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Influence of different Carbon and Nitrogen Sources on the Production of Single Cell Biomass from Potato Peels

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ABSTRACT

Potato peel can be converted into various value-added compounds, such as enzymes, bio sorbents, biohydrogen, and biogas. **Objective:** To evaluate the influence of different Carbon and Nitrogen Sources on the Production of Single Cell Biomass from potato peels. **Methods:** The process of fermentation was carried out in mix broth with different concentrations of carbon, nitrogen, and different nitrogen sources to determine the effect of these factors on the production of SCP. **Results:** Maximum yield of dry cell biomass (0.251 and 0.245 g/100 ml) was achieved with organic nitrogen source peptone and inorganic nitrogen source ammonium sulphate, respectively. Ammonium sulphate is more suitable to use as peptone is expensive organic nitrogen source. Next optimization of nitrogen concentration was done with ammonium sulphate with different concentrations and best yield (0.190 g/100 ml) was obtained with 1.5% nitrogen concentration. **Conclusions:** In conclusion, the study suggests that ammonium sulphate is a more suitable nitrogen source than peptone for maximizing the yield of dry cell biomass. Additionally, optimization of nitrogen concentration with ammonium sulphate showed that a 1.5% concentration is the best for achieving the highest yield. These findings have important implications for improving the efficiency and cost-effectiveness of industrial-scale production of dry cell biomass.

INTRODUCTION

Using emerging scientific approaches, developing countries can expand their economies by converting their low-cost industrial and agricultural wastes into more valuable products [1]. As potato peel waste increases due to increased consumption of manufactured edible potato products, it can also be converted into various value-added compounds, such as enzymes, bio sorbents, biohydrogen, and biogas [2]. Rapid increase in population is damaging the quality of life and causing shortage of food and resources. Researchers all over the world are putting efforts to control this issue by technological progress. Currently in this global village technology enables us to make precise estimates of how much resources we shall need in future for human livelihood [3, 4]. Two basic factors that will increase demand of food and water in future are: First, increase in population at rate of 1.4, so by 2050 the human population will grow to nearly 9.3 billion people; Second, the development in standard of living which means by 2050 about 3 billion people will join widening middle class, mainly due to economic progress in developing countries [1]. Which will result in changed life style and different food requirements. These changes will cause 50% hike in protein demand and 102% rise in demand of meat products in future. Consequently, tackling such global issues several research studies are focusing new ways of protein production. Making single cell proteins (SCPs) by using agricultural waste source through fermentation is one of the most beneficial approaches [5, 6]. According to literature "SCPs are the dried cells of microorganisms such as fungi, algae and bacteria that are

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used as protein supplement in human foods or animal feeds". By using cheap feedstock and waste products as source of carbon and energy, one can use microorganisms to produce biomass and protein concentrates[7]. Potatoes are produced in large quantity throughout the world. Substantial amount of potato waste is created due to its broad use in different food industries. Potato waste can be used to produce environment friendly industrial goods. Various "green chemistry" techniques can help in extracting polyphenols from potato peels which can have both environmental and economic benefits. Anyhow, further research is necessary to improve processing lines like Investment of capital, use of energy, yield, nature of solvent and integration [8, 9]. Till now no unconventional processes carry out all these requirements and it is still a challenge to produce cost effective products at industrial level [10]. Huge amount of potato crops is produced every year throughout the world and is one of the most important components of human nutrition. Potato peel waste has zero worth as by-product of potato-processing industries. Almost 15-40 % of total potato weight turns into potato peel waste depending upon the different peeling process used by food industries [11]. Such large quantity of food waste creating worries for many scientists especially in Europe and their scientific research provides multiple solutions for the problem. The article focused on summarizing the research work on how potato peel waste can be utilized in food producing industries [12]. Results show that potato peel waste can be used as antioxidant in food chain as it has large phenol content, partial flour substitute and in fermentation as solid substrate [11, 13].

