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A CRITICAL REVIEW ON SINGL CELL PROTEIN PRODUCTION USING DIFFERENT SUBSTRATES

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ABSTRACT

Single cell protein or other related substances are basically proteins derived from organic matter using microbial organisms for the industrial production of SCPs various kind of substrates and microbes are used. The main purpose behind this selectivity is search of low cost substrates to reduce production cost and finally the product for better production and cost efficiency many substances were tried by several scientists in laboratory and later on as a pilot project. Present paper deals with the available literature and presents critical review on the subject.

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INTRODUCTION

The worldwide, large-scale development of SCP process has contributed greatly to the advancement of present day biotechnology. Research and development of SCP process has involved work in the fields of microbiology, biochemistry, genetics, chemical and process engineering, food technology, agriculture, animal nutrition, ecology, toxicology, medicine and veterinary science and economics. This involved large number of different kinds of substrates and variety of microbes for the production of single cell protein. Research on single cell protein technology started a century ago when Max Delbruck and his colleagues found out the high value of surplus brewer's yeast as a feeding supplement for animals. During world War first Single cell protein technology proved to be more than useful as Germany used it to replace more than half of its imported protein sources by yeast. In 1919, Sak in Denmark and Hay duck in Germany invented a method named "Zulaufverfahren" in which sugar solution was fed to an aerated suspension of yeast instead of adding yeast to diluted sugar solution. In the 1960s, researchers at British Petroleum developed a technology called "protein-from-oil process" for producing single cell protein by yeast fed by waxy n-paraffins, a product produced by oil refineries (Ageitos *et al*., 2011). The increasing world deficiency of protein is becoming a major problem for humankind. Since early fifties, efforts have been made to explore new, alternate and unconventional protein. For this reason, in 1996, new sources mainly bacteria, yeast, fungi and algae were used to produce protein biomass named single cell protein (SCP). The term SCP was coined in 1966 by Carol L. Wilson. Single cell protein is dried cells of micro-organism, which are used as protein supplement in human foods or animal feeds. Besides high protein content (about 60-82% of dry cell weight), SCP also contains fats, carbohydrate, nucleic acids, vitamins and minerals. Another advantage with SCP is that it rich in certain essential amino acids likes lysine and methionine which are limiting in most plant and animal foods. Micro-organism like bacteria, yeast, fungi and algae utilize inexpensive feedstock and waste to produce biomass, protein concentrate or amino acids. Conventional substrates such as starch, molasses, fruit and vegetable wastes have been used for SCP production, as well as unconventional ones such as petroleum by-products, natural gas, ethanol, and methanol and lignocellulose biomass. The protein obtained from microbial source is designated as "*Single Cell Protein*" (SCP).

In microbial protein production, several natural products have been tested. The use of natural cheap substrates and waste industrial products for cultivating micro-organisms appear to be general trend in studies of applied nature (Grewal *et al*., 1990; Osho, 1995). For the same purposes Haider and EL-Hassy (2000) tested date extract supplemented with nitrogen source as a suitable substrate whereas, cashew and apple juice was used by Osho (1995). Several investigations were carried out using cellulose and hemicellulose waste as a suitable substrate for increasing single cell protein production. Many raw materials have been considered as substrate (carbon and energy sources) for SCP production (Nasseri *et al*., 2011). Further in many cases, these raw materials have been hydrolysed by physical, chemical and enzymatic methods before use. Various hydrocarbon, nitrogenous compounds, polysaccharides and agricultural wastes such as hemicelluloses and cellulose waste (Azzam, 1992; Zubi, 2005) from plants and fibrous proteins such as horn, feather, nail, and hair from animals have thus been used for the production of SCP (Ashok e*t al*.,2000). Another investigation clearly revealed that for *S. cerevisiae*, banana skin was the best substrate followed by that of rind of pomegranate, apple waste, mango waste and sweet orange peel. Various forms of organic waste such as cellulose hemicelluloses, hydrocarbon and different types of agricultural waste were used in the production of SCP (Adedayo *et al*., 2011).

