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Bioremediation of lignocellulosic wastes of food industries by *Aspergillus flavus* as food and feed additive protein by solid-state fermentation process

Fatemeh Ardestanii*, Ali Shokuhi Rad

Department of Chemical Engineering, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

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ABSTRACT

The lignocellulosic wastes produced in food industries are suitable raw materials for the production of biological products. In this study, the solid state fermentation of *Aspergillus flavus* on lignocellulosic wastes was evaluated for microbial protein production. The fraction of the full factorial method was applied for experiment design and process optimization. The results analysis was performed through signal to noise statistical index using the Taguchi approach via Qualitek-4 software. Glycine, ammonium sulfate and iron sulfate concentration as well as temperature were considered as effective parameters. The maximum biomass concentration of 45.7 g/kg containing 55.75% (w/w) pure protein was obtained at optimal conditions including 0.5, 0.02, and 2 g/kg of ammonium sulfate, iron sulfate and glycine, respectively, at 25 °C. Ammonium sulfate (33.78% (w/w) contribution) and culture temperature (31.98% contribution) were evaluated as the most effective factors on biomass and microbial protein production. The highest interaction occurred between ammonium sulfate and glycine with an interaction severity index of 50.03%. The low deviation of 3.94% was determined between optimum theoretical biomass concentration (43.9 g/kg) and the experimentally measured one (45.7 g/kg). Due to the high protein content of 55.75% (w/w), *Aspergillus flavus* was introduced as a suitable strain for industrial protein production.

1. Introduction

Microbial cell protein production has been considered by researchers in recent years because of the limited sources of animal and plant proteins and the need to meet the world's food and animal feed demands [1]. This type of protein is defined as the dried cell biomass of microorganisms grown on a large scale. This type of protein has some advantages such as low cost, use of the waste materials as the main substrate, and an acceptable nutritive value depending on the amino acid composition [2]. Microbial protein is produced with different microbial strains including microalgae, bacteria, yeasts and filamentous fungi [3,4]. Various agricultural and food industries wastes have been employed to provide the required nitrogen and carbon sources for protein producing strains. Some researches applied ammonium salts as nitrogen sources and molasses as a carbon source for yeast strains such as *Saccharomyces cerevisiae* [5]. Microbial protein is produced by various microorganisms [6-8] on different substrates such as whey [9,10], starch processing wastewater [11], cellulose [12], pineapple waste [13], beet pulp [14], cassava [15] and rice polishing [16]. Lignocellulosic residues containing agricultural wastes have been introduced as cheap and abundant raw materials for protein production in solid state fermentation processes. Applying these materials in such processes results in a major decrease in environmental contamination as well as producing a valuable and low cost protein source with high nutritional value [1]. The medium composition and

*Corresponding author. Tel.: +989113234890
E-mail address: ardestani_fatemeh@yahoo.com
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environmental condition of the microbial protein production process are important and effective parameters on microbial cell growth and protein content in cell biomass [17]. The *Aspergillus* species are a group of the most important microorganisms used to produce food and pharmaceutical products such as such as prebiotics [18], pectin by endo-polygalacturonase [19], volatile flavor components [20], and ligninolytic enzymes [21]; they are also used in some valuable processes such as biodegradation [22] in solid state fermentation. These are also suitable microorganisms for producing single cell protein [17,23]. Fungal protein is actually a dehydrated cell biomass consisting of proteins, lipids, nucleic acids, carbohydrates, inorganic compounds and vitamins [24]. Fungal biomass has high protein content and fairly rapid and continuous biomass production depending on the environmental conditions. Due to its lower nucleic acid content, fungal protein is also more suitable for human foodstuff and animal feed enrichment in comparison to bacterial extracted protein. The waste materials of fruits processing industries are known as suitable substrates for biotechnological products [25]. In this work, the production of food and feed additive protein from *Aspergillus flavus* cell biomass was studied and optimized in a solid state fermentation process using the lignocellulosic wastes of food industries. A fraction of the full factorial methodology was used to optimize medium composition and environmental conditions of the solid state fermentation process in order to reach the highest cell biomass and its protein content.

