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Production of Single Cell Protein from Fruit Waste

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Abstract: ***The fast growing population of the world has led to an increased demand for protein rich foods. The conversion of fruit waste into single cell protein is an alternative solution to the worldwide protein deficiency and environmental pollution concerns. Dried cells of bacteria, algae, yeast and fungi which are rich in protein and could be used as dietary supplements are called single cell protein. In the present study single cell protein represent microbial cells grown in mass culture and harvested for use as protein source in foods and animal feed. Fruit waste was used as sole carbon source for preparation of fermentation on used for the growth of bacteria, fungi, algae and yeast. The increased incubation time of different fruits hydrolaysate enhanced the biomass yield and the protein formation within the cell. The bioconversion of fruit waste by microbial fermentation into single cell protein has potential use for solving the worldwide protein deficiency in food. The finding in this study indicated that fruit waste could serve as a good substrate for single cell protein production.***
Keywords: Single cell protein, fruit waste, biomass, microorganism, fermentation process.

I. INTRODUCTION

The rapidly increasing world population generates the challenge of providing necessary food sources. In particular, protein supply poses a problem since essential amino acids cannot be replaced. 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 proteins. 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 microorganisms, which are used as protein supplements in human foods or animal feeds. Besides high protein content (about 60 82% of dry cell weight), SCP also contains fats, carbohydrates, nucleic acids, vitamins and minerals. Another advantage with SCP is that it is rich in certain essential amino acids like lysine and methionine which are limiting in most plant and animal foods. Microorganisms 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, methanol and lignocellulosic biomass. The protein obtained from microbial sources is designated as Single Cell Protein (SCP).

The worldwide, large-scale development of SCP processes has contributed greatly to the advancement of present day biotechnology. Research and development of SCP processes 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. In developing SCP processes new technical solutions for other related technologies in wastewater treatment, production of alcohol, enzyme technology and nutritional science also improves.

II. HISTORICAL CONTENT

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 with 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. Moreover, Candilaarborea and C. Utilis were used during the Second World War and about 60 percent of the country replaced the food input (Arora *et al.*, 1991).

In the 1960s, researchers at British Petroleum developed a technology called proteins-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). Initial research work was done by Alfred Champagnatar and BP s Lavera, Oil Refinery in France; a small pilot plant there started operations in March 1963, and the same construction of the second pilot plant, at Grange Mount oil refinery in Britain was authorised (Bamberg *et al.*, 2000).

In microbial protein production, several natural products have been tested.

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The use of natural cheap substrates and waste industrial products for cultivating microorganisms appears to be a general trend in studies of applied nature (Grewal *et al.*, 1990; Osho *et al.*, 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 were used by (Osho *et al.*, 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 substrates (carbon and energy sources) for SCP production (Nasseri *et al.*, 2011). Further in many cases, these raw materials have been hydrolyzed by physical, chemical and enzymatic methods before use. Various hydrocarbon, nitrogenous compounds, polysaccharides and agricultural wastes such as hemicelluloses and cellulose waste (Azzam *et al.*, 1992; Zubi *et al.*, 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 *et al.*, 2000). These waste products have been converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms and are rich in some growth factors required by microorganisms.

Another investigation clearly revealed that for *S. cerevisiae*, banana skin was the best substrate followed by that 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 *et al.*, 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 *Saccaharomyces cerevisiae* pineapple 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 SCP production using cheap energy sources like Eichornia and Banana peel. (Jaganmohan *et al.*, 2013). The cladodes of Opuntia ficus-indica (cactus pear) were one such lignocellulosic raw material that has the potential for production of SCP in arid and semi-arid regions (Gabriel *et al.*, 2014).

The classical raw materials are substances containing mono and disaccharides, since almost all microorganisms can digest glucose, other hexose and pentose sugars and disaccharides (Richmond *et al.*, 2004). 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. 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 which the carbon and energy source is derived from. Many companies producing SCP including BP (UK), Kanegafuichi (Japan) and Liquichimica (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 source of carbons and energy for the micro-organisms. Other potential substrates for SCP include bagasse, citrus wastes, sulphite waste liquor, molasses, animal manure, starch, sewage, etc.