METHODS

The process of fermentation was carried out in mix broth with different concentrations of carbon, nitrogen, and different nitrogen sources to determine the effect of these factors on the production of SCP. To study the effect of different carbon concentrations on the mix broth (100 ml) with different carbon concentration (%) of 1, 2, 3, 4 and 5 were prepared in flasks. These flasks were properly plugged and autoclaved at 121 °C for 15 minutes after adjusting thier pH at 5.5. These flasks were inoculated with *R. oligosporous* under aseptic conditions. These flasks were then incubated at 35 °C for 3 days. After three days, inoculated flasks were filtered with whatmann filter paper and biomass was separated. All the experiments were carried out in triplicates. The influence of diffrent organic (urea, peptone and yeast extract) and inorganic (Ammonium sulphate and Ammonium nitrate) nitrogen sources was tested on the production of single cell biomass by adding each of the nitrogen source into the growth media at 0.5% w/v. The pH of each flask was aadjusted at 5.5 with 1M HCl/ 1M NaOH and then plugged with the cotton. These media were sterlized by autoclaving at 121 °C for 15 minutes. After autoclaving, inoculum prepared from slants of *Rhizopus oligosporous* was added into each flask at 2% v/v in the laminar air flow and incubated at 35°C for three days. After three days, biomass was separated from filtrate by filration by using whatmann filter paper No. 1. All the experiments were carried out in triplicates. In the previous experiments best yield of SCP was achieved with Ammonium sulpahte, therefore in this parameter the effect of different concentarions of Ammonium sulphate was explored. The experiment was carried in triplicate flasks by adding Ammonium sulphate in concentrations (%) of 0, 0.5, 1 and 1.5. The pH of all the media was adjusted with 1M HCL and 1M NaOH at 5.5 and plugged with cotton properly before autoclaving. The media were sterilized by autoclaving at 121°C for 15 minutes and then 1 ml of inoculum was added in each flask under aseptic conditions. All the flasks were incubated at 35 °C and after three days the biomass was collected by the filtration process. The wet biomass was weighed and dried before taking the dry biomass weight. All the experiments were carried out in triplicates.

RESULTS

The effect of different carbon concentrations (0%, 1%, 2%, 3%, 4%, 5%) was evaluated in the fermentation process to get the best quantity of dry cell biomass. The results obtained were described in Table 1. The best yield of single cell protein (0.556 g/100 ml) was acquired with carbon concentration of 3% after three days of fermentation period. The maximum crude protein content of dried biomass obtained was 45-55 %. The stastical analysis showed that the dry biomass with respect to the different concentrations of glucose was highly significant ($p < 0.001$). The clustered bar graph respresnting the total dry biomass, biomass yield and consumed sugar of each concentration of glucose was shown in Figure 1.

Sr. No.	Glucose (%)	Dry biomass (%) Mean ± SD	Consumed sugar (%) Mean ± SD	Biomass yield (g/g) Mean ± SD
1.	1.	$0.33^{d} \pm 0.003$	$1.87^{e} \pm 0.007$	$0.18^{b} \pm 0.003$
2.	2.	$0.40^{c} \pm 0.018$	$2.15^{d} \pm 0.005$	$0.19^{b} \pm 0.005$
3.	3.	$0.56^{a} \pm 0.007$	$2.55^{c} \pm 0.004$	$0.22^{a} \pm 0.004$
4.	4.	$0.45^{b} \pm 0.017$	$4.43^{a} \pm 0.003$	$0.10^{c} \pm 0.013$
5.	5.	$0.45^{b} \pm 0.015$	$4.34^{b} \pm 0.004$	$0.10^{c} \pm 0.006$
	Significance level (95%)	$p<0.001$		

Means that do not share a letter are significantly different in a column

Table 1: Effect of different carbon concentrations on the biomass growth

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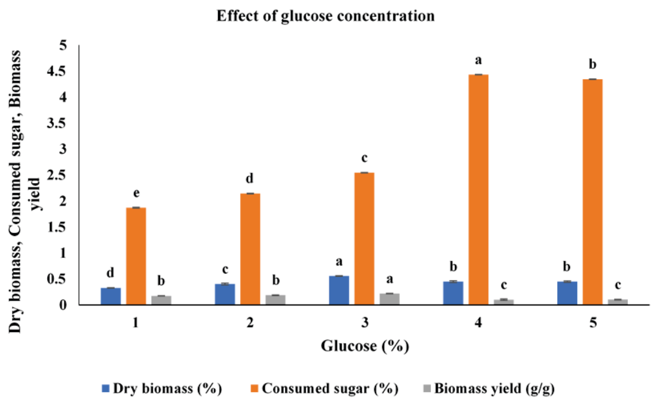