2. Materials and methods

2. 1. Materials

Aspergillus flavus PTCC5004 was provided by the Iranian Research Organization for Science and Technology. Lignocellulosic apple waste was provided by the *Nooshe Mazandaran* Co. (Nashtarood, Iran) and hydrolyzed by enzymatic decomposition pre-treatment [26] using cellulase enzyme (Powder, more than 0.3 units per mg solid, Sigma-Aldrich Co, Germany). All chemicals used for culture medium composition and analytical assays were purchased from the Sigma-Aldrich Company (Germany).

2. 2. Methods

2. 2. 1. Microorganism and inoculums

In order to prepare the inoculums, lyophilized cells were cultured in a medium that contained (g/l): peptone, 9; yeast extract, 5; potassium hydrogen di-phosphate, 1; magnesium sulfate, 1 and glucose, 40. Incubation was conducted for 72 h at 27 °C and 150 rpm agitation speed. Then, the solid cultures of *A. flavus* were prepared on PDA plates. Some slants were also cultured as stock cultures and stored at -20 °C. The fungi colonies were transferred from the surface of the plates to distilled water using a sterile loop under sterile conditions. The spore suspensions were used as inoculums in the next stages [17].

2. 2. 2. Solid state fermentation

The solid bed cultures were prepared in perforated metal trays equipped with humidity (Digital Humidity Controller, FOX-1H, Korea) and temperature (Digital Temperature Controller (Samwon Eng, SU-105IP, Korea) controlling systems. 1000 g of sterile hydrolyzed lignocellulosic waste was placed on the tray. In order to save bed moisture and prevent closing of the tray holes, thin wet tissues were placed on the underside of the solid bed. Also, a water injection system was installed at the top of the substrate bed to keep the bed humidity in the desired point (70-75%). The fermentation process was performed for 120 h. An air injector system installed at the bottom of the trays, inserted 2 l/min of air to the bed to avoid anoxia and control the bed temperature and humidity.

2. 2. 3. Medium composition

Four key parameters including ammonium sulfate, iron sulfate, glycine concentrations and culture temperature, each one at four different levels, were considered for the optimization process (Table 1). 100 ml of a salt solution containing potassium di-phosphate, 2 g; magnesium sulfate, 1 g; manganese sulfate, 1 g; and zinc sulfate 1 g was added to each of the solid state cultures placed on each metal tray. After that, 10 ml of fresh spore suspension (containing about 10^8 spores per ml) was added to each solid bed. Then the solid bed was stirred slowly by a sterile small metal shaft until it reached a full uniform composition. The initial bed moisture was adjusted to 70% after adding the salt solution. The composition of the cultures was based on the L-16 orthogonal array designed by the Qualitek-4 software to determine an optimized medium composition to obtain the highest fungal biomass (Table 1). Each experiment was repeated twice and the mean results were used for statistical analysis.

Table 1. Key factors and their levels assigned to different columns in optimization of microbial protein Production of *A. flavus* PTCC5004 in solid state batch culture medium

Factor	Level 1	Level 2	Level 3	Level 4
Ammonium sulfate Con. (g/kg)	0.3	0.4	0.5	0.6
Iron sulfate Con. (g/kg)	0.02	0.05	0.1	0.15
Glycine Con. (g/kg)	0.5	1	1.5	2
Culture temperature (˚C)	20	25	30	35

2. 2. 4. Measurements

At the end of each fermentation process, the fungal biomass was separated from the solid substrate through washing with distilled water while shaking at 250 rpm inside an Erlenmeyer flask; then, the resulting suspension was centrifuged at 10000 rpm for 15 min. The *Aspergillus flavus* PTCC5004 biomass was applied for the measurement of the protein content after drying at 60 ˚C for at least 24 h and reaching a constant weight. The cell dry weight was determined by measuring the differences in the dried and fresh fungal biomass. The total nitrogen was analyzed by the Micro-Kjeldahl (Sama Tajhiz Chimi Azma, IRAN) methodology based on the Iran National standard Method, INSO 19052. A mixture of TiO_2, K_2SO_4 and $CuSO_4.5H_2O$ was used as the digestive catalyst [27]. The amino acid composition of the produced microbial protein was determined using high performance liquid chromatography (HPLC, Shimadzu, auto sampler: SIL-20A, degasser: DGU-20 A, pump: LC-20 AD, detector: SPD-20 A, Japan) with a C18 column and serine as the internal standard.

