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Optimization of Single Cell Protein Production by *Aspergillus niger* Using Taguchi Approach

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ABSTRACT: World population has been continuously raised and therefore due to this fact new source of foods, particularly proteins, are in demand. In recent years, single cell protein has been considered as an accepted substitute for animal and plant proteins. In this study, single cell protein production was studied in a batch submerged culture using *Aspergillus niger* PTCC5012. Experimental design was performed by Qualitek-4 software using Taguchi as a fractional factorial statistical method. Glucose, magnesium sulfate and potassium hydrogen phosphate concentrations as well as the pH were considered to be the four key parameters, each one at four different levels. Optimal conditions were created to achieve the maximum single cell protein of *Aspergillus niger* PTCC5012 in respect of the optimum concentrations of the mentioned parameters. An acceptable consistency of 94% was observed between optimum single cell protein proposed by the software and the experimentally measured one. Glucose concentration was evaluated as the most effective parameter on single cell protein production yield with 40.95% contribution. The concentration of potassium hydrogen phosphate was introduced as the less effective factor on single cell protein production with 13.99% contribution.

Keywords: *Aspergillus niger, Batch Submerged Culture, Culture Composition, Single Cell Protein, Taguchi Method.*

Introduction

According to the increasing world population and the limitations in animals and plants protein sources, single cell protein (SCP) production has attracted the attention of investigators in recent years. Single cell protein or microbial protein is introduced as a good protein source to provide required supplements for human food and animal feed. The first time, this term was used in the Massachusetts Institute by Carol Wilson. Using the term "SCP" was aimed to avoid microbial or bacterial words in nutritional field (Schultz *et al*., 2006). British oil industry has been innovated a method by which yeasts, separate the branchless hydrocarbons from the branched hydrocarbons via converting them to microbial the biomass. SCP production from hydrocarbons was considered as an independent field of the petrochemical industry (Raja *et al.,* 2008). Petroleum Co. produced several thousand tons of SCP in a small commercial scale. Imperial Chemical Industry Co. was a successful large-scale SCP producer for several years. All the developed countries made some efforts to produce SCP. In Germany, Hoechst Ajay Co. performed a series of fundamental research and development programs in large scale production supported by the Ministry of Research and Technology. In Japan, some developing activities were performed at industrial scale (Rajoka *et al.,* 2006).

In mid1930s and World War II, this matter was more focused and SCP

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production reached to 15 thousand tons per year. Research team of British Petroleum Co. obtained microorganisms able to grow on normal paraffin. In 1965, a setup was designed to produce about 400 tons SCP per year. Finally in 1970, SCP production with about one hundred thousand tons per year was produced (Anupama and Ravindra, 2000). Imperial Chemical Industry Co. produced a *Fusarium graminearum* fungal SCP similar to meat, structurally. The contents of nucleic acids were low, because using some additional operations to reduce the existed cell RNA. The product is allowed for human usage in England. Initial production was about 1000 tons in 1985 (Paraskevopoulou *et al.,* 2003).

In Iran, a factory was established to produce SCP from sugar cane molasses in Boroujerd. Also, SCP production from methanol is designed at Jahad Research Institute (Gellissen, 2000). Many research activities were carried out for SCP production. For example, SCP production by *Torula* yeasts on whey (Ghaly *et al.,* 2005), industrial scales SCP productions (Ardestani, 2011; Gandhimathi *et al.,* 2012) SCP from cassava plant (Becker, 2007; Bhalla *et al.* 2007; Gregory, 1977), potato starch (Calleja *et al.,* 1986) and cellulosic sulfite wastes and wastewaters (Rasoul-Amini *et al.* 2009). Some of other substrates such as beet pulp (Ghanem, 1992), carbohydrates (Nasseri *et al.* 2011), rice straw (Ghorbani *et al.,* 2012), sugarcane bagasse (EI-Nawwi and EI-Kader, 1996) and pineapple wastes (Dhanasekaran *et al.,* 2011) were used for SCP production too.

