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Evaluation of Chitin-Glucan Complex Production in Submerged Culture of Medicinal Mushroom of Schizophilum commune: Optimization and Growth Kinetic

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ABSTRACT

Background: *Schizophyllum commune*, is one of the important medicinal-fungi foods in the world. Due to its important constituents such as extracellular and intracellular polysaccharides, it is widely used in industry and medicine. One of the important polysaccharides of this fungus is chitin-glucan complex (CGC). The aim of this study was to investigate the growth of native fungus *Schizophyllum commune* isolated from northern forests of Iran and to optimize its CGC production in submerged cultivation.

Materials & Methods: Growth kinetics studies of native *Schizophyllum commune* fungi of Iran and CGC production were performed and growth curves were plotted. In order to increase CGC production, optimization of culture medium was done by investigating independent variables of pH, inoculum percentage and aeration percentage by response surface methodology.

Results: The results showed that the specific growth coefficient of Iranian native *Schizophyllum commune* ($_{max}\mu$) was 0.991-day. Tenth day was also selected as the best time for growth and production in the submerged medium. In optimum conditions, initial pH of 8.92, percentage of inoculum 9.99 and aeration percentage of 150 was obtained. After 10 days, the amount of dry cell weight was 13.05 g/L and the amount of chitin-glucan complex produced was 2.9 g/L.

Conclusion: Investigation of kinetic parameters of growth and production showed that the experimental data are in accordance with the logistic growth model with R2=0.9665 and the Luedeking and Piret model for production with R2 = 0.9439. The results also show that the initial pH has a significant effect on the growth of this fungus.

Keywords: *Schizophyllum commune*, Chitin glucan complex, Optimization, Submerged Cultivation

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Introduction

In recent years, various metabolites such as, chitin, chitosan and CGC have been prepared from *Schizophyllum commune* due to their antibody properties and antiviral activity and have been used in pharmaceutical, cosmetic and hygienic fields **(8،9)**. *S. commune* is a fungal and edible fungus belonging to the basidiomycetes family **(14)**. CGC is a major component of the cell wall in yeast and fungi that stabilizes and strengthens cells and is a copolymer of diglucosamine, N-acetylglucosamine, and glucose **(2, 1)**. In 2010, Hao *et al.* investigated the optimization of variables affecting the growth and production of *S. commune* polysaccharide **(17)**. Chagas *et al.* (2014) investigated the effect of pH and temperature on the production of CGC from the

yeast *Pichia pastoris* (2). The purpose of this study was to investigate the variables affecting the growth of native *S. commun*e fungi in Iran and increasing the production of CGC using response surface methodology and growth kinetic model And CGC production using Logical and Luedeking and Piret model.

Materials and Methods

A) Cultivation *Schizophyllum commun*e and CGC Extraction

The fungus was cultured in culture medium PDB for 10 days. Then 2 g of dried mycelium was mixed with 60 mL of 4.2 mol NaOH. This mixture was incubated at 90°C for 3 h. The mixture was centrifuged. The precipitate was then dissolved in 300 mL of sterilized distilled water and centrifuged again. The process was continued until pH=7. The precipitate was then mixed with 60 mL of 0.25 mol HCl and placed in a 50°C oven for 2 h. The precipitate was then mixed with 60 mL of 0.25 mol HCl and placed in the incubator at 50°C for 2 h. The precipitate was dissolved in 300 mL of distilled water and continued until pH=7. CGC was dehydrated with isopropanol and placed at 60°C for 24 h (18).

B) Experiment Design

Response surface methodology was used to estimate the effect of independent variables on cell mass growth and CGC production of *S. commun*e. The parameters affecting the production of CGC (pH, aeration percentage, percentage of primary inoculum) of *S. commune* were designed based on the BBD method (Box Behnken Design) of Design Expert 11 software.