3. Results and discussion

3. 1. Biomass production

The fungal biomass concentration produced was measured for each sample at the end of the incubation time and are presented in Table 2. The results showed that the maximum cell biomass (26.92 g/kg in the first trial and 26.97 g/kg in the second trial) was obtained in experiment number 9 in which ammonium sulfate, iron sulfate and glycine concentrations as well as culture temperature were adjusted on 0.5, 0.02, 1.5 g/kg and 35 °C, respectively (Table 2). The minimum cell biomass (3.84 g/kg) in the first trial and 4 g/kg in the second trial) was recorded for experiment number 4 where ammonium sulfate, iron sulfate and glycine concentrations as well as solid bed temperature were adjusted on 0.3, 0.15, 2 g/kg and 35°C, respectively (Table 2). Thus, it can be concluded that an increase in ammonium sulfate concentration had a positive effect on *Aspergillus flavus* PTCC5004 reproduction while a vise-versa impact was observed for iron sulfate concentration.

3. 2. The Mean effect of each factor on the biomass production

Signal to noise ratio (S/N) analysis was applied to determine the mean effect of each factor. The calculated values based on the Eq. 1 (n is the frequency of each test repeating and y_i (g/kg) is the biomass concentration produced in each repeat) are presented in Table 2.

$$\frac{S}{N} \equiv -10 \, \mathrm{Log} \left[\frac{\sum_{i=1}^{n} (1/y_i)^2}{n} \right] \quad (1)$$

Table 2. The layout of the L-16 orthogonal arrays, experimental results and calculated S/N in optimization of microbial protein production of *A. flavus* PTCC5004 in solid state fermentation

Factor	Ammonium sulfate Con.	Iron sulfate Con. (g/kg)	Glycine Con. (g/kg)	Culture Temperature (°C)	Cell biomass Con. (g/kg)		S/N
Trial		Factor level			Repeat 1	Repeat 2	
1	1	1	1	1	6.98	7.02	16.90
2	1	2	2	2	9.92	9.74	19.85
3	1	3	3	3	11.4	11.64	21.23
4	1	4	4	4	3.84	4	23.89
5	2	1	2	3	15.53	15.79	26.50
6	2	2	1	4	21.02	21.27	21.66
7	2	3	4	1	12.09	12.13	18.58
8	2	4	3	2	17.87	17.95	25.06
9	3	1	3	4	26.92	26.97	28.61
10	3	2	4	3	25.77	25.80	28.23
11	3	3	1	2	23.81	23.87	27.55
12	3	4	2	1	13.86	13.91	22.85
13	4	1	4	2	24.96	25	27.95
14	4	2	3	1	7.76	7.79	17.82
15	4	3	2	4	11.62	11.67	21.32
16	4	4	1	3	9.49	9.51	19.55

The investigation of the average effects of each factor at the designed levels on the *A. flavus* PTCC5004 biomass production (Figure 1A) showed that an increase in the ammonium sulfate concentration from 0.3 to 0.5 g/kg had about a 32% positive impact on the biomass production. An 11.28% loss of cell dry weight was recorded with an increase in the iron sulfate concentration from level 1 to level 3 (0.02 to 0.1 g/kg) based on the obtained results (Figure 1B). Thus, the highest average S/N ratio of 28.61 was obtained for experiment number 9 with level 3 of ammonium sulfate and level 1 of iron sulfate. Also, adding more amounts of glycine to the culture medium showed a reinforcing growth impact on *A. flavus* PTCC5004. An increase of glycine concentration from level 1 to level 4 (0.5 to 2 g/kg) led to improved cell

growth and a biomass concentration of about 16% (Figure 1C). The best results in regard to cell biomass production of *A. flavus* PTCC5004 were observed at 25 °C (level 2 of temperature factor). An increase in the level of culture temperature from level 1 to 2 caused about 32% cell growth amplification. However, more increases in temperature from level 2 to levels 3 and 4 (25 °C to 30 °C and 35 °C) led to about a 5% loss in cell mass (Figure 1D).