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 conventionally applied genetics together with recombinant DNA technology (Omar *et al.*, 1991). Single cell proteins have application in animal nutrition as: fattening calves, poultry, pigs and fish breeding in the foodstuffs area as: aroma carriers, vitamin carrier, emulsifying aids and to improve the nutritive value of baked products, in soups, in ready-to-serve meals, in diet recipes and in the technical field as paper processing, leather processing and as foam stabilizers.

III. CHOICE OF MICROORGANISM FOR SCP PRODUCTION

A. Bacteria

Characteristics that make bacteria suitable for SCP production include rapid growth of bacteria, short generation time and 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 (Bamberg, 2000) 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 a mineral nutrient supplement to the bacterial culture medium to fulfill the deficiency of nutrients that may be absent in natural waters in a concentration sufficient to support growth. Potential phototrophic bacterial strains are recommended for single cell protein production. Some researchers also suggest use of methanotrophic and other bacterial species for single cell protein production (Arora *et al.*, 1991). The generation time of Methylophilusis about 2 hours is used in animal feed and in general produces a more favorable protein composition than yeast or fungi.

Therefore large quantities of single cell protein animal feed can be produced using bacteria like Brevibacterium (Adedayo et al., 2011) *Methylophilus methylitropous, Acromobacter delvaevate, Acinetobacter calcoacenticus, Aeromonas hydrophilla, Bacillus megaterium, Bacillus subtilis (*Gomashe *et al.*, 2014*), Lactobacillus species, Cellulomonas species, Methylomonas methylotrophus (*Piper*, 2004), Pseudomonas fluorescens, Rhodopseudomonas capsulate, Flavobacterium species, Thermomonospora fusca* (Dhanasekaran *et al*., 2011). Algae Since ancient times, Spirulina was cultivated by people near lake chad in Africa and the Aztecs near Texcoco in Mexico. They used it as food after drying it. Spirulina is the most widely used algae so much that even astronauts take it to space during their space travel. Similarly, biomass obtained from Chlorella and Senedessmus has been harvested and used as a source of food by tribal communities in certain parts of the world. Alga is used as a food in many different ways and its advantages include simple cultivation, effective utilization of solar energy, faster growth and high protein content. The algae Spirulina has been considered for use as a supplementary protein (Raja *et al*., 2008). It is a blue green algae having strong antioxidant activity and provokes a free radical scavenging enzyme system.

A diet enriched with Spirulina and other nutraceuticals may help protect the stem/progenitor cells. Spirulina maxima prevent fatty liver development induced by carbon tetrachloride (CCl4). It is concluded that the use of Spirulina should be encouraged in patients suffering from malnutrition, immune suppression, hepatic and neural compromise, etc. although further investigations on the antiviral effects of this alga and its clinical implications are strongly needed. Single cell protein (SCP) production by five strains of Chlorella species (M109, M121, M122, M138, and M150), isolated from different habitats, and was studied under the influence of eight environmental factors (*Mahasneh et al*., 2005).

B. Yeast

Yeast single-cell protein (SCP) is a high- nutrient feed substitute (Burgents et al, 2004). Among these, the most popular are yeast species Candida (Bozakouk, 2002), *Hansenula, Pitchia, Torulopsis and Saccharomyces.* The production of single cell protein using Saccharomyces cerevisiae grown on various fruit waste (Tanveer, 2010). The typical oily yeasts genera include *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon and Lipomyces*. pineapple and orange peels were evaluated for the production of single cell protein using Saccharomyces cerevisiae by submerged fermentation (Sengupta *et al*., 2006).

C. Fungi

Many fungal species are used as a source of protein rich food (Bhalla *et al*., 2007). Many other filamentous species are also used as a source of single cell protein. In 1973, in second international conference convened at MIT, it was reported that Actinomycetes and filamentous fungi produced protein from various substrates. During the world war II, trials were made to utilize the cultures of Fusarium and Rhizopus (Yousuf, 2012) grown in fermentation as a source of protein food. The inoculums of Aspergillus oryzae (Anupama and Ravindra, 2000) or Rhizopus arrhizus were selected because of their non-toxic nature. Saprophytic fungi grow on complex organic compounds and convert them into simple structures. The high amount of fungal biomass is produced as a result of growth. Mycelia yield varies greatly which depends upon organisms and substrates.

