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Single cell protein: Management and utilization of agricultural and horticultural waste and production of supplemental protein

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Abstract
Major portion of the world's population is mainly depends on the agriculture. The high production of agricultural commodity also causes the high production of waste like peels of fruits, bagasse in sugarcane, waste remains in vegetables after use, parts remained after harvesting the crop, waste produced during of agricultural production. The disposal of agricultural and horticultural waste is a serious problem and their deposition poses health hazard for all the living beings. People in the third world and developing countries are suffering from menace of protein deficiency in their diet resulting in serious protein energy malnutrition problems. The situation demands exploration of unconventional protein source to fortify human food. The protein from microbes is cheap, easy to obtain in crude form, nutritive and can be made available as food or feed to increase its nutritive value. Therefore the bioconversion of agricultural and horticultural waste into certain valuable products like single Cell protein has the ability to solve the world wide food protein deficiency by obtaining an economical product for food or feed. However using waste as substrate for the production of high nutritious product may also alleviate the problems related to waste management.

Keywords: single cell protein, agricultural and horticultural waste, waste management and utilization

Introduction

Major portion of the world's population depends on agriculture and every year million tones of agricultural commodities are produced. The utilization of these commodities in different forms (processed form) leads to production of tones of agricultural waste (polishing of grains, bagasse in sugarcane, pods in pulses, plant part remains after harvest, etc) and horticultural waste (peels of fruit, annual plants, vegetables waste, annual crop remains after harvest, damaged fruits and vegetables, etc). The disposal of waste is a serious problem and their deposition poses health hazard for all the living beings [Khan, M. *et al*., (2009, 2010)] [6, 7]. Proteins are the essence of life processes and are important for proper growth and development of all the living beings. Its deficiency may lead to a number of health hazards in an individual especially those who are living below poverty line is suffering from malnutrition. There is a huge gap between the demand of protein-rich food and its supply to the ever increasing world population. In order to achieve this gap, Single cell protein is an innovative and an alternative way to this direction. Although animal proteins are considered to be best quality proteins [Saima, *et al*., (2008)] [13]. However microbial protein also known as single cell protein grown on agricultural wastes is one of the important optional proteins because of higher protein content and very short growth cycle [Bekatorou, *et al*., (2006)]. The novelty of unwanted waste product consumption added a new economic incentive to Single Cell Protein production, as the idea of zero cost substrates, or even the generation of additional revenues through the concept of waste treatment were argued and incorporated to reduce the production cost estimates Khan, M. *et al*., (2010) [7]. The benefits of Single Cell Protein production were thus extended from the production of food to the preservation of the environment. It is therefore thought imperative to make use of these affordable agri-horti cheap substrates for culturing fungal species which can be harvested as a source of Single Cell Protein to be used as such for human and animal consumption or as a supplement in the food and fodder. Thus, if the waste is utilized regularly and systematically for the production of Single Cell Protein, it will be a source of protein at affordable cost.

Single cell protein introduction

The production of Single cell protein began in the late 1960s. The term single cell protein (SCP) refers to dead, dry cells of micro-organisms such as yeast, bacteria, fungi and algae which grow on different carbon sources. The name "single cell protein" was used for the first time, twenty years ago by the M.I.T. professor Carol Wilson to give a better image than "microbial protein". It can also be called biomass, bioprotein or microbial protein. The single cell protein is a dehydrated cell consisting of mixture of proteins, lipids, carbohydrates, nucleic acids, inorganic compounds and a variety of other non-protein nitrogenous compounds such as vitamins [Dhanasekaran D. (2011)] [3]. Using microorganisms for SCP production showed that the protein content is higher compared to plant and animal proteins with good nutritional value. The usefulness of SCP from microorganisms is of great significance such as, food additives, protein supplement, as sources of human and animal feeds, additives in some chemicals and pharmaceutical products [Ukaegbu-Obi, K.M. (2016)] [16]. The advantages of producing SCP through fermentation process as against the conventional methods are as follows; the process is not affected by weather condition, short generation time, it utilize cheap agricultural residues and limited area of land mass [Yunus, F. *et al.*, (2015)] [19]. Another advantage with SCP is that it is rich in certain essential amino acids like lysine, methionine which are limiting in most plant and animal foods. This protein can be used as additive added to the main diet instead of sources known very expensive such as soybean and fish.

Micro Organisms used in single cell protein production

The selection of certain microbial strain is very important, some of the criteria are: [Ukaegbu-Obi K.M. (2016).] [16]

1. Performance (growth rate, productivity, and yield) on specific low-cost substrates to be used Temperature and pH tolerance
2. Oxygen requirements, heat generation during fermentation and foaming characteristics
3. Growth morphology and genetic stability in the fermentation
4. Ease of recovery, and requirements for further downstream processing
5. Structure and composition of the final product, in terms of protein

Different types of microbes such as bacteria, fungi, mold, algae and yeasts can be used as the sources of SCP.