In this work, single cell protein production was studied in a batch submerged culture of *Aspergillus niger* PTCC5012. Optimization process was performed with using a fractional factorial statistical method. Qualitek-4 software was used for the experimental design and data analysis. In this process, four separate factors; glucose, magnesium sulfate and potassium hydrogen phosphate concentrations as well as the pH were considered as the most effective parameters, each at four different levels. The aim of this investigation is to optimise the process for the production of more single cell protein.

Materials and Methods

- *Microorganism and inoculums*

Aspergillus niger PTCC5012 was provided from Iranian Research Organization for Science and Technology. Primary culture of potato dextrose agar medium was used. After inoculation, the culture medium was incubated for 4 days at 27°C for the fungi to grow in the form of white and then black colonies. After three days from the incubation, the black spores of *Aspergillus niger* PTCC5012 covered the surface of the plates completely. In order to prepare inoculums, the spores were added to distilled water using a sterile loop under the sterile conditions. The spore suspensions were used as inoculums.

- *Culture medium*

Using a fraction of full factorial approach, 16 shaking flasks were prepared to identify the optimized medium composition to reach the most yields in SCP production. In this work, four factors, each one in four different levels were studied (Table 1). In each flask, medium composition was adjusted based on the proposed orthogonal array by Qualitek-4 software as shown in Table 2. Also, 0.1 mL of trace elements mixture, which included ($g.L^{-1}$) of $FeSO_4 \cdot 7H_2O$ (2.5) $CuSO_4 \cdot 5H_2O$ (0.5), $ZnSO_4 \cdot 7H_2O$ (1), $MnSO_4 \cdot 4H_2O$ (0.5) and $CoSO_4$ (0.5) were added to each shaking flask.

- *Measurements*

Produced single cell protein in each flask was determined quantitatively by weighting the dried filtered biomass for each 24 h of total incubation time (200 h). Biomass was

Table 1. Key factors and their levels assigned to different columns in optimization of SCP production of *Aspergillus niger* PTCC5012 in submerged batch culture medium

Serial number	Factor	Level 1	Level 2	Level 3	Level 4
1	Glucose Con. (g /L)	50	60	70	80
2	$MgSO_4$ Con. (g /L)	0.5	1	1.5	2
3	KH_2PO_4 Con. (g /L)	4	5	6	7
4	pH	5	5.5	6	6.5

Table 2. The layout of the L-16 orthogonal arrays designed by Qualitek-4 software and the obtained results of experimental fermentation with the designed cultures of *Aspergillus niger* PTCC5012.

Factor	Glucose Con.	$MgSO_4$ Con.	KH_2PO_4 Con.	pH
Trial	Factor level			
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	1	4	4	4
5	2	1	2	3
6	2	2	1	4
7	2	3	4	1
8	2	4	3	2
9	3	1	3	4
10	3	2	4	3
11	3	3	1	2
12	3	4	2	1
13	4	1	4	2
14	4	2	3	1
15	4	3	2	4
16	4	4	1	3

filtered using a micro filter with a pore size of 0.2 micron. Filtered biomass was dried in the oven at 65°C for 20-24 h to reach a constant weight. The liquid part of the filtered samples was centrifuged for 5 min at 5000 rpm and then was used for further analysis of glucose concentration.

Results and Discussion

- *SCP Production in Batch Fermentation*

SCP production results are presented in Table. 3. SCP concentration was varied from the lowest amount (33.45 g. $L^{-1)}$ for trial number 3 to the highest value (44.1 g. L^{-1}) at trial number 9. At trial number 9, glucose, $MgSO_4$ and KH_2PO_4 concentrations and pH were in levels 3, 1, 3 and 4, respectively. In other word the concentrations were 70, 0.5, 6 g. L^{-1} for glucose, $MgSO_4$ and KH_2PO_4, respectively with the pH at 6.5.

