OPTIMIZATION OF SINGLE CELL PROTEIN PRODUCTION FROM CASSAVA PROCESSING WASTEWATER BY MIXED CULTURE OF *Endomycopsis fibuligera* TISTR 5097 AND *Candida utilis* TISTR 5046

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ABSTRACT

Optimization of single cell protein production from cassava processing wastewater by mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 was studied. *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 were mixed with a volume ratio of 1 : 4 and corn steep liquor was used as nitrogen source. The results showed that the maximum cell dry weight of mixed culture was 4.89 g/L and protein content was 0.47 g protein/g dry weight at initial pH 4.0 in a shake flask culture at 25 °C and shaking speed of 200 rpm. The optimum agitation speed, aeration rate for the growths of these yeasts in a fermenter were 300 rpm, 1.5 vvm with cell dry weight of 6.97 g/L

KEYWORDS: single cell protein, cassava processing wastewater, *Endomycopsis fibuligera, Candida utilis*, optimization.

1. INTRODUCTION

Thailand is one of the biggest cassava producers in the world. Cassava production has been produced mainly in the North Eastern region. The processing capacity is more than 20 – 21 million tons each year. Most of total products have been exported as animal feed to European countries. For domestic uses, mostly cassava is served as raw materials for flour production, feedstock in fermentation industries such as glucose, dextrin and sugar alcohol. The wastewater volumes discharged by the cassava starch industry are vast and comprise considerable organic carbon with Chemical Oxygen Demand (COD) values ranging from 6,000 – 10,000 mg/L causing serious environmental pollution. The possibility of utilizing this effluent as a substrate for the culture of amylolytic yeasts and single cell protein production. has been studied [1]. Cassava wastewater can be converted into glucose by amylolytic enzyme from amylolytic yeasts, for example, *Endomycopsis* sp., *Saccharomyces cerevisiae,* followed by non-amylolytic yeast, *Candida utilis*. These yeasts have been used in the process to produce single cell protein. A mix culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 was used in the present study for single cell protein production from cassava processing wastewater. The aim of the investigation was to study the factors influencing SCP production from the two yeasts.

2. MATERIALS AND METHODS

2.1 Microorganism

E. fibuligera TISTR 5097 and *C. utilis* TISTR 5046 were obtained from Thailand Institute of Scientific and Technological Research(TISTR), Bangkok Thailand.

2.2 Media

The media for stock and starter cultures of the two yeast strains were YM agar slants and YM broth, respectively. Cassava wastewater medium prepared from cassava processing wastewater by filtration using Whatman No.1. Experiments were conducted in 250 ml flasks containing 50 ml of cassava processing wastewater. The pH was adjusted with 1 N HCl, 1 N NaOH and autoclaved at 121 °C for 15 min.

2.3 Inoculum preparation

Cell suspension was prepared from culture grown on YM agar slant. One loop of the culture was inoculated into 250 ml flask containing 50 ml of YM broth. The flask was incubated on an orbital shaker with a shaking speed of 150 rpm at 30 °C for 24 h, then measured O. D. = 0.5 (A_{540})absorbance [2].

2.4 Cultivation in shake flask

Optimization of SCP was attempted by varying factors that might affect SCP production including pH (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0), fermentation temperatures (20°C, 25°C, 30°C and 35°C) and shaker speed (150, 200 and 250 rpm). The experiments were carried out in 250 ml flasks containing 50 ml of wastewater.The pH adjusted with 1 N HCl, 1 N NaOH, autoclaved at 121°C for 15 min and inoculated with *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 at a volume ratio of 1 : 4 (5% inoculum). *Candida utilis* TISTR 5046 was first inoculated into the culture for 18 h before *E. fibuligera* was inoculated. The flasks were incubated on an orbital shaker with a shaking speed of 150 rpm at 30°C for 66 hour [3]. Cell dry weight and protein content were then analysed.

2.4 Cultivation in fermenter

Preculture of mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 grown under optimal conditions derived from the shake flask was inoculated (5% inoculum) into 3 litres of the same medium in a 5 litre -fermenter at various agitation speed of 200 and 300 rpm with aeration rates of 1.0 1.5 and 2 vvm. Cell dry weight and protein content were then analysed.

2.5 Analysis of cell dry weight and protein content

The biomass or cell dry weight in the medium was harvested by centrifugation, washed twice with distilled water and followed by drying to constant mass at 80°C for 16 h. Protein content of the dry biomass was estimated according to the Lowry method [4].

