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PRODUCTION OF SINGLE CELL PROTEIN FROM AGRICULTURAL WASTE USING MICROORGANISMS

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Abstract: Protein accounts for the major requirement of the human body. Single cell protein (SCP) represents microbial cells grown in mass culture that are harvested as dried cell mass in the form of natural protein. Agricultural waste used for SCP proves to be a cheap and economic source and also helps management of waste . In the present study, orange and beetroot fruit waste was used as a substrate to grow *Saccharomyces cerevisiae* and *Aspergillus niger* to obtain SCP. Protein estimation was done by Folin Lowry method and determination of wet and dry weight was estimated and SCP produce calculated. The SCP produced will be a convenient feeding source for poultry, fisheries and animal farms.

Keywords: SCP, Media optimization, Fermentation, Folin Lowry, Fruit hydrolysate media

1) INTRODUCTION

Single cell protein (SCP) refers to the edible protein extracted from pure microbial cultures. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as an ingredient or a substitute for protein-rich foods, and is suitable for human consumption or as animal feeds (Mahmood Khan Yousuf., 2012). It can be used as a protein supplement for both humans or animals. It can provide the best protein supplemented food for undernourished children.

In India, most of the protein is derived from cereals with relatively low digestibility and quality unlike SCP produced by microbes. Unicellular microorganisms like algae, yeast, bacteria, have very high nutritive value due to high concentration of essential aa in their biomass. These microbes can be grown using inexpensive substrates like agricultural waste. SCPs are the dried cells of microorganisms, which are used as protein supplements. Another advantage with SCP is that it is rich in essential amino acids like lysine, methionine which are limiting in most plants and animals' foods (Gour Suman et al., 2015).

Microorganisms use the agricultural waste as a carbon and nitrogen source and convert them into high-quality proteins. SCP offers appreciable solution to the problem of protein deficiency which is increasing as the population increases (Omar and Sabry, 1991). Algal protein is widely known but is not easily digestible and requires warm conditions for growth along with plenty of sunlight. Thus yeast protein is widely acceptable as it has a larger size, low N.A content, high lysine content and less accumulation of toxins (Singh Nee Nigamet al., 2009). So it will be beneficial to focus on yeast single cell protein rather than bacterial and algal single cell protein. The agricultural waste is approximately 50 million tonnes per annum which is a rich source of carbohydrates that support the growth of microorganisms (Adoki.A et al, 2008). Agricultural waste used for SCP proves to be a cheap and economic source and also helps management of waste Therefore, the present investigation was carried out to assess the potential of various fruit and vegetable waste for cost effective SCP

production. Orange and beetroot peels were used as a source of agricultural waste or substrate for SCP production.

The aim is to manage and reuse fruit and vegetable waste to manufacture SCP. The protein can be used as an additive added to the main diet instead of sources known very expensive such as soya bean and fish. Some of the objectives are as follows, to overcome the shortage of protein-rich food. To reuse agricultural waste as a substrate to control waste disposal problem to some extent. To produce inexpensive protein-rich food for humans and animals. Commercial production of SCP to meet the increasing demand for protein-rich food.

The consumption of microorganisms by humans is not a revolutionary step because intentionally or unintentionally the intake of microorganisms has been an old practice in commonly fermented products such as cheese, alcohol, yoghurt. The population demands for good economic products and waste management.

2) MATERIALS AND METHODS

2.1) Sample collection

Orange peels and beetroot peels were collected from local market. 20g of each fruit peels were weighed and further used for extraction purpose

2.2) Culture preparation

The microorganisms used to ferment fruit wastes was Saccharomyces cerevisiae and Aspergillus niger. The yeast used was baker's yeast in the form of beads and A.niger was sub cultured on SAB Slant.

2.3) Media

SAB Slant was used for sub culturing A.niger . Two media parameters were checked w.r.t SCP production from microorganisms. 1) Fruit hydrolysate media (with and Without glucose) – minimal media where carbohydrate source was studied and 2) Nitrogen source (Fruit hydrolysate media with urea) was prepared.

