH for growth and protein production of *Sc. occidentalis* using cassava starch as a sole carbon source were observed to be 30° C and 5.5, respectively (data not shown). The results are in close agreement with the findings of Calleja et al. (1986) and Yinbo et al. (1992) who demonstrated maximum yield of protein in *Sc. alluvius* and Trichosporon cutaneum at 30 ° C and pH 5.5. On the contrary, higher temperature and pH optima were noted in *Saccharomyces uvarum, Saccharomycopsis fibuliger* and Enterobacter aerogenes (Ghanem et al. 1986; Ravindra, 2000).

Effect of starch concentration

Fig. 1 shows the effect of starch concentration on the growth and protein production of *Sc. occidentalis*. Generally, the high concentration of starch is effective in enhancing growth and protein production by the organism. The maximum protein was produced (2.5 g/l) with 4% (w/v) starch in 48 h of fermentation. No substrate inhibition was occurred at high starch concentration, suggesting that the organism produced sufficient amylases to convert all of the starch to the assimilable oligosaccharides and simple sugars. Our result was similar to that of Calleja et al. (1986) who examined the effect of the initial starch concentration on the growth of *Sc. alluvius* and reported the maximum yield of 0.51 g dried biomass/g starch at 4% (w/v) initial starch concentration. However, the maximum yield was also reported in Sc. alluvius and *Sc. castellii* using 1 and 2% (w/v) starch, respectively (Wilson and Ingledew, 1982; Hongpattarakere and H-Kittikun, 1995).

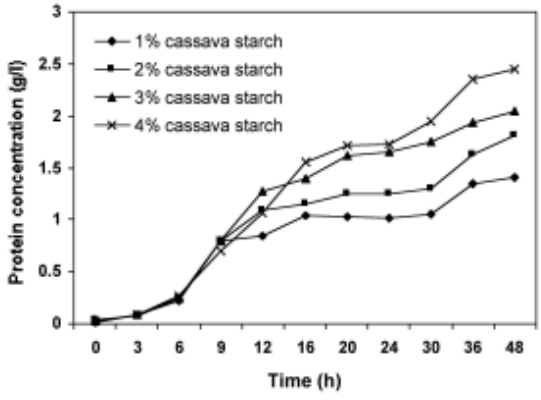


Fig. 1 Effect of cassava starch concentration on protein production of Sc. occidentalis TISTR5555.

Effect of nitrogen and inorganic phosphate sources

Nitrogen is an essential compound in protein synthesis and as a component of cell wall polymers. A number of publications have indicated that the optimal nitrogen source for growth and protein production was dependent on the tested organisms. For instance, supplementation of the growth medium with 4 g/l of $(NH_4)_2SO_4$ improved the yield of biomass, carbohydrate consumption and protein content of *Candida utilis* (Zayed and Mostafa, 1992).

Another example is that supplementation of diluted vinasse medium with corn steep liquor (CSL) and urea resulted in a marked increase in the biomass and protein content of *Debaryomyces hansenii, Kluyveromyces fragilis* and *S. cerevisiae* (Selim et al. 1991). We, therefore, investigated the effect of the nitrogen sources on the growth and protein production of the *Sc. occidentalis.* The results are showed in Fig. 2. Of the four different nitrogen sources at various concentrations, NH_4NO_3 at 0.5% (w/v) exhibited maximum protein yield (4.73 g/l) as compared with the other nitrogen sources.

No obvious stimulation of growth was found by supplementing various concentrations of KH2PO4 (data not shown). Thus, only small amount of nitrogen compounds needed to be added for the preparation of the growth medium for SCP production by *Sc. occidentalis.*

