http://dx.doi.org/10.22037/afb.v5i4.23139

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2018, 5 (4):243-252
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

Mycoprotein Production from Date Waste Using *Fusarium venenatum* in a Submerged Culture

Seyedeh. Fatemeh Seyed. Reihani, Kianoush Khosravi-Darani*

Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background and Objective: Production of single cell protein has various outstanding advantages, e.g., it can be grown on waste and it is environmental friendly as it helps in upgrading agricultural wastes. In the present study, the influence of process parameters on the biomass formation (g l^{-1}), protein production (% w w^{-1}) and volumetric productivity (g l^{-1} h^{-1}) of *Fusarium venenatum* IR372C was determined.

Material and Methods: The Vogel medium was used with glucose as the carbon source for pre culture cultivation and date sugar as the carbon source for production medium. In the first phase of the study, submerged fermentation was conducted in 500 ml flasks and a 3l stirred-tank bioreactor was exploited to conduct the submerged fermentation in the second phase. Plackett-Burman Design with eleven factors, i.e., date sugar concentration, $NH_4H_2PO_4$, peptone, $MgSO_4$, KH_2PO_4, temperature, time, shake rate, inoculate age, inoculate size, pH in two levels and Response Surface Methodology with three variables, i.e., date sugar concentration, time and inoculate size were employed to determine the fermentation condition by which the maximum biomass, protein and productivity were achieved.

Results and Conclusion: Based on obtained results, by using the selected levels of influencing process variables, a relatively high amount of total protein (*ca*. 4 g l^{-1}, 65.3% in the first phase using flasks and 5.5 g l^{-1}, 76% in the second phase by using the bioreactor, respectively) was achieved. The amino and fatty acid profiles of mycoprotein and its relatively high fibre content (6%) imply that mycoprotein could be incorporated in various types of foods as a functional ingredient.

Conflict of interest All authors have declared that they don't have any conflict of interest for publishing this research.

Article Information

Article history:

Received	24 Oct 2018
Revised	12 Nov 2018
Accepted	22 Dec 2018

Keywords:
- Date waste
- Food grade
- *Fusarium venenatum*
- Inoculate size
- Mycoprotein
- Submerged fermentation

***Corresponding author:**
Kianoush Khosravi-Darani, Prof, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Tel: +98-21-22376473
Fax: +98-21-22376473
E-mail: k.khosravi@sbmu.ac.ir

1. Introduction

Lower life quality, penury and starvation will be inevitable by excessive global population growth. Thus, humankind has been trying to prevent this disaster through technological advances for a global access to food. Facing such worldwide issues, protein production has been the subject of various research investigations. One of the most advantageous approaches among those research studies is production of single-cell proteins (SCP) through fermentation by using agricultural waste [1-3]. Among various microorganisms, *Fusarium (F.) venenatum* is the most common species to be successfully utilized in food industry. It has been used to produce mycoprotein as food being sold under the trade name Quorn [4-6]. This product with a fibrous texture is a rich source of high quality protein including essential amino acids. It is also less energy dense than equivalent meat products and does not contain animal fats and cholesterol [1,3]. Mycoprotein shows satiation properties which can be a solution for overweight by enabling people to achieve a healthier diet with low fat and high fibre content. The fibre composition is about one-third chitin and two-thirds β-1, 3- and β-1, 6-glucans. The fat content of the produced biomass is typically 2-3.5%, and the fatty acid composition is similar to that of vegetable oils [7]. As pointed out in literature, mycoprotein presents an attractive food product that can improve appetite regulation and postprandial glycaemic and insulin responses in overweight and obese individuals at risk of developing type 2 diabetes mellitus [8-10]. In addition, it is well demonstrated that mycoprotein from *F.*

venenatum acutely reduces energy uptake and improves glycaemic profile [8].

Owing to its β-glucan and chitin content (about 6% w w^{-1}), mycoprotein can work as a prebiotic that can selectively stimulate beneficial bacteria in the colon and therefore, improve health. It has been suggested that mycoprotein could now be of use either in breakfast cereals and puffed snacks, or be added to yoghurt and ice-cream [7]. Producing SCP with highest protein content and digestibility at the lowest cost requires seeking economic substrates [11,12]. Agricultural wastes are being used as economic carbon and energy sources for mycoprotein production [13]. Date fruit with high content of carbohydrates, minerals and vitamins, is extensively produced in Middle East, and a large amount is wasted during the picking, package and commercialization process. Therefore, it could be utilized as a good source of carbon and energy, leading in cheap fermentation processes [14].

According to the reported studies, a vast range of microorganisms are capable of producing SCP [12,15,16]. However, solely several ascomycetes such as *Neurospora* spp., *F. venenatum* and *Monascus* spp. are well-known as generally regarded as safe microorganisms [4]. Technically, many studies are based on submerged fermentation in recent years [17-26] maybe due to its better heat and mass transfer in addition to its culture homogeneity that leads to more reliable, and reproducible attributes in comparison to solid state fermentation setups. Considering the importance of the influence of process parameters on the outcome of submerged fermentations, the identification of the main process parameters and their optimization in mycoprotein production seems to be a necessity. In addition, evaluation of nutritional value of the product is of great interest.

Here, the influence of process parameters on the biomass or cell dried mass (g l^{-1}), protein production (% w w^{-1}) and volumetric productivity (g l^{-1} h^{-1}) of *F. venenatum* IR372C were investigated by submerged fermentation with date waste sugar as the carbon source. Initially, a Plackett-Burman design (PBD) was conducted to screen the main process variables. Then, a central composite design (CCD) was performed based on PBD results to optimize the levels of effective variables toward the best response. Furthermore, ribonucleic acid contents of produced mycoprotein were reduced, and the amino and fatty acid profiles of the final product were investigated.

