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PRODUCTION OF SINGLE CELL PROTEIN FROM SUGAR APPLE PEEL USING *Saccharomyces cerevisiea*

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Abstract: *Single cell proteins are the dried cells of microorganisms, which are used as protein supplement in human foods or animal feeds. The increasing world demand for protein rich food led to the search for the formulation of alternative protein sources to supplement the conventional protein sources. SCP is one of the most important steps for this goal, and is an alternative and an innovative way to successfully solve the global food problem. Research on single cell protein has been stimulated by a concern over the eventual food crisis or food shortages that will occur if the world population is not controlled. Many scientists believe that use of microbial fermentations and the development of an industry to produce and supply single cell protein from agricultural waste are insufficient. The sample was washed under running tap water and oven dried for further treatment. The dried sample was pre-treated by grounding to a fine powder using mortar and by soaking the biomass in dilute sulphuric acid H_2SO_4 (2% v/v). This slurry was then subjected to high pressure steam at 121°C for 120 minutes. After pre-treatment, steam of autoclave was quickly released and the solid and liquid were seperated by filtration. The solid fraction was dried and stored untill further use.The sugar apple peel gave a maximum growth of on the 7th day however, a fall in growth was observed on the 6th day. There was a higher yield of biomass compared to the YEPD medium which served as the control, having a constant increase in growth with a maximum cell density. It can be concluded from the results of this experiment that sugar apple peel is a good substrate for production of single cell protein and Saccharomyces cerevisae can be used for large scale production of single cell protein which can be used as an enrichment for carbohydrates based foods as animal feeds.*

Keywords: Sugar, Biomass, Microorganisms, Fermentations, Food

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INTRODUCTION

The worldwide large-scale development and process of single cell protein (SCP) has contributed greatly to the advancement of present-day biochemistry and biotechnology. A large population of the world, especially those who are living below poverty line are suffering from malnutrition (Dhanasekeran *et al.*, 2011). There is a big gap between the demand of protein rich food and its supply to the ever-increasing world population. In order to bridge this gap, single cell protein (SCP) is an innovative and an alternative way to this direction. SCP may be used as human food supplement or as animal feed (Singh, 1998). The name “single cell protein” was used for the first by the Massachusetts Institute of Technology Professor Carol Wilson to give a better image than “microbial protein” (Ware, 1977). The single cell protein is a dehydrated cell, which consist of mixture of proteins, lipids, carbohydrates, inorganic compounds, nucleic acids and a variety of other non-

protein nitrogenous compounds such as vitamins. Agricultural wastes are important substrate for production of microbial protein, but there must meet the following criteria; it should be non-toxic, cheap, abundant and able to support multiplication and rapid growth of the organism, which result in high quality biomass (Dhanasekeran *et al.*, 2011).

The production of SCP technologies arose as a promising way in solving the problem of protein shortage worldwide. They evolved as bioconversion processes that turned low value by-products, often waste into products with added nutritional and market value (Ugalde *et al.,* 2002). The novelty of unwanted waste products consumption has added a new economic incentive to SCP production, as the idea of zero cost substrates, or even generation of additional revenues through the concept of waste product treatment were argued and incorporated favorably thereby reducing the production cost estimates. The benefits of SCP production were thus extended from the production of food to the preservation of the environment at large.

Different microorganisms can be used as source of SCP such as bacteria, yeasts Moulden algae. The bioconversion of fruit wastes into SCP production has the potential to solve the worldwide food problem deficiency by obtaining an economical product for feed and food. By using food processing leftovers in the production of SCP as substrate alleviate pollution (Mondal *et al.,* 2012).

This work was carried out using agricultural waste [Sugar apple peel], which was obtained after the fruit have been consumed. Generated waste is either given to animals as feed or discarded along with other waste products, most of which are treated physically, resulting in the pollution of the environment.

Single cell proteins are dried cells of microorganisms, which can be used as protein supplement in animal feeds or human food. Microorganisms such as fungi, algae, yeast and bacteria utilize inexpensive feedstock and wastes as sources of carbon and energy for the growth in the production of biomass (Nasseri *et al*., 2011).

The future of SCP will be heavily dependent on reducing production costs and improving quality by fermentation, downstream processing and improvement in the producer organisms as a result of conventional applied genetics together with recombinant DNA technologies (Omar and Sabry,1991). The necessary factor considered for use of SCP is the demonstration of the absence of toxic and carcinogenic compounds originated from the substrates, biosynthesized by the microorganisms or formed during processing.

Different types of microorganisms can be used as source of SCP which includes bacteria, algae, yeast and filamentous fungi.

The production of SCP from various microbes, particularly from fungi and bacteria has received many considerable attention, in contrast, only a few studies have dealt with the feasibility of using SCP from microalgae (Mahasneh, 1997). Algal proteins are of high quality and comparable to conventional vegetable proteins. However, due to high production costs as well as technical difficulties, cultivation of algae as protein is still in evaluation (Rasoul-Amini *et al.,* 2009).