3. RESULTS AND DISCUSSION

3.1 Optimum conditions for SCP production in shake flasks

Growth of mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 at different initial pH, temperature and shaking speeds was studied. When the media were adjusted at different initial pH, the result showed that the maximum cell dry weight (4.60 g/L) and protein content (0.46 g protein/g dry weight) were obtained at pH 4.0 after 66 hour of incubation (Fig 1). When the cultures were incubated at different temperatures, it was found that the most favorable for growth was obtained at 25°C with the highest cell dry weight of 4.70 g/L and protein content of 0.46 g protein/g dry weight after 66 hour of incubation (Fig 2). The optimum speed for cell dry weight (4.82 g/L) and protein content (0.47 g protein/g dry weight) was at 200 rpm (Fig 3). Mixed culture of *E. fibuligera* TISTR 5097 and *C .utilis* TISTR 5046 were then cultivated under the above optimal conditions. The result showed that maximum cell dry weight of 4.89 g/L and protein content of 0.47 g protein/g dry weight were achieved after 66 hour of incubation (Table 1). Lemmel *et al* [5] reported that potato processing wastewater was a good medium for propagating *S. fibuligera* and *C .utilis*. Analyses showed that high dilution rates resulted in increased the rate of carbon consumption, the amount of reducing sugar consumed, the rate of protein production and indicated that pH of 4.3 to 4.8 increased the amount of protein produced. Jin *et al* [1] reported that fungus *Rhizopus oligosporus* DAR 2710 has the ability to convert more than 95% starch materials in starch processing wastewater (SPW) to produce 4.5 – 5.2 g of dry fungal biomass from a litre of SPW cultivation at 35 °C and initial pH 4.0.The fungal biomass contained 46% protein and was safe for human and animal consumption. Trien *et al* [6] reported that single cell protein from the cultivation of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5001 using cassava as carbon source in a mixed culture could be obtained from dry cassava with a yield of 0.55 g of dry biomass (48.8% protein content) per 1 g of cassava or 0.89 g dry biomass (38.4% protein content) per 1 g cassava.

3.2 Optimum conditions for SCP production in fermenter

Growth of mixed culture of *E. fibuligera* TISTR 5097 and *C.utilis* TISTR 5046 was studied in a fermenter at agitation speeds of 200 and 300 rpm with aeration rates of 1.0, 1.5 and 2.0 vvm. The results indicated that the agitation speed of 300 rpm and aeration rate of 1.5 vvm were effective for growth.(Fig 4). Maximum cell dry weight of 6.97 g/L was obtained at 42 h, while the maximum cell dry weight from shake flask was 5.22 g/L at 48 h. The cultivation time in fermenter was reduced from 48 h to 42 h. when compared to the cultivation time in shake flask (Fig 5). Hongpattarakere and H – Kittikun [7] reported that *Schwanniomyces castelli* B 5285 produced 7.12 g cell dry mass/litre, with protein yield of 6.4 g/100 g starch which was grown with 2% w/v cassava starch as sole carbon source. The optimum agitation speed and aeration rate for growth of this yeast in a fermenter were 400 rpm, 1.67 vvm.The cell dry mass to 8.90 g/L, cell yield to 44 g/100 g starch and protein yield to 7.4 g/100 g starch.

4. CONCLUSION

Mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 was capable to grow in cassava processing wastewater. Maximum cell dry weight and protein content gained under shake flask and fermenter conditions were not much different.

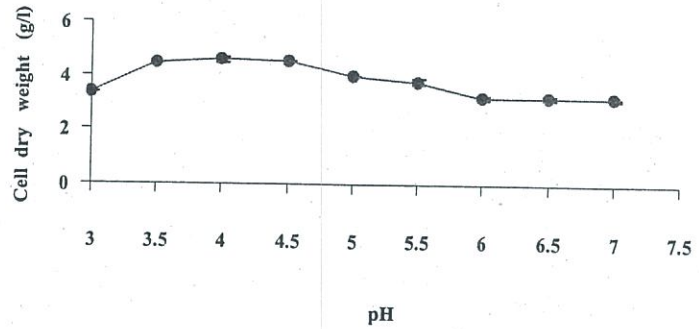

Fig 1. Growth of mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 under various initial pH of the medium.