The degree of SCP production depends on the type of substrate used and also on media composition (Mondal, 2006). The highest amount of protein (690 mg) was obtained from *Saccharomyces cerevisiae* when grown on beetroot extract supplemented with basal media, and the highest biomass yield per gram of substrate (16%) was obtained from *Aspergillus niger* when grown on banana extract supplemented with basal media. For *Saccharomyces cerevisiae* cucumber waste has been proved better substrate followed by orange peels. *Aspergillus terreus* possesses a high protein value and has been used as a better choice for single cell protein production using cheap energy sources like Eichornia and Banana peel. (Jganmohan *et al*., 2013). The cladodes of *Opuntia ficus-indica* (cactus pear) were one such lignocellulose raw material that has potential for production of single cell protein in arid and semi-arid regions (Gabriel et al., 2014). Apart from this, Agricultural wastes, Kefir yeast, Maize and millet, Mangrove sediment, Kilka fish, Oil production, Banana peels, Utilization of various Fruit waste, Fruit of beles peels, Bioprotein production from coconut dregs, Orange peels, Pre-treated rice husk, Bamboo using rice bran, Sugar can bagasse, Bengal gram husk by yeast, Pineapple waste, Removal of 'COD' from dairy waste water, Solid state fermentation, Beet pulp, Papaya extract, Corn COB, Soymilk residue etc. has also been explored.

PRODUCTION OF SCP

The process of SCP production from any micro-organism or substrate would have the following basic steps:

- The selected micro-organism is inoculated in pure state.
- The microbial biomass is recovered from the medium.
- SCP processes are highly aerobic (except those using algae). Therefore, adequate aeration must be provided. In addition, cooling is necessary as considerable heat is generated.
- Prevented of contamination by maintaining sterile or hygienic conditions. The medium components may be heated or sterilized by filtration and fermentation equipments may be sterilized.
- Addition, to the carbon source, of sources of nitrogen, phosphorus and other nutrients needed to support optimal growth of the selected micro-organism.
- Provision of a carbon source; it may need physical and/or chemical pre-treatment.
- Processing of the biomass for enhancing its usefulness and/or storability.

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 will recombinant DNA technologies (Omar and Sabry, 1991). The classical raw materials are substances containing mono and disaccharides, since almost all micro-organisms can digest glucose, other hexose and pentose sugars and disaccharides. These materials also are utilized in other branches of industry with a high price level, which puts the economic aspect of the production of microbial biomass in doubt (Oura, 1983). The choice of substrates that are normally abundant has determined the design and strategy of SCP processes. The most widespread and commonly used substrates for SCP production have been those where the carbon and energy source is derived from. Many companies producing SCP including BP (UK), Kanegafuichi, Japan and Italy appeared on the scene. In the United States less than 15% of the plants making SCP were said to rely on hydrocarbons as the sources of carbons and energy for the micro-organisms. Other potential substrates for SCP include bagasse, citrus wastes, and sulphite waste liquor. Molasses, animal manure, whey, starch, sewage, etc.

PRODUCTION OF SCP USING AGRICULTURAL WASTES

Agricultural wastes are basically the most useful substance for the production of single cell protein. In recent years, wastes such as pineapple peels, rice bran, saw dust, brewer's waste and other industrial wastes has been used as substrates for growing single cell protein (SCP) and its production. Agricultural wastes have been found to be very cheap in the production of high quality protein. They are also easy to obtain. Single cell protein (S.C.P) is generated from various agricultural waste and they are potentially valuable nutritional constituent which when processed could yield food, feed, fuel, chemicals and minerals (Anupama and Ravindera, 2000; Han, 1975). Single Cell Protein (SCP) are seen as microbial organism which are allowed to grow on waste products especially agricultural wastes as well as agro based Industries and they produce large quantity of protein and store them in their cell bodies.

PRODUCTION OF SCP USING KEFIR YEAST FROM WHEY

Kefir is a light alcoholic beverage, having its ancient origins in the Caucasian area. It is prepared by inoculation of raw milk with existing kefir grains consist of a polysaccharide matrix in which yeast and bacteria are embedded and live symbiotically. The origin, the storage condition and the mode of handling (Marsall and Cole, 19855; Duitschaever *et al*., 1987; Motaghi *et al*., 1997) affect the microflora of kefir grains and beverages which are predominantly yeasts of the genera *Saccharomyces, Candida, Torula*, and lactic bacteria of the genera *Lactobacillus* and *Streptococcus*. This microbial population of kefir, which ferments lactose, seems to have a potential for alcohol production using milk whey to produce potable or fuel grade alcohol. The production of an alcoholic drink from milk whey should be accompanied by the formation of volatile by products that are constituents of traditional alcoholic beverages and contribute to a typical organoleptic character. In the frame of this purpose a novel system consisting of kefir yeast has developed to produce kefir cell granules after their aerobic incubation on whey.