2. Materials and Methods

2.1. Microorganism, media, inoculum and culture condition

F. venenatum IR372C was purchased from Iranian Research Institute of Plant Protection (IRIPP) and used throughout this investigation. The strain was maintained at 4°C on agar-solidified Vogel slants. The defined medium of Vogel was used with glucose as the carbon source for pre culture cultivation [7]. The pH of the Vogel's medium was equal to 5.7. Inoculum was prepared in 250 conical flasks containing 100 ml Vogel's medium. Flasks were inoculated with single microbial colonies from slants and incubated on a shaker incubator-cooling at 25°C, 200 rpm, for 48 and 72 h (Jaltajhiz Co. Ltd, Iran). Date sugar purchased from Dombaz Co. (Bandar Abbas, Iran) and contained about 70% total solids (fructose 33.1%, glucose 31.05%, ash 2.5%, protein 1.58%, fibre 0.28 and fat 0.1) with a moisture content of 30 % (pH 4.7). Production medium components were the same as in the inoculum medium except for date sugar, which was used instead of glucose. Inocula were prepared in 5% v v^{-1}, containing 1.7×10^5 CFU ml^{-1} (OD measured at 600 nm, R^2=0.97) in 250 conical flasks with 100 ml Vogel's medium and incubated in the shaker incubator at 30°C, 150 rpm for 72 h.

2.2. Fermentation

In the first phase of study, submerged fermentation was conducted in 500 ml flasks each containing 200 ml of production medium. The culture medium was inoculated with fungal suspension, and incubated at 26-32°C as defined by experimental design. After fermentation, biomass was obtained by filtration of culture medium through pre-dried and weighted Whatman filter papers (No.1, UK) and washed two or three times with cold distilled water, then dried by an oven (Memmert, USA) at 60ºC to a constant weight (24-48 h). The cell dry mass was quantified gravimetrically (Sartorius BA210S, Germany) [7,14]. In the second phase of this study, a 3l stirred-tank bioreactor (Winpact, USA) was exploited to conduct the submerged fermentation. Information on pH, temperature, stirring rate and aeration were monitored throughout the running experiment by the bioreactor system.

2.3. Analytical Methods

The crude protein content was measured using the modified Lowry method [27] and the absorbance of each sample was read by spectrophotometer at 600 nm (Optima, Japan). Calibration curves were prepared for each assay with a 1-5 mg ml^{-1} bovine serum albumin (BSA), (R^2=0.97) in Microsoft Excel 2013. The protein content was represented by % w w^{-1} protein per total dry mass. The RNA content of biomass was reduced in order to meet required safety standards by exposing the biomass to heat shock at 65°C for 25 min (before filtration) [5]. A guanidine/phenol solution (RNX-plus, from Sinaclon, Iran) was used to measure the total RNA. The concentration of RNA was determined by a spectrophotometer (UV/VIS spectrophotometer, Optima, Japan) at 260 nm [14]. Amino acid profile of produced mycoprotein was determined by

reversed phase HPLC. Sample was injected onto a Zorbax Eclipse-AAA column, 4.6×150 mm, 5 µm, (Agilent, USA) at 40°C with a fluorescence detector. A 40 mM NaH_2PO_4 was used as mobile phase A and a ratio of 45: 45: 10 v v^{-1} acetonitrile: methanol: water was used as mobile phase B [28].

Fatty acid composition of biomass was determined by a DSQ GC-MS, Trace GC ultra (Thermo, USA) using a capillary column (Thermo, TR-5) (30 m per 0.25 mm ID, film thickness 0.25 µm) operated with Helium as the carrier gas (0.2 ml per min). 1 µl of methylated sample was injected with injector temperature of 280°C. For the first minute oven temperature kept at 50°C, and then increased to 270°C by a rate of 7°C per min [28].

2.4. Experimental design

In the present study, PBD was used as a screening method to estimate the most significant parameters by using Design Expert 10.

After performing PBD, the effect of main factors and their interactions that significantly influence the mycoprotein production were analyzed and optimized by a central composite design (CCD, α =1.68) with 8 cube points, 6 center points in cube and 6 axial points.

Cell dry mass (CDM) of produced biomass, protein production and productivity were considered as the three responses (Y_1, Y_2, Y_3). The general form of second order polynomial, which coefficients were analyzed by Minitab 14, were calculated according to Eq 1:

$$Y_i=\beta_0 +\sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \qquad \text{Eq 1}$$

Where Y_i is the predicted response, X_iX_j are input variables which influence the response variable Y; β_0 is the offset term; β_i is the i^{th} coefficient; β_{ii} the j^{th} quadratic coefficient and β_{ij} is the ij^{th} interaction coefficient. Statistical analysis of the model was performed by the analysis of variance (ANOVA). The optimum levels of variables (within the experimental range) for maximum biomass, protein production and productivity were determined, and the defined optimum point was confirmed by running the final trial.

3. Results and Discussion

3.1. PBD

The parameters and their levels were chosen based on preliminary experiments and reports in literature. Thus a date sugar concentration of 25 and 30 g l^{-1} were chosen as the two levels of carbon source [7,18,23,24]. For the nitrogen source, based on literature, the two levels for peptone concentration were selected as 0 and 5 g l^{-1} [23,29] and for ammonium dihydrogen phosphate as 0 and 4.5 g l^{-1} as organic and inorganic nutrient sources [7,8,14]. Magnesium sulfate (0.05, 0.15) and potassium dihydrogen phosphate (0.5, 1.6) were used [7,13,24] as metal salts. The temperature were set to 26°C and 32°C [7,24,29]. Fermentation time [7,18], shaking rate during incubation, [23,30-31] inoculum age, inoculum size and pH [7,14,17,32-36] were selected according to the literature. As known, in PBD two levels can be considered for each factor. The selected factors that include medium components (*i.e.*, carbon, nitrogen, and phosphorous source concentration), environmental factors (*i.e.*, incubation temperature and time) and inoculum condition (inoculate age and size) are presented in Table 1.

Table 1. Levels of 11 selected experimental factor for PBD

	Variables	Low	High
A	Date sugar (gl^{-1})	25	30
B	$(NH_4)\ H_2PO_4$ (gl^{-1})	0	4.5
C	Peptone	0	5
D	$MgSO_4$	0.05	0.15
E	KH_2PO_4 (gl^{-1})	0.5	1.6
F	Temperature (ºC)	26	32
G	Time (h)	48	72
H	Shake Rate	100	200
J	Inoculate age (h)	24	48
K	Inoculate size (% v v^{-1})	8	12
L	pH	4.5	5.5

Selected experimental factors and a PBD for conducting twelve experimental trials are shown in Table 2. As shown, (+1) and (-1) represent the two different levels of the independent factors examined. The statistical results of the variables considered as the main factors based on their contribution are shown in Table 3.