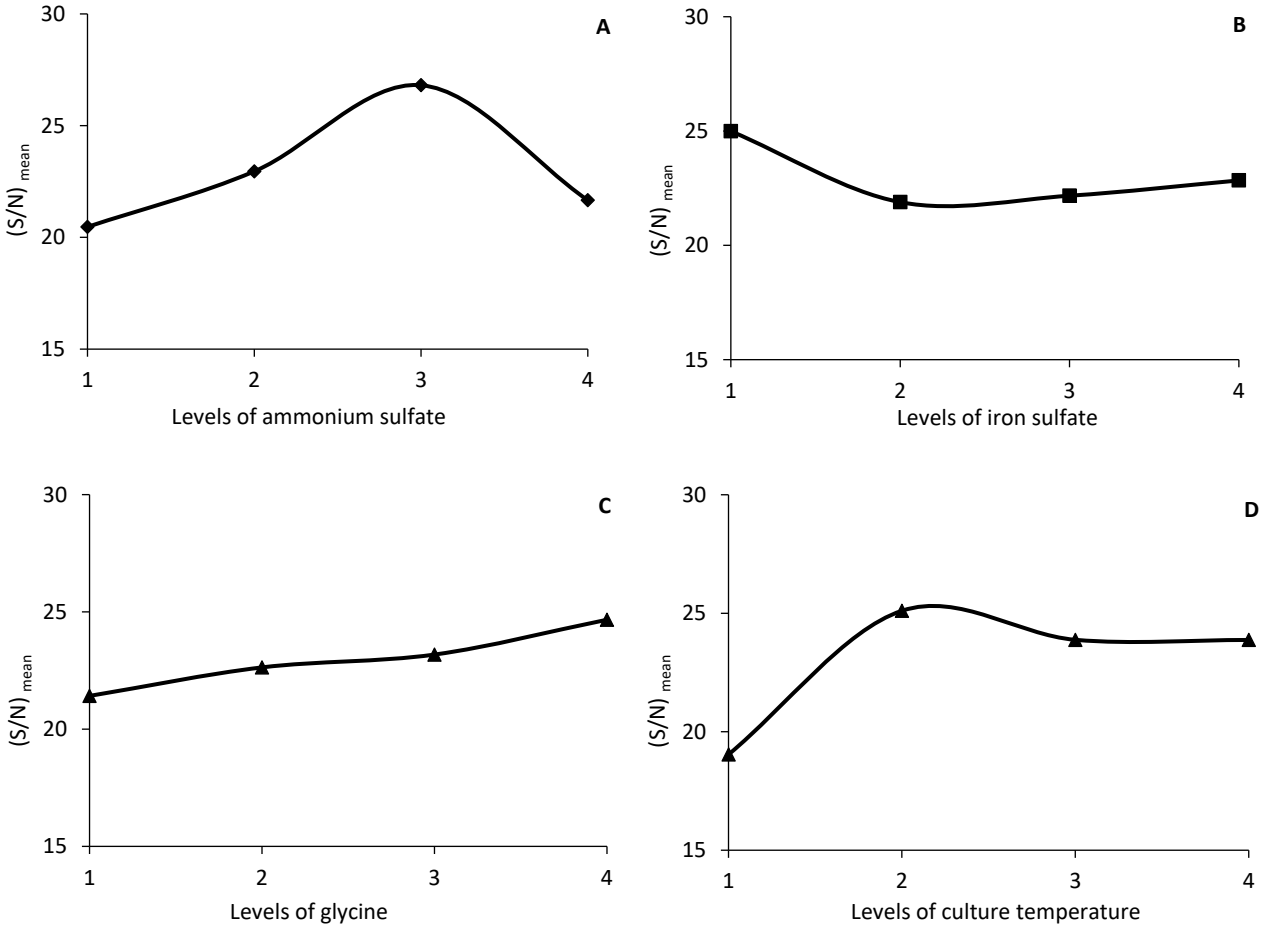

Fig. 1. The mean effect of ammonium sulfate concentration (A), iron sulfate concentration (B), glycine concentration (C) and solid bed temperature (D) on the cell growth in optimization of microbial protein production from *A. flavus* PTCC5004 in solid state fermentation using lignocellulosic substrate