There are some species of molds, for example, *Aspergillus niger* (Yabaya and Ado, 2008), *A. fumigates, Fusarium graminearum* which are very dangerous to humans, therefore, such fungi, must not be used for toxicological evaluations should be done before recommending to use as SCP. Very recently, SCP technology is using fungal species for bioconversion of lignocellulosic wastes (Lenihan *et al.*, 2010). The filamentous fungi that have been used include *Chaetomium celluloliticum, Fusarium graminearum (*Zubi, 2005*), Aspergillus fumigates, A. niger, A. oryzae, Cephalosporium cichorniae, Penicillium cyclopium, Rhizopuschinensis, Scytalidum aciduphlium, Tricoderma viridae,* and *Tricoderma alba Paecilomyces varioti* (Jaganmohan *et al*., 2013).

IV. POTENTIAL SUBSTRATES FOR SCP PRODUCTION

Single Cell Protein can be produced by a number of different substrates, often this is done to reduce biological oxygen demand of the effluent streams leaving various types of agricultural processing plants. The two main strategies with regard to substrate were to consider low grade waste material and very high quality of protein in it (Reed and Nagodawithana, 1995).

Many raw materials have been considered as substrate for SCP production (Nasseri *et al*., 2011). 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, methanol and lignocellulosic biomass (Martin, 1991; Bekatorou *et al*., 2006). Carbohydrate substrates are the most widely used for SCP production due to the fact that carbohydrates are natural microbial substrates and also because carbohydrates constitute a renewable feedstock (Ugalde and Castrillo, 2002).

The availability of the substrate and its proximity to the production plant are the major factors that determine the design and strategy of an SCP production process. Molasses, the residual liquid obtained after crystallization of sugar from the concentrated sugar solution obtained from the milling of sugar cane or sugar beet, contains 45-55 % sugars, namely sucrose, glucose, fructose, raffinose, melibiose and galactose. It is estimated that for every 100kg of cane milled for sugar production, some 3.5 to 4.5 kg of molasses is obtained.

The use of molasses for the production of SCP is determined by its availability and low cost, its composition and the absence of toxic substances and fermentation inhibitors (Bekatorou *et al*., 2006). Though molasses is a suitable carbon feedstock for SCP production, it requires supplementation with ammonia salts and phosphorus salts (Ugalde and Castrillo, 2002). Cassava is a tropical root crop produced in more than 80 countries and it is a rich source of starch for SCP production (Ejiofor *et al*., 1996).

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, as well as unconventional ones such as petroleum by-products, natural gas, ethanol, methanol and also human and animal excreta can be used (Suman *et al.*, 2015). Lignocellulosic biomass such as cellulose and hemicellulose waste is used as a suitable substrate for increasing single cell protein production (Nasseri *et al*., 2011).

V. FRUIT WASTE

The content of fruit processing waste is highly dependent on the type of fruit and the part of the fruit that forms the main mass of the waste. If the waste is mainly whole fruit, then a large number of monosaccharides and disaccharides will be available in the waste, as is in the case with banana wastes, where 5 to 30% of harvested bananas are discarded as waste due to export regulations (Baldensperger *et al.*, 1985). A similar situation exists for figs where, due to incorrect transport, storage and market changes, large quantities of figs are not realized, and they need to be disposed of when they begin to deteriorate (Hashem *et al.*, 2014).

Similarly, as for sugar and starch production waste, also fruit processing residues can be used as feed for cattle, however, this is often not possible, or the transportation of the residues is too expensive (Bhalla *et al.*, 1994). Consequently, in order to reduce production costs, usually the waste from fruit processing is simply discarded (Upadhyay *et al.*,1988). Mainly dietary fibre rich fruit processing wastes come from juice and essential oil production factories (De Gregorio *et al.*, 2002). Pomace and juice pulps make up about 25 to 65% of the total fruit volume used for juicing(Bhalla *et al.*, 1994). Considering that about 25% of the harvested fruits are used in industrial processing(Bhalla *et al.*, 1994), the global annual amount of pomace and juice pulp produced from apples and citrus fruits is estimated at around 15 million tonnes (FAO, 2018a). From a nutritional point of view, fruit remnants are not suitable for monogastric animal feeds because of their low digestibility and low protein content (Bhalla *et al.*, 1994). Due to a large amount of generated waste and its limited use of it, researchers have looked at ways to improve the nutritional value of fibrous fruit wastes by using them as substrates for the production of SCP (De Gregorio *et al.*, 2002).