2.4) Preparation of sample hydrolysate (extract)

The collected fruit peels (Orange and beetroot) were cut into small pieces and were used as substrate for production. 20g of each sample were weighed using a weighing balance. 100ml of 10% HCl (10ml conc HCl +90ml Distilled water) was added to each waste respectively. The solution was placed in water bath for 1hr. After being allowed to cool, it was filtered using Muslin cloth. The prepared sample hydrolysates were stored in brown bottles for further analysis. The hydrolysates thus prepared were used as carbon and Nitrogen source for biomass production. These hydrolysates were used as the substrates for the microorganisms selected.

2.5) Preparation of Fruit Hydrolysate medium (For 1L)

2.5.1) Fruit hydrolysate media (without glucose)- Ammonium sulphate(4g), Monopotassium phosphate(2g), magnesium sulphate heptahydrate(1g), sodium chloride(0.1g) and Calcium chloride(0.2g). Distilled water was added to make final volume of 1 litre and the pH was adjusted to 5.5.

2.5.2) Fruit hydrolysate media (with glucose)- Ammonium sulphate(4g), Monopotassium phosphate(2g), magnesium sulphate heptahydrate(1g), sodium chloride(0.1g), Calcium chloride(0.2g) and Glucose(Dextrose)(2g). Distilled water was added to make final volume of 1 litre and the pH was adjusted to 5.5.

2.5.3) Fruit hydrolysate medias with nitrogen source, with urea and without a nitrogen source were also prepared. Medias were autoclaved at 121 °C/15 psi/ 15 minutes and cooled.

2.6) Fermentation and Harvesting of SCP:

2.6.1) *Saccharomyces cerevisiae*: The supplemented fruit hydrolysate medium was prepared. The sample extracts were prepared, taking 2ml fruit hydrolysate sample in 50ml fruit hydrolysate medium .The pH was adjusted to 5.5 . The medium was autoclaved at 121°C for 15 minutes and then cooled. Each medium was added with little amount of S.cerevisiae beads and incubated at 28°C in rotary shaker for 8 days.

2.6.2) *Aspergillus niger*: The supplemented fruit hydrolysate medium was prepared. The sample extracts were prepared, (taking 2ml fruit hydrolysate sample in 50ml fruit hydrolysate medium) .The pH was adjusted to 5.5 . The medium was autoclaved at 121°C for 15 minutes and then cooled. Each medium was added a loopful culture of A.niger and incubated at 28°C in rotary shaker for 8 days.

2.7) Determination of wet weight and dry weight :

Determination of wet weight and dry weight was done to know the better yield and to compare the SCP production in terms of weight. Wet weight was determined on 8th day by taking 10ml of fermented extract in a centrifuge tube and centrifuging at 3000rpm for 20mins. Later the supernatant was discarded and pellet was vortexed and wet weight was measured in a petri plate. Further it was kept for Air dry at RT and the dry weight was Measured the next day.

2.8) Protein Estimation by Folin Lowry :

The protein amount obtained was determined using Folin Lowry's method. Standard Bovine serum albumin was used as a standard, 5 standards were prepared ranging from concentration 40ug/ml to 200 ug/ml.

1) Alkaline C solution

- Solution A = Na2CO3 and NaOH
- Solution B = 0.5% CuSO4 and 1% Sodium Potassium tartarate

2) Folin reagent

Appearance of blue colour and optical density was recorded at 660nm.

3) RESULTS

Fruit Hydrolysate from orange and beetroot peel waste using 10% HCl- All samples were finely crushed and treated with 10%HCl, later keeping them in boiling water bath and purification was done using Muslin cloth . For further purpose they were stored in plastic brown bottles.

3.1) Saccharomyces cerevisiae (with and without glucose)- Inoculation of S. cerevisiae

Taking 50ml media with 2ml fruit hydrolysate and adding little beads of S.cerevisiae. It was further kept for incubation for 8 days for SCP fermentation.

