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Conversion of Potato Peels into Single Cell Protein

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ABSTRACT

The economic expansion in developing countries can be achieved by converting their low cost industrial and agricultural wastes into more valuable resultants with the help of emerging scientific approaches. **Objective:** to produce single cell protein from microorganism (fungus) through the process of submerged fermentation utilizing the agro-industrial waste (potato peel) as substrate. **Methods:** Four broths (control, glucose broth, potato peel broth and mix broth) were prepared. The maximum dry cell biomass (0.523 g/100 ml) was obtained with mix broth which was unitized for further research. All the broths were supplemented with potassium dihydrogen phosphate, magnesium sulphate, sodium chloride, and yeast extract. **Result:** The growth of fungal biomass in stirred tank and bubble column fermenter was compared and optimum yield was obtained with bubble column fermenter (5.45 g/100ml). This bioconversion will not only supply protein rich food but also help in control of environmental pollution. **Conclusion:** It is concluded that potato peels can be an attractive substrate for the production of single cell protein as they are good source of sugar and other nutrients required for the support of microorganisms.

INTRODUCTION

In developing countries, the economic expansion can be achieved by using their low cost industrial and agricultural wastes into more profitable resultants with the aid of emerging scientific approaches. In the same way, potato peel waste can be converted into various value-added compounds such as enzymes, biogas, bio-sorbents, biohydrogen and various other products as this waste is increasing due to increased usage of manufactured edible potato products [1]. Biodegradable wastes can be an important source for making of highly nutritious and high-quality products without any harm. Different types of waste materials add pollution to the environment; however, they can be transformed into low quality products. Many approaches have been appearing to convert these waste materials into high quality products [2, 3]. Increasing aquaculture farming practices has been putting pressure on the greater demand of protein in animals feed which is reduced by converting low-cost agricultural waste into single cell protein (SCP) by using the bacteria or yeast [4, 5]. A large amount of waste and by-products from food industries are not only a source of environmental pollution but also dangerous for all living organisms. Alongside, a huge number of people around the world have been suffering from malnourishment [6, 7]. To fulfill the global protein deficiency, different kinds of wastes can be bio-converted into single cell protein which can be used as a solution to environmental pollution as well as feed for animals [8]. Potato peels are weighty material in potato-processing industries that are not much used and considered as waste, while they can be a good source of lignin, protein, lipids, cellulose, ash, starch and non-starch carbohydrates (pectin, cellulose and hemicellulose), although the fermentable sugars are low in quantity [9-11]. Potato is the 4th basic crop being cultivated thought out the globe after, wheat, rice and maize. The fast-growing chips and fries' industries are involved in making of huge

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piles of potato peels waste which is highly considered a pollution issue since it can spread, the leaf roll and late blight like diseases in adjacent plants in close vicinity, due to high moisture content. This waste has anti-inflammatory, antioxidant, apoptotic, antibacterial and chemo preventive like extraordinary properties [9-12]. The breed, variety and composition of potato varies to some extent depending on the geographical region but usually its composition shows that the raw potato peel consists of 83.3–85.1% of water, 1.2–2.3% of protein, 8.7–12.4% of carbohydrates, 0.1–0.4% of lipids, 7.8% of starch, 1.02–2.92% of phenolic compounds, 2.5% of dietary fiber, 0.51–0.96% of flavonoids and 0.9–1.6% of ash [1, 11]. The peeling methods also affect the potato peels composition, since the abrasion peeling results in more starch and less lignin content while the opposite case exists in steam peeling [1]. With increasing demand of protein, the pressure on conventional protein sources has been increased which urges the researchers to find alternate protein sources to meet the demand. The aim of this experimental work is to obtain single cell protein from microorganism using cheap and easily available low-cost substrate that are of no use but cause environmental pollution.

