

**Original Article****Effect of pH and different Fermentation Time Intervals on the Production of Single Cell Proteins (SCPs) from Potato Peels**Hira Nadeem^{1*}¹Department of Zoology, University of the Punjab, Lahore, Pakistan

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ABSTRACT

Dried cells of microorganisms such as fungi, algae, and bacteria, known as Single-Cell Proteins (SCPs), are utilized as a source of protein supplements in animal feed or human food. These SCPs can be produced through the use of low-cost feedstocks and waste materials as sources of carbon and energy, which can be converted into biomass and concentrated proteins.

Objective: To optimize the yield and growth of dry cell biomass through the manipulation of fermentation conditions. **Methods:** A batch fermentation process was used to produce dry cell biomass from a microorganism. Different pH values, fermentation times, and reactor configurations were tested, and the resulting biomass was analyzed for its protein content.

Results: The maximum yield of dry cell biomass was achieved at pH 4.5, with a yield of 1.951 g/100 ml. The maximum dry biomass was achieved after 72 hours of fermentation, with a yield of 2.824 g/100 ml. The maximum yield of dry biomass was achieved with an Airlift fermenter at an aeration rate of 1.0 vvm and a temperature of 35°C for 72 hours, resulting in a yield of 5.452 g/L. The protein content of the dried cell biomass was found to be in the range of 45-55%.

Conclusions: This study demonstrates that the yield and growth of dry cell biomass can be optimized by controlling the fermentation conditions, specifically pH, fermentation time, and reactor configuration. These findings may have implications for the industrial-scale production of dry cell biomass, as they offer insight into how to maximize yield and protein content.

INTRODUCTION

Developing countries can expand their economies by converting low-cost industrial and agricultural waste into valuable products using emerging scientific approaches [1]. Potato peel waste, which is generated due to increased consumption of manufactured edible potato products, can be converted into value-added compounds such as enzymes, biosorbents, biohydrogen, and biogas [2]. The global population is expected to reach 9.3 billion by 2050, and an increase in the standard of living will cause a 50% hike in protein demand and a 102% rise in demand for meat products [3]. Researchers all over the world are making efforts to control these issues by making technological progress [4]. One of the most beneficial approaches for protein production is the production of single-cell proteins (SCPs) from agricultural waste sources through

fermentation [5]. SCPs are dried cells of microorganisms such as fungi, algae, and bacteria that are used as protein supplements in human foods or animal feeds [6]. By using cheap feedstock and waste products as a source of carbon and energy, microorganisms can produce biomass and protein concentrates [7]. Potatoes are one of the most important components of human nutrition and are produced in large quantities worldwide. Substantial amounts of potato waste are created due to its broad use in different food industries [8]. Potato peel waste, which accounts for 15-40% of the total weight of potatoes depending on the peeling process used, has zero worth as a by-product of potato-processing industries [9]. Potato peel waste can be utilized as an antioxidant in the food chain due to its large phenol content, partial flour

substitute, and as a solid substrate in fermentation [10]. "Green chemistry" techniques can be used to extract polyphenols from potato peels, which can have both environmental and economic benefits [8]. However, further research is necessary to improve processing lines, including the investment of capital, use of energy, yield, nature of solvent, and integration, to produce cost-effective products at the industrial level [11].

METHODS

The process of fermentation was carried out in mix broth with different pH and fermentation time intervals to determine the effect of these factors on the production of SCP. The effect of different pH levels on the growth of single cell biomass was investigated by adjusting the pH of growth media at 4.5, 5, 5.5, 6, and 6.5. All the flasks were then plugged and autoclaved at 121 °C for 15 minutes. Afterward these flasks were inoculated by adding inoculum at 2% v/v and incubated at 35 °C for 3 days. After three days the biomass was harvested and dried for further study. All the experiments were carried out in triplicates. To study the influence of fermentation time period on the cell biomass growth, seven flasks of growth media were prepared and adjusted their pH at 5.5 with 1M HCl/ 1M NaOH. These media were then autoclaved for 15 minutes at 121 °C and inoculated with 2 ml of inoculum prepared from *Rhizopus oligosporus* subcultures. These media were incubated at 35 °C for further study. The biomass from each flask was collected after the gap of 24 hours for subsequent seven days through the process of filtration and both wet as well as dry weight of biomass was measured each time. All the experiments were carried out in triplicates. For determination of reducing sugars Benedict's quantitative test was used [12]. The crude protein of single cell protein was determined by the Kjeldhal procedure [13]. The total protein in the growth media was estimated by following the Lowrey method [14].