Fig 1: Inoculation of fruit hydrolysate in Glucose.

Fig 2: Inoculation of fruit hydrolysate media containing in media containing no Glucose.

3.1.1) Determination of wet weight and dry weight

Sample	Wet weight (grams)	Dry weight (grams)
Orange with glucose	0.18	0.01
Orange without glucose	0.12	0.01
Beetroot with glucose	0.16	0.01
Beetroot without glucose	0.24	0.01

Fig 3: Dry Weight.

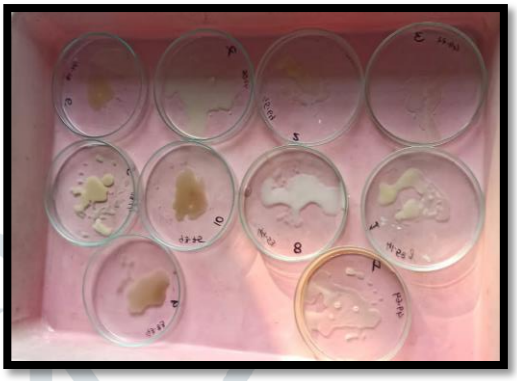
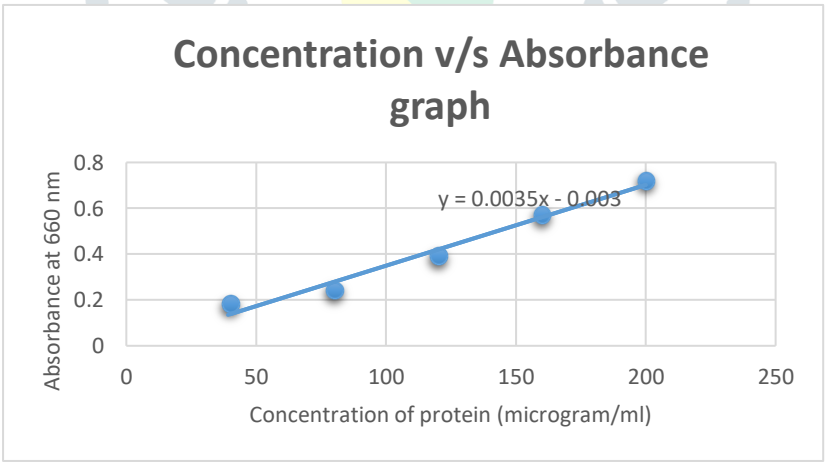


Fig 4: Wet weight

After Incubating the extract for 8 days, for preparing the sample for chemical estimation pellet was obtained by centrifuging 10ml extract at 3000rpm for 20 mins. Determination of wet weight and dry weight was carried out of the pellet obtained and was further used for Folin Lowry-chemical estimation of SCP.

3.1.2) Chemical estimation by Folin Lowry- 5 standards of concentration 40 to 200 ug/ml with an interval of 40 were prepared . Absorbance was recorded at 660nm. Samples were 1:10 diluted. The dried pellets are dissolved in 1ml supernatant to perform Folin Lowry on 8th day. The sample was 1:10 diluted (0.9ml distilled water + 0.1ml sample). Standard used was Bovine serum albumin.



Concentration (ug/ml)	1:10 dilution	Conc.x10 (ug/ml)
Orange with glucose	95.14	951.4
Orange without glucose	32.28	322.8
Beetroot with glucose	89.42	894.2
Beetroot without glucose	95.14	951.4