Algae

They used it as food after drying it. Spirulina is the most widely used algae. Similarly, biomass obtained from Chlorella and Senedessmus is harvested and used as source of food by tribal communities in certain parts of the world. Algae are used as a food in many different ways and its advantages include simple cultivation, faster growth and rich in protein content. Algal single cell protein has limitations such as the need for warm temperatures and plenty of sunlight in addition to carbon dioxide, and also that the algal cell wall is indigestible [Mondal, A.K. (2006)] [10]. Algae grown in ponds can produce 20 tons of protein, per acre, per year [Nigam, N.M. (2000)] [11].

Yeasts and fungi

Yeast is suitable for single protein production because of its superior nutritional quality. The supplementation cereals with single cell proteins, especially yeast, make them as good as animal protein. It has been calculated that 100 lbs of yeast will produce 250 tons of protein in 24h [Nigam, N.M. (2000)] [11]. Many fungal species are used as sources of protein rich food. Among these, most popular are yeast species, *Candida*, *Hansenula*, *Pitchia*, *Torulopsis* and *Saccharomyces*. Many other filamentous species are also used as sources of single cell protein. Cultures of *Fusarium* and *Rhizopus* have been grown in fermentation as a source of protein food. The inoculums of *Aspergillus oryzae* or *Rhizopus arrhizus* is selected because of their non-toxic nature. Saprophytic fungi grow on complex organic compounds and convert them into simple structures. High amount of fungal biomass is produced as a result of growth. Mycelial yield vary greatly which depends upon organisms and substrates. There are some species of moulds, for example, *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium graminearum* which are very dangerous to human, therefore, such fungi must not be used or toxicological evaluations should be done before recommending to use as Single cell protein [Nigam, N.M. (2000)] [11]. Yeasts are probably the most widely accepted and used microorganism for single cell protein. So it will be beneficial to focus on yeast single cell protein rather than bacterial and algal single cell protein [Mondal, A.K. (2006)] [10].

Bacteria

Among bacterial species, Cellulomas and Alcaligenes are the most frequently used bacterial species as a single cell proteins source [Gad, A. S. *et al* (2010)] [5]. Potential phototrophic bacterial strains are recommended for single cell protein production. Generation time of Methylophilus is about 2 hours and this bacterium is used in animal feed; in general produce a more favorable protein composition than yeast or fungi. Therefore the large quantities of single cell protein animal feed can be produced using bacteria [Gad, A. S. *et al* (2010)] [5]. Characteristics that make bacteria suitable for this application include rapid growth of bacteria, short generation times of bacteria - almost can double their cell mass in 20 minutes to 2 hours. They are also capable of growing on a variety of raw materials that range from carbohydrates such as starch and sugars to gaseous and liquid hydrocarbons which include methane and petroleum fractions; to petrochemicals such as methanol and ethanol; nitrogen sources which are useful for bacterial growth include ammonia, ammonium salts, urea, nitrates, and the organic nitrogen in wastes, also it is suggested to add mineral nutrient supplement to the bacterial culture medium to fulfill deficiency of nutrients that may be absent in natural waters in concentrations sufficient to support growth [Suman G.(2015)] [14]. Bacteria are capable of growth on a wide variety of substrates, have a short generation time and have high protein content. Their use is somewhat limited by poor public acceptance of bacteria as food, small size and difficulty of harvesting and high content of nucleic acid on dried weight basis [Mondal A.K. (2006)] [10].Bacteria are usually high in protein (50-80%) and have a rapid growth rate [Nigam N.M. (2000)] [11].

Table 1: Average different composition of the main group of microbes (% dry weight)

Composition	Fungi	Algae	Yeast	Bacteria
Protein	30-40	40-60	45-55	50-65
Fat	2—8	7—20	2--6	1—3
Ash	9—14	8—10	5--10	3—7
nucleic acid	7—10	3—8	6--12	8---12