PRODUCTION OF SCP USING YEAST BIOMASS IN MAIZE AND MILLET

Maize and millet bran were used to enhance the growth of the organism using control and complex media. It could be seen that the maximum yield in yeast biomass was achieved using maize bran which was supported with complex media. Therefore, the use of maize bran for cheaper biomass production in large quantity is highly encouraged.Yeast biomasses are widely used in good processing feed, drug and many laboratory culture media. Appreciable amount of yeast biomass are product of brewing industries. Propagation of yeast for biomass production has become a common practice in many part of the world. The choice of substrate and yeast strains is dependent on locality as well as other consideration it's quite reasonable to ensure that isolates are from edible substrate and be non-pathogenic, non-oxygenic and should be able to produce the desired product in large quantity (Emejuaiwe *et al*., 2000).

PRODUCTION OF SCP USING FROM CORN COB

Micro-organisms are capable of utilizing the organic matter of such residues as a source of energy for their growth. They require carbon compounds for the synthesis of cell biomass for which they have the ability to covert inorganic nitrogen into their body proteins (Khan, 1992). The protein from micro-organisms is cheap and competitive with other protein sources. It may have good nutritive value depending, however. Upon amino acid composition such low cost agro-industrial lignocellulose wastes must be treated by physical/chemical method to liberate cellulose from lignins. Since cellulose in lignin-hemicellulose-cellulose complex is not accessible to enzymatic hydrolysis (Bungay, 1982). Corn cob is very hard lignocellulose waste of corn industry and cannot be utilized efficiently by microbes without pre-treatment.

PRODUCTION OF SCP USING STICKWATER OF KILKA FISH

One of the most important problems in kilka meal factories is stick water production, as waste, from meal production process. Five hundred litters of stick water are produced for each 1000kg of kilka fish. This waste has rather high protein and might be used as substrate for the growth of bacteria. Due to the rapid growth of bacteria, the quality and amount of protein (up 70%), bacteria had a significant advantage over other micro organisms for SCP production (Anupama and Ravindra, 2000). Among the most important bacteria that used for the production of SCP, it can be noted the various species of *Pseudomonas*, *Bacillus* and *lactic acid bacteria* (Erdaman *et al*., 1997).

In most cases, single cell protein was produced from waste of feed in industries and few studies related to SCP production from waste and residues aquatic done. Aquatic organisms due to their high protein and essential fatty acids have been extremely valuable and these wastes can be used as a substrate for the SCP production (Lunar *et al*., 2006).

PRODUCTION OF SCP USING SINGLE CELL OILS

Single cell oils in combination with stable isotopes have become indispensable to study metabolic pathways. Oils derived from microbial sources are named microbial oils, unicellular oils or single cell oils. The term SCO is used as a parallel to single cell protein (SCP) to denote oils of microbial origin. It indicates a triacyl -glycerol type of oil, similar to that found in plant and animal edible oils and fats (Kyle *et al*., 1992, Ratledge, 1993, Boswell *et al*., 1996).

PRODUCTION OF SCP USING WASTE BANANA PEEL

Banana (*Musa paradisiaca*) fruit peel is an organic waste that highly rich in carbohydrate content and other basic nutrients that could support microbial growth. In tropical climates, such as in India, the banana trees continue bearing fruit throughout the year. Sugar represents that part of the fruits which is used by micro-organisms for single cell protein for food and feed applications. The use of such as cheap and readily available substrate is desirable to lower the cost of production, reduce waste disposal and management problems, conserve natural resources and provide feed for livestock purposes. Therefore, the use of banana peel extract as a fermentation medium was taken into consideration as an alternative raw material for the production of *Trichoderma harzianum* biomass in this investigation. The effect of various carbon and nitrogen sources on biomass production was also studied.

PRODUCTION OF SCP USING FRUIT WASTES BY USING *Aspergillus oryzae* and *Rhizopus oligosporus*

The present investigation was carried out to utilize various fruit wastes in the production of single cell protein by using two standard food fungi viz. *Aspergillus oryzae* and *Rhizopus oligosporus*. Proteins are present in all living tissues as building block components of the body. Proteins are therefore also called as building blocks of life. Proteins are important dietary constituent for the supply of nitrogen as well as sulphur. Proteins are major structural components that provide mechanical support to the body. In other words proteins are the essence of life processes and are important for proper growth and development of all the living beings.