3.3. Factors interaction

The existing interactions between each of the two factors are presented in Figure 2 and Table 3. The results indicated that the most interaction was observed between ammonium sulfate and glycine concentrations with an interaction severity index equal to 50.03% (Table 3). Also, the least interaction was recorded between glycine and culture temperature with an interaction severity index equal to 21.91%. The interaction severity index for each of the two investigated factors is presented in Table 3. Based on the results, ammonium sulfate and iron sulfate performance in the fungi growth medium was significantly impressed at the presence of glycine. A considerable high interaction severity index of 49.34% was recorded between iron sulfate concentration and the culture temperature too, indicating a remarkable relationship between the iron sulfate turnover and the medium temperature. The results showed a noticeable interaction between all two investigated factors (Table 3).

Table 3. Interaction between investigated factors in optimization of microbial protein production of *A. flavus* PTCC5004 in solid state fermentation using lignocellulosic substrate

Factors	Interaction Severity index (%)
Ammonium sulfate * Glycine	50.03
Iron sulfate * Glycine	49.96
Iron sulfate * Culture temperature	49.34
Ammonium sulfate * Culture temperature	28.5
Ammonium sulfate * Iron sulfate	26.6
Glycine * Culture temperature	21.91

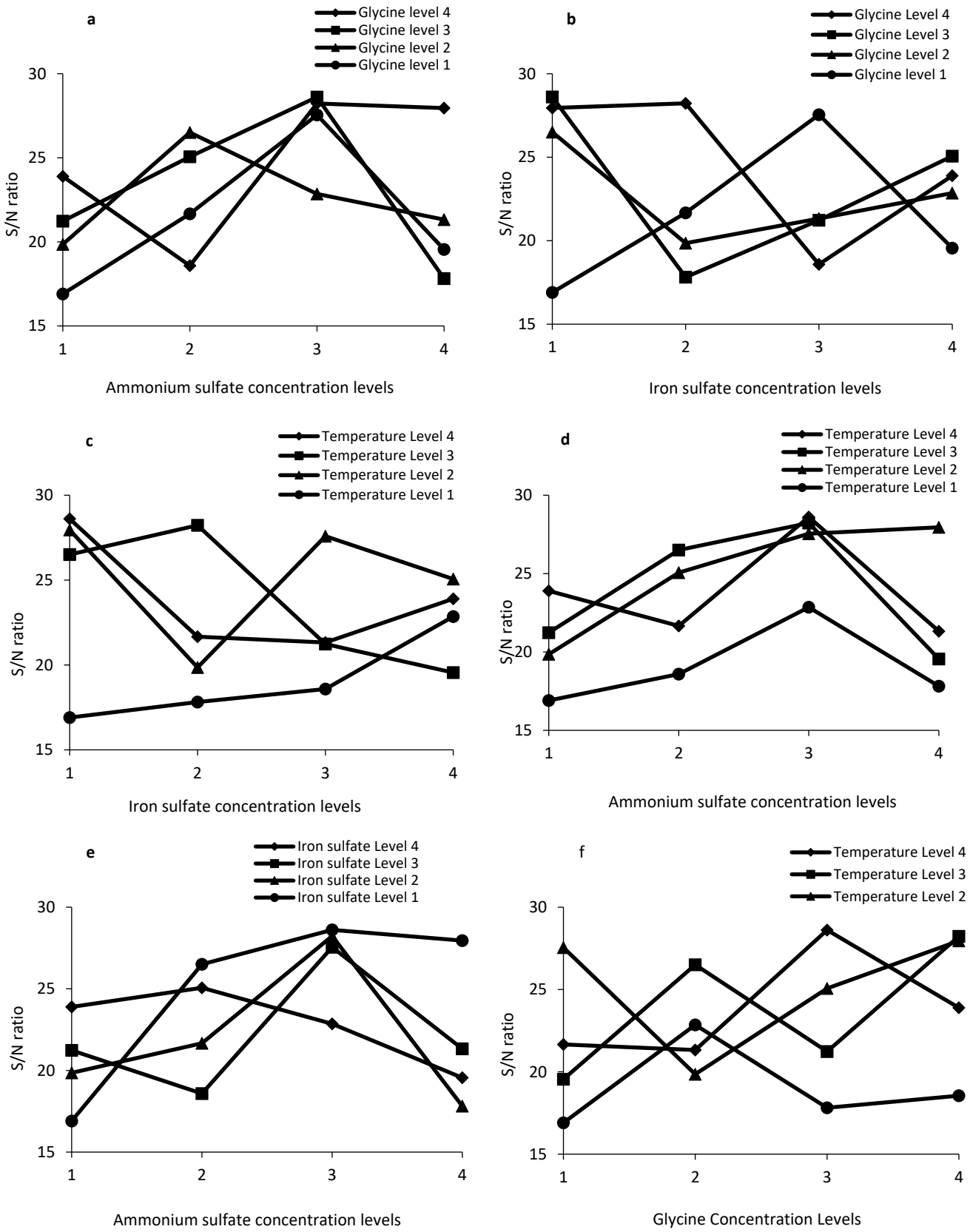

Fig. 2. Interaction between each of the two investigated factors in the optimization of fungal protein production of *A. flavus* PTCC5004 in solid state fermentation using lignocellulosic substrate

3. 4. Optimum culture composition

The analysis of the experimental data using the statistical index of signal to noise ratio showed that at optimum conditions, the ammonium sulfate, iron sulfate and glycine concentrations should be adjusted to 0.5 (level 3), 0.02 (level 1) and 2 (level 4) g/kg, respectively, and the culture temperature must be set on 25 ˚C (level 2). Therefore, it can be concluded that ammonium sulfate has an important role as a nitrogen source in fungal growth and reproduction. The role of each factor in *Aspergillus flavus* PTCC5004 biomass production was calculated via the software and indicated its contribution percentage. The