VI. CULTIVATION METHOD

The production of single cell protein takes place in a fermentation process (Chandrani- Wijeyaratne and Tayathilake, 2000). This is done by selected strains of microorganisms which are multiplied on suitable raw materials in the technical cultivation process directed to the growth of the culture and the cell mass followed by separation processes. Process development begins with microbial screening, in which suitable production strains are obtained from samples of soil, water, air or from swabs of inorganic or biological materials and are subsequently optimized by the selection, mutation, or other genetic methods. Then the technical conditions of cultivation for the optimized strains are done and all metabolic pathways and cell structures will be determined. Besides, process engineering and apparatus technology adapt the technical performance of the process in order to make the product ready for use on a large technical scale. Here is where the economic factors (energy, cost) come into play. Safety demands and environmental protection is also considered in the production of SCP in relation both to the process and to the product. Finally, safety and the protection of innovation throw up legal and controlled aspects, namely operating licenses, product authorizations for particular applications and the legal protection of new processes and strains of microorganisms. Single cell protein can be produced by Fermentation processes, namely:

A. Submerged fermentation

In submerged process (Varavinit *et al*., 1996), the substrate used for fermentation is always in a liquid state which contains the nutrients needed for growth. The fermentor which contains the substrate is operated continuously and the product biomass is continuously harvested from the fermentor by using different techniques then the product is filtered or centrifuged and then dried.

Aeration is an important operation in cultivation, heat is generated during cultivation and it is removed by using a cooling device. The microbial biomass can be harvested by various methods (Kargi *et al.*, 2005). Single cell organisms like yeast and bacteria are recovered by centrifugation while filamentous fungi are recovered by filtration. It is important to recover as much water as possible prior to final drying done under clean and hygienic conditions.

B. Semisolid fermentation

In semisolid fermentation (Adedayo *et al.*, 2011), the preparation of the substrate is not cleared and it is also more used in solid state e.g. cassava waste. Submerged culture fermentations require more capital investment and have high operating cost. The cultivation involves many operations which include stirring and mixing of a multiphase system, transport of oxygen from the gas bubbles through the liquid phase to the microorganisms and the process of heat transfers from liquid phase to the surroundings (Anderson and Jorgensen, 2005). A special bioreactor is designed for identifying mass and energy transportation phenomena, called U-loop fermentor (Jorgensen, 2010). Production of single cell protein involves basic steps of preparation of a suitable medium with a suitable carbon source, prevention of the contamination of the medium and the fermentor, production of microorganisms with desired properties and separation of synthesized biomass and its processing (Soland, 2005). Carbon source used can be n-alkenes, gaseous hydrocarbons, methanol and ethanol, renewable sources like carbon oxide molasses, polysaccharides, effluents of breweries and other solid substances (Talebnia, 2008).

C. Solid state fermentation

Solid state fermentation (SSF) has been extensively studied with thousands of publications describing various types of bioreactor designs, process conditions and microorganisms for the production of various value added products like SCP, feeds, enzymes, ethanol, organic acids, B- complex vitamins, pigments, flavours, (Singhania *et al.*, 2009). This process consists of depositing a solid culture substrate, such as rice or wheat bran, on flatbeds after seeding it with microorganisms; the substrate is then left in a temperature-controlled room for several days. Liquid state fermentation is performed in tanks, which can reach 1,001 to 2,500 square metres (10,770 to 26,910 sq ft) at an industrial scale. Liquid culture is ideal for the growing of unicellular organisms such as bacteria or yeasts. To achieve liquid aerobic fermentation, it is necessary to constantly supply the microorganism with oxygen, which is generally done via stirring the fermentation media. Accurately managing the synthesis of the desired metabolites requires regulating temperature, soluble oxygen, ionic strength and pH and control nutrients (Capalbo *et al.*, 2001).