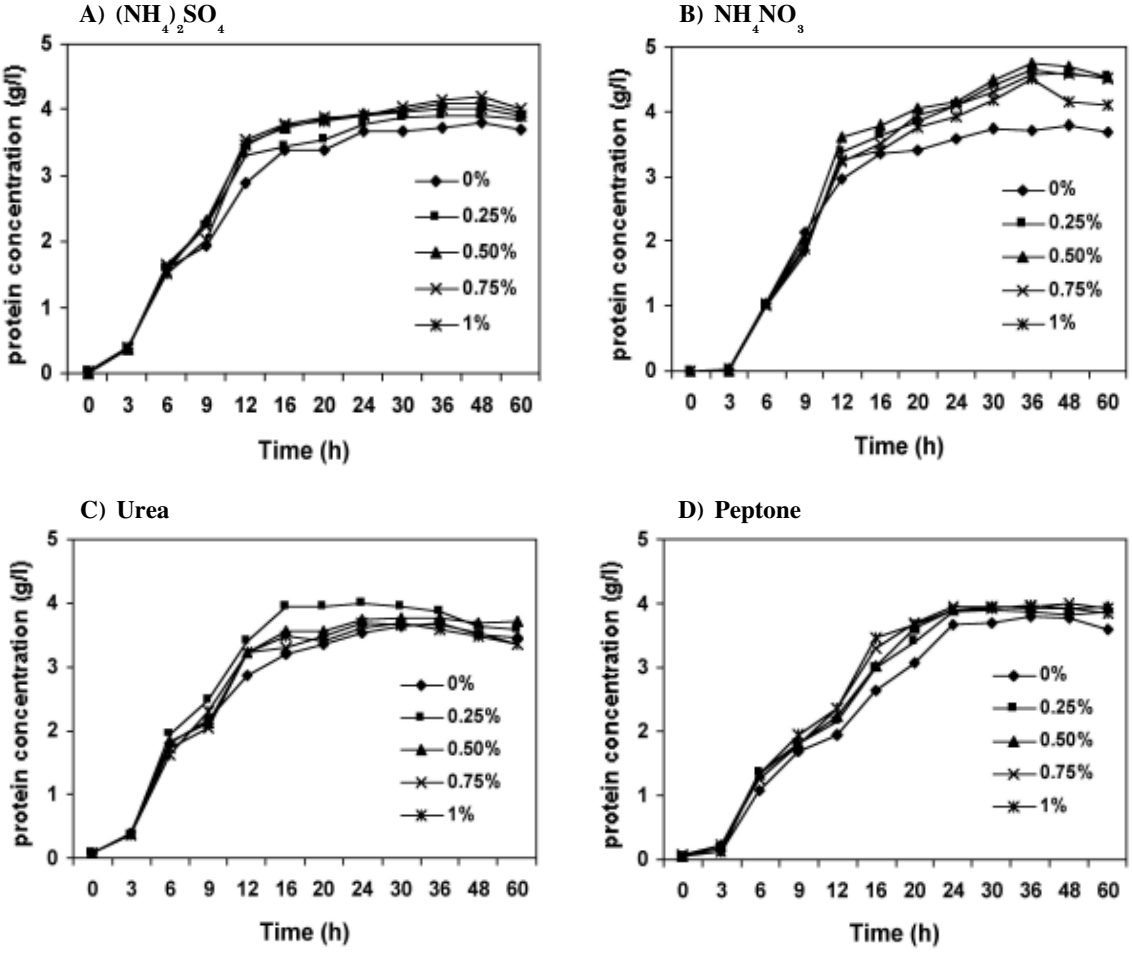


Fig. 2 Effect of $(NH_4)_2SO_4$ (A), NH_4NO_3 (B), urea (C) and peptone (D) at various concentrations on protein production of *Sc. occidentalis* TISTR5555

Effect of yeast extract

In addition to carbon and nitrogen sources, the effect of yeast extract on protein production was also tested. As shown in Fig. 3, supplementation of the growth medium with yeast extract improved the protein content of *Sc. occidentalis*. The maximum protein concentration of 2.8 g/l was obtained when *Sc. occidentalis* was grown in the medium containing 0.5% (w/v) yeast extract. Our results are in accordance with that obtained by Zayed and Mostafa (1992) who observed increased in the yield of biomass, carbohydrate consumption and protein content when growing *C. utilis* in the growth medium supplemented with yeast extract. Our results, together with that of Zayed and Mostafa (1992) suggested that yeast extract may contain some growth factors, such as vitamin, inosinic acid and guanylic acid which promote microbial growth and protein production (Aeschlimann and Stockar, 1990).

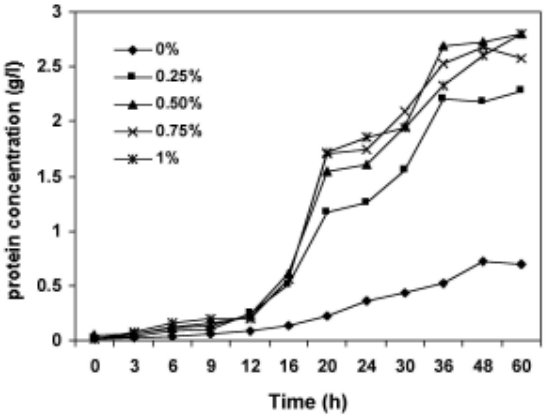


Fig. 3 Effect of yeast extract at various concentrations on protein production of *Sc. occidentalis* TISTR5555

SCP production in bioreactor

The production of SCP in large-scale was carried out using 5 and 10-1 bioreactor with a working volume of 3 and 6-1, respectively. Under optimum cultural conditions, 2.43 and 2.84 g/l of protein were obtained (Fig. 4). The biomass concentration changes followed a pattern almost similar to the protein concentration with the maximum number of cells observed closely at the maximum concentration of produced protein. The concentration of residual sugars increased during the first 9-h after cultivation, and then decreased. This is due to the degradation of cassava starch by amylolytic enzymes produced by *Sc. occidentalis* during fermentation.

