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Production of Single Cell Protein from Mix Fruits Waste using *Lactobacillus*

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Abstract

The word Single Cell Protein typically refers to dead, dry cells of microorganisms such as Bacteria, yeast, fungi which growth on different carbon source. Single Cell Protein (SCP) production has the potential for feeding ever increasing world population at cheaper rates. The protein comes from a number of vegetable, fruits and cereals often not affordable by a common man and therefore microbial protein can be an alternative source to feed economically down communities in the world in general and India in particular. The use of molasses for the production of SCP is determined by its availability and low cost, its composition and absence of toxic substance and fermentation inhibitors. SCP also contains fats, carbohydrate, nucleic acids, vitamins and minerals. Microorganism like bacteria, yeast, fungi and algae utilize inexpensive feedstock and waste to produce biomass, protein concentrate or amino acids. A mixture of materials can be used as a substrate for producing as pineapple peel residue, pomegranate waste, apple waste, and pear waste. In this work we intended to investigate the possibility of bioconversion of fruits wastes in to SCP by using *Lactobacillus* on media containing fruit wastes. The mix fruits waste was found good amount of reducing and total sugar respectively 4.47±0.08 mg% and 5.33±0.08 mg%. It was also found to 24.67±0.67 mg% protein content. The degree of microbial biomass growth depends on the types of substrate used. The increase in biomass contents were observed when there was increase in mix fruits waste concentration. Thus the present investigation mix fruit wastes were used for production of biomass. The biomass thus produced can be further used as food or feed.

Keywords

SCP, *Lactobacillus*, Biomass, fruit waste, food and feed.

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INTRODUCTION

Microorganism, such as lactic acid bacteria, yeast have been used in human house hold for thousand years. In the last decade, new techniques for using microbiological fermentation products in human food or animal feed have been developed. Some products obtained from such production methods are called Single Cell Protein (SCP). The word Single

Cell Protein typically refers to dead, dry cells of microorganisms such as Bacteria, yeast, fungi which growth on different carbon source[1]. The dry cells of unicellular microorganisms produced commercially as 'microbial protein' or 'Single Cell Protein'. SCP can be produced from cheap waste material available in large quantities which minimize the environmental pollution. SCP also contains fats, carbohydrate, nucleic acids, vitamins and minerals. Microorganism like bacteria, yeast, fungi and algae utilize inexpensive feedstock and waste to produce biomass, protein concentrate or amino acids.

Single Cell Protein (SCP) production has the potential for feeding ever increasing world population at cheaper rates[2]. The protein comes from a number of vegetable, fruits and cereals often not affordable by a common man and therefore microbial protein can be an alternative source to feed economically down communities in the world in general and India in particular. The SCP Biomass produced may supply as an ideal supplement for aqua feeds[3]. Microbes have 46% of crude protein content processing large quantities of essential amino acids[4]. The agro industrial wastes including fruits and vegetables peels, bagasse and molasses are locally produced in higher amounts and increases the pollution level to a significant level, thus these waste can be utilized for SCP, thereby causing a reduction in pollution level as well as producing SCP biomass.

The bacteria are also capable of growing on a variety of raw material that range from Carbohydrates such as starch and sugars to gaseous and liquid hydrocarbons which include methane and petroleum fractions[5]. Single Cell Protein can be produced by a number of different substrate, 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[6]. The use of molasses for the production of SCP is determined by its availability and low cost, its composition and absence of toxic substance and fermentation inhibitors[7]. The future of SCP will be heavily dependent on reducing production costs and improving quality by fermentation, downstream processing and improvement in the producer organism as a result of conventional applied genetics together with recombinant DNA technology[8]. A mixture of materials can be used as a substrate for producing as pineapple peel residue, pomegranate waste, apple waste, and pear waste. In this work we intended to investigate the possibility of bioconversion of fruits wastes in to SCP by using *Lactobacillus* on media containing fruit wastes.

