

# Comparative Study of Production of Single Cell Protein from Different Agricultural Waste Substrates using *Aspergillus Niger*

Abhishikta Dasgupta, Jasmine Chughasrani

Thadomal Shahani Engineering College, University of Mumbai, Mumbai, Maharashtra, India

## ABSTRACT

Single-cell protein (SCP) also referred as microbial protein is defined as protein derived from cells of microorganisms such as yeast, fungi, algae, and bacteria, which are grown on various carbon sources for synthesis. The dried cells of microorganisms or the whole organism is harvested and consumed. In this work SCP was produced from different agricultural waste substrates like food and vegetable waste, rice husk, pulses husk, bagasse and wheat straw using *Aspergillus niger*. These substrates not only act as nutritive supplement but also ensure good waste management. Also, carbohydrate content of each sample was determined. For maximizing the yield of SCP, some factors were optimized. Various buffers were used like phosphate buffer, carbonate-bicarbonate buffer and 0.1N NaOH. The sample that shows the best result for SCP was identified to be MCD and fruit and vegetable waste in 50:50 ratio and rice husk. In the future SCP could be produced to not only be used to produce protein but multiple products rich in carbohydrate, vitamins, lipids and other amino acids. Also yield could be increased by genetically modifying SCP organisms.

**KEYWORDS:** Single cell protein, substrates, *Aspergillus niger*

**How to cite this paper:** Abhishikta Dasgupta | Jasmine Chughasrani "Comparative Study of Production of Single Cell Protein from Different Agricultural Waste Substrates using *Aspergillus Niger*" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-2, February 2021, pp.99-102, URL: www.ijtsrd.com/papers/ijtsrd38339.pdf



IJTSRD38339

Copyright © 2021 by author(s) and International Journal of Trend in Scientific Research and Development Journal. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0) (<http://creativecommons.org/licenses/by/4.0>)



## INTRODUCTION

Ever increasing demand for food and feed protein for human and animal consumption has led to research and production of single cell protein (SCP) or also referred to as microbial protein. SCP is defined as protein derived from microbial such as yeast, fungi, algae & bacteria which are grown on various carbon substrates for synthesis. The dried cells of micro-organisms or whole organism is consumed.

SCP could be an option for food consumption, because it is relatively safe and could be mass produced under harsh conditions. [1] Substrates that are used by micro-organisms as a carbon source could be from waste products like whey, sulphite waste liquor and agricultural waste or high energy substrates like n-alkanes, natural gas (methane), methanol and ethanol.[2]

In this paper SCP was produced from different agricultural waste substrates like food and vegetable waste substrates like food and vegetable waste, rice husk, pulses husk, bagasse and wheat straw as a carbon source and using *Aspergillus niger* as a microbial source. These agricultural waste substrates not only act as a nutritive supplement but also ensure good waste management.

## 1. Methodology

### 1.1. Media Preparation

Modified Czapek Dox Medium was made by dissolving in 1000 ml of distilled water and was then autoclaved at 121°C

for 15 mins. The media was cooled down to room temperature and was kept in the fridge till further use.

### 1.2. Pretreatment methods

#### 1.2.1. For Fruit and vegetable waste

Fruit and vegetable wastes like Peels of banana, orange, and pumpkin were collected. All the fruit peels were taken and allowed to dry in the sun for a week and later were further dried in an oven. The fruit peels were transferred to an electric grinder and crushed well into a paste. These food wastes which include their peels or mesocarp were washed several times with sterile, distilled water and dried before weighing and blending with distilled water in the ratio 1:4. The blended fruit wastes were passed through muslin cloth to trap solids residues, and leave behind the fruit waste broth. 100 mL of each food waste filtrate was transferred in to 250mL Erlenmeyer flasks. The samples prepared were autoclaved at 121°C for 15 min. The samples were prepared in duplicates and designated as Food Waste Medium (FWM).[3]

#### 1.2.2. For Rice Husk

Rice husk powder was procured from a store. It was pre-treated to remove lignin and to expose the inner cellulose fibers to cellulolytic attack by the organism. The pre-treatment process was carried out by adding one litre of 1% sodium hydroxide to 100 g of rice husk powder and the pH was adjusted around 5.6 by adding the required acid or base.