3.2) Aspergillus niger (with and without glucose)- Inoculation of A.niger

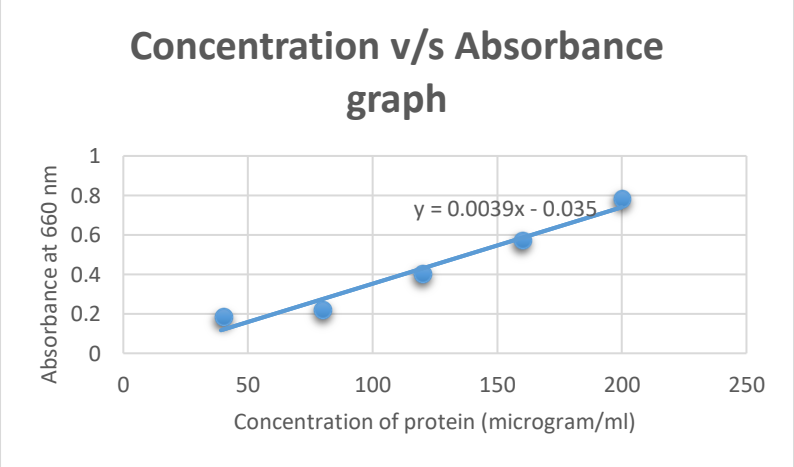
Taking 50ml media with 2ml fruit hydrolysate and a loopful of A.niger culture was Incubated for 8 days. Dense Growth was seen in all flasks in the form of fungal spores. It shows that organism has utilized all the nutrients along with fruit hydrolysate and was able to produce SCP.

Fig 5: Dense growth seen after fermentation

3.2.1) Determination of wet and dry weight

Sample	Wet weight (grams)	Dry weight (grams)
Orange with glucose	0.76	0.02
Orange without glucose	0.70	0.02
Beetroot with glucose	0.89	0.03
Beetroot without glucose	0.70	0.02

3.2.2) Chemical estimation by Folin Lowry-



Concentration (ug/ml)	1:10 dilution	Conc.x10 (ug/ml)
Orange with glucose	96.15	961.5
Orange without glucose	80.76	807.6
Beetroot with glucose	170.5	1705
Beetroot without glucose	157.6	1576

3.3) Saccharomyces cerevisiae (for nitrogen source)- Inoculation of S. cerevisiae

Nitrogen parameter was studied by two sources, fruit hydrolysate media without ammonium sulphate (instead of that 2ml urea was used) and another where fruit hydrolysate media without ammonium sulphate and Without fruit hydrolysate. Beads of S.cerevisiae were inoculated in the medium and kept for incubation for 8days. Growth was seen in all flasks as the solution in the flask became turbid showing growth if microorganism.