Figure 1: Effect of different glucose concentrations on total biomass yield, total dry biomass and sugar consumed of media

The effect of different organic (urea, peptone, yeast extract) and inorganic (ammonium sulphate, ammonium nitrate) nitrogen sources on the production of single cell protein was studied during the process of submerged fermentation. The results obtained were mentioned in Table 2. which showed that best yield (0.253 g/100 ml) was obtained with ammonium sulphate followed by peptone (0.240 g/100 ml). The total crude protein content of dried cell biomass estimated was in range of 45-55%. The stasistical study showed that dry biomass yield was highy significant with respect to the different nitrogen sources ($p < 0.001$). The clustered bar graph respresnting the total dry biomass, biomass yield and consumed sugar of different nitrogen sources was shown in Figure 2.

Sr. No.	Nitrogen Sources	Dry biomass (%) Mean ± SD	Consumed sugar (%) Mean ± SD	Biomass yield (g/g) Mean ± SD
1.	Ammonium Sulphate	$0.25^{a} \pm 0.007$	$2.59^{a} \pm 0.006$	$0.09^{a} \pm 0.003$
2.	Ammonium Nitrate	$0.23^{b} \pm 0.010$	$2.59^{b} \pm 0.007$	$0.09^{a} \pm 0.007$
3.	Peptone	$0.24^{ab} \pm 0.005$	$2.66^{b} \pm 0.004$	$0.09^{a} \pm 0.004$
4.	Yeast Extract	$0.12^{c} \pm 0.008$	$2.45^{d} \pm 0.003$	$0.05^{b} \pm 0.008$
5.	Urea	$0.06^{d} \pm 0.010$	$2.47^{c} \pm 0.004$	$0.02^{c} \pm 0.005$
	Significance level (95%)	$p<0.001$		

Means that do not share a letter are significantly different in a column

Table 2: Effect of different nitrogen sources on the biomass yield

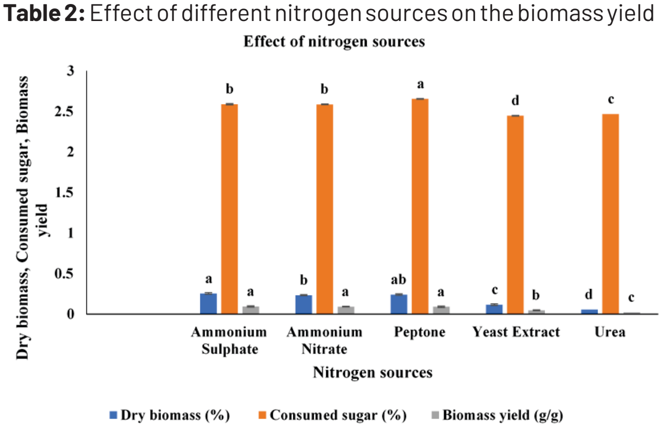

Figure 2: Effect of different nitrogen sources on the yield of dry cell biomass and total sugar consumed

The influence of different nitrogen concentrations (0%, 0.5%, 1%, 1.5%) were studied to oobtain maxium yield. The results obtained were mentioned in Table 3. the results showed that maxium yield of biomass (0.190 g/100 ml) was obtained with nitrogen contentaion of 1.5 %. The crude protein content of dried cell biomass was in range of 45-55%. The statistical study showed that the effect of different nitrogen concentrations on the dry biomass yield was highly significant ($p < 0.001$). The clustered bar graph respresnting the total dry biomass, biomass yield and consumed sugar of different concentrations of nitrogen (ammonium sulphate) was shown in Figure 3.