It was then filtered using filter paper and transferred to conical flasks. The medium was then autoclaved at 121°C and at 15 psi for 15 min. [4]

### 1.2.3. For Wheat Straw

The wheat straw was washed three times with tap water to remove some of the surface dust, possible contaminants and water soluble materials, then dried at 80°C. 27 g of dried straw was taken and powdered in an electric grinder. The powder was treated with 1% NaOH and was washed with water. The pH was adjusted around 5.6 by adding the required acid or base. The medium was autoclaved at 121°C and at 15 psi for 15 min. The flasks were kept in shaker incubator for 1 week.[5]

### 1.2.4. For sugarcane bagasse

Substrate sugarcane bagasse used in this study was collected from a local sugarcane juice vendor. It was washed with distilled water several times and dried in an oven at 80 °C for 24 h and then ground in an electric grinder. To study the influence of alkaline pre-treatment of sugarcane bagasse on destruction of its lignocellulosic structure and thus enhancement of protein production, the substrate was pre-treated using NaOH solution. The powder obtained was strained twice through a muslin cloth. 25 grams of this powder was taken in a 500ml conical flask and 400ml distilled water was added to it. The flask was placed on a shaker at 150 rpm for 1 week. After a week the contents of the flask were filtered through filter paper. The filtrate was taken and 8 g of sucrose powder was added to it. 8. The pH was adjusted around 5.6 by adding the required acid or base. The medium was autoclaved at 121°C and at 15 psi for 15 min. [6]

### 1.2.5. For Pulses Husk

The waste of green gram and Bengal gram husk was collected. It was then powdered in an electric grinder. 50 ml of 10% (w/v) HCL was added to the each sample (40 gm) in conical flask respectively. The mixture/solution was placed in water bath at 100° C for one hour. After allowing it to cool, it was filtered through Whatman filter paper. The pulses husk solution was diluted with sterile distilled water. The pH was adjusted around 5.6 by adding the required acid or base. The medium was autoclaved at 121°C and at 15 psi for 15 min. The sterile solution/broth thus prepared was used as carbon and nitrogen source for biomass production.[7]

### 1.3. Inoculum

Freshly prepared culture of *A.niger* containing mycelia was used.

## 2. Fermentation and Analytic methods

### 2.1. Standardization of protein extraction buffer

Different buffers namely; citrate (pH 5.0), phosphate (pH 7.0) and carbonate-bicarbonate (pH 10.0) were used for the extraction of total proteins from *A. niger*.

#### 2.1.1. Fermentation process

First 11 conical flasks were taken and labelled properly. Into each flask media was transferred aseptically such that the total volume was 100ml in each flask using sterilised pipettes as follows:

In the first flask 100ml of Modified Czapek Dox (MCD) was added which was considered as the standard.

In the next flask 50ml of Fruit waste media was taken and 50ml of MCD media was added to it. In another flask only 100ml of Fruit waste media was taken.

Similarly, in the next flasks 50 ml of MCD and 50 ml of other agricultural waste substrates (rice husk, wheat straw, pulses husk and bagasse) were added separately and 100 ml of each of the other substrates were added separately.

Freshly prepared *A.niger* culture grown in SDB media was taken using pre-sterilised tips and 2ml of it was transferred maintaining aseptic LAF conditions into each of the 11 flasks mentioned above. All the flasks were kept on a shaker at 120-150 rpm and 28±2° C for eight days before harvesting.

After 8 days, the fungal mycelia was harvested by centrifugation at 4000 rpm for 5 mins and then the pellet was collected.

Using phosphate buffer, the pellet was homogenised at 4000 rpm by centrifuging it for 45 mins. The supernatant was collected for estimating protein content. The same procedure was followed using carbonate-bicarbonate buffer. In case of NaOH buffer, first 0.1 NaOH was added to the mycelial mass followed by boiling it at 80°C for 5 mins. Then it was centrifuged at 4000 rpm for 45 mins and the supernatant was collected.[4]

## 3. Protein Estimation

The extract obtained for each substrates was used to estimate its protein content. Protein content was estimated using Folin-Lowry method.[8]

## 4. Carbohydrate estimation

Crude extract obtained for each of the substrates was used to estimate its carbohydrate content. Carbohydrate content was estimated using Anthrone's test.[9]