Fig 6: Inoculation of *S.cerevisiae* in Nitrogen source media

3.3.1) Determination of wet and dry weight

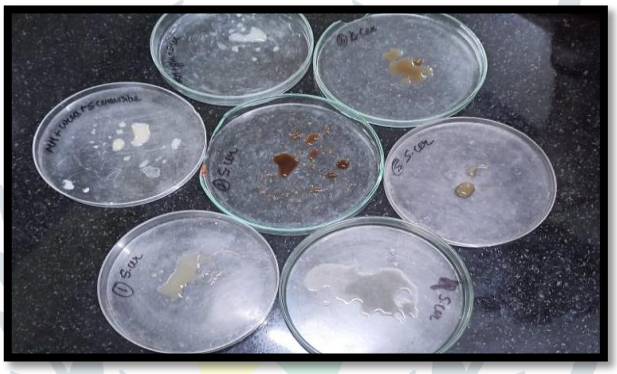


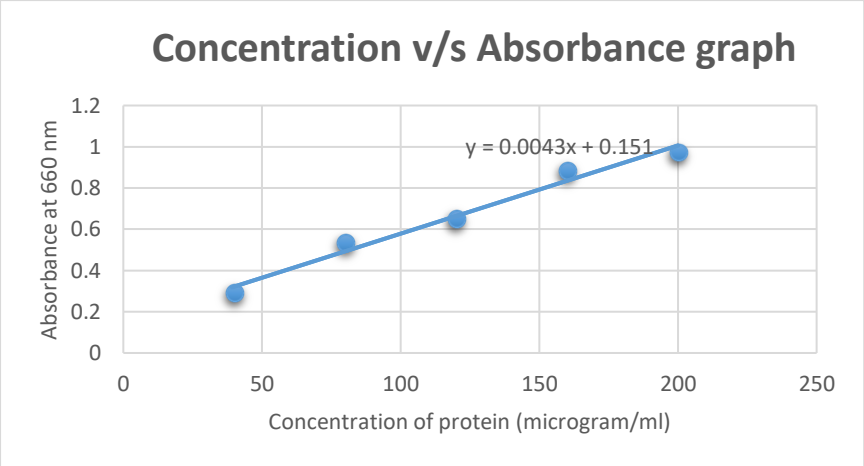
Fig 7: Wet weight

As all flasks showed turbidity, growth of organism was confirmed. For Weight determination, pellet was obtained by centrifuging the extract and was weighed (wet weight) and further was dried overnight for dry weight determination.

Sample	**Wet weight (grams)**	**Dry weight (grams)**
Orange hydrolysate	0.17	0.03
Beetroot hydrolysate	0.13	0.03
Media with urea	0.16	0.04
Media with NH4SO4	0.20	0.03

3.3.2) Chemical estimation by Folin Lowry

5 standards of concentration 40 to 200 ug/ml with an interval of 40 were prepared. Absorbance was recorded at 660nm. Samples were 1:5 diluted. The dried pellets are dissolved in 1ml supernatant to perform Folin Lowry on 8th day. The sample was 1:5 diluted (0.4ml distilled water + 0.1ml sample). Standard used was Bovine serum albumin.



Concentration (ug/ml)	1:5 dilution	Conc.x5 (ug/ml)
Orange hydrolysate	71.86	359.3
Beetroot hydrolysate	127.6	638
Media with urea	11.39	56.95
Media with NH4SO4	4.41	22

3.4) Aspergillus niger (for nitrogen source)- Inoculation of A niger

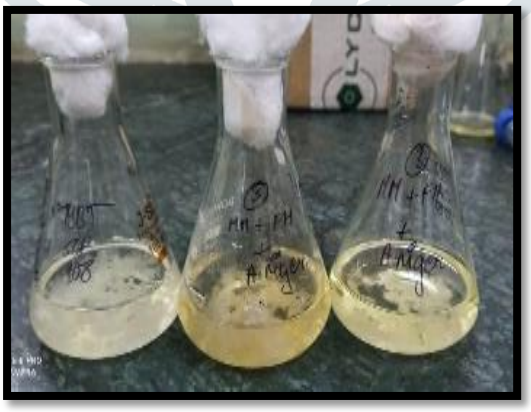


Fig 8: Growth of *A.niger* after fermentation

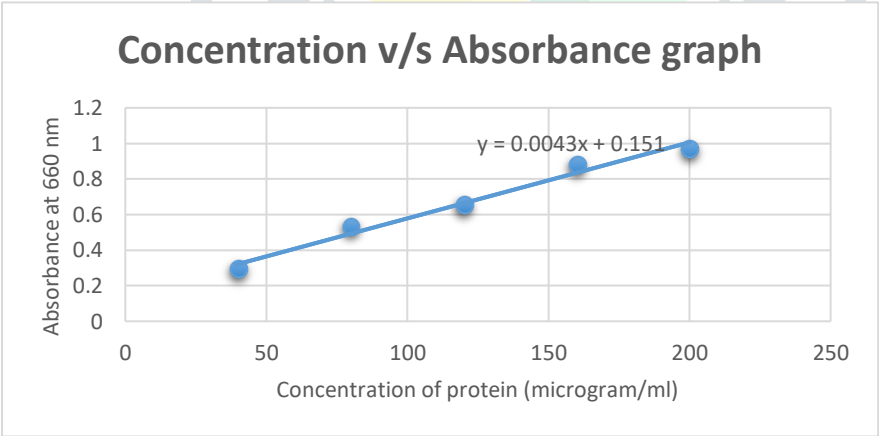
3.4.1) Determination of wet weight and dry weight

As all flasks showed turbidity, growth of organism was confirmed. For chemical estimation, pellet was obtained by centrifuging the extract and was weighed (wet weight) and further was dried overnight for dry weight determination.