C) Investigation of Growth Kinetics and Production of CGC for *Schizophyllum commun*e

To obtain the growth curve of *S. commun*e, this fungus was cultured in PDB medium for a period of 20 days and biopsy was performed every two days and biomass and CGC were recorded (22).

$$dC/dt = \mu_m (1 - C/C_m) C \quad \text{Equation 1}$$

In Equation 2, cell viability (gr/L), μ_m is the maximum specific growth rate (in terms of day^{-1}) and C_m of the maximum cell mass concentration obtainable (gr/L). This equation is widely used to predict metabolites.

$$(dP)/dt = \alpha (dC)/dt + \beta C \quad \text{Equation 2}$$

Here P is the concentration of CGC (gr/L), C the cell mass (gr/L), α in β, ($g \times g^{1}$) in ($g \times (g \times h)^{-1}$) values are constant. This model is experimental in which dC / dt indicates CGC production rate relative to growth rate and C represents CGC production regardless of growth.

Results

A) Optimization of Parameters Affecting CGC Production

Table 1 shows the results of cell dry weight and CGC values produced in each of the 17 experiments by combining different levels of parameters.

Analysis of variance of the results of the experiments in terms of cell mass and CGC level using Design Expert software is shown in Tables 2 and 3, respectively. R^2 values for cell dry weight and CGC were 9653% and 0.9584% respectively indicating a good correlation coefficient. According to software analysis based on dry weight of biomass produced, aeration percentage and pH with P-values of 0.0097 and <0.0001, respectively and based on CGC weighted values of 0.0149 and <0.00011, respectively it had a significant effect on biomass growth and CGC. According to this analysis, there is no interaction between the variables studied. Also, the second power of pH variable with P- value of 0.009 and 0.0023 had significant effect on growth rate of biomass and CGC production, respectively.

Table 1. Experimental Design (BBD) and Results Based on Cellular Dry Weight and CGC Amount

Response		Parameters			Number the experiment
CGC rate (g/L)	The amount of mycelium (g/L)	Percentage of inoculum (C)	(B) pH	Percentage of aeration	
2.5	11.33	5.5	7	100	1
2.9	12.94	10	7	150	2
2.6	12.04	1	7	150	3
2.88	12.9	5.5	9	150	4
2.55	11.46	5.5	7	100	5
2.73	12.24	1	9	100	6
1.6	7.14	1	5	100	7
2	8.96	5.5	5	50	8

Response		Parameters			Number the experiment
2.6	11.68	5.5	9	50	9
2.2	10.1	1	7	50	10
2.5	11.38	5.5	7	100	11
1.84	8.2	10	5	100	12
1.97	8.82	5.5	5	150	13
2.53	11.28	10	7	50	14
2.6	11.96	10	9	100	15
2.51	11.44	5.5	7	100	16
2.48	11.39	5.5	7	100	17

Table 2. Analysis of Variance and Regression Coefficients Estimated Cell Dry Weight Production

Expression	Sum of squares	Mean square	Degrees of freedom	F-Value	P-Value
Model	43.01	4.78	9	21.63	**0.0003**
A	2.74	2.74	1	12.40	**0.0097**
B	30.65	30.65	1	138.79	**<0.0001**
C	1.02	1.02	1	4.63	**0.0685**
AB	0.46	0.46	1	2.09	**0.1912**
AC	0.02	0.02	1	0.089	**0.7744**
BC	0.45	0.45	1	2.03	**0.1970**
A^2	0.84	0.84	1	3.82	**0.0916**
B^2	6.66	6.66	1	30.14	**0.0009**
C^2	0.28	0.28	1	1.26	**0.2980**
residua	1.55	0.22	7		

Table 3. Analysis of variance and regression coefficients estimated CGC weight production

Expression	Sum of squares	Mean square	Degrees of freedom	F-Value	P-Value
Model	2.04	0.33	9	17.92	**0.0005**
A	0.13	0.13	1	10.31	**0.0149**
B	1.45	1.45	1	114.51	**<0.0001**
C	0.068	0.068	1	5.42	**0.0527**
AB	0.024	0.024	1	1.90	**0.2101**
AC	2.250	2.250	1	0.018	**0.8975**
BC	0.034	0.034	1	2.71	**0.1436**
A^2	0.051	0.051	1	4.02	**0.0850**
B^2	0.27	0.27	1	21.74	**0.0023**
C^2	0.015	0.015	1	1.21	**0.3075**
residual	0.088	0.22	7		