The results showed that the incubation time, date sugar concentration, inoculate size and shaking rate had significantly ($P \leq 0.05$) positive effects on protein production. The other parameters showed significant impact on the other two responses. The optimum result of protein production obtained under conditions as follow: date sugar concentration 25 g l^{-1}, $(NH_4)H_2PO_4$ 4.5 g l^{-1}, peptone 0 g l^{-1}, $MgSO_4$ 0.15 g l^{-1}, KH_2PO_4 1.6 g l^{-1}, temperature 26°C, time 72 h, shake rate 200 rpm, inoculate age 48 h, inoculate size 8% v v^{-1} and finally pH 4.5. Under the optimum conditions, 70.02% crude protein in the dry product was produced.

Table 2. Results of performed runs using PBD[a]

Run	A	B	C	D	E	F	G	H	J	K	L	CDM ($g\ l^{-1}$)	Protein % (ww^{-1})	Productivity ($g\ l^{-1}\ h^{-1}$)
1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	2.95±0.01	49.35±0.52	1.02±0.01
2	1	1	1	-1	-1	-1	1	-1	1	1	-1	3.32±0.02	44.87±0.13	0.62±0.02
3	1	1	-1	1	1	1	-1	-1	-1	1	-1	3.11±0.02	28.20±1.40	0.58±0.02
4	1	-1	-1	-1	1	-1	1	1	-1	1	1	2.67±0.03	41.34±0.73	0.57±0.03
5	1	-1	1	1	1	-1	-1	-1	1	-1	1	3.31±0.01	44.87±0.43	0.93±0.01
6	-1	1	1	-1	1	1	1	-1	-1	-1	1	2.67±0.00	57.69±0.47	0.80±0.00
7	1	1	-1	-1	-1	1	-1	1	1	-1	1	3.06±0.00	47.56±0.23	0.99±0.00
8	-1	1	1	1	-1	-1	-1	1	-1	1	1	1.35±0.01	48.14±0.76	1.00±0.01
9	-1	-1	-1	1	-1	1	1	-1	1	1	1	2.10±0.00	58.97±1.50	0.81±0.00
10	-1	-1	1	-1	1	1	-1	1	1	1	-1	2.70±0.00	55.02±0.85	1.27±0.00
11	-1	1	-1	1	1	-1	1	1	1	-1	-1	6.63±0.02	70.02±1.34	1.19±0.02
12	1	-1	1	1	-1	1	1	1	-1	-1	-1	2.06±0.03	60.71±1.06	1.02±0.03

Table 3. Analysis Of Variance of the main factors in PBD for Y_2: Protein%

Source	Sum Squares	Df	Mean Sq	F	P
A-date sugar conc.	541.92	1	541.92	24.54	0.0158
D-$MgSO_4$ amnt.	120.82	1	120.82	5.47	0.1013
E-KH_2PO_4	1.00	1	1.00	0.045	0.8452
G-Time	580.49	1	580.49	26.28	0.0144
H-Shake rate	454.44	1	454.44	20.58	0.0201
J-Inoc. age	167.79	1	167.79	7.60	0.0704
K-Inoc. amnt.	489.81	1	489.81	22.18	0.0181
L-pH	165.88	1	165.88	7.51	0.0713
Residual	66.26	3	22.09	----	----
Cor Total	2588.40	11	----	----	----

3.2. RSM

Based on PBD results, main factors contributing in the production of protein were further chosen to be assessed by RSM. Shake rate was one of the factors with significant effect on protein production at its higher level, *i.e.*, 200 rpm. Nevertheless, examination of higher rates of shaking was not possible due to technical constraints and thus, this factor was fixed at 200 rpm.

3.2.1. Regression model and statistical testing

A central composite design (CCD) with three independent variables was used. Twenty runs were required to cover all possible combinations of factor levels. The experiments were run in a random order to minimize the effects of unexpected variability in the observed responses. The experimental range for each independent variable was defined regarding the results of performed PBD (Table 2).

A model describing the behavior of each response was created by finding the best setting of variables in order to optimize the fermentation process. Second order models for the three responses in terms of coded variable are given by Eq. 2, Eq. 3, and Eq. 4, respectively:

$$Y_1=4.27 + 2.00\ X_1 + 0.75\ X_2 + 0.30\ X_3 + 1.47\ X_{11} - 0.64\ X_{22} - 0.21\ X_{33} - 0.64\ X_1X_2 - 0.09\ X_1X_3 - 0.47\ X_2X_3 \quad \text{Eq. 2}$$

$$Y_2=67.27 + 8.36\ X_1 + 5.5\ X_2 + 5.05\ X_3 - 16.04\ X_{11} + 3.31\ X_{22} - 14.78\ X_{33} - 4.07\ X_1X_2 - 1.76\ X_1X_3 - 0.48\ X_2X_3 \quad \text{Eq. 3}$$

$$Y_3=0.058 + 0.029\ X_1 - 0.022\ X_2 + 0.005\ X_3 + 0.023\ X_{11} - 0.002\ X_{22} + 0.0003\ X_{33} - 0.022\ X_1X_2 - 0.002\ X_1X_3 - 0.0099\ X_2X_3 \quad \text{Eq.4}$$

Where X_1, X_2 and X_3 represent the date sugar concentration, time and inoculate size, and Y_1, Y_2 and Y_3 represent the CDM, protein and productivity, respectively. Among these second-order regression models, Eq. 3 was more satisfactory since its coefficient of determination value (R^2) was relatively high and closer to 1 (0.935). The *P*-value for lack of fit was 0.097 that implies the validity of quadratic model for protein production [14]. The R^2 for CDM and productivity models were 85.9% and 82.3%, respectively. The main and interaction effects of date sugar concentration, incubation time and inoculate size were analyzed and the predicted and obtained results for all the three responses are presented in Table 4.