VII. NUTRITIONAL ADVANTAGES OF SCP

Single cell protein basically comprises proteins, fats carbohydrates, ash ingredients, water, and other elements such as phosphorus and Potassium. Aside from the nutritional benefits of single cell protein, another benefit of single cell protein technology is there throughout the year of production. Also, it plays its role in waste management as waste materials are used as substrates. A small area of land is required and SCP is made in less time. To access the nutritional value of single cell protein, many factors must be considered which include nutrient composition, amino acid profile, vitamin and NA content as well as allergies and gastrointestinal effects. To access toxicological and carcinogenic effects, long term feeding trails are also required. The process of drying, harvesting and processing has an effect on the nutritive values of the finished products. Single cell protein is basically composed of protein, fats, carbohydrates, ash ingredients, water and other elements such as potassium and phosphorous. The composition of SCP depends on the nature of the substrate and also on the organism used. Proteins not only provide nutritional value but also perform the number of other functions.

Single cell protein from yeast and fungi has 50-55% protein it has a high protein- carbohydrates ratio (Mchoi and park, 2003). It contains more lysine and less amount of methionine and cysteine. It also has a good balance of amino acids and it has high B-complex vitamins and more suitable as poultry feed. Some yeast strains with probiotic properties such as *Saccharomyces cerevisiae* and *Debaryomyces hansenii* improve larval survival either by colonizing the gut of fish larvae, which triggers the early maturation of the pancreas. Single cell proteins produced by using bacteria contain more than 80% protein although they have a small amount of sulphur containing amino acids and are high in nucleic acid content (Attia *et al.*, 2003).

Nutritive and food values of SCP vary with the microorganisms used. The method of harvesting, drying and processing has an effect on the nutritive value of the finished product (Bhalla *et al.*, 2007). Single cell protein basically comprises proteins, fats carbohydrates, ash ingredients, water, and other elements such as phosphorus and Potassium (Jamel *et al.*, 2008). The composition depends upon the organism and the substrate upon which it grows. Proteins not only provide a nutritional component in a food system but also perform a number of other functions (Mahajan and Dua, 1995).

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With excellent nutrient profiles and capacity to produce mass economically, SCPs have been added to aquaculture diets as partial replacement for fishmeal (Olvera-Novoa *et al*., 2002) and for fortification of rotifer and Artemia (McEvoy *et al*., 1996). Yeast single-cell proteins (SCPs) are playing a greater role in the evolution of aquaculture diets (Gao *et al*., 2008). Some yeast strains with probiotic properties, such as Saccharomyces cerevisiae (Oliva-Teles and Goncalves, 2001) and Debaryomyces hansenii (Tovar *et al*., 2002), boost larval survival either by colonizing the gut of fish larvae, thus triggering the early maturation of the pancreas, or via the immune stimulating glucans derived from the yeast cell wall (Campa-Cordova *et al*., 2002 and Burgents *et al*., 2004). The idea that the single cell protein could help the less developed countries in future food shortages was gaining research interest among scientists in universities and industry. For the future success of SCP, first, food technology problems have to be solved in order to make it similar to familiar foods and second, the production should compare favourably with other protein sources.