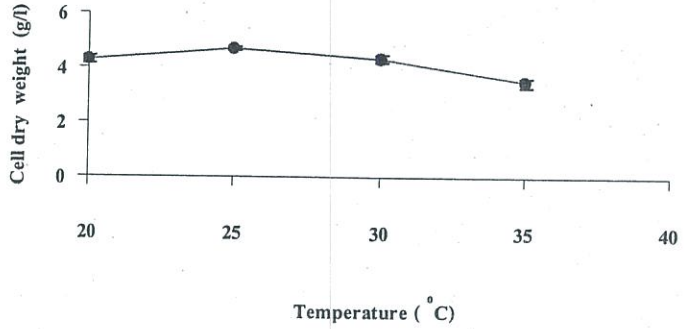

Fig 2. Growth of mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 under various temperatures.

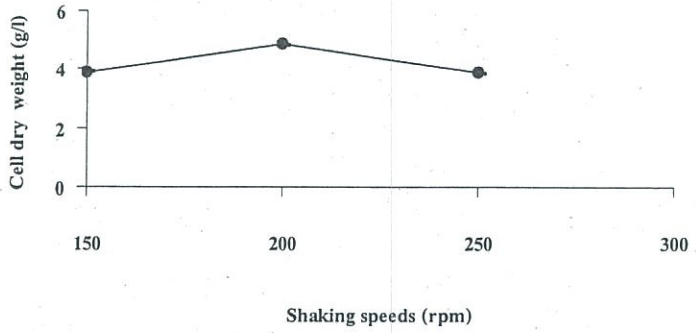

Fig 3. Growth of mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 under various shaking speeds.

Table 1 Composition of single cell protein under optimal conditions in shake flasks

Compositions	concentrations
Cell dry weight	4.89 g/l
Protein	0.47 g protein/g dry weight
Lipid	0.15 %
Moisture	2.30 %
ash	7.49 %

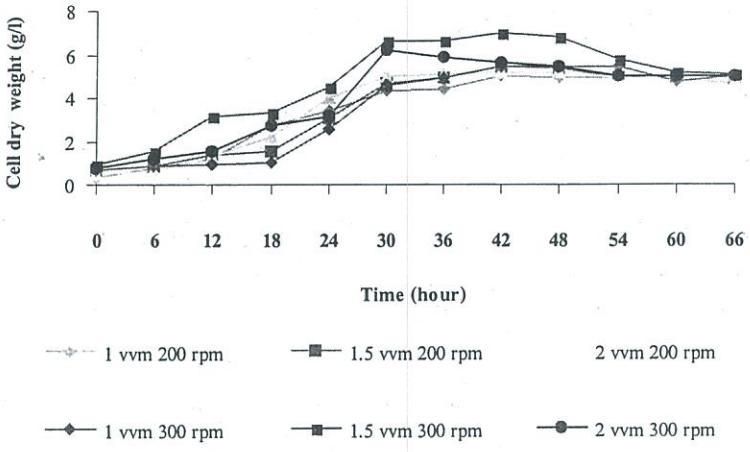

Fig 4. Growth of mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 under growth conditions in fermenter.

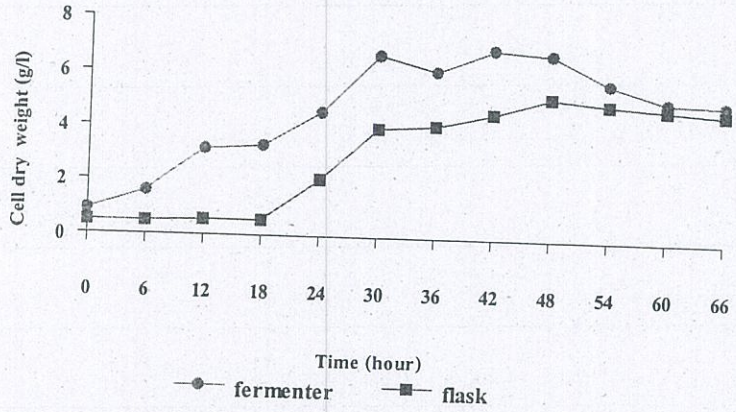

Fig 5. Growth of mixed culture of *E. fibuligera* TISTR 5097 and *C. utilis* TISTR 5046 under optimal conditions in shake flasks and fermenter.

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