MATERIALS AND METHOD

Isolation and Preservation of *Lactobacillus*

The medium which was selected for the lactic acid bacteria was de Man, Rogosa and Sharpe (MRS) agar medium (Tryptone 10gm, Meat extract 10gm, Yeast extract 4gm, Tri-ammonium citrate 2gm, Sodium acetate 5gm, Magnesium sulfate ($MgSO_4.7H_2O$) 0.20gm, Manganese sulfate 0.050gm, Di-potassium phosphate 2gm, Glucose 20gm, Tween80 1.080ml, Agar-Agar 15gm, D/W 1000ml, pH (at 28°C) 5.7 $\pm$ 0.1). A loopful of the curd samples was streaked on the sterile MRS agar Petri plate by streaking method, under aseptic conditions. After streaking the Petri plate, they were incubated at 37°C for 24 to 48 hrs. After the incubation, colonies were re-streaked on the MRS agar Petri plate for the formation of isolated colonies. Then from these plates isolated colonies were restreaked on MRS agar slants and stored at 4°C.

Phenotypic characterization

Characterization of all the isolates was performed on the basis of their morphological and biochemical characteristics as described[9].

Morphological examination of culture

Morphological and cultural examination was carried out by using Gram's staining method described by Hans Christian Gram (1884) [9].

Identification of the pure culture

Pure culture isolated on MRS agar slant was identified with the help of biochemical tests like endospore test, Hugh and Leifson's test, motility test, catalase test and sugar fermentation test[9].

Endospore test

Bacterial smear was made on microscopic slide under aseptic conditions and heat fixed. Then slide was placed over the steaming water bath and malachite green (primary stain) was applied for 5 min. Slide was removed from the water bath and rinsed with water until water run clear. Then the slide was flooded with the counter stain safranin for 20 s and rinsed with water. After these slides were blot dried, they were observed under the light microscope[9].

Hugh and Leifson's test

The purpose of this test was to determine whether an organism is an oxidizer or a fermenter on the basis of production of acid in aerobic and anaerobic conditions. Hugh and Leifson's medium was prepared into culture tubes. Then these test tubes were autoclaved at 121°C for 15 min. A filter sterilized solution of 10% carbohydrate (glucose) was

aseptically added to the medium to a final concentration of 1%[9].

Medium was cooled and inoculated by stabbing with the test organism. After stabbing, all the culture tubes were kept in incubator under aerobic and anaerobic conditions at 37°C for 24 to 48 h. After incubation, all the test tubes were observed for fermentation[9].

Motility test

Hugh and Leifson's medium was also used for the testing if the bacteria were motile or non-motile through stab inoculation[9].

Catalase test

This test was used to check the production of enzyme catalase. For this test a clean microscopic slide was taken. A drop of 3% H_2O_2 was taken on the microscopic slide aseptically. A loopful of bacterial culture was taken and mixed with 3% H_2O_2 solution on the slide and the presence of the bubble production observed[9].

Starch hydrolysis test

This test was used to check Organism producing amylase utilize starch in the vicinity of the colony, hence when the medium is flooded with iodine solution colorless zone is seen surrounding the colony, the remaining portion of the medium turns blue[9].

Collection and Preparation of fruits waste extract

The fruit wastes like Apple waste, Pear waste, Pineapple waste, Pomegranate waste were collect from fruit market yard of Visnagar, Gujarat.

Take the 500gm of mix fruits waste like Apple waste, Pear waste, Pineapple waste, Pomegranate waste containing of peels, pulp, seeds. Then they were subjected to thorough washing under running tap water. Then cleaned initially with 2% H_2SO_4 and further washed with sterile distilled water. Then take 100ml of distilled water for crushing the fruits waste was macerated in blender. The fruits extract was obtained filtered with the use of cheese cloth again filter with filter paper.

Take 100ml filtrate in 250ml Erlenmeyer flask. Other than control, were prepared consisting of the basal media D-Glucose (2%W/V), Ammonia sulfate $(NH_2)_2SO_4$), $MgSO_4.7H_2O$ (0.5gm), KH_2PO_4 (1gm). The Erlenmeyer flask was plugged with sterile cotton wool and aluminum foil. The flask was then autoclaved at standard temperature of 121°C, pressure of 15 psi for a time period of 15minutes.

Production of SCP

After autoclaving, in aseptic condition inoculate 1.0ml (3×10^8 cfu/ml) *Lactobacillus* culture in flask then incubate on mechanical shaker at 200rpm for 45hrs at 25°C. After incubation period centrifuge this extract at 12000rpm for 15 minute. Transfer a pellet on filter paper (Whatman filter paper No. 1). The filter paper containing biomass was dried at 90°C for 24 hrs to get moisture free bacterial contents. The dried powdered was use as further analytical process.