Regression analysis of the experimental data showed that all the three factors had positive linear effect on mycoprotein production ($P \leq 0.05$) as shown in Table 5. Among the three variables, date sugar concentration had highest impact on protein production as given by highest linear coefficient [7], followed by time (5.50) and inoculum size (5.05). This result is in accordance with what was reported in similar studies, *i.e.*, date sugar [14,17] and inoculum size [19] significantly influenced the mycoprotein production. Chemical structure and type of the carbon source can directly affect the cell growth and composition as well as protein content of the final product [20]. A similar relationship between the carbon source and protein production regardless of the type of microorganism was reported in previous studies [25,30,37].

Table 4. Central composite design with the experimental data and predicted values

Run	Date Sugar (gl^{-1}) X_1	Incub. Time X_2	Inoc. Size (%) X_3	CDM (g) Y_1		Protein % w w^{-1} Y_2		Productivity g l^{-1} h^{-1} Y_3	
				experimental	predicted	experimental	predicted	experimental	predicted
1	35	50	5	5.45	5.15	62.09	58.14	0.109	0.098
2	15	50	10	3.93	3.01	53.01	51.66	0.078	0.061
3	25	73	7.5	4.4	4.27	67.58	67.27	0.060	0.058
4	41.81	73	7.5	8.07	7.75	55.27	59.59	0.110	0.111
5	25	73	3.29	3.45	3.75	44.12	47.44	0.047	0.053
6	25	73	7.5	4.35	4.27	66.04	67.27	0.059	0.058
7	25	73	7.5	4.41	4.27	67.34	67.27	0.060	0.058
8	8.18	73	7.5	2.72	3.75	42.65	42.87	0.037	0.052
9	15	96	5	4.21	3.93	53.96	53.83	0.043	0.042
10	35	50	10	6.023	5.78	66.34	63.24	0.12	0.110
11	15	96	10	4.25	4.03	60.02	60.74	0.044	0.043
12	15	50	5	3.01	2.25	46.32	44.06	0.060	0.046
13	35	96	5	5.53	5.92	64.02	62.14	0.057	0.063
14	35	96	10	5.65	5.89	67.53	66.56	0.058	0.061
15	25	111.7	7.5	4.73	4.39	76.32	76.09	0.042	0.033
16	25	73	7.5	4.27	4.27	67.30	67.27	0.058	0.058
17	25	34.31	7.5	1.82	2.88	60.31	65.08	0.053	0.078
18	25	73	7.5	4.12	4.27	66.15	67.27	0.056	0.058
19	25	73	7.5	4.25	4.27	70.04	67.27	0.058	0.058
20	25	73	11.70	3.95	4.37	56.31	57.54	0.054	0.064

Table 5. Estimated regression coefficients of process variables for % Protein

Variable	Coef.	SE Coef.	*t*-value	*P*-value[a]
constant	67.2781	1.301	51.693	0.000
X_1	8.3609	1.452	5.757	0.000
X2	5.5041	1.452	3.790	0.004
X3	5.0504	1.452	3.478	0.006
X1X1	-16.040	2.378	-6.747	0.000
X2X2	3.3142	2.378	1.394	0.194
X3X3	-14.7858	2.378	-6.219	0.000
X1X2	-4.0765	3.191	-1.277	0.230
X_1X_3	-1.7642	3.191	0.553	0.593
X_2X_3	-0.4844	3.191	-0.152	0.882

3.2.2. Interaction between factors influencing mycoprotein production

The interaction effects and optimal levels of the variables were illustrated by the response surface and contour plots. Considering its better fit, the surface plots of the main response, *i.e.*, protein production, are shown, and the influence of date sugar and time interaction on protein production is illustrated (Figure 1). Nevertheless, for better addressing the issue, contour plots of the other two responses are also presented in Figure 2a.

The surface plot showed increasing trend for mycoprotein production with increased date sugar and in a more moderate way with increase in time. As illustrated, an increase in protein production can be observed with higher levels of date sugar; however, concentrations of more than 30 g l^{-1} seem to cause reduction in protein formation. The amount of carbon source consumption varies from species to species and from strain to strain [38]. Moreover, incubation time seemed to have a direct relationship with protein production. As shown in Figure 2a by a moderate slope, there will be more protein production with a longer incubation time.

Date sugar as the carbon and energy source function as the main factor in biomass and protein production. However, for maximum protein production, it should be limited (Figure 2a) to a concentration not higher than about 30 g l^{-1}, while CDM (Y_1) still keeps increasing at levels higher than 30 g l^{-1} (Figure 3).

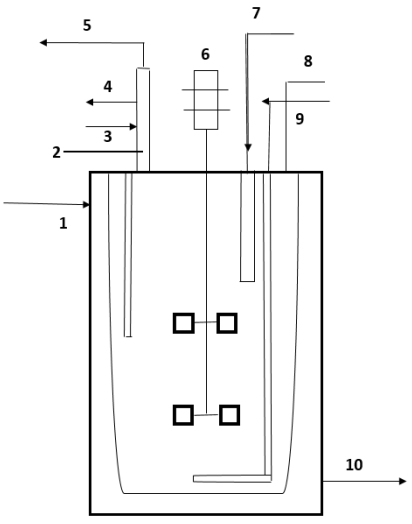

Figure 1. Scheme of the stirred-tank bioreactor:1 Cooling water inlet, 2 Condenser, 3 Water in, 4 Water out, 5 Gas out, 6 Motor, 7 Feed in, 8 Inoculum in, 9 Air in, 10, Cooling water out.