Example of some bacteria used for single cell protein (SCP) production includes: *Acromobacter delvacvate, Acinetobacter calcoacenticus, Aeromonas hydrophila, Thermomono sporafusca, Lactobacillus* sp*, Methylomonas methylotrophus, Methylomonas clara, Pseudomonas fluorescens.* Others include *Rhodopseudomonas capsulate, Bacillus megaterium, Bacillus subtilis, Cellulomonas* sp. and *Flavobacterium* sp. (Bhalla et al., 2007).

Yeast is a group of microorganisms that is regarded as the most widely accepted for SCP production. It have some advantages such as larger size, which makes it easier to harvest, lower nucleic acid content, high lysine content and ability to grow at acidic pH. However, the most important advantage of yeast is familiarity and acceptability because of the long history of its use in traditional fermentations (Becker, 2007).

MATERIALS AND METHODS

MATERIAL /REAGENTS

i. FRESH Sugar apple peels
ii. Microscope
iii. spectrophotometer
iv. Incubator
v. Petri dish
vi. Marccartney bottle
vii. Filter paper
viii. Autoclave
ix. Distilled water
x. Synthetic medium yeast extract pepton D glucose (YEPD)

REAGENT

i. Ammonium sulphate
ii. Sodium chloride
iii. Calcium chloride anhydrous
iv. Potassium bisulphate
v. Hydrochloric acid

METHODS

Sample Preparation and Pre-Treatment

The sample obtained was washed under running tap water and oven dried for further treatment. The dried sample was further pre-treated by grounding to fine powder using mortar and thereby soaking the biomass in dilute sulphuric acid H_2SO_4 (2% v/v). The slurry was then subjected to high pressure steam at 121°C for 120 minutes. steam of autoclave was quickly released,solid and liquid were seperated by filtration after pre-treatment. The solid fraction dried and stored untill further use (Dhabhai *et al.,*2012)

Preparation of Inoculum

Preparation of inoculum was carried out by 1 week PDA slant culture of the strain. The inoculum was prepared by pouring 5ml of sterile distilled water in marccartney bottle where there was 1 week old slant culture of the organism (Adhikari and Baral, 1998).

Culture Condition for SCP Production.

A complex media of the pre-treated sugar apple peel was used for the single cell protein production, the complex media was prepared with the sample (sugar apple peel) at a concentration of 70g/l, supplemented with 5.0g $(NH_2)_2SO4$, 1.0g KH_2PO_4, 0.5g $MgSO_4.7H_2O$, 0.1g Nacl, 0.1g $CaCl_2$, pH 5.5 (Phaff *et al.,* 1996). Two hundred millitres (200ml) dispensed

into a 250ml cornical flask and sterilized at 121^0C for 15 minutes. One ml of the inoculum was inoculated in the medium (Numbhn, 2006).

A synthetic medium, yeast extract peptone D-glucose (YEPD) medium which served as a control was prepared by dissolving 10g of yeast extract and 20g of peptone in 1L of distilled water. 20 gram per litre of the D-glucose was prepared separately. The two solutions were then autoclaved for 15 minutes at 121^0C. After, autoclaving the two solutions were then mixed (Al-Mhanna, 2012).Two hundred ml was aseptically dispensed into a 500ml Erlenmeyer flask and inoculated with 1ml of the inoculum. The two flasks were then incubated on an orbital shaker for 7 days at room temperature (Numbhn, 2006).

Determination of the Single Cell Protein Yield

This was determined based on the concentration of the organism in the culture at the interval of 24 hours for a period of 7 days. A 1:10 dilution was made with distilled water using 10 ml of the medium. Same procedure was carried out on a blank sample containing only the pre-treated sample which was used in blanking the spectrophotometer and absorbance measured at 670nm.The absorbance value was used to drive the density which represents the yield of the organism (SCP) (Al-Mhanna, 2012).

Determination of Protein Content of the Single Cell Protein

The SCP sample was weighed (100mg) and placed into digestion test tube and 5ml of concentrated H_2SO_4 was added. This was then heated for 30-45mins to digest, until the content appeared clear. Ten ml of 40% NaOH solution was added to 100ml of the digest, the mixture was steamed, distilled to liberate NH_3 into 5ml boric acid containing 4 drops of mixed methyl red and bromocresol green. The distillate was removed and titrated against standard HCl. The end point was noted i.e., a change of color from grey to pink. The amount of acid consumed represents the titer value, which was used to calculate percentage nitrogen using the formula: (Al-Mhanna, 2012).

$$\text{Percentage Nitrogen } (\%N_2) = \frac{(A-B)\ xNx14x100}{W}$$

Where

A= quantity of HCl used to neutralize the test sample

B= quantity of HCl used to neutralize the blank

N= normality of HCl

14= relative atomic mass of nitrogen

W= weight of sample (mg)

Percentage protein = percentage nitrogen x protein factor (6.25).

RESULTS AND DISCUSSION

RESULT

The results obtained during the study are presented in tables 1 – 4 below and followed by respective discussion.

The colony appearance and vegetative morphology of *Saccharomyces cerevisae* was studied and presented in the table 1 below.