The software proposed a nonlinear regression model for biomass production and a nonlinear regression model for CGC production based on code variables as follows:

$$\text{Biomass} = 11.40 + 0.59\,A + 1.96\,B + 0.34\,AB - 0.070\,AC - 0.33\,BC + 0.45\,A^2 - 1.26\,B^2 - 0.26\,C \quad \textbf{Equation 3}$$

$$\text{CGC} = 2.51 + 0.13\,A + 0.42\,B + 0.092\,C + 0.077\,AB - 7.500\text{E-}003\,AC - 0.092\,BC + 0.11\,A^2 - 0.26\,B^2 - 0.060\,C^2 \quad \textbf{Equation 4}$$

In Figure 1-A, two-dimensional diagrams (A) the effect of acidity on aeration percentage; (B) the effect of aeration percentage on acidity; (C) Effect of acidity inoculum on cell dry weightand 1-B (3D) Effect of acidity to aeration percentage; (B) Effect of aeration percentage to acidity; (C) Effect of acidity percentage to acidity on cellular dry weight. In Figure 2-A, the two-dimensional diagram (A) the effect of acidity on aeration percentage; (B) the effect of aeration percentage on acidity; (C) Effect of inoculum percentage on acidity and 4-B three-dimensional diagrams (A) Effect of acidity on percentage aeration; (B) Effect of percentage of inoculum on acidity; (C) The effect of the percentage of inoculum acidity on CGC weight is given.

The data obtained in this study showed that there is a linear relationship between cell mass production and CGC.