METHODS

Potato peels were collected to use as substrate from local market Lahore. Initially, potato peels were washed with tap water to remove dust particles, dried at room temperature and stored in zipper bag at 4 °C. The fungus, *Rhizopus oligosporous* was obtained from PCSIR testing laboratories complex, Lahore and used to produce single cell protein. The *R. oligosporus* was grown and maintained on potato dextrose agar (PDA) plates and then slants were stored at 4 °C. Potato peels were chopped/ cut into small pieces then, 20 g potato peels were taken in 250 ml Erlenmyer flask and 100 ml distilled water was added. The pH of this mixture was adjusted 3.5 with conc. HCl and then autoclaved at 121 °C for 15 minutes. After cooling, it was filtered with muslin cloth to separate potato chunks. The filtrate was designated as potato peel extract (PPE). For fermentationn, innoculum was prepared from subculture of *R. oligosporous* grown on PDA slants. The slants were flodded with 20 ml sterilzed distilled water to dislodge spores from the fungal hyphae. The inoculum size was adjusted to 10^{6-7} spore/ml with the help of hemocytometer for inoculation in all experiments. The fresh inoculum was prepared every time for the investigation of each parameter. Four broths were prepared named as control, potato peels extract broth, glucose broth and mix broth. The control broth comprising of yeast extract, peptone, glucose, sodium chloride and magnesium sulphate prepared. The pH of this solution was kept at 6.5 with HCl/NaOH. This control was then shifted to fermentor and autoclved alongwith fermentor at 121 ℃ for 15 min. The control was inoculated with 2% (v/v) *Rhizopus oligosporus*. After 3 days fermented material was harvested and measured the wet weight of biomass. It was dried by keeping in oven for 24 hrs at less than 80 ℃ until constant mass was achieved. Then dried biomass was recorded. Potato peels extract broth contains 100 ml of previously described pretreated potato peel extract. Glucose broth (100ml) was prepared by adding 3 g glucose in 100ml distilled water. Mix broth comprising of 100 ml potato peels extract broth and 1.2 g glucose. The potato peels extract, glucose and mix broth were supplemented with chemicals mentioned in The pH of all broths were adjusted with dil. HCl/ NaOH at 5.5. These broths were autoclaved at 121 °C for 15 min. After cooling, the broth were inoculated with 2% (v/v) freshly prepared inoculum of *R. oligosporous*. These culture were placed in incubator at 35 °C for 3 days. After three days, biomass was filtered by using Whatmann filter paper and weighed the wet biomass by using digital balance. The biomass was kept in oven at 80 °C for 24 hours until constistant weight. All the experiments were carried out in triplicates. Fermenters are the vessels that provide optimum conditions for the growth of microorganisms and are used to produce a variety of products. Stirred tank fermenters are better known for their property of agitation with the help of impellers while the airlift fermenter lacks the impeller system for agitation and use air for the purpose of mixing [13]. The temperature in both the fermenter types was maintained at 35 ℃ with aeration speed of 1.0 vvm. The mix media was fermented with *Rhizopus oligosporus* for upto 3-4 days in each fermenter. After that cell biomass was separated from filtrate by filtering the fermented media. This wet cell biomass was then kept in oven at 70-75 °C temperature until the constant weight was achieved. All the experiments were carried out in triplicates. For determinition of reducing sugars Benedict's quantitaive test was used [14]. The crude protein of single cell protein was determined by the Kjeldhal procedure [15]. The total protein in the growth media was estimated by following the Lowrey method [16].

RESULTS

The purpose of utilizing waste potato peels was to reduce the burden on conventional protein sources. Based on this fact, a series of experiments were conducted to produce single cell protein from *Rhizopus oligosporos* from waste potato peels through submerged fermentation. This waste is selected beacuse it is easily available in enormous qauntity and cheap source to utilize for developmental purposes. Three broths (glucose broth, potato peels broth

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and mix broth containing both potato peels as well as glucose) were formulated with slight difference in their composition and same suplements were added in all of them. The results described in table 1, that maximum product of SCP (0.523 g/100 ml) was achieved with mix media which is followed by potato peels media. The crude protein content of dried biomass was in range of 45-55%. The results of control medium was 0.045 g/100 ml dry cell biomass. The purpose of control was to compare the results of mix broth with it. The comparison showed that potato peels were better substrate to provide necessary elements for the fungus. The statistical analysis showed that dry biomass yield produced with all of the three media was significantly different ($p < 0.001$).

