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Full Length Research Paper

Production and rheological evaluation of mycoprotein produced from *fusarium venenatum* ATCC 20334 by surface culture method

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***Fusarium venenatum* (ATCC 20334) was used for fungal protein production. Date syrup was selected as a carbon source.The critical variables that affected mycoprotein production were identified by Plackett–Burman design (PBD).The significant parameters were the carbon source concentration (10 g/l), temperature (28 ˚C), seed size (10% v/v), nitrogen and phosphorus sources concentration (3.5 and 1.6 g/l) respectively. The rheological behaviour of filtrated biomass of *Fusarium venenatum* was studied under steady and dynamic shear conditions.This product showed shear-thinning behaviour with yield stress in the steady shear measurements.With respect to the dynamic shear measurements,the filtrated biomass showed gel-like behaviour.In addition,the viscosity at 100 1/s showed Arrhenius type temperature dependence, with activation energy 2.904 KJ/mol.**

Key words: *Fusarium venenatum*, mycoprotein, Plackett–Burman design, rheological properties, surface culture**.**

INTRODUCTION

Fusarium venenatum has been used for production of Qourn mycoprotein in the UK since 1985 (Wiebe, 2004). Mycoprotein is a good source of protein and fiber. The composition of the fiber is about one-third chitin and two-thirds β-1, 3 and 1, 6 glucan. The fat content of the harvested material is typically 2-3.5% and the fatty acid composition is similar to vegetable fat (Rodger, 2001). This product may function as a prebiotic in the lower gut. Wiebe used *Fusarium venenatum* A3/5 to produce mycoprotein in 150,000 liter pressure reactors in continues flow process. In this work glucose is provided as the carbon source and ammonium as the nitrogen source (Wiebe, 2002). In 2008 Ahangi et al used *Fusarium oxysporum* for production of mycoprotein. Under optimum condition the fungal biomass contained 42% protein. While this microorganism is toxic and the productivity was still low (5 g/l biomass contain of 42% protein). Also the results showed that decreased agitation speed increases the production of mycoprotein. So application of surface culture method for production of mycoprotein is investigated in this work. Also there is not any investigation on rheological evaluation of this product.

Evaluation of the rheological properties of fungal biomass from *Fusarium venenatum* under different conditions of temperature and shear seems essential. These information are used for final product development, quality control, the design of processing equipment and calculation of fluid flow e.g. pump sizing, extraction, filtration, and purification (Tonon et al.,2009).There are several approaches to carry out rheological characterizations. The selected technique depends on specific product and the functional characteristics that need to be analyzed. To understand the relationship between structural and rheological properties of foods, rheological tests

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Abbreviations

Plackett–Burman design (PBD).
Linear viscoelastic (LVE)

Table 1. Upper and lower levels assayed for the 7 variables at PBD

	variables	(+)	(-)
A:	Date syrup (g/l)	14	7
B:	$(NH_4)\ H_2PO_4$ (g/l)	3.5	2.88
C:	KH_2PO_4 (g/l)	2.5	1.6
D:	Temperature (˚C)	30	25
E:	Time (h)	96	72
F:	Seed age (h)	72	48
G:	Seed size (% v/v)	10	5

in the linear viscoelastic (LVE) region is necessary.The response of a material subjected to harmonically varying stress or strain is used to construct mechanical spectra that can provide information about the viscous and elastic nature of the sample over a range of frequencies (Steffe, 1996).

Several numbers of statistical experimental designs are used for evaluation of fermentation variables. PBD (Plackett and Burman, 1946) is a well established and widely used statistical technique for screening and selection of critical culture variables (Khosravi-Darani and Zoghi, 2008).

In this work, PBD is applied to determine of the critical variables affecting mycoprotein production in surface culture.The