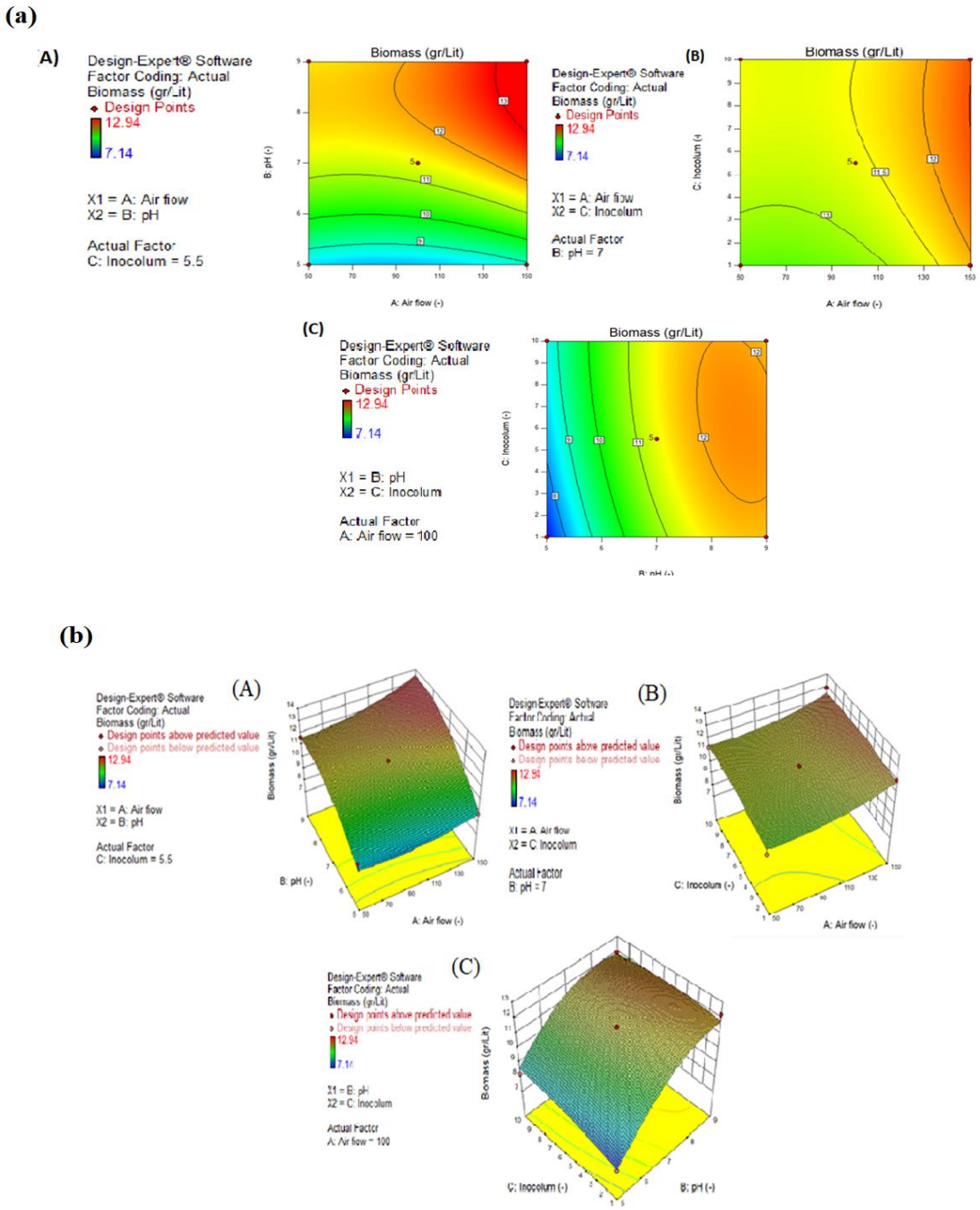


Figure 1. (a) Two-dimensional graph (A) Effect of acidity on aeration percentage; (B) Effect of aeration percentage on acidity; (C) Effect of acidity on aeration percentage; and (B) Three-dimensional graph (A) Effect of acidity on aeration percentage; (b) Effect of aeration percentage on aeration; (C) Effect of acidity percentage on acidity on cell dry weight.