## 5. Result

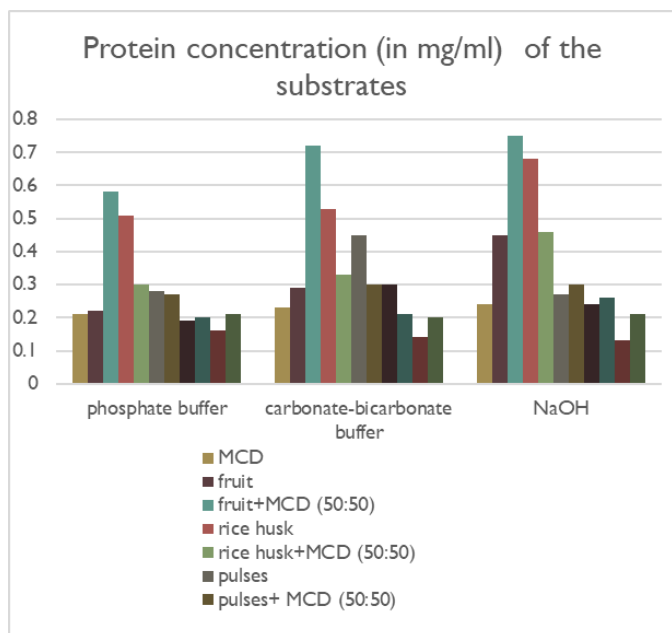
### 5.1. Protein concentration

Table 1 represents the concentration of protein found in phosphate buffer, carbonate-bicarbonate buffer and 0.1N NaOH in mg/ml.

Substrate	Phosphate buffer	Carbonate-bicarbonate buffer	0.1N NaOH
MCD	0.21	0.23	0.24
FWM	0.22	0.29	0.45
MCD+FWM (50:50)	0.58	0.72	0.75
Rice husk	0.51	0.53	0.68
MCD+Rice husk (50:50)	0.3	0.33	0.46
Pulses husk	0.28	0.45	0.27
MCD+Pulses husk (50:50)	0.27	0.3	0.3
Bagasse	0.19	0.3	0.21
MCD+ Bagasse (50:50)	0.2	0.21	0.26
Wheat straw	0.16	0.14	0.13
Wheat straw+ MCD (50:50)	0.21	0.2	0.21

**Table 1: Concentration of Protein in phosphate, carbonate-bicarbonate and 0.1N NaOH buffer**

The figure 1 shown shows three graphs explaining the comparison of protein concentration of various substrates in phosphate, carbonate-bicarbonate and 0.N NaOH buffer.



**Figure 1: Three graphs showing the comparison of protein concentration of various substrates in phosphate, carbonate-bicarbonate and 0.N NaOH buffer.**

It was observed that the highest protein concentration was in the substrate containing 50% fruit and 50% MCD in 0.1N NaOH and the least protein concentration was seen in the case of wheat straw in 0.1N NaOH.

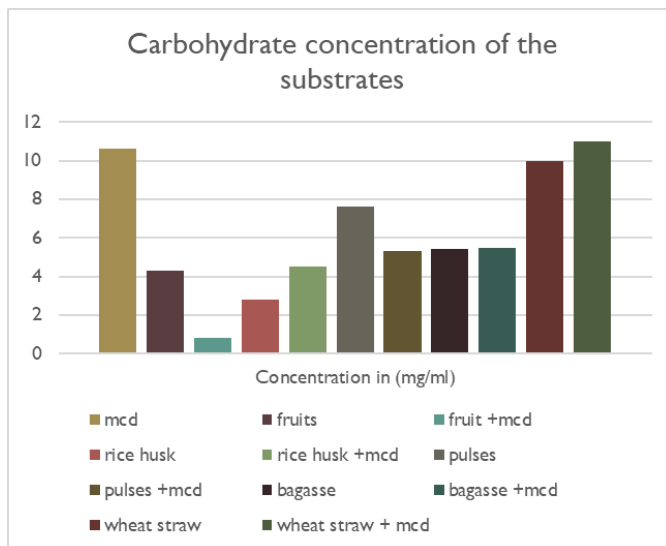
**5.2. Carbohydrate estimation**

Table 2 represents concentration of carbohydrate found for each substrate in mg/ml.