ammonium sulfate concentration and culture temperature were determined as the most effective factors on fungal biomass production with 33.78% and 31.98% contribution, respectively. Thus, these two factors had a significant role in *A. flavus* PTCC5004 growth under the applied conditions in this research. An increase in the iron sulfate from level 1 to the higher levels caused a decrease in cell proliferation, which demonstrated an inhibitory effect. The contribution of iron sulfate and glycine concentrations in biomass production was determined to be only 5.133% and 4.332%, respectively. This means that the changes in iron sulfate and glycine concentrations within the limits defined in this study had no significant effects on fungal cell growth and proliferation. The results of Jaganmohan et al. (2013) on *Aspergillus terreus* growth in solid state fermentation indicated that substrate type has a striking role; they found that the obtained cell biomass concentration was 100 mg/g using rice bran and 80 mg/g using banana peel as the main substrate, which was higher than the results of the present work (45.7 g/kg at optimum condition) [28]. The theoretical expected fungal biomass concentration at optimum conditions was estimated at 43.9 g/kg and the actual experimental obtained value was equal to 45.7 g/kg with a very low deviation of about 3.94%. The perfected compatibility between the optimum theoretical biomass concentration (proposed by software) and actual one obtained experimentally under optimized conditions, verifies the sustainability of the applied statistical method for the studied optimization case. The findings indicated that solid state fermentation is a more suitable system for filamentous fungi such as *Aspergillus* species. The best pH and temperature for *Aspergillus terreus* growth was recognized at pH=5-6 and 35 ˚C, respectively, which is almost similar to *A. flavus* PTCC5004 [28]. Ardestani and Alishahi (2015) reported a 42 g/L cell biomass concentration for *Aspergillus niger* using an optimal culture composition that contained 70 g/L initial glucose concentration in a submerged fermentation bioprocess, which is in accordance with the present work findings for *A. flavus* PTCC5004 [17]. However, differences between the fungal species and culture composition should be considered