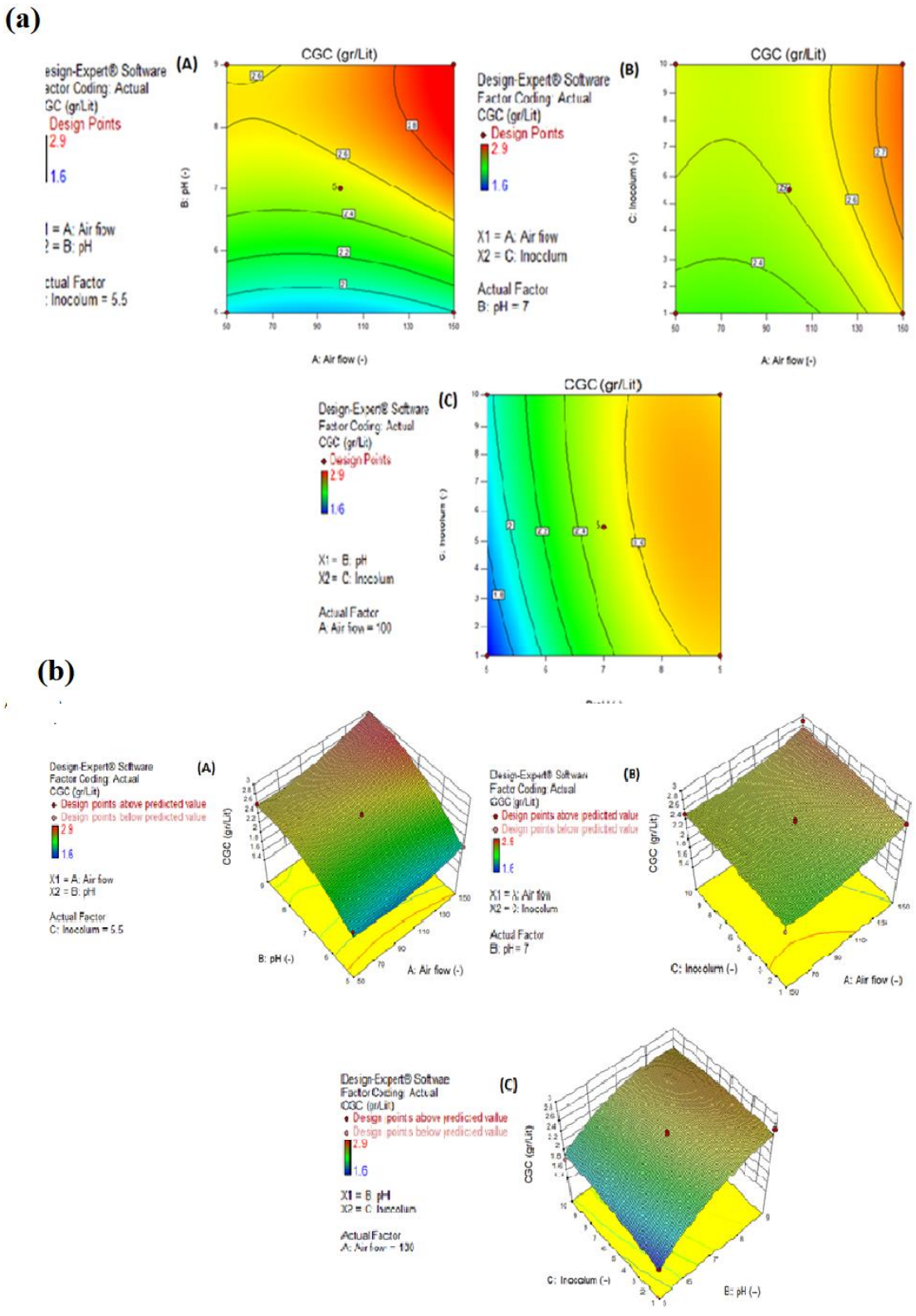


Figure 2. (a) Two-dimensional diagram (A) Effect of acidity on aeration percentage; (B) Effect of aeration percentage on aeration; (C) Effect of inoculum percentage on acidity and (b). Three-dimensional graph (A) of the effect of acidity on aeration percentage; (B) Effect of aeration percentage on aeration; (C) Effect of acidity percentage on acidity on CGC weight.