Media	Dry biomass (%) Mean ± SD	Consumed sugar (%) Mean ± SD	Biomass yield (g/g) Mean ± SD
Glucose media	$0.29^{c} \pm 0.006$	$1.37^{c} \pm 0.008$	$0.21^{c} \pm 0.002$
Potato peel media	$0.42^{b} \pm 0.003$	$1.42^{b} \pm 0.003$	$0.30^{b} \pm 0.005$
Mix media	$0.52^{a} \pm 0.002$	$1.65^{a} \pm 0.004$	$0.32^{a} \pm 0.004$
Significance level (95%)	$P < 0.001$		

Table 1: Screening of best yield giving media based on total biomass quantity

Means that do not share a letter are significantly different in a column. Initial sugar: Glucose=3%, Potato peel media=1.8%, Mix media=3% Mix medium and potato peels medium yielded more biomass as compared to the glucose medium and mix medium was selected as best yield-producing medum. The clustered bar graph respresenting the total dry biomass, biomass yield and consumed sugar of each medium was shown in figure 1.

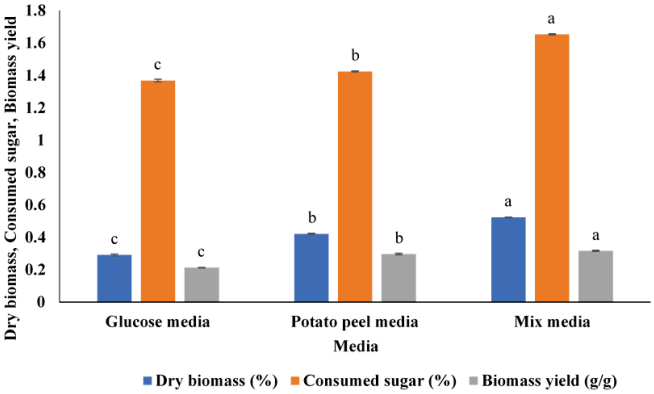

Figure 1: Screening of medium with different carbon sources for biomass production.

The effect of fermenter type on the growth of SCP was studied by fermenting one litre of mix media in both STR and Bubble column fermenter shown in Figure 2.3. and 2.4, respectively. The biomass from both fermenters were harvested after three days. The maximum yield of dry cell biomass (5.452 g/L) was obtained from Airlift fermenter. The results were mentioned in table 2.

Biomass	Stirred-Tank Bioreactor	Bubble Column Fermenter
Dry mass (g/L) ± SD	$4.52^{b} \pm 0.020$	$5.45^{a} \pm 0.010$
Significance level (95%)	$P < 0.001$	

Table 2: Effect of fermenter type on the yield of biomass

Means that do not share a letter are significantly different

The total quantity of dry cell biomass was significantly different with STR and bubble column fermenter ($p < 0.001$). The change in total dry biomass with respect to the fermenter type depicted in Figure 2.

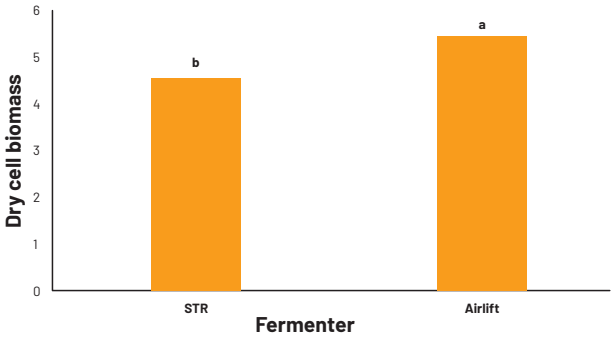

Figure 2: Dry cell biomass production in different bioreactors

Total nitrogen and crude protein in *Rhizopus oligosporous* fungal biomass was determined with micro-Kjeldhal method and it was found that it was found that the dry mass contained 50% crude protein.