Substrate	Concentration	Substrate	Concentration
MCD	10.6	Pulses husk+MCD (50:50)	5.3
FWM	4.3	Bagasse	5.4
MCD+FWM (50:50)	0.84	Bagasse+MCD(50:50)	5.5
Rice husk	2.8	Wheat straw	10
Rice husk+MCD (50:50)	4.5	Wheat Straw +MCD(50:50)	11
Pulses husk	7.6		

**Table 2: concentration of carbohydrate found for each substrate.**

The highest concentration of carbohydrate was seen in 50% wheat straw and 50% MCD whereas 50% Fruit and 50% MCD had the least amount of carbohydrate. Figure 2 shows a bar graph representing the comparison of carbohydrate concentration of various substrates.



**Figure 2: Bar graph showing the comparison of carbohydrate concentration of various substrates**

**6. Conclusion and Discussion**

The best buffer overall for most substrates was found to be 0.1N NaOH. The highest protein concentration observed was seen in 50% Fruit and 50% MCD, which also had the lowest carbohydrate concentration. Also, the highest concentration of carbohydrate was seen in 50% wheat straw and 50% MCD. It is also observed that 50% wheat straw and 50% MCD had the lowest protein concentration. Hence, it can be concluded that protein and carbohydrate concentrations are inversely proportional to each other.

For our report the SCP Production could be optimised by exposing the substrates to different pH conditions or changing the source of organism for example yeast.

Microalgae are currently used mainly in the form of supplements, available in tablet, capsule or liquid form, but they are increasingly also processed as ingredients which can be included in pastas, baked goods, snacks, and so on. The most accessible commercial products are derived primarily from *Arthrospira platensis* and *Arthrospira maxima* (sold as spirulina), *Chlorella*, *Dunaliella salina* and *Aphanizomenon flos-aquae*. Yeast is another micro-organism that is extensively used for production of SCP

In future, SCP production could be a new doorway for profitable possibilities. Such as in some operations the production of the metabolite may be the primary reason for conducting the fermentation. In this sense, the SCP produced can almost be considered a by-product whose sale helps improve the economics of the operation.

Applications of SCP Production in the future may involve:

**6.1. Multiproduct Fermentations**

There are various by-products released from SCP fermentations they could be rich sources of carbohydrates, lipids, amino acids and other co-factors. The kinds of by-products vary with the type of micro-organism and substrate involved in the fermentation process of SCP Production.

**6.2. Genetic Modification of SCP Organisms**

**6.2.1. Mutation, Selection, and Breeding**

Numerous microbial products of present and future commercial importance could be produced from SCP-quality microorganisms. Mutation and selection techniques can also

be used to improve the protein content of SCP organisms. This is an important task because the value of the biomass will be largely determined by its "crude protein" content. The development of techniques to select high protein mutants has been difficult because there are very few markers for overall higher intracellular protein concentration. It is observed that *Saccharomyopsis lipolytica* mutants with higher overall protein content than the wild type were produced by the use of continuous n-alkane fermentation. By using highly aerated culture conditions in a chemostat, a mutant with a more efficient phenotype appeared that displaced the wild-type strain in as little as a few hundred hours after inoculation of the system with the wild type. The cells of the more efficient mutant phenotype contained 10% more nitrogen than wild type cells and had lost the capacity to form a pseudomycelium — a property which subsequently proved to be easily detectable in sugar-containing agar plates.

### 6.2.2. Protoplast Fusion

Under laboratory conditions, the fusion process is usually enhanced by adding polyethylene glycol (PEG) to the protoplast suspension. PEG causes extensive aggregation and membrane contact between adjacent protoplasts. Fusion follows soon thereafter, and within hours the cytoplasm integrate to form a recombinant entity having nuclei derived from different sources — a heterokaryon. As cell walls form, the resulting cell having a mixture of nuclei is often referred to as a heterokaryocyte. This technique is nonspecific, inasmuch as protoplasts from identical cells, different species, different genera, and even plant-animal, animal-fungal, and plant-fungal interkingdom fusions have been successfully accomplished. The fusion products are then selected for desired phenotypes by techniques similar to those used in mutation programs. This form of genetic improvement of phenotypic versatility is exemplified by the fusion of *Saccharomyces cerevisiae* with *Saccharomyopsis lipolytica*.