RESULTS

Different pH values (4.5, 5, 5.5, 6, 6.5) of fermented media were investigated to obtain best yield of *Rhizopus oligosporus* biomass. The results mentioned in Table 1, showed that best quantity of dry biomass (1.048 g/100 ml) was yielded at pH of 5.5. The total crude protein produced was 45-55 %. The statistical analysis confirmed that the results of yield of dry biomass were significantly different at 4.5 pH ($p < 0.001$). The clustered bar graph representing the total dry biomass, biomass yield and consumed sugar of mix media at different pH was shown in Figure 1.

Sr. No.	Nitrogen Sources	Dry biomass (%) Mean ± SD	Consumed sugar (%) Mean ± SD	Biomass yield (g/g) Mean ± SD
1.	4.5	1.95 ^a ± 0.004	2.77 ^a ± 0.004	0.71 ^a ± 0.004
2.	5	1.01 ^b ± 0.015	2.81 ^a ± 0.003	0.36 ^b ± 0.006
3.	5.5	1.05 ^b ± 0.007	2.88 ^a ± 0.005	0.36 ^b ± 0.004
4.	6	0.71 ^c ± 0.006	2.72 ^a ± 0.009	0.26 ^d ± 0.005
5.	6.5	0.90 ^d ± 0.012	2.86 ^b ± 0.005	0.31 ^e ± 0.005.
	Significance level (95%)	$p < 0.001$		

Means that do not share a letter are significantly different

Table 1: Effect of different pH value on the biomass growth

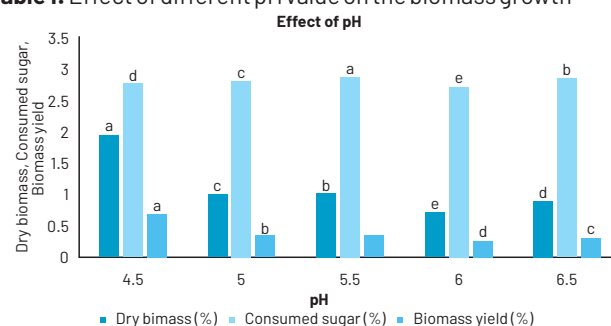


Figure 1: Effect of different pH levels on the total production of dry cell biomass and total sugar consumed in media

Seven flasks of mix media were inoculated to study the effect of different time periods on the process of fermentation. The biomass was harvested from each flask after 24 hours time intervals. The results of bioprotein yield obtained from different time period was shown in Table 2. The maximum dry biomass (2.824 g/100 ml) was obtained after three days of fermentation. The statistical study showed that dry cell biomass yield was significantly different at different time period ($p < 0.001$). The crude protein production after third day was not significantly different. The clustered bar graph representing the total dry biomass, biomass yield and consumed sugar of each medium was shown in Figure 2, while the variation in total crude protein content with respect to fermentation time period was depicted in Figure 3.

Sr. No.	Nitrogen Sources	Dry biomass (%) Mean ± SD	Consumed sugar (%) Mean ± SD	Biomass yield (g/g) Mean ± SD
1	0.26g ± 0.013	0.79d ± 0.019	0.33d ± 0.004	23.67c ± 0.501
2	0.48f ± 0.004	1.48c ± 0.004	0.33d ± 0.008	36.37b ± 0.39
3	2.82a ± 0.004	2.34b ± 0.007	1.21a ± 0.005	50.21a ± 0.615
4	1.28b ± 0.006	3.00a ± 0.006	0.43b ± 0.009	50.32a ± 0.142
5	1.16c ± 0.008	3.00a ± 0.009	0.39c ± 0.007	50.37a ± 0.072
6	1.02d ± 0.013	3.00a ± 0.006	0.34d ± 0.005	50.40a ± 0.043
7	0.98e ± 0.026	3.00a ± 0.005	0.33d ± 0.007	50.45a ± 0.046
	Significance level (95%)	$P < 0.001$		