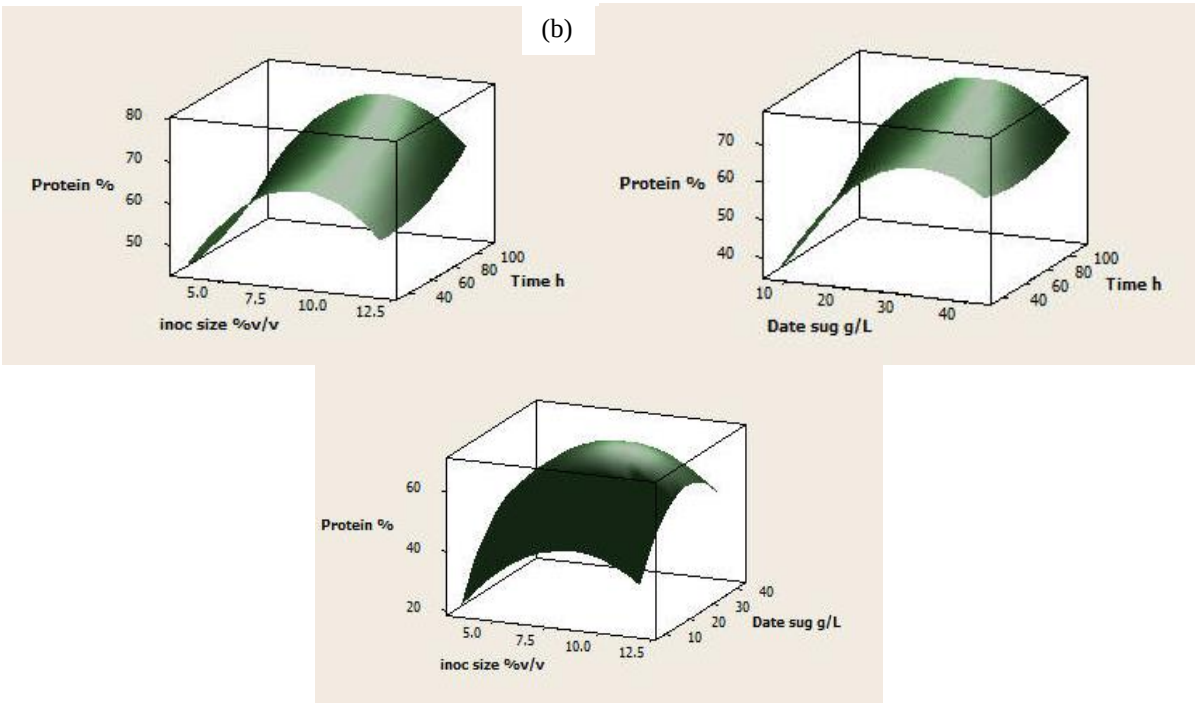

Figure 2. Response surface of interaction effect between a. date sugar concentration and time; b. inoculate size and date sugar concentration; c. inoculate size and time, on mycoprotein production

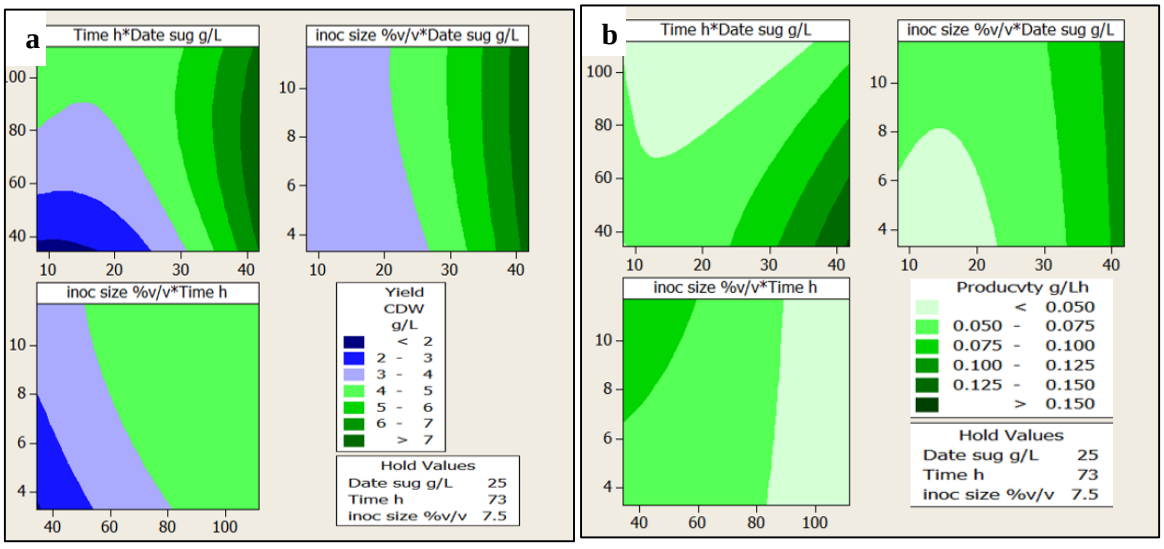

Figure 3. Response contours of interaction effect between (a) time and date sugar concentration, inoculate size and date sugar concentration and inoculate size and time on biomass (CDW) production (Y_1); (b) time and inoculate size with date sugar concentration, and inoculate size with time, on productivity (Y_3).

This difference in behavior of the two responses resulted in difficulty in determination of optimum point where the two or three responses are at their highest level.

Figure 2b represents the interaction between date sugar concentrations and inoculum size. As shown, increase in the level of inoculate size and the level of date sugar leads to higher protein production. Results indicated that an inoculum amount of 7-9% and date sugar concentration of 25-30 g l^{-1} contribute to higher protein production. Increase of inoculum size up to 10% v v^{-1} can result in higher protein production.

A similar result has been reported for protein production from starch waste by *Aspergillus oryzae*, where a value of 7.5% (v v^{-1}) was reported as the optimum level of inoculum size in a data range of 1 to 15.5% (v v^{-1}) [39]. Further increase of inoculum size with constant concentration of carbon source caused decrease in protein production; this may be due to limitation of medium

components and resulted in competition between cells. High production of protein was observed by increase in time; however; by rising the inoculum size to more than 8%, protein production drops with a strong slope (Figure 2c). As stated before, with constant carbon source concentration, by longer incubation time, high levels of inoculation can lead to competition between cells for energy uptake. In other words, high inoculum size acts as a limiting factor against the cell growth. In Figure 3, contour plots of the other two responses, *i.e.*, biomass (CDM) and productivity are presented.

As shown in Figure 3a, by using higher levels of date sugar along with time, biomass production increased with a strong slope and there was no limitation observed. For productivity as response (Figure 3b), date sugar levels higher than 30 g l^{-1}, beside a limited incubation time, productivity can increase.