### 6.2.3. Plasmid-Assisted Molecular Breeding (PAMB)

The greatest advantage of applying the techniques used in molecular biology to strain improvement programs is the specificity with which a particular cellular component can be improved or introduced de novo. The combination of endogenous plasmid transfer, recombinant DNA technology, and more conventional selection methods (e.g., chemostat selection). The greatest advantage of applying the techniques used in molecular biology to strain improvement programs is the specificity with which a particular cellular component can be improved or introduced de novo. The combination of endogenous plasmid transfer, recombinant DNA technology, and more conventional selection methods (e.g., chemostat selection).

The first application reported for recombinant DNA technology outside the biomedical field: the improvement of the nitrogen-assimilation efficiency of *Methylophilus methylotrophus* by replacing the endogenous system with the efficient *E. coli* glutamate dehydrogenase gene. Subsequent work led to the expression in *M. methylotrophus* of the chicken ovalbumin and mouse dihydrofolate reductase genes, and of a chemically synthesized human  $\alpha$ 1 interferon gene. These results were originally made possible by the development of small, high-copy-number, wide-host-range plasmids, and similar vectors may find use in other SCP-

quality microorganisms. The excellent results reported in these studies with *M. methylotrophus* are very encouraging in that they allow us to envision the near-term, industrial-scale use of SCP organisms with a high level of wholesome protein that coproduce high-value products such as fine chemicals and pharmaceuticals.

### References

- [1] Anneli Ritala, Suvi T. Häkkinen, Mervi Toivari and Marilyn G. Wiebe\*Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016, Front. Microbiol., 13 October 2017, <https://doi.org/10.3389/fmicb.2017.02009>. J. Clerk Maxwell, A Treatise on Electricity and Magnetism, 3rd ed., vol. 2. Oxford: Clarendon, 1892, pp.68–73.
- [2] K. Spalvins, K. Ivanovs and D. Blumberga, Single cell protein production from waste biomass: review of various agricultural by-products, Agronomy Research 16(S2), 1493–1508, 2018 <https://doi.org/10.15159/AR.18.129>.
- [3] OSHOMA, CE; EGUAKUN-OWIE, SO Conversion of Food waste to Single Cell Protein using *Aspergillus Niger* J. Appl. Sci. Environ. Manage. Vol. 22 (3) 350 – 355. March 2018K. Elissa, "Title of paper if known," unpublished.
- [4] ANUPAMA and RAVINDRA, Pogaku. Studies on production of single cell protein by *Aspergillus niger* in solid state fermentation of rice bran. Braz. arch. biol. technol. [online]. 2001, vol.44, n.1 [cited 2019-10-24], pp.79-88.
- [5] D. S. Chahal, M. Moo-Young, G. S. Dhillon, Bioconversion of wheat straw and wheat straw components into single-cell protein, Canadian Journal of Microbiology, 1979, 25:793-797, <https://doi.org/10.1139/m79-116>
- [6] Ashfaq M, Solomon S and Pathak N. Enhanced Protein Production by *Saccharomyces cerevisiae* by Exploitation of Pretreated Sugarcane Bagasse as Inexpensive Substrate. Int J Anim Sci. 2019; 3(1): 1042.
- [7] S. Anbuselvi, Surabhi Mahalanobis, Manas Jha Optimization of Single Cell Protein Using Green Gram Husk and Bengal Gram Husk Using Yeast Int. J. Pharm. Sci. Rev. Res., 28(1), September – October 2014; Article No. 35, Pages: 188-190
- [8] Chang-Hui Shen, Chapter 8 Quantification and Analysis of Proteins, Diagnostic Molecular Biology, Elsevier, 5 April 2019, <https://doi.org/10.1016/B978-0-12-802823-0.00008-0>
- [9] Timothy R. Parsons, Yoshikaki Maita, Carol M. Lalli, Chapter: 3.2 Determination of Total Particulate Carbohydrate by Anthrone Reagent, A Manual of Chemical & Biological Methods for Seawater Analysis, Elsevier, 1984.
- [10] Tusé D. Single-cell protein: current status and future prospects. Crit Rev Food Sci Nutr. 1984;19(4):273-325. doi:10.1080/10408398409527379