3.5. Protein content of dried cell biomass

The maximum protein content of the *A. flavus* PTCC5004 dried cell biomass was evaluated at 55.75% using optimal culture composition. Jaganmohan *et al.* (2013) reached a 35% protein content in the dried cell biomass of *Aspergillus terreus* under the optimal condition that was more than 37% lower than *A. flavus* PTCC5004 using our proposed optimum culture composition [28]. The protein content of *A. flavus* PTCC5004 cell biomass was also significantly more than the *Aspergillus niger* AS-101 reported by Singh et al. (1991) which was equal to 30.4% [29]. Anupama and Ravindra found a maximum protein yield of 41.68 mg/g of rice bran for *Aspegillus niger* proliferated in an optimized culture composition that was nearly the same as findings of this work [23].

4. Conclusions

This work is the first report on the optimization of the microbial protein production bioprocess by *A. flavus* biomass using the fraction of full factorial statistical method. An optimal culture composition was determined to achieve the highest cell biomass and protein content of *A. flavus* PTCC5004 at a 150 rpm agitation speed. The culture temperature and ammonium sulfate concentration were introduced as the most effective factors on *A. flavus* PTCC5004 growth and reproduction. Iron sulfate and glycine concentrations had no considerable contribution in the biomass and protein production. The addition of more amounts of iron sulfate from level 1 and a culture temperature of more than level 2 showed an inhibitory effect on fungal biomass production, whereas adding more amounts of ammonium sulfate and glycine improved fungal biomass production. Thus, *A. flavus* is sensitive to high amounts of iron sulfate as well as high temperatures. The applied statistical method was recognized to have good efficiency in the studied optimization case, which was based on low manifested deviation. The produced cell biomass of *A. flavus* under optimum conditions contained significant protein content that is suitable for commercialization.

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References

[1] Ravindra, P. (2000). Value-added food: Single cell protein. *Biotechnology advances*, *18*(6), 459-479.

[2] Nasseri, A. T., Rasoul-Amini, S., Morowvat, M. H., Ghasemi, Y. (2011). Single cell protein: production and process. *American journal of food technology*, *6*(2), 103-116.

[3] Raja, R., Hemaiswarya, S., Kumar, N. A., Sridhar, S., Rengasamy, R. (2008). A perspective on the

biotechnological potential of microalgae. *Critical reviews in microbiology*, *34*(2), 77-88.

[4] Rasoul-Amini, S., Ghasemi, Y., Morowvat, M. H., Mohagheghzadeh, A. (2009). PCR amplification of 18S rRNA, single cell protein production and fatty acid evaluation of some naturally isolated microalgae. *Food chemistry*, *116*(1), 129-136.

[5] De Kock, S. H., Du Preez, J. C., Kilian, S. G. (2001). The effect of growth factors on anoxic chemostat cultures of two *Saccharomyces cerevisiae* strains. *Biotechnology letters*, *23*(12), 957-962

[6] Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology advances*, *25*(2), 207-210.

[7] Gellissen, G. (2000). Heterologous protein production in methylotrophic yeasts. *Applied microbiology and biotechnology*, *54*(6), 741-750.

[8] Paraskevopoulou, A., Athanasiadis, I., Kanellaki, M., Bekatorou, A., Blekas, G., Kiosseoglou, V. (2003). Functional properties of single cell protein produced by kefir microflora. *Food research international*, *36*(5), 431-438.

[9] Schultz, N., Chang, L., Hauck, A., Reuss, M., Syldatk, C. (2006). Microbial production of single-cell protein from deproteinized whey concentrates. *Applied microbiology and biotechnology*, *69*(5), 515-520.

[10] Ghaly, A. E., Kamal, M., Correia, L. R. (2005). Kinetic modelling of continuous submerged fermentation of cheese whey for single cell protein production. *Bioresource technology*, *96*(10), 1143-1152.

[11] Jin, B., van Leeuwen, H. J., Patel, B., Doelle, H. W., Yu, Q. (1999). Production of fungal protein and glucoamylase by *Rhizopus oligosporus* from starch processing wastewater. *Process biochemistry*, *34*(1), 59-65.

[12] El-Nawwi, S. A., El-Kader, A. A. (1996). Production of single-cell protein and cellulase from sugarcane bagasse: effect of culture factors. *Biomass and bioenergy*, *1*(4), 361-364.