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

Table 2: Effect of different fermentation periods on SCP production

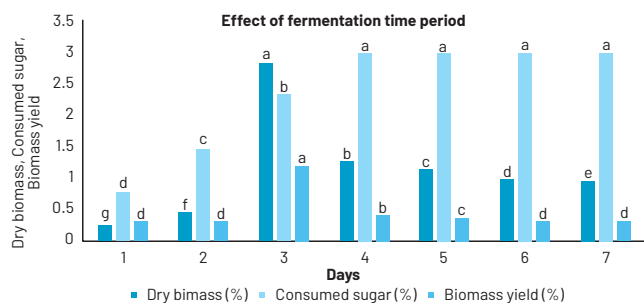


Figure 2: Effect of fermentation time period on the total dry cell biomass and consumed sugar as well as biomass yield

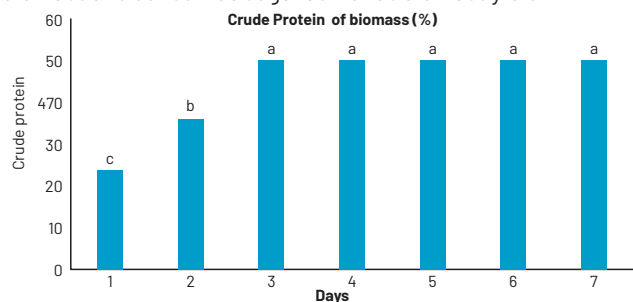


Figure 3: Crude protein variation with respect to the fermentation time period

DISCUSSION

The aim of this study was to investigate the effect of different pH values and fermentation time periods on the production of biomass. The study found that the best yield of dry biomass was obtained at pH 5.5, with a total crude protein produced ranging from 45–55%. The highest yield of dry biomass was achieved after three days of fermentation, with no significant difference in crude protein production after this point. The results of this study are consistent with previous research on the effect of pH on microbial growth. For example, a study by Qin *et al.*, found that the optimal pH for mycelial growth of *Rhizopus nigricans* was 5.5 [15]. Similarly, a study by Dinarvand *et al.*, reported that the maximum biomass production of *Aspergillus niger* was obtained at pH 5.5 [16]. The findings regarding the effect of fermentation time on biomass production are also consistent with prior research. For example, a study by Carboue *et al.*, (2012) found that the maximum biomass production of *Rhizopus oryzae* was achieved after three days of fermentation [17]. Similarly, a study by Zhu *et al.*, reported that the highest biomass yield of *Panus conchatus* was obtained after three days of fermentation [18]. However, the study did not investigate the effect of factors such as temperature or substrate concentration on biomass production, which could be important to consider in future research. Overall, the findings of this study suggest that pH and fermentation time are important factors to consider in the production of biomass. By optimizing these parameters, it may be possible to increase the yield of biomass and reduce the

cost of protein-rich meals used as feed for animals, while minimizing environmental pollution [19, 20].

CONCLUSIONS

This research investigated various factors that impact the production of biomass from potato peels. Potato peels are a valuable substrate for producing single cell protein because they contain essential nutrients like sugar that microorganisms need to survive. To increase the yield of dry cell biomass, nitrogen can be added to the basic media. Moreover, using single cell protein from less expensive agro-industrial sources to feed animals can lower the cost of protein-rich animal feed, reduce waste, and decrease environmental pollution. Compared to traditional agricultural protein sources, single cell proteins offer a superior alternative.

Conflicts of Interest

The author declare no conflict of interest.

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REFERENCES

- [1] Department of Economic and Social Affairs. World population projected to reach 9.7 billion by 2050. 2015. [Last cited: 20th Feb 2023]. Available from: <https://www.un.org/en/desa/world-population-projected-reach-98-billion-2050-and-112-billion-2100>.
- [2] Javed A, Ahmad A, Tahir A, Shabbir U, Nouman M, Hameed A. Potato peel waste—its nutraceutical, industrial and biotechnological applications. *AIMS Agriculture and Food*. 2019 Sep; 4(3): 807–823. doi: 10.3934/agrfood.2019.3.807.
- [3] Khan MK, Asif M, Razzaq ZU, Nazir A, Maan AA. Sustainable food industrial waste management through single cell protein production and characterization of protein enriched bread. *Food Bioscience*. 2022 Apr; 46: 101406. doi: 10.1016/j.fbio.2021.101406
- [4] Sheikh RA, Al-Bar OA, Soliman YM. Biochemical studies on the production of biofuel (bioethanol) from potato peels wastes by *Saccharomyces cerevisiae*: effects of fermentation periods and nitrogen source concentration. *Biotechnology & Biotechnological Equipment*. 2016 May; 30(3): 497–505. doi: 10.1080/13102818.2016.1159527
- [5] Reihani SF and Khosravi-Darani K. Influencing factors on single-cell protein production by submerged fermentation: A review. *Electronic Journal of Biotechnology*. 2019 Jan; 37: 34–40. doi: 10.1016/j.ejbt.2018.11.005