PRODUCTION OF SCP USING FRUIT WASTE BY USING YEAST (*Saccharomyces cerevisiae*)

India is the second major producer of fruits and vegetables in the world. It contributes 10% of world fruit production. According to India Agricultural Research Data Book 2004, the total waste generated from fruit and vegetables comes to 50 million tons per annum. Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth thus fruit processing wastes are useful substrate for production of microbial proteins. The utilization of fruit wastes in the production of SCP will help in controlling pollution and also solving waste disposable problem to some extent in addition to satisfy the world shortage of protein rich food.

Single cell protein represents microbial cells (primary) grown in mass culture and harvested for use as protein sources in foods or animal feeds. In the present study, Pomegranate waste, Orange waste, Banana waste, Watermelon waste, was used as sole carbon source for preparation of fermentation media on which strains of yeasts, *Saccharomyces cerevisiae* used.

PRODUCTION OF SCP USING FRUIT OF BELES

Since the mid-nineteenth century, cactus pear (Beles) has been one of the more popular plants in the southern part of the Globe. For more than 0 years, the cactus pear fruit has been considered an important forage crop and major source of income and food for peasant growers in the period from June to August. Cactus pear is an important part of the cultural heritage and a food resource for people in the Tigray region.

The cactus pear's important ecological role in the region is exemplified such as combating desertification, soil remediation, as a refuge and a food source for wild fauna, as well as providing fruit and cladodes for people, forage for cattle and biomass for energy. Studies were carried out to assess to potential of beles' fruit peels for cost effective yeast biomass production. In this kind of work, beles' fruit peels were introduced as a potential substrate for fermentation to produce bio protein which can be used in food as such or as animal feed.

PRODUCTION OF SCP USING PAPAYA EXTRACT MEDIUM

Papaya (*Carica papaya* L.) is a sugar crop with soluble saccharides in the form of glucose, fructose and sucrose (Solvaraj and Pal, 1982), and it is widely cultivated in several countries (Samson, 1980). In tropical climates the papaya tress continue bearing fruit throughout the year , and the fruit in turn follow the same pattern of maturity so that it is possible to pick the fruit at any desired stage of maturity (Cobley and Stele, 1976; Samson, 1980). It displays rapid growth and a high yield of 100kg plant per year or 154,000 kg per hectare per year, even during the fourth year of growth (Allan, 1981).

The average yield per hectare is about 22000 fruits weighing 34 tons. Sugar represents that part of the fruits which is used by micro-organisms for single cell protein and alcohol production (Oura, 1983). The potential of papaya as an energy crop was estimated by Ayanru et al. (1985) who showed that it has a capacity of generating ethanol by microbial conversion of sugars in the papaya fruit. Sugar crops like papaya fruit have the advantage over starch and cellulose waste in single-cell protein programmes, as the latter require extensive supply of sugars and digestion prior to their utilization for cell growth and biomass production.

RODUCTION OF SCP USING ORANGE PEELS

In another study an evaluation of the possible use of orange peels (OP) as substrate to produce single-cell protein and mini-food by *Aspergillus niger, Rhizopus oryzae* and *Saccharomyces cerevisiae* was performed. Since many of the SCP-producing organisms are multicellular and, as their biomass consists of more than just protein, it has been suggested than an alternative name such as novel protein or mini-food is used to better describe this product.

PRODUCTION OF SCP USING BEET PULP

A mixed culture of *Trichoderma reesei* and *Kluyveromyces marxianus* was found to be more efficient for single cell protein (SCP) production (51%) from beet pulp than the other tested mixed cultures containing *T. reesei* or a monoculture of *T. reesei* (49%). In Egypt, surplus quantities of beet pulp (BP), a by-product of sugar production from sugar beet, are available. Some scientists succeeded in obtaining high protein yield (49.3%) using treated BP as the sole carbon source by a monoculture of *Trichoderma reesei*. The present article aims to further increase the protein outputs from beet pulp using mixed cultures. The dried beet pulp was physically treated by milling in Wiley mill and passed through a 60 gauge mesh sieve, followed by chemical pre-treatment with 3% NaOH, to provide the most suitable beet pulp for microbiological studies with *T. reesei*.