3.3. Optimization and verification

As results show, maximum levels for all the three responses could not be achieved. Even though, considering the mycoprotein production as the main response, the defined optimum conditions and levels of each of the main factors would differ with those of the two other responses. In fact, in order to obtain a higher total protein production considering the cell growth as an important response is inevitable. Thus, although a maximum protein of 70% was achievable, selecting the fermentation condition and experimental factors led to a lower total amount of protein. Based on this fact, optimization was conducted by considering a moderate level of desirability for all the three responses. On this basis, a 36.3 g l^{-1} date sugar, 60 h incubation time and 8.2% inoculum size were identified as the optimized conditions in order to achieve an overall higher mycoprotein concentration (4.04 g l^{-1}) with a protein and CDM content of 65.3% and 6.2 g l^{-1}, respectively, and a volumetric productivity of 0.1023 g l^{-1} h^{-1}. To validate the optimum combination of the process variables, confirmatory experiments were carried out. The selected combinations of the three variables resulted in a good accordance with the predicted response optimization and obtained values.

Nevertheless, achieved data from bioreactor showed a 13% increase in produced biomass and 22% increase in protein production compared to the results of the first phase of the study. Applying the optimized condition in the bioreactor (n=10) resulted in an average biomass of 7.2± 0.64 g l^{-1} and 76 ± 6.56 protein% (w w^{-1}) production, with productivity amounting to approximately 0.12 g l^{-1} h^{-1}.

3.4. RNA reduction and analytical results

Heat treatment at 65°C for 25-30 min reduced the RNA content from 10.01 to 1.06%, which is considered a safe level (≤ 2 g per day) for human consumption [5,8]. This result is in agreement with previous studies on *F. venenatum* [5,14].

Amino and fatty acids composition of fungal biomass are presented in Table 6. As evident, the amino acid profile of biomass almost included the essential amino acids (except for aspartic acid and proline). This finding confirm other similar studies that also reported the presence of essential amino acids in SCP from *F. venenatum* [14]. Analysis of fatty acids profile indicated that the ratio of unsaturated to saturated fatty acid was about 3 to 2. Hosseini and Khosravi-Darani reported a ratio of 3.2-3.5 to 1 for unsaturated to saturated fatty acids [14]. As a matter of fact, consumption of high amounts of unsaturated fatty acids provides health benefits [40].

Table 6. Amino and Fatty acid composition of mycoprotein of *F. venenatum*

Amino Acid	Content (%ww^{-1})	Fatty Acid	Content (%ww^{-1})
L-Alanine	0.24±0.01	Decane, dodecane, undecane	2.02±0.003
L-Arginine	0.71±0.02	tetradecanoic	0.52±0.000
L-Cystine	0.21±0.01	Pentadecanoic	0.14±0.000
L-Glutamic	2.23±0.09	Hexadecanoic	0.29±0.000
Glycine	0.35±0.01	Margaric	2.18±004
L-Histidine	0.72±0.02	Nonadecanoic	2.65±0.006
L-Isolucine	0.15±0.01	Eicosanic	1.48±0.002
L-Leucine	0.19±0.01	Myristic	0.52±0.000
L-Lysine	0.26±0.00	Pentadecanoic	0.14±0.000
L-Methionine	0.42±0.01	Palmitoleic	2.21±0.001
L-Phenylalanine	0.30±0.01	Palmitic	23.24±0.29
L-Serine	15.54±0.56	Isostearic	0.73±0.001
L-Theronine	0.33±0.01	Linoleic	39.4±0.353
L-Tyrosine	0.27±0.01	Oleic	18.01±0.365
L-Valine	0.29±0.01	Stearic	5.79±0.161

4. Conclusion

Based on obtained results, by using the selected levels of influencing process variables, a relatively high amount of protein was achieved (*ca*. 4 g l^{-1}, 65.3% in first phase by using flasks and 5.5 g l^{-1}, 76% in second phase by using the bioreactor). As expected, using a bioreactor led to a better control of fermentation conditions and also aeration that caused considerably higher levels of biomass, protein and productivity. Further research and development will ensure the usage of mycoprotein as a non-animal protein or as a diet supplement especially in developing countries.

5. Acknowledgements

The financial support provided by Iran's National Elites Foundation is gratefully acknowledged.

6. Conflict of Interest

All authors have declared that they don't have any conflict of interest for publishing this research.

References

1. Verstraete W, Clauwaert P, Vlaeminck SE. Used water and nutrients: Recovery perspectives in a 'panta rhei' context. Bioresour Technol. 2016; 215:199-208. doi:10.1016/j.biortech.2016.04.094

2. Yunus F, Nadeem M, Rashid F. Single-cell protein production through microbial conversion of lignocellulosic residue (wheat bran) for animal feed. J Inst Brew. 2015; 121: 553-557. doi: 10.1002/jib.251

3. Rasoul-Amini S, Morowvat MH, Younes Gh. Single cell protein: Production and process. American J Food Technol. 2011; 6: 103-116. doi: 10.3923/ajft.2011.103.116.

4. Ferreira, JA, Mahboubi A, Lennartsson PR, Taherzadeh MJ. Waste biorefineries using filamentous ascomycetes fungi: Present status and future prospects. Bioresour Technol. 2016; 215, 334-345. doi: 10.1016/j.biortech.2016.03.018

5. Wiebe M. Myco-protein from *Fusarium venenatum*: A well-established product for human consumption. Appl Microbiol Biotechnol. 2002; 58 (4): 421-427. doi: 10.1007/s00253-002-0931-x

6. Asgar MA, Fazilah A, Huda N, Bhat R, Karim AA. Nonmeat protein alternatives as meat extenders and meat analogs. Compr Rev Food Sci Food Saf. 2010; 9:513-529. doi: 10.1111/j.1541-4337.2010.00124.x

7. Hosseini SM, Khosravi-Darani K, Mohammadifar MA, Nikoopour H. Production of mycoprotein by *Fusarium venenatum* growth on modified vogel medium. Asian J Chem. 2009; 21(5): 4017-4022.