DISCUSSION

Fermentation is the process by which single cell protein can be yielded and there are three types of fermentation: solid state, semi-solid and submerged fermentation. The main difference in all types is of moisture content of substrate being fermented. Each type has its own pros and cons [2, 3]. The process of fermentation is often followed by the harvesting, filtration, centrifugation, washing, cell disruption, protein extraction and purification steps [2]. Substrates provide nutrition for microorganism which participate in the fermentation process because these are rich in organic compounds essential for the fermentation. In submerged fermentation all the nutrients are completely dissolved in liquid medium and factors like pH and temperature can be controlled easily [17]. In our study, potato peels were used as substrate in submerged type of fermentation and the process of single cell protein (SCP) production was carried out at 35 ℃ temperature as well as at 5.5 pH. One study reported that different fungi produced different quantity of crude protein (*A. niger*-6.26%, *R. stolonifera*-7.25%, *R. pusillus*-6.63%, *A. fumigatus*-6.28%) on jackfruit peels [18]. According to many studies, the 1.0 vvm rate of oxygen flow accounts for better output. According to this review the most frequently used temperature and pH are in the range of 25-38 ℃ as well as 3.5-5.0 respectively [19]. This study is also conducted with

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1vvm aeration rate in both STR and airlift fermenter at 35 ℃ and pH 5.5. The results of our study indicates that maximum yield of dry biomass (0.523 g/100 ml) was obtained when the potato peel media was supplemented with glucose. Similar results were reported by Mondal *et al*. (2012) where glucose was added in fruit hydrolysate to enhance the biomass production from *S. cerevisiae* because glucose acts as a carbon source [20]. The total crude protein obtained from orange peels (30 %) was lower than that of obtained with cucumber peels which was 53.4 %. In our study almost 50 % crude protein was obtained with *Rhizopus oligosporus*. The findings of our study were in line with that of Khan *et al*. (2009) who obtained the crude protein of 59.5 mg from papaya waste, 57.3 mg from cucumber peels, 51.6 mg from pomegranate waste, 48 mg from pineapple skin and 43.2 mg from skin of watermelon through the process of fermentation by using *Rhizopus oligosporus* [21]. In one research conducted by Yousufi (2012a), Okara and wheat grit were used as substrates to produce SCP by using *Rhizopus oligosporus* and *Aspergillus oryzae* [22]. The substrates were managed in ratio 3: 1, 1: 1 and 1: 3 and fermented at different pH (3,4,5,6,7) at 30 C. The results showed that maximum SCP was achieved at pH 5. Oshoma *et al*. (2017), made three media: glucose banana peel medium, supplemented banana peel medium and un-supplemented banana peel medium [23]. Media were autoclaved and inoculated with *Aspergillus niger*. After that the media were fermented for 8 days at 28 ℃. The highest biomass obtained was 3.05 g/L in supplemented banana peel media. The protein content of supplemented banana peel medium was 0.68 g/L, that of glucose banana peel medium was 0.67 g/L and of un-supplemented medium was 0.57 g/L. The results of this study are contrary to our research work as we obtained a good yield of biomass and total crude protein. The reason may be a difference of substrate or microorganism (fungus) as the factors that influence the production of dry cell biomass are mainly the type of substrate and the type of microorganisms [18].

CONCLUSIONS

It is concluded that potato peels can be an attractive substrate for the production of single cell protein as they are good source of sugar and other nutrients required for the support of microorganisms. Supplementation of basic media with a nitrogen source can also enhance the yield of dry cell biomass. Likewise other cheaper agro-industrial sources can be utilized to produce SCP that can be used as feed for animals. Utilization of cheaper and waste resources will not only help in minimizing the environmental pollution issue but also aid in reducing the cost of protein rich meals used as feed in animal culturing. Single cell protein is a better substitute for proteins which are being obtained from agriculture sector.

Conflicts of Interest

The authors declare no conflict of interest

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