Agricultural and horticultural waste as a substrate

Wastes can be defined as unwanted materials which are discarded from a variety of sources. Waste refers to anything considered useless, but produced by the same action that produces something useful. It could be a by-product of households, industries, agriculture, mining, commercial, and sundry other ventures, activities or sources. When something is unwanted and no longer serves a purpose, it is generally thought of as waste and discarded. However, the word waste may have different connotations, since what one considers as waste may not be waste to another person. In other words, waste is not completely useless, since what is considered as waste can be recycled to produce another product (Suman, G. (2015) [14]. In recent years, it has been observed that, the global intensification of food production has resulted in producing large quantities of food and generating lot of agricultural wastes (Elijah A.I and Edem V.E. (2017) [4] Improper management of these

wastes can constitute a public health risk and environmental problems, such as diseases and air pollution (Yazid, N.A., *et al* (2017) [18]. Nevertheless, this agricultural waste is rich in organic matters that are natural substrate to microorganisms (Azam, S. *et al* (2014) [2].
For waste, to be a useful substrate for production of microbial protein, it must meet the following criteria [Ukaegbu-Obi K.M. (2016) [16]

1. It should be non-toxic
2. Abundant
3. Totally regenerable
4. Non-exotic, and cheap
5. Able to support rapid growth and multiplication of the organisms resulting in a biomass of high quality.

Over the last few years, a lot of research has been done for reprocessing and reuse of different agricultural wastes for the conversion of valuable and nutritive products. Therefore the present investigation was carried out to assess the potential of various agriculture wastes for cost effective biomass production which can be used in food as such or as animal feed [Mondal, A.K. (2006)] [10]. A number of agricultural and agro industrial waste products have been used for the production of SCP and other metabolites, including orange waste, mango waste, cotton salks, kinnow-mandarin waste, barley straw, corn cops, rice straw, corn straw, onion juice and sugar cane bagasse (Nigam, N.M. (2000) [11], cassava starch, wheat straw, banana waste capsicum powder and coconut water. (Zhao, G. *et al.*, (2010) [20]. The usage of such wastes as a sole carbon and nitrogen source for the production of SCP by microorganisms could be simply attributed to their presence in nature on large scale and their cheap cost [Dhanasekaran, D. (2011)] [3]. The production of single cell protein can be done by using waste materials and inexpensive feedstock as the substrate, specifically agricultural wastes such as wood shavings, sawdust, corn cobs. Conventional substrates such as starch, molasses, fruit and vegetable wastes have been used for single cell protein production [Nigam N.M. 2000)] [11]. Lignocellulosic biomass such as cellulose and hemicelluloses waste is used as a suitable substrate for increasing single cell protein production [Nigam, N.M. (2000)] [11]. These raw materials have been hydrolyzed by physical, chemical and enzymatic methods before. The degree of single cell protein production depends on the type of substrate used and also on media composition [Mondal, A.K. (2006)] [10]. The classical raw materials are substances containing mono and disaccharides, since almost all microorganisms can digest glucose, other hexose and pentose sugars and disaccharides. Agricultural activities and food industry generate considerable quantities of wastes which are rich in organic matter and could constitute new materials for value added products. To this effect, their valorization by the biotechnical processes represents a solution of choice insofar biomass production. A large quantity of solid waste is generated from fruit processing industries. Among these, the orange production is predicted to reach 66.4 million tons by 2010 (Talebnia, F. (2008) [15]. Around half of this amount after extraction of the juice consists of orange peels, segment membranes and seed. The peels hold a range of carbohydrate polymers; makes it idyllic as a source of renewable energy through anaerobic digestion (Talebnia, F. (2008) [15]. Apple pomace, including peels and seeds constitute 25–35% of the quantity of the processed apples. Besides other nutrients, apple pomace is a rich source of pectin and is being used as a natural substrate for pectinase production. Similarly, potatoes are one of the most important staple crops for human consumption. Potato peels are good sources of quality plant carbohydrate. Regarding citrus fruits, it is estimated that out of total annual domestic production (2.02 Million MT) 1.7 Million MT is available for consumption after post-harvest losses.

Following table shows the biomass Production by *Saccharomyces cerevisiae* in various fruit wastes taken in the present investigation [Khan M *et al* (2010)] [7].

Table 2

Substrate	Crude protein percentage (%)
Rind of pomegranate	54.28
Mango waste	39.98
Banana skin	58.62
Sweet orange peel	26.26
Apple waste	50.86

Following table shows the biomass production by *Rhizopus oligosporus* in various fruit wastes taken in the present investigation [Khan M *et al* (2009)] [6].

Table 3

Substrate	Biomass Production (mg/100g of substrate)
Papaya waste	59.5 mg
Cucumber peelings	57.3 mg
Pineapple fruit skin	48.0 mg
Rind of pomegranate	51.6 mg
Skin of watermelon	43.2 mg

Following table shows the biochemical analysis of pineapple waste [Dhanasekaran D. (2011)] [3].