Sr. No.	Nitrogen Concentration ($(NH_4)_2SO_4$) (%)	Dry biomass (%) Mean ± SD	Consumed sugar (%) Mean ± SD	Biomass yield (g/g) Mean ± SD
1.	0	$0.01^{c} \pm 0.006$	$2.27^{d} \pm 0.005$	$0.00^{a} \pm 0.004$
2.	0.5	$0.16^{b} \pm 0.004$	$2.66^{c} \pm 0.005$	$0.06^{a} \pm 0.008$
3.	1	$0.15^{b} \pm 0.014$	$2.69^{b} \pm 0.004$	$0.06^{a} \pm 0.005$
4.	1.5	$0.19^{a} \pm 0.007$	$2.74^{a} \pm 0.009$	$0.07^{a} \pm 0.006$
	Significance level (95%)	$p < 0.001$		

Means that do not share a letter are significantly different in a column

Table 3: Effect of different nitrogen concentrations on the SCP yield

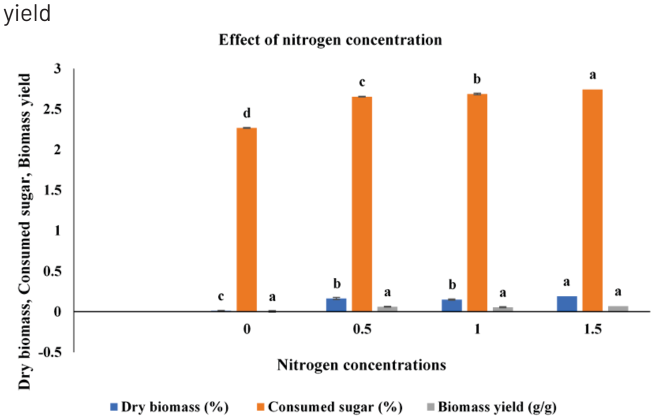

Figure 3: Effect of different concentration of Ammoinium sulphate on the production of dry cell biomass and total sugar consumed

DISCUSSION

The factors that influence the production of dry cell biomass are mainly the type of substrate, the type of microorganisms, inoculum age and size, composition of media, incubation temperature, pH, incubation time, nitrogen sources, carbon concentration, heat removal, aeration as well as agitation speed *etc.*, [14, 7]. Different studies showed that particle size and quantity of nitrogen source are the factors that greatly influence biomass production and its protein content [15]. In our study different nitrogen sources were tested to achieve better quantity of dry cell biomass and ammonium sulphate gave the best yield at 1.5 % w/v. In case of inorganic nitrogen source ammonium sulphate gave the highest yield while in

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case of organic nitrogen source peptone provided with best growth. Similar case happened when ammonium sulphate was added into the fermentation media then maximum yield of biomass was obtained with *A. niger* and *S. cerevisiae*. Ammonium sulphate not only provide nitrogen but also Sulphur to the medium for better growth [16]. Yousufi (2012), attained the maximum content of protein (61.2 mg/100 g) on fruit waste through solid state fermentation from *Rhizopus oligosporus* while in our study 45-55% crude protein was achieved from dry cell biomass of *R. oligosporus* [17]. The increase in our protein content may be due to supplementation with nitrogen source. Nguyen *et al.*, obtained maximum protein content of (39.71%) *Rhizopus oligosporus* biomass by using the cassava as a substrate with different quantities of macro and micro nutrients under variable conditions [18]. In our study 50% crude protein content was obtained with same fungus but different substrate with diverse compositions and under different conditions. The difference may be due to change in substrate, micro, macro supplements and conditions[19, 20].

CONCLUSIONS

Different factors affecting production of biomass from potato peels was studied in this research. Since potato peels are a good source of sugar and other nutrients required for microorganisms to survive, potato peels can be considered an attractive substrate for the production of single cell protein. In addition to adding nitrogen to basic media, it is also possible to boost the yield of dry cell biomass by supplementing it with nitrogen. Additionally, animals can be fed SCP produced from other cheaper agro-industrial sources. As a result of reducing the cost of protein-rich meals used as feed for animals through the use of cheaper and waste resources, the environmental pollution issue will be minimized. In comparison to proteins obtained from agriculture, single cell proteins are a better alternative.

Conflicts of Interest

The author declare no conflict of interest.

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