Chemical composition of SCP

The chemical composition analysis of the biomass of *Sc. occidentalis* revealed 43.87% total protein, 0.67% fiber, 0.18% calcium, 0.77% phosphorus, and 0.43% ash (Table 1). Its protein content is highly comparable to that of Pichia pinus (39% dry weight) and *C. utilis* (49.8% dry weight) (Rashad et al. 1990; Kurbanoglu, 2001). The detailed amino acid composition of *Sc. occidentalis* and FAO reference protein (Algur and Gokalp, 1991) are given in Table 2. The biomass obtained from the yeast contained all of the essential amino acids. The essential amino acid concentrations were somewhat lower than that of FAO reference protein. Among the amino acids,

glutamic acid was the most abundant. It was reported that the potential nutritional value of SCP is determined with amount of lysine and methionine amino acids (Tauk, 1982; Malathi and Laddha, 1989). With proper methionine supplementation, SCP from *Sc. occidentalis* could be an acceptable protein supplement for human and animal consumption.

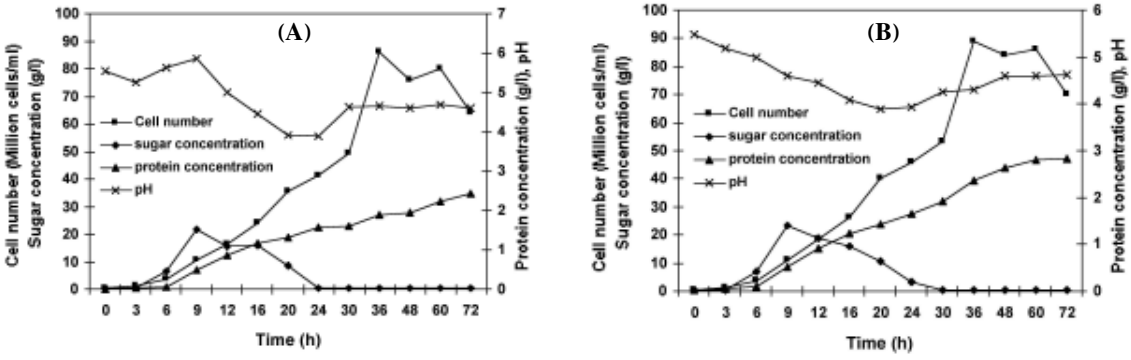


Fig. 4 Time course of SCP production from cassava starch by *Sc. occidentalis* TISTR5555 in a 5-l (A) and 10-l bioreactor (B)

Table 1 The chemical composition of SCP from *Sc. occidentalis* TISTR5555 on a dry weight basis

Components	g/100 g dry weight
Total protein	43.87
Fatty acid:	
16:0	0.34
16:1	0.11
18:0	0.45
18:1	1.44
18:2	0.40
20.0	0.24
Minerals:	
Calcium	0.18
Phosphorus	0.77
Fiber	0.67
Ash	4.31
Moisture	9.32

Table 2 Amino acid composition of SCP from *Sc. occidentalis* TISTR5555

Amino acid	g/100 g protein	FAO (g/100 g protein)
Aspartic acid	2.86	-1
Threonine	1.35	2.80
Serine	1.39	-
Glutamic acid	3.65	-
Proline	1.07	-
Glycine	1.34	-
Alanine	2.20	-
Cysteine	0.02	2.00
Valine	1.44	4.20
Methionine	0.21	2.20
Isoleucine	1.20	4.20
Leucine	2.08	4.80
Tyrosine	0.83	-
Phenylalanine	1.51	2.80
Histidine	0.68	-
Lysine	2.34	4.20
Arginine	1.02	-
Trytophan	0.28	1.0

[1] not reported.