- *Analysis of the results*

Table 4 presents the average effects of each factor and interactions on the designed

levels of SCP production. The difference between the average value of each factor at level 2 and 1 indexed the relative influence of the effect, the larger the difference greater was the influence. The sign of the differential value indicates whether the change from level 1 to level 2 or 3 increased (+) or decreased (-) the result (Table 4).

Thus, it can be seen that the glucose concentration showed the highest influence to that of other factors and the least contribution was noticed with assigned levels of pH. Based on the adjustment of the optimum levels of influenced factors higher SCP production was achieved. Proposed optimum conditions to achieve the highest SCP production are given in Table 5. Based on the results, the optimum conditions for the glucose concentration was at its level 3 (70 g. L^{-1}). Two other factors ($MgSO_4$ and KH_2PO_4 concentrations) must be adjusted at their level 4 (2, 7 g. L^{-1}, respectively) to achieve the optimum condition for SCP production. The optimum pH value was obtained at level 1. Thus the best pH value for this process must be adjusted at 5. The contribution of each factor to reaching this product yield is presented in the mentioned table too. The results showed that glucose concentration had a significant contribution and KH_2PO_4 concentration played the least role in SCP production in the investigated process.

As might be observed in the table, glucose concentration was the most influencing factor in SCP production with 41% confidence level and a considerable higher level in comparison with other factors. Next to glucose, magnesium sulfate with 27% had the most contribution share in *Aspergillus niger* PTCC5012 SCP production. The sum of the contribution of the other three factors ($MgSO_4$ and KH_2PO_4 concentrations and pH) was about 59%.

Table 3. The obtained results of SCP production in experimental batch fermentation with using the designed cultures of *Aspergillus niger* PTCC5012

Run Number	SCP Concentration (g/L)		
	Repeat 1	Repeat 2	Mean
1	38.75	38.70	38.725
2	33.50	33.65	33.575
3	35.50	35.40	33.45
4	39.00	39.20	39.10
5	39.40	39.50	39.45
6	38.00	38.20	38.10
7	41.50	41.40	41.45
8	40.50	40.60	40.55
9	44.20	44.00	44.10
10	40.20	40.40	40.30
11	41.50	41.20	41.35
12	42.60	42.50	42.55
13	41.40	41.50	41.45
14	40.60	40.50	40.55
15	38.90	39.00	39.95
16	43.00	43.20	43.10

Figure 1 represents the average effects of each factor and interactions at the designed levels on SCP production. The difference between the average value of glucose concentration (factor A) at levels 1, 2 and 3 indexed the relative positive influence of the effect. At level 4 of this factor, a relative negative impact on SCP production was observed. For $MgSO_4$ concentration (factor B), with the change the level from 1 to 2, a decrease in average SCP production was obtained. While at the levels 3 and 4 of this factor, an increasing profile was resulted again. Investigation of the effects of KH_2PO_4 concentration (factor C) showed a similar profile as factor B. In the case of culture medium pH (factor D), as shown in Figure 1, changing the factor level from 1 to 2, followed a relative remarkable drop in average SCP production. However, the changes of pH from level 2 to levels 3 and 4, had not any significant impacts on the mean SCP production.

The results of analysis by qualitek-4 software based on the fraction of the full factorial method indicated that the theoretical expected SCP concentration at optimum conditions was 45.44 $g.L^{-1}$. Next, SCP production under the theoretical optimum conditions was performed experimentally in a batch submerged culture of *Aspergillus niger* PTCC5012. Experimental SCP concentration was obtained as 42 g. L^{-1}. Therefore there is an acceptable consistency equal to 92.43% between the theoretical SCP concentration and the experimental value obtained at optimum conditions.