Table 4

Characteristics	Percentage by weight
Moisture	85.00%
Reducing sugar	10.80%
Non reducing sugar	13.00%
Protein	0.60%

Production of single cell protein from waste and waste management

Steps involved in the conversion of Agro-waste into Single Cell Protein

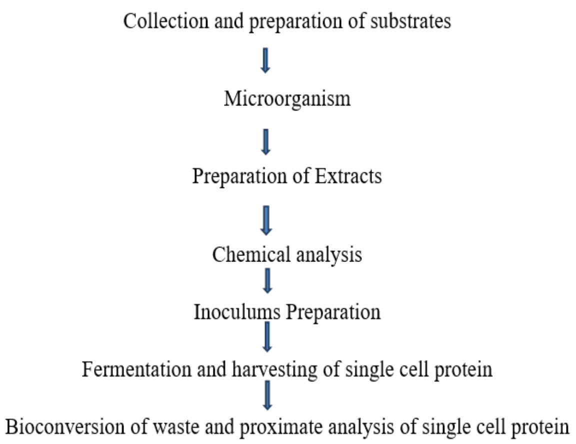

Fig 1

Collection and preparation of substrates

The agricultural and horticultural waste like sugarcane byproducts, pulp, and peel from oranges and lemons, potatoes, apples, carrots, cucumber and oranges were procured from the local markets, collected, washed several times with sterile water and frozen at − 20°C until ready for use. The peels were separated, oven dried, ground and sieved through 1-mm mesh screen. Or wastes were neutralized to pH 7.0 and autoclave-sterilized with 0.1% (final) nitrogen content before use [Mahan, K.M *et al.*, (2018)] [9]. The food wastes which include peels or mesocarp were washed several times with sterile, distilled water and dried before they were weighed and blended with distilled water in the ratio1:4. Theblended fruit wastes were passed through muslincloth to trap solids residues, leaving behind the fruit waste broth. A 100 mL of each food waste filtrate was transferred in to 250mL Erlenmeyer flasks. The samples thus prepared were autoclaved at 121^{o}C for15 min [Oshoma C.E (2018)] [12]. The samples thus prepared were packed in transparent Zip-lock polythene bags and stored at room temperature.

Microorganism

The selection of microorganism is depending on the type of substrate and amount of biomass produced by the microorganisms. Identification of the microorganism's isolate was based on cultural and microscopy characterization following procedures from Barnet and Hunter (1972); and maintained on specific media with respect to microorganisms and stored at 4^{o} [35]. The microorganisms used to ferment wastes are obtained from different sources [Mondal A.K. (2006)] [10]. The selected microbe is cultured on the suitable medium.

Preparation of Extracts

If wastes which are rich in cellulose where used as substrate for production of SCP. Wastes were degraded to convert cellulose content into more available sugars by chemical treatments with little modification to the procedure of Lenihan et al. required amount of 10% (w/v) HCl was added to the each waste in conical flask respectively. The mixture/solution was placed in water bath at 1000C for one hour. After being allowed to cool, it was filtered through Whatman filter paper. Filtrates were diluted with sterile distilled water at varying concentrations and autoclaved at 121^{0}C for 15 mints. The sterile solution/broth thus prepared was used as carbon and nitrogen source for biomass production [Mondal A.K. (2006)] [10].

Chemical analysis

The determination of crude fat, crude fibre and total soluble solids are done. The chemical analysis is done on a small sample of waste. The method for the determination of crude fat, crude fibre and total soluble solids were determined as described by AOAC (2006). Total carbohydrate content was determined using Anthrone reagent. Ash content was obtained by igniting the fruit wastes in a muffle furnace at 550C as previously described by Pearson. Moisture content was determined by the method based on the principle of drying to constant weight. The biomass was expressed in terms of total protein content. The protein estimation was determined according to the method described by Lowry et al [Lowry O.H et al (1951)] [8].

Inoculums Preparation

The inoculums of the selected or suitable microorganism with are going to be used in the fermentation process are prepared and kept ready before fermentation process.

Fermentation

The fermentation of the waste is done in large fomenters, where the microbial inoculums are culture on the agricultural waste. The favorable conditions for fermentation process are maintained.

Bioconversion of fruit waste and harvesting of single cell protein

After fermentation biomass was separated from culture by vacuum filtration and Washed with sterile water. Before taking the weight of the biomass, it was transferred into an aluminum disk and was oven dried at 105^{0}C for one hr followed by cooling in desiccators to balance the temperature and weight. The biochemical constitutes of biomass such as crude protein, total carbohydrate content, reducing sugar and mineral content were separated and collected [Mondal A.K. (2006)] [10].

Conclusion

The management of the agricultural and horticultural waste can be done by single cell protein production. The studies showed that the waste produced from agriculture can be used for protein production as a substrate. The utilization of waste as a substrate for the single cell protein production is solution for both waste management and the protein scarcity.

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