Sample	Wet weight (grams)	Dry weight (grams)
Orange hydrolysate	2.76	0.04
Beetroot hydrolysate	1.85	0.04
Media with urea	0.04	0.04
Media with NH4SO4	0.07	0.03

3.4.2) Chemical estimation by Folin Lowry :

Concentration (ug/ml)	1:5 dilution	Conc.x5 (ug/ml)
Orange hydrolysate	139.7	698.5
Beetroot hydrolysate	201.2	1006
Media with urea	73.07	365.35
Media with NH4SO4	80.76	403.8



4) CONCLUSION

At earlier times, wastes were treated with various chemicals for their decomposition but this method was not satisfactory for better disposal. Now it is possible to convert various waste into valuable food or feed products for humans and animals which is not only an environment friendly way but also a healthy business activity. SCP is a bulk of dried cells produced by unicellular microorganisms. It can be termed as bio protein, microbial protein etc. Unlike plant sources, their production is independent of seasonal and climatic variations and can be produced throughout the year.

In this experiment, 2ml of sample extracts were added in the fruit hydrolysate media and the pH was adjusted to 5.5. 1ml of microbial culture was added to the same and kept for incubation for 8 days in a rotary shaker. Fungi and yeast were used for this which are Aspergill1us niger and Saccharomyces cerevisiae respectively.

The ability of these microorganisms to utilize glucose and nitrogen from fruit hydrolysate and produce desired product was checked. Quantitative estimation of the protein produced by the microbes was done by performing Folin Lowry assay and the graphs for concentration of std. protein v/s absorbance at 660 nm were plotted. 2 samples were taken namely, orange peels and beetroot peels.

From the results obtained, it is seen that, S. cerevisiae produced 359.3 micrograms/ml protein by using orange peel hydrolysate and 638 micrograms/ml protein using beetroot peel hydrolysate as a nitrogen source which is far greater than the media supplemented with urea (56.95 micrograms/ml), and media supplemented with NH4S04 (22.05 micrograms/ml) and it produced 951.4 micrograms/ml of protein by using beetroot fruit peel as a glucose source which is greater than he protein produced by media supplemented with glucose (894.2 micrograms/ml). A. niger produced 698.5 micrograms/ml protein using orange peel hydrolysate and 1006 micrograms/ml protein using beetroot peel hydrolysate as a nitrogen source which is again greater than the protein produced by the organism in media supplemented with urea (365.35 micrograms/ml), and media supplemented with NH4SO4 (403.8 micrograms/ml). Therefore, it can be concluded that Saccharomyces cerevisiae and Aspergillus niger can efficiently use orange and beetroot waste as their main substrate for protein production.

We can say that agricultural waste can be efficiently used as an inexpensive substrate for production of SCP from fungi. SCP so produced can be further purified, dried and made into a powdered form to be commercially used as a protein supplement for humans and feed for animals.

5) FUTURE PROSPECTS

The production of Single Cell protein from fruits and vegetable waste is cost beneficial and environment friendly. As there is an increase in Population the demand for proteinaceous food will also increase. To meet such demand, SCP i.e protein derived from microorganisms can be a useful method. Further many tests can be done in future related to SCP such as confirmatory quantification tests through HPLC where all information related to protein can be studied. Also PAGE can be performed to know the number of proteins present because more than one protein can be present. It will also help to reduce pollution. Malnourished people can benefit from protein supplement. A convenient feeding source for poultry, fisheries, and animal farms. SCP is currently produced from a limited number of microbial species, particularly when considering human consumption. The range of sources for SCP used in animal feed is broader than that approved for human consumption and is expanding. SCP is frequently seen as a potential coproduct that could strengthen the economic potential of an otherwise unprofitable bio refinery process, as well as a means of reducing the downstream processing costs required to dispose of process waste.

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