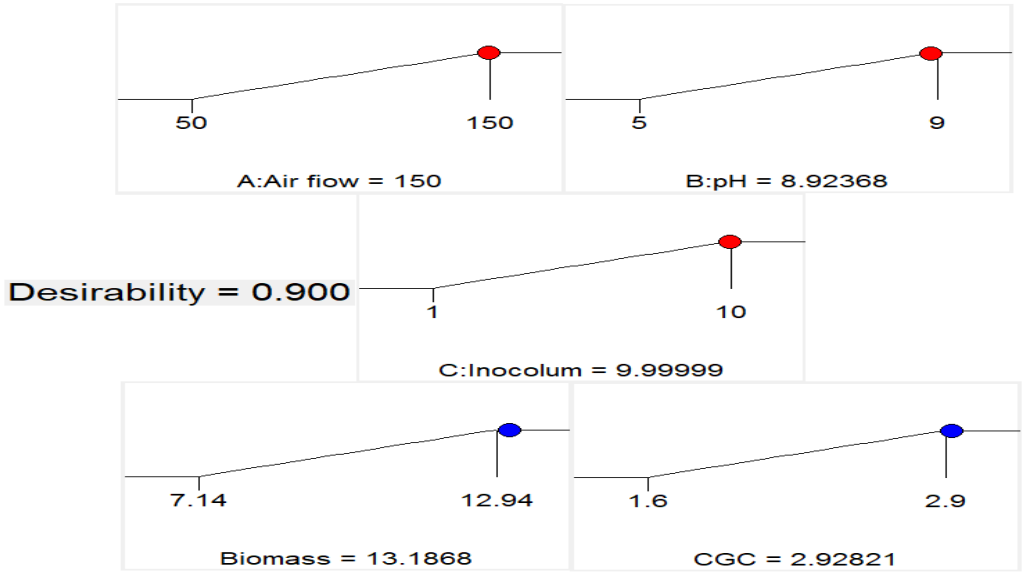


Figure 3. Optimum point to enhance fungal growth and CGC production

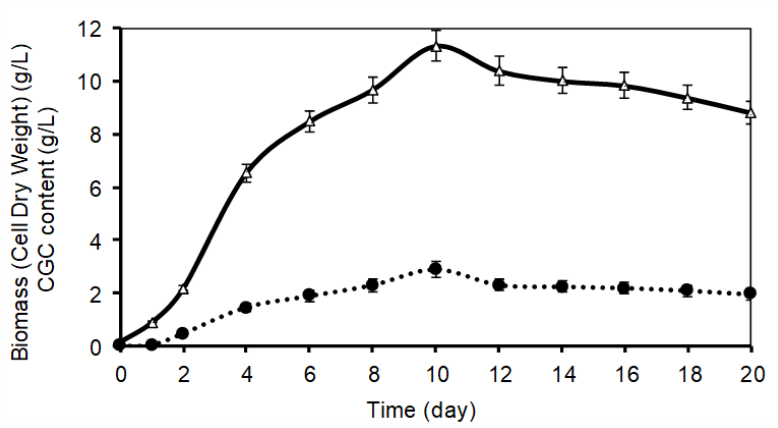
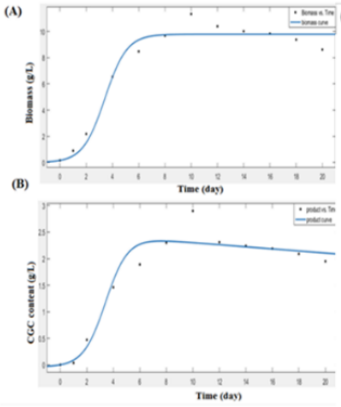


Figure 4. Temporal changes of cellular dry weight growth, production of CGC in PDB medium; *Schizophyllum commun*e, CGC production curve (●), mycelium growth curve (Δ)



(c)

Culture Medium / Model kinetic parameters	PDB
C_m (g/l)	9.793
μ_{max} (day^{-1})	1.193
α ($g \times g^{-1}$)	0.2524
B ($g \times (g \times h)^{-1}$)	-0.002004
R^2 (growth)	0.9665
Adjusted R^2 (growth)	0.9631
R^2 (production)	0.9439
Adjusted R^2 (production)	0.9383

Figure 5. Laboratory data and regression curves of (A) biomass and (B) Figure 5. Laboratory data and regression curve (A) biomass and (B) CGC production in mycelial form of Schizophyllum commune inea fungus fermentation in PDB medium (.) laboratory data and (-) regression curve and (C) Coefficients of mycelial growth and CGC production obtained from the model.

Discussion

Based on the Figures 1-5, it can be said that as the aeration progresses and the alkaline pH increases, the CGC level increases. In 2001, Amorim *et al.* investigated the preparation of CGC at identical conditions and initial pH ranging from 5 - 6.5 and found that at pH = 6.5 the highest amount of CGC was produced **(24)**. According to a 2006 study by Feofilova *et al.* about changing CGC content and composition during the development of ascites in submerged cultures, they found out that the composition and content of CGC clearly depend on the fungal development stage and the maximum amount of CGC production. Mycelium was observed in acidic medium in liquid culture **(7)**.

It can be said that the present study is consistent with the studies of Amorim and Feofilova in 2001 and 2006, and as the alkaline pH progresses, CGC production increases **(7,24)**. The important influence of the initial pH of the environment can be justified by the fact that the pH of the environment can affect membrane function, cell morphology, nutrient uptake and production of extracellular and intracellular products **(25)**.

Specific growth coefficient of *Schizophyllum commun*e native kumina (maxμ) is 0.991-day. In 2018, Moraditanha *et al.* studied the kinetics of growth and production of CGC from gonoderma leucidum in the immersed medium. After investigating the changes; the specific growth coefficient (maxμ) was found to be 0 5274 day-1 **(22)**. According to the specific growth coefficient obtained, *Schizophyllum commun*e has a faster growth and production of CGC than *Gonoderma leucidium*.

Also, the kinetic model of growth and production of CGC from *S. commun*e in this study is in line with the model of Tang *et al.* (2004) on *G. leucidium* and Feng *et al.* (2010) study on structural model of polysaccharide production from fungi *Shita*ka **(28)**.

Conclusion

Based on the optimization of the optimum conditions, aeration percentage of 150, pH of 8.92 and inoculation percentage of 9.99 were obtained. The kinetic parameters of growth and production have been investigated and matching of experimental data with logistic growth model with R2=0.9665 and Luedeking and Piret s model for production with R2=0.9439 showed good agreement. According to the curve plotted on day 10, it was selected for having the highest cellular dry weight and CGC.

Acknowledgment

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Conflict of Interest

Authors declared no conflict of interests.