- [6] Ritala A, Häkkinen ST, Toivari M, Wiebe MG. Single cell protein—state-of-the-art, industrial landscape and patents 2001–2016. *Frontiers in Microbiology*. 2017 Oct; 8: 2009. doi: 10.3389/fmicb.2017.02009
- [7] Sindhu R, Binod P, Pandey A. Biological pretreatment of lignocellulosic biomass—An overview. *Bioresource Technology*. 2016 Jan; 199: 76–82. doi: 10.1016/j.biortech.2015.08.030
- [8] Gaudino EC, Colletti A, Grillo G, Tabasso S, Cravotto G. Emerging processing technologies for the recovery of valuable bioactive compounds from potato peels. *Foods*. 2020 Nov; 9(11): 1598. doi: 10.3390/foods9111598
- [9] Sepelev I and Galoburda R. Industrial potato peel waste application in food production: a review. *Research for Rural Development*. 2015 May; 1: 130–6.
- [10] Food and Agriculture Organization of the United Nations. *World Agriculture: towards 2015/2030*. 2002. [Last Cited: 20th Feb 2023]. Available at: <https://www.fao.org/3/y3557e/y3557e00.pdf>.
- [11] Delgado CL. Rising consumption of meat and milk in developing countries has created a new food revolution. *The Journal of Nutrition*. 2003 Nov; 133(11): 3907S–10S. doi: 10.1093/jn/133.11.3907S
- [12] Hernández-López A, Sanchez Felix DA, Zuñiga Sierra Z, Garcia Bravo I, Dinkova TD, Avila-Alejandre AX. Quantification of reducing sugars based on the qualitative technique of Benedict. *ACS Omega*. 2020 Dec; 5(50): 32403–10. doi: 10.1021/acsomega.0c04467
- [13] Sáez-Plaza P, Michałowski T, Navas MJ, Asuero AG, Wybraniec S. An overview of the Kjeldahl method of nitrogen determination. Part I. Early history, chemistry of the procedure, and titrimetric finish. *Critical Reviews in Analytical Chemistry*. 2013 Oct; 43(4): 178–223. doi: 10.1080/10408347.2012.751786
- [14] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*. 1951; 193: 265–75. doi: 10.1016/S0021-9258(19)52451-6
- [15] Qin G, Li G, Li P, Wang M, Hong X, Wang G. Optimization of fermentation process of exopolysaccharides from *Rhizopus nigricans* by response surface methodology. *Mycosystema*. 2019; 38(9): 1570–7.
- [16] Dinarvand M, Rezaee M, Foroughi M. Optimizing culture conditions for production of intra and extracellular inulinase and invertase from *Aspergillus niger* ATCC 20611 by response surface methodology (RSM). *Brazilian Journal of Microbiology*. 2017 Jul; 48: 427–41. doi: 10.1016/j.bjm.2016.10.026
- [17] Carboué Q, Tranier MS, Perraud-Gaime I, Roussos S. Production of microbial enzymes by solid-state fermentation for food applications. In *Microbial enzyme technology in food applications 2017* Mar: 437–451. doi: 10.1201/9781315368405-28
- [18] Zhu M, Han Y, Hu X, Gong C, Ren L. Ergothioneine Production by Submerged Fermentation of a Medicinal Mushroom *Panus conchatus*. *Fermentation*. 2022 Aug; 8(9): 431. doi: 10.3390/fermentation8090431
- [19] Mondal AK, Sengupta S, Bhowal J, Bhattacharya DK. Utilization of fruit wastes in producing single cell protein. *International Journal of Science, Environment and Technology*. 2012; 1(5): 430–8.
- [20] Ismail S. Production of Single Cell Proteins (SCP) by *Cupriavidus necator*: Impact of environmental parameters, carbon and nitrogen sources (Doctoral dissertation, INSA de Toulouse). 2022. Available at: <https://theses.hal.science/tel-03718434/>.