Analytical procedure

The moisture, Protein estimation by folin Lowary's method, reducing sugar estimation by DNS method, Total sugar estimation by Phenol sulfuric acid method were determine by AOAC methods (AOAC, 1975).

Statistical analysis

The Statistical analysis was carried using Microsoft Excel.

RESULTS AND DISCUSSION

The curd sample lactic acid producing *Lactobacillus* strain was isolated. Colonies were observed on the surface of MRS agar Petri plate. More than one type of colony was observed on surface of MRS agar Petri plate. Creamy to white color, Round and rod shape, small and large size, flat and light convex, entire, smooth and rough surface tics observed. The cultural and morphological characteristics were further resolved on the basis of microscopic examination. Majority of the microorganisms were Gram positive rods and cocci shaped bacteria. The strains were phenotypically characterized on the basis of their morphological, cultural, and biochemical characteristics. Gram's staining of the bacterial culture showed they were gram positive and their cell morphology was rod shaped and some of them were coccid shaped. Endospore test showed that the bacteria were non-endospore forming, showing negative result (red colour) instead of forming positive result (green colour).

Hugh and Leifson's test showed that the bacterial culture was capable of producing fermentation. And when these medium culture tubes were used to determine the motility of bacteria, then it was found that the bacteria were non-motile, growing in a confined stab line instead of making the whole medium turbid.

Catalase test showed that the isolate was able to produce bubbling when mixed with 3% H_2O_2. This showed that there was presence of catalase enzyme. Starch hydrolysis test observe transparent zone surrounding the colony. Flood the pale with Lugol's iodine and read immediately, because the blue fades rapidly.

Analysis of *Lactobacillus* biomass is shown in Table 1. The mix fruits waste like Apple waste, Pear waste, Pineapple waste, Pomegranate waste containing of

peels, pulp, seeds were found good amount of reducing and total sugar respectively 4.47±0.08 mg% and 5.33±0.08 mg%. It was also found to 24.67±0.67 mg% protein content.

Constituents	mg%
Moisture content	10.67±0.33
Total sugar	5.33±0.08
Reducing Sugar	4.47±0.08
Protein	24.06±0.63

Table 1. Analysis of *Lactobacillus* Biomass

The Pearson's matrix correlation analysis of *Lactobacillus* biomass production is shown in Table 2 and shows positive correlations (p=5%) among parameters studied. The highest positive correlation was observed between the presence of moisture and reducing sugar (r=0.945) followed by total sugar and reducing sugar (r=0.929), then moisture and total sugar. The strong correlation between moisture and total sugar has demonstrated their positive influence on increase of the rate of SCP.

	Moisture content	Total sugar	Reducing Sugar	Protein
Moisture content	1			
Total sugar	0.756	1		
Reducing Sugar	0.945	0.929	1	
Protein	0.052	0.693	0.376	1

Table 2. Pearson's matrix correlation analysis of *Lactobacillus* Biomass

DISCUSSION

According to Potnis *et al.*[10] in their investigation used of supplements for the Lactobacillus growth on fruit waste materials like Watermelon, Sweet lime, Custard apple their protein content respectively 0.50%, 2.35 % and 2.45% and carbohydrate content respectively 0.50%, 11.20% and 18.75%.

According to Yousufi[11], Biomass production with *Aspergillus oryzae* and *Rhizopus oligosporus*. Several different fruit wastes have utilized as a substrate like apple waste, papaya waste, orange waste, pineapple waste, pomegranate waste, watermelon waste, mango waste, guava waste and banana waste their protein content respectively 49.1, 60.1, 40.6, 47.2, 50.9, 44.3, 46.5, 41.6, 43.2 mg/100gm.

According to Dhanasekaran *et al.*[12], Yeast biomass production with use of pineapple waste as a substrate. Their protein, reducing and non-reducing sugar contents respectively 0.6%, 10.8% and 13%.

CONCLUSION

The use of mix fruits waste as substrate for the production of microbial biomass protein. The degree of microbial biomass growth depends on the types of substrate used. The increase in biomass contents were observed when there was increase in mix fruits waste concentration. The utilization of substrate was increased with the increase in the concentration of protein. Thus the present investigation mix fruit wastes were used for production of biomass. The biomass thus produced can be further used as food or feed.

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