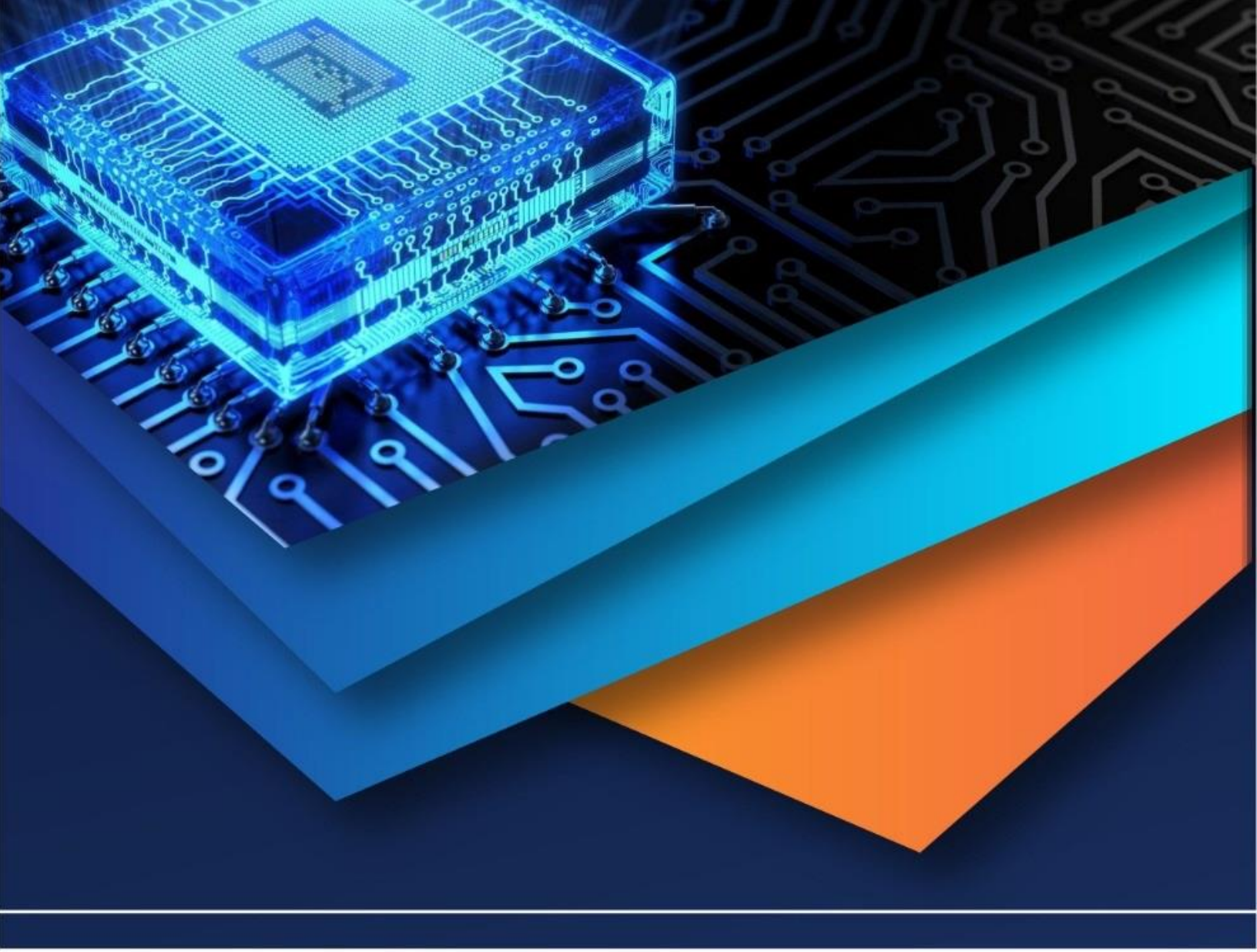
In conclusion, SCP shows very attractive features as a nutrient supplement for humans. It basically comprises of protein, carbohydrates, fats, water and elements like phosphorous and potassium. SCP has various benefits over animal and plant proteins in that its requirement for growth are neither seasonal or climatic dependent, therefore, it can be produced all around the year.SCP has a high protein content with a wide amino acid spectrum, low fat content and higher protein carbohydrate ratio than forages. It can be grown on waste and is, therefore, environment friendly. Thus, the use of SCP as an alternative nutrient supplement can solve the problem of food scarcity in a rapidly growing population, especially in a developing country like India. However, despite of all these benefits, SCP production has gained less importance because of the lack of acceptability of SCP as a nutrient supplement among people. Moreover, high nucleic acid content, presence of non-digestible cell wall, unacceptable colours and flavours and a high risk of contamination and cell recovery further restricts their use as a global food. Therefore efforts should be made to find alternative substrates and methods which can minimize the pitfalls of the substrates and methods currently in use for the production of SCP and thus lead to acceptance of this valuable nutrient supplement on a global basis.

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