[13] Dhanasekaran, D., Lawanya, S., Saha, S., Thajuddin, N., Panneerselvam, A. (2011). Production of single cell protein from pineapple waste using yeast. *Innovative Romanian food biotechnology*, *8*, 26-32.

[14] Ghanem, KM (1992) Single cell protein production from beet pulp by mixed culture. *Qatar university science journal,12,* 85-88

[15] Ubalua, A. O. (2007). Cassava wastes: treatment options and value addition alternatives. *African journal of biotechnology*, *6*(18), 2065-2073.

[16] Rajoka, M. I., Khan, S. H., Jabbar, M. A., Awan, M. S., Hashmi, A. S. (2006). Kinetics of batch single cell protein production from rice polishings with *Candida utilis* in continuously aerated tank reactors. *Bioresource technology*, *97*(15), 1934-1941.

[17] Ardestani, F., Alishahi, F. (2015). Optimization of single cell protein production by *Aspergillus niger* using Taguchi approach. *Journal of food biosciences and technology*, *5*(2), 73-79.

[18] Kim, S. Y., Jeong, H. S., Ahn, S. W., Shin, K. S. (2014). Prebiotic effects of structurally identified galacto-oligosaccharides produced by β-galactosidase from *Aspergillus oryzae*. *Food science and biotechnology*, *23*(3), 823-830.

[19] Contreras-Esquivel, J. C., Voget, C. E., Vita, C. E., Espinoza-Perez, J. D., Renard, C. M. G. C. (2006). Enzymatic extraction of lemon pectin by endo-polygalacturonase from *Aspergillus niger*. *Food science and biotechnology*, *15*(2), 163-167

[20] Shon, S., Kim, J., Oh, H., Ha, J. (2003). Volatile flavor components of Kochujang prepared with *Aspergillus oryzae*, *Bacillus licheniformis* and *Saccharomyces rouxii*. *Food science and biotechnology*, *12*(1), 18-22.

[21] dos Santos, T. C., dos Santos Reis, N., Silva, T. P., Machado, F. D. P. P., Bonomo, R. C. F., Franco, M. (2016). Prickly palm cactus husk as a raw material for production of ligninolytic enzymes by *Aspergillus niger*. *Food science and biotechnology*, *25*(1), 205-211.

[22] Rhim, J. W., Gennadios, A., Lee, J. J., Weller, C. L., Hanna, M. A. (2003). Biodegradation of cast soy protein films by *Aspergillus oryzae* and *Bacillus subtilis*. *Food science and biotechnology*, *12*(1), 96-99.

[23] Ravindra, P. (2001). Studies on production of single cell protein by *Aspergillus niger* in solid state fermentation of rice bran. *Brazilian archives of biology and technology*, *44*(1), 79-88.

[24] Bekatorou, A., Psarianos, C., Koutinas, A. A. (2006). Production of food grade yeasts. *Food technology and biotechnology*, *44*(3), 407-415.

[25] Linke, B., Schröder, K., Arter, J., Gasperazzo, T., Woehlecke, H., Ehwald, R. (2010). Benchmarks. *Biotechniques*, *49*, 655-657.

[26] Yang, B., Dai, Z. Y., Ding, S. Y., Wyman, C. E. (2011). Enzymatic Hydrolysis of Cellulosic Biomass. *Biofuels*, 2, 421-450.

[27] Rahnamaei, M. (2015). Cereals and pulses-Determination of the nitrogen content and calculation of the crude protein content- kjeldahl method. *Iran National Standard Organization*, INSO 19052, 2-9.

[28] Jaganmohan, P., Daas, B. P., Prasad, S. V. (2013). Production of single cell protein (SCP) with *Aspergillus terreus* using solid state fermentation. *European journal of biological sciences*, *5*(2), 38-43.

[29] Singh, A., Abidi, A. B., Agrawal, A. K., Darmwal, N. S. (1991). Single cell protein production by *Aspergillus niger* and its evaluation. *Zentralblatt für mikrobiologie*, *146*(3), 181-184.