Conclusion

In conclusion, the optimum temperature and initial pH for growth and protein production of Sc. occidentalis were found to be 30° C and 5.5, respectively. The maximum protein content was obtained when *Sc. occidentalis* was cultured in the growth medium containing 4% cassava starch as a sole carbon source. Supplementation of the growth medium with 0.5% NH_4NO_3 and 0.5% yeast extract, but not with KH_2PO_4, improved the yield of biomass and protein content of *Sc. occidentalis.* The biomass of the *Sc. occidentalis* contained 43.87% total protein which was comparable to those from other organisms. In addition, the yeast biomass contained all of the essential amino acids and when compared with FAO reference protein it showed a good profile.

Acknowledgements

The authors thank Assoc. Prof. Sanha Panichajakul and Assist. Prof. Pakawadee Narong for their guidance and helpful during the laboratory work. We also thank the National Research Council for funding of the project. Technical assistance from Ms. Vipawan Seangdung and undergraduate students of Biotechnology Department are gratefully acknowledged.

References

Aeschlimann A. and U.V. Stockar. 1990. The effect of yeast extract supplementation on the production of lactic acid from whey permeates by *Lactobacillus helviticus*. Appl. Microbiol. Biotechnol. 32: 398-402.

Algur, O. and H.Z. Gokalp. 1991. Some fermentation parameters influencing single cell protein production by *Rhizopus arrhizus* and *Actinomucor elegans.* Doga-Tr. J. Biol. 15: 190-197.

AOAC. 1984. Official methods of analysis, 14th edition. Assocoation of official analytical chemists, Washington, DC.

Azoulay, E., F. Jouanneau, J.C. Bertrand, A. Raphael, J. Janssens, and J.M. Lebeault. 1980. Fermentation methods for protein enrichment of cassava and corn with *Candida tropicalis*. Appl. Env. Microbiol. 39 : 41-47.

Calleja, G.B., M. Yaguchi, S. Levy-Rick, J.R.H. Seguin, C. Roy, and C.V. Lusena. 1986. Single cell protein production from potato starch by the yeast *Schwanniomyces alluvius*. J. Ferment. Technol. 64 : 71-75.

Ghanem, K.M., A.E. Refai, and M. El-Gazaerly. 1986. Some fermentation parameters influencing single cell protein production by *Saccharomyces uvarum* Y-1347. Agric. Wastes. 15 : 113-120.

Grace, M.R. 1986. Cassava Processing. Rome : FAO.

Hongpattarakere, T. and A. H-Kittikun. 1995. Optimization of single-cell protein production from cassava starch using *Schwanniomyces castellii.* World J. Microbiol. Biotechnol. 11 : 515-518.

Kurbanoglu, E.B. 2001. Production of single cell protein from ram horn hydrolysate. Turk. J. Biol. 25 : 371-377.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193 : 265-275.

Ma, T.S. and G. Zuaga. 1941. Micro–Kjeldahl determination of nitrogen. Ind. Eng. Chem. 14 : 280–282.

Malathi, S. and G.S. Laddha. 1989. Single cell protein from defatted mango (*Mangifera indica*) kernels. Ind. J. Exp. Biol. 27 : 792–794.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. 31 : 426–428.

Nojiri, M. 1979. SCP production process from tapioca. Proceedings of the International Conference on Global Impacts of Applied Microbiology. P. 320–333.

Rashad, M.M., S.A. Moharib, and E.W. Jwanny. 1990. Yeast conversion of mango waste or methanol to single cell protein and other metabolites. Biol. Wastes. 32 : 277–284.

Ravindra, A.P. 2000. Value–added food: single cell protein. Biotechnol. Adv. 18 : 459–479.

Reade, A.E. and K.F. Gregory. 1975. High–temperature production of protein–enriched feed from cassava by fungi. Appl. Microbiol. 30 : 897–904.

Selim, M.H., A.M. Elshafei, and A.I. El–Diwany. 1991. Production of single cell protein from yeast strains grown in Egyptian vinasse. Bioresource Technol. 36 : 157–160.

Tauk, S.M. 1982. Culture of *Candida* in vinasse and molasses: Effect of acid and salt addition on biomass and raw protein production. Eur. J. Appl. Microbiol. Biotechnol. 16 : 223–227.

Wilson, J.J. and W.M. Ingledew. 1982. Isolation and characterization of *Schwanniomyces alluvius* amylolytic enzymes. Appl. Environ. Microbiol. 44 : 301–307.

Yinbo, Q., C. Hongzhang, and G. Peiji. 1992. SCP production from steam exploded hemicellulose autohydrolysate by Trichosporon cutaneum. J. Ferment. Bioeng. 73 : 386–389.

Zayed, G. and N. Mostafa. 1992. Studies on the production and kinetic aspects of single cell protein from sugar cane bagasse saccharified by *Aspergillus niger*. Biomass and Bioenergy. 3 : 363–367.