8. Bottin J, Swann JR, Cropp E, Chambers ES, Ford HE, Ghatei M A, Frost GS. Mycoprotein reduces energy intake and postprandial insulin release without altering glucagon-like peptide-1 and peptide tyrosine-tyrosine concentrations in healthy overweight and obese adults: a randomised-controlled trial. Brit J Nutr. 2016; 116: 360-374. doi: 10.1017/S0007114516001872

9. Fiinnigan TJA. Mycoprotein: Origins, Production and Properties. In: Philips GO, Williams PA, Handbook of Food Proteins, Cambridge: Woodhead publishing limited. 2011: pp. 335-352. doi: 10.1533/9780857093639.335

10. Joshi VK, Kumar S. Meat analogues: Plant based alternatives to meat products-a review. Int J Food Ferment Technol. 2015; 5(2): 107-119. doi: 10.5958/2277-9396.2016.00001.5.

11. Uçkun Kıran E, Trzcinski AP, Liu Y. Platform chemical production from food wastes using a biorefinery concept. J Chem Technol Biotechnol. 2015; 90, 1364-1379. doi: 10.1002/jctb.4551

12. Suman G, Nupur M, Anuradha S, Pradeep B. Single cell protein production: A review. Int J Curr Microbio App Sci. 2009; 4 (9): 251-269.

13. Ukaegbu-Obi KM. Single cell protein: A resort to global protein challenge and waste management. J Microbiol Microbial Technol. 2016; 1 (1), 1-5.

14. Hosseini SM, Khosravi-Darani K. Response surface methodology for mycoprotein production by *Fusarium venenatum* ATCC 20334. J Bioprocess Biotech. 2011; 1: 102. doi: 10.4172/2155-9821.1000102.

15. Akanni G, Ntuli V, du Preez J. Cactus pear biomass, a potential lignocellulose raw material for Single Cell Protein production (SCP): A Review. Int J Curr Microbiol Appl Sci. 2014; 3(7): 171-197.

16. Srividya AR, Vishnuvarthan VJ, Murugappan M, Dahake PG. Single cell protein-a review, Int J Pharm Res Scholars. 2014; 472-485.

17. Prakash P, Namashivyam SKR, Narendrakumar G. Optimization of growth parameters for elevated production of mycoprotein-*Fusarium venenatum* Using RSM. J Pure Appl Microbiol. 2014; 8(6): 4843-4849.

18. Prakash P, Karthick RNS, Swetha S. Design of medium components for the enhanced production of mycoprotein By *Fusarium venenatum* using placket barman model. Res J Pharm Biol Chem Sci. 2015; 6(1): 1251-1255.

19. Adoki A. Factors affecting yeast growth and protein yield production from orange, plantain and banana wastes processing residues using *Candida* sp. Afr J Biotechnol. 2008; 7(3), 290-295. doi: 10.4314/ajb.v7i3.58406.

20. Srivastava S, Pathak N, Srivastava P. Identification of limiting factors for the optimum growth of *Fusarium oxysporum* in liquid medium. Toxicol Int. 2011; 18(2): 111-116. doi: 10.4103/0971-6580.84262.

21. Ghaly AE, Kamal M, Correia LR. Kinetic modelling of continuous submerged fermentation of cheese whey for SCP production. Bioresour Technol. 2005. 96, 1143-1152. doi:10.1016/j.biortech.2004.09.027.

22. Haddish K. Production of single cell protein from fruit of beles (*Opuntia ficus-indica* L.) peels using *Saccharomyces cerevisiae*. J Microbiol Exp. 2015; 3 (1).00073. doi: 10.15406/jmen.2015.02.00073.

23. Pradeep FS, Pradeep BV. Optimization of pigment and biomass production from *Fusarium moniliforme* under

submerged fermentation conditions. Int J Pharm Pharm Sci. 2013; 5(3): 526-535.

24. Ferreira JA, Lennartsson PR, Taherzadeh MJ. Production of ethanol and biomass from thin stillage using food-grade zygomycetes and ascomycetes filamentous fungi. Energies 2014; 7, 3872-3885. doi: 10.3390/en7063872.

25. Ardestani F, Alishahi F. Optimization of single cell protein production by *Aspergillus niger* using taguchi approach. J Food Biosci Technol. 2015; 5 (2): 73-79.

26. Fazenda M, Seviour R, McNeil B, Harvey LM. Submerged culture fermentation of ‘‘higher fungi”: The macrofungi. Adv Appl Microbiol. 2008; 63, 33-103. doi: 10.1016/S0065-2164(07)00002-0

27. Slocombe SP, Ross M, Thomas N, McNeill S, Stanley MS. A rapid and general method for measurement of protein in micro-algal biomass. Bioresour Technol. 2013; 129, 51-57. doi: 10.1016/j.biortech.2012.10.163

28. Bartolomeo MP, Maisano F. Validation of a reversed-phase HPLC method for quantitative amino acid analysis. J Biomol Tech. 2006; 17(2): 131-137.

29. Zhao CH, Chi Z, Zhang F, Guo FJ, Li M, Song WB, Chi ZM. Direct conversion of inulin and extract of tubers of Jerusalem artichoke into single cell oil by co-cultures of *Rhodotorula mucilaginosa* TJY15a and immobilized inulinase-producing yeast cells. Bioresour Technol. 2011; 102, 6128-6133. doi: 10.1016/j.biortech.2011.02.077

30. Rajoka MI, Khan SH, Jabbar MA, Awan MS, Hashmi AS. Kinetics of batch single cell protein production from rice polishings with *Candida utilis* in continuously aerated tank reactors. Bioresour Technol. 2006; 97, 1934-1941. doi: 10.1016/j.biortech.2005.08.019

31. Anvari M, Khayati G. Submerged yeast fermentation of cheese whey for protein production and nutritional profile analysis. Adv J Food Sci Technol. 2011; 3(2): 122-126.