Conclusion

The optimum conditions concerned with the production of single cell protein based on *Aspergillus niger* PTCC5012 were investigated. The results showed that glucose concentration was the most effective factor on the single cell protein production. Potassium hydrogen phosphate concentration and the pH of medium showed less roles in single cell protein production. The optimum conditions to reach the

Table 4. The main effects of each factor in optimization process of SCP production in experimental batch fermentation with using the designed cultures of *Aspergillus niger* PTCC5012

Serial Number	Factor	Level 1	Level 2	Level 3	Level 4	L2-L1
1	Glucose Concentration (g. L^{-1})	31.282	32.012	32.475	32.257	0.730
2	$MgSO_4$ Concentration (g. L^{-1})	32.235	31.604	31.870	32.317	- 0.632
3	KH_2PO_4 Concentration ($g.L^{-1}$)	32.099	31.710	32.050	32.167	- 0.389
4	pH	32.212	31.848	31.927	32.040	- 0.365

Table 5. Proposed optimum conditions to achieve the maximum *Aspergillus niger* PTCC5012 SCP production

Serial Number	Factor	Optimum Level	Level Description	Contribution	Contribution percent
1	Glucose Con. (g. L^{-1})	3	70	0.468	40.95
2	$MgSO_4$ Con. (g. L^{-1})	4	2	0.310	27.12
3	KH_2PO_4 Con. (g. L^{-1})	4	7	0.160	13.99
4	pH	1	5	0.205	17.94
	Total			1.143	100

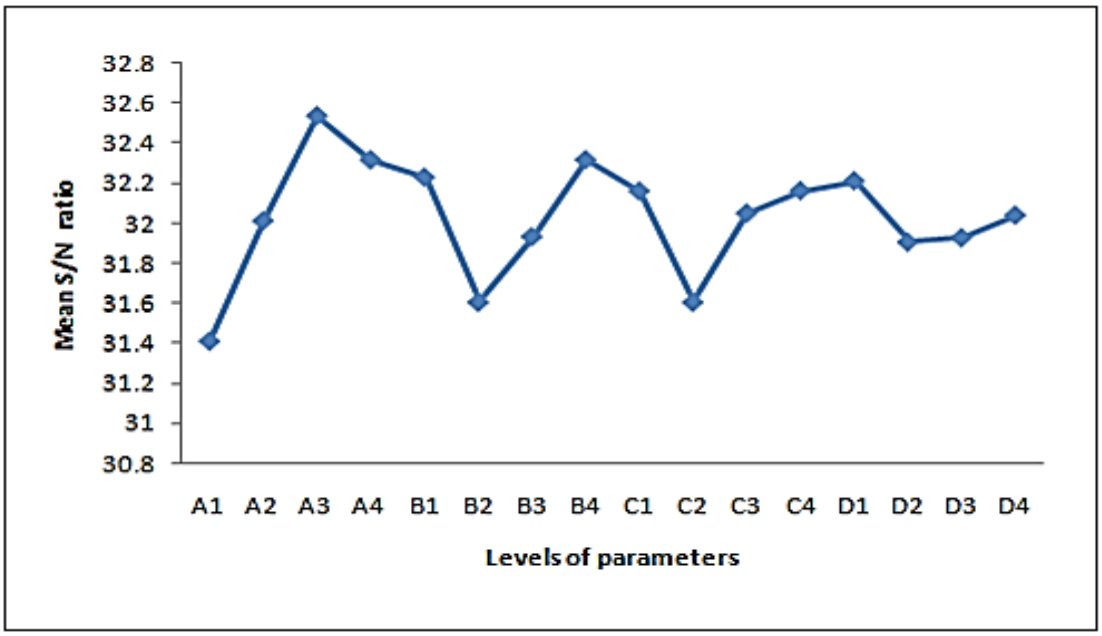

Fig.1. The main effects of each factor in optimization process of *Aspergillus niger* PTCC5012 SCP production

maximum single cell protein yield was based on the glucose, $MgSO_4$ and KH_2PO_4 concentrations to be equal to 70, 2 and 7 g. L^{-1}, respectively with the culture medium pH of 5. An acceptable consistency was observed between the theoretical expected SCP concentration at optimum conditions and the experimental yield obtained under the proposed optimum conditions.

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