Table 1. The Optical Density Of SCP (Sugar Apple Peel) And YEPD Medium Taking At 670nm Wavelength

Media	Absorbance							
	Zero Hr	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Complex	0.912	1.361	1.727	2.379	2.841	2.861	2.566	2.004
	0.924	1.358	1.729	2.380	2.799	2.801	2.562	2.013
Synthetic	0.786	1.144	1.580	1.890	2.416	2.420	2.108	1.612
	0.763	1.139	1.582	1.868	2.420	2.433	2.009	1.654

Key: Hr = Hour

The single cell produced during the experiment within the period of study is as presented in table 2 below.

Table 2. Content of Single Cell Protein Produced From Sugar Apple Waste

Media	% Cell Protein cfu/ml(10^3) production for seven days at 24 hours interval						
	Zero hour	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Complex	6.563	10.000	13.125	19.688	24.660	31.750	33.906
	5.469	10.000	14.250	20.781	25.180	32.810	33.906
Synthetic	5.469	8.750	11.875	15.313	17.500	22.969	27.380
	5.469	8.750	10.000	14.250	18.594	24.069	28.440

The result obtained within an interval of 24 hours daily for a period of seven days for yeast count is as presented below

Table 3. The Log of Yeast Count for Seven Days at 24 Hours Interval

Media	Log of Yeast Count							
	Zero Hr	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Complex	3.233	3.300	3.397	3.531	3.613	3.613	3.544	3.462
Synthetic	3.204	3.255	3.398	3.477	3.578	3.578	3.531	3.415

Key: Hr = Hour

The morphological characteristics of the yeast obtained during the experiment was carefully studied and presented in the table 4 below.

Table 4. Morphological Characteristics of Saccharomyces cerevisae

	Colonial appearance			**Vegetative morphology**		**Inference**
Isolate	**Pigmentation**	**Size**	**Form**	**Shape**	**Budding**	
Y1	Creamy	Large	Smooth	Elongated	Positive	***Saccharomyces cerevisae***

KEY: Y1 = large creamy colony.

DISCUSSION

Production of single cell protein

The substrate tested for single cell protein production can be used in place of some commercially available synthetic media such as yeast extract peptone D-glucose (YEPD) medium from the result obtained.

Dhanasekeran*et al.,* (2011) reported that the substrate is rich in cellulose, hemicelluloses and other carbohydrates. It also contain a good amount of reducing and non-reducing sugars (10 and 13% respectively), which is most favorable for the growth of microorganisms. It was further found to contain 0.6% protein and this is most suitable for yeast fermentation.

Essentially, the increased cell density is due to the nutrients in the substrate, while the decrease in growth is due to a lack of available carbon and energy sources for metabolic activity. The increase in growth after a period of decline on the seventh day indicates that the organism can utilize its metabolic byproducts, which can be inhibitory or toxic if they build up.

Many researchers in their studies used inorganic supplements for the growth of myceliaon waste materials. Ojokoh and Uzeh (2005) utilized glucose (2% w/v) and $(NH_4)_2HPO_4$ (0.25% w/v) as a nitrogen source supplement for the production of*Saccharomyces cerevisae*biomass in papaya extract medium. Similarly, Adoki (2008) studied various factors influencing cell biomass production with *Candida* species using citrus fruit wastes and found that the test strain was capable of meeting its amino acid requirements in culture when supplied with inorganic nitrogen sources. As such, addition of nutrient supplement to the media provided available nitrogen and other nutrients for the organism and therefore enhances growth.

In essence, the agricultural waste performed better as a microbial growth substrate than the yeast extract peptone D-glucose medium, likely due to the presence of a higher concentration of sugars, carbohydrates and proteins. This makes the agricultural waste a more suitable source of nutrients for microbial growth.

Dhanasekeran *et al.,* (2011) reported appreciable yields of yeast (*Saccharomyces cerevisae*and *Candida tropicalis)* biomass using pineapple waste as substrate. In a study by Al-Mhanna (2012), *Saccharomyces cerevisae*was cultivated in date juice as single cell protein compared to yeast extract peptone D-glucose medium.

In summary, the study found that the highest amount of biomass was achieved on the 7th day of fermentation, which was consistent with previous research by Dhanasekeran *et al*., (2011) on the production of single cell protein from pineapple waste using yeast (*Saccharomyces cerevisae* and *Candida tropicalis*).

The percentage crude protein value obtained in this study was close to the crude protein value (31.2) obtained in a study by Yousufi (2012) on the production of single cell protein from pineapple skin and mango peel using *Aspergillus oryzae*.

Essien *et al*., (2005) utilized banana peel as a substrate for Mould growth and biomass production. As such, production of fungal biomass on fruit and other agricultural wastes shall not only minimize loads of pollutants but at the same time, the malnourished people can have protein supplement at an affordable cost.

CONCLUSION

It can be concluded from the results of this experiment that Sugar apple waste is a good substrate for production of single cell protein and *Saccharomyces cerevisae*can be used for large scale production of single cell protein which can be used as an enrichment for carbohydrates based foods as animal feeds.

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