32. Sen R, Swaminathan T. Application of response surface methodology to evaluate the optimum environmental conditions for the enhanced production of surfactin. Appl Microbiol Biotechnol. 2004; 47: 358-363. doi: 10.1007/s002530050940

33. Mnif I, Chaabouni-Ellouze S, Ghribi D. Optimization of inocula conditions for enhanced biosurfactant production by *Bacillus subtilis* SPB1, in submerged culture, using box-Behnken design. Probiotic Antimicrobial Proteins. 2013; 5(2): 92-98. doi: 10.1007/s12602-012-9113-z

34. Onwosi CO, Odibo FJC. Use of response surface design in the optimization of starter cultures for enhanced rhamnolipid production by *Pseudomonas nitroreducens*. Afr J Biotechnol. 2013; 12(19): 2611-2617. doi: 10.5897/AJB12.2635

35. Farkya S, Pandey AK, Rajak RS. Mycoherbicides potential of *Fusarium* spp. against *Parthenium*: Factors affecting in vitro growth and sporulation. Bioved. 1996; 7, 1–9. doi: 10.2478/v10045-010-0076-3

36. Gupta VK, Misra AK, Gaur RK. Growth characteristics of *Fusarium* spp. causing wilt disease in *Psidium Guajava L* in India. J plant Prot Res. 2010; 50(4): 452-462. doi: 10.2478/v10045-010-0076-3.

37. Hezarjaribi M, Ardestani F, Ghorbani H. Single cell protein production by *Saccharomyces cerevisiae* using an optimized culture medium composition in a batch submerged bioprocess. Appl Biochem Biotechnol. 2016; 179(8): 1336-1345. doi: 10.1007/s12010-016-2069-9.

38. Khosravi-Darani K, Zoghi A, Fatemi SSA. Application of Plackett-Burman design for citric acid production from pretreated and untreated wheat straw. Iran J Chem Chem Eng. 2008; 27: 91-104.

39. Jin B, Van Leeuwen HJ, Patel B, Yu Q. Utilisation of starch processing wastewater for production of microbial biomass protein and fungal α-amylase by *Aspergillus oryzae***.** Bioresour Technol. 1998; 66: 201-206. doi: 10.1016/S0960-8524(98)00060-1.

40. Li Y, Hruby A, Bernstein AM, Ley SH, Wang DD, Chiuve SE, Sampson L, Rexrode KM, Rimm EB, Willett WC, Hu FB. Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: A prospective cohort study. J Am Coll Cardiol. 2015; 66(14):1538-1548. doi: 10.1016/j.jacc.2015.07.055

http://dx.doi.org/10.22037/afb.v5i4.23139

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2018, 5 (4): 243-252
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

تولید پروتئین قارچی از ضایعات خرما با استفاده از *فوزاریوم وناتوم* در کشت غوطه‌ور

سیده فاطمه سید ریحانی، کیانوش خسروی دارانی*

گروه تحقیقات صنایع غذایی، انستیتو تحقیقات تغذیه ای و صنایع غذایی کشور، دانشکده علوم تغذیه و صنایع غذایی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران

چکیده

سابقه و هدف: تولید پروتئین تک سلولی مزایای چشمگیر بسیاری دارد از جمله اینکه می تواند بر روی ضایعات و رشد کند، دوستدار محیط زیست است و به بهبود دفع زباله های کشاورزی کمک کند. در مطالعه حاضر، اثر پارامترهای فرایند بر تولید زیست توده[1] (gL^{-1})، تولید پروتئین ($w\ w^{-1}\%$) و بهره وری حجمی *فوزاریوم وناتوم* IR372C ($gL^{-1}h^{-1}$) تعیین شد.

مواد و روش‌ها: محیط وگل به همراه گلوکز به عنوان منبع کربن برای پیش کشت تلقیح و با قند خرما به عنوان منبع کربن برای محیط کشت تولید استفاده شد. در مرحله اول مطالعه، تخمیر غوطه‌ور در یک فلاسک‌های ۵۰۰ میلی لیتری انجام و در فاز دوم از یک بیوراکتور ۳ لیتری با تانک همزن‌دار برای انجام تخمیر غوطه ور استفاده شد. برای تعیین شرایط بهینه تخمیر که از آن حداکثر زیست توده، پروتئین و بهره وری به دست آید از طراحی پلاکت برمن با ۱۱ عامل، یعنی غلظت قند خرما، $NH_4H_2PO_4$، پپتون، $MgSO_4$، KH_2PO_4، درجه حرارت، زمان، سرعت همزدن، سن تلقیح، میزان تلقیح، pH در دو سطح و روش سطح پاسخ با سه متغیر غلظت قند خرما، زمان و میزان تلقیح برای تعیین شرایط تخمیر با بیشترین میزان زیست توده، پروتئین و بهره‌وری به‌کار گرفته شد.

یافته‌ها و نتیجه‌گیری: براساس نتایج به‌دست آمده، با استفاده از مقادیر انتخابی برای متغیرهای فرایند، مقدار نسبتا زیادی پروتئین (به‌ترتیب حدود $4\ g\ l^{-1}$، ۶۵/۳٪ در مرحله اول با استفاده از فلاسک و $5/5\ g\ l^{-1}$، ۷۶٪ در مرحله دوم با استفاده از بیوراکتور) به‌دست آمد. پروفایل‌های اسیدهای چرب و آمینو اسیدهای پروتئین قارچی و میزان نسبتا بالای فیبر آن (۶٪) دلالت بر این دارد که پروتئین قارچی می‌تواند در انواع گوناگون غذاها به‌عنوان ترکیبی فراسودمند مورد استفاده قرار گیرد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله

دریافت ۲۴ اکتبر ۲۰۱۸
داوری ۱۲ نوامبر ۲۰۱۸
پذیرش ۲۲ دسامبر ۲۰۱۸

واژگان کلیدی

- ضایعات خرما
- درجه غذایی
- *فوزاریوم وناتوم*
- میزان تلقیح
- پروتئین قارچی
- تخمیر غوطه‌ور

***نویسنده مسئول**

کیانوش خسروی دارانی، گروه تحقیقات صنایع غذایی، انستیتو تحقیقات تغذیه ای و صنایع غذایی کشور، دانشکده علوم تغذیه و صنایع غذایی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران.

تلفن: ۲۲۳۷۶۴۷۳-۲۱-۹۸+
دورنگار: ۲۲۳۷۶۴۷۳-۲۱-۹۸+
پست الکترونیک:
k.khosravi@sbmu.ac.ir

[1] Biomass