BIOPROTEIN PRODUCTION OF SCP USING COCONUT DREGS

This increasing world demand for food and feed protein led to the search for non-conventional protein sources to supplement the conventional protein sources. Animal feed industry also suffering from inadequate and high cost availability of conventional ingredients. Supplementation of vegetable protein with animal proteins results in higher feeding cost. Other than that, there is also a strong competition between human beings and livestock for conventional protein source. The utilization of coconut dregs waste provides as alternative substrate and also help in solving waste disposal problems has never been exploited. Coconut dregs are the leftover fibre from coconut milk production is traditionally used as animal food and finds no other applications. Therefore, in order to solve the problems of protein shortage and proper waste management of coconut milk production's waste, coconut dregs have been chosen as potential substrate for bio protein production by solid state fermentation. In addition, this substrate is easily available in Malaysia and other part of the world and it also has high nutritional value, high carbohydrates content and available in low cost or no cost.

PRODUCTION OF SCP USING PRETREATED RICE HUSK

Several studies have involved various species in producing single cell protein from waste celluloses and fugal mycelial biomass is an acceptable source of edible protein (2-5). Rice husk is a paddy field waste and contain minerals (ash matter), Carbohydrate (cellulose, non-cellulose), nitrogen and lipid. The object of the present work is to examine the efficiency of pre-treated (Chemical and enzymatic) rice husk as a medium for the growth of *Penicillium javanicum* and production of single cell protein. The growth of a strain of *Penicillium javanicum* on pre-treated rice husk was studied by batch wise fermentation in shake flasks. The rice husk was pre-treated with various concentrations of acid/alkalis and enzyme. It was observed that the growth on perchloric acid treated rice husk was higher. However nitrogen and protein content was found higher in fungal biomass when sulphuric acid treated rice husk was used as a substrate for the growth of *Penicillium javanicum*

PRODUCTION OF SCP USING BENGAL GRAM HUSK BY YEAST

Thus in the wake of considerable advancement in biotechnology yeast based single cell protein production stands as the best alternative to supplement the requirements of food and feed-grade protein, vitamins and amino acids. The main objective of this work was to compare the production of SCP from Bengal gram husk can be used as substrate for fermentation using yeast.

SCP POTENTIAL OF ENDOPHYTIC FUNGI ASSOCIATED WITH BAMBOO FROM USING RICE BRAN AS SUBSTRATE

The study to identify the endophytic fungi associated with bamboo and to determine their potential as sources of single cell protein using dried rice bran as substrate. Endophytic fungi were isolated during shoot proliferation of the micro propagated lateral stem of bamboo. Nine species of endophytic fungi were described, characterized and identified. These include three species of *Aspergillus (Aspergillus niger, Aspergillus flavus* and *Aspergillus ochraceus*), three species of *Fusarium* (*Fusarium semitectum, Fusarium* sp1, *Fusarium* sp 2), *Monascus ruber, Penicillium citrrinum* and *Cladosporium cladosporioides*. This corresponds to recent studies wherein species of *Aspergillus, Fusarium, Penicillium* and *Cladosporium* were endophytes of several crops. Similarly, shen *et al*. reported that the same genera of fungi were isolated from several species of bamboo.

PRODUCTION OF SCP USING SUGAR CANE BAGASSE

Bagasse, the residue left after extraction of sucrose from sugar cane, contains ca. 30-35% hemicelluloses (Robert *et al*. 1994), which has the advantage of being of the few lignocellulose wastes which become available in large localized quantities in the course of normal agricultural practice. Mild acid treatment yields a mixture of monosaccharides, mainly pentose, with D-xylose as the main component (Du Toit *et al*. 1984). Besides sugar, the hydrolysate contains acetic acid, furfural, hydroxy methyl-furfural and soluble lignin. Sugar cane hemicelluloses bagasse can be easily hydrolysed and the resulting sugar solution can be used as a cultivation medium for the production of single cell protein.

PRODUCTION OF SCP USING DAIRY WASTE WATER

The aim of study was to produce single cell protein (SCP) i.e. Biomass from dairy waste water. Dairy waste water was collected and analysed for Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Total suspended solids (TSS). The main composition of dairy waste water is Lactose (about 70%), lactose being largely responsible for the high organic load in dairy waste water. Thus, dairy waste water is particularly suitable for the production of SCP, using lactose utilizing micro-organisms. *Saccharomyces cerevisiae* and *Humicola* species were evaluated for their ability to grow and produce biomass and reduce the organic load of the production of dairy waste water. *Saccharomyces cerevisiae* and *Humicola* species were able to reduce COD by about 62% and 93% respectively, with continuos biomass production. The decrease in lactose i.e. Organic load and increase in biomass (SCP) occurred in parallel and growth rate also increased simultaneously with increasing lactose consumption rate. Finally, cost effective SCP process and product can bev performed in an industrial scale and the product can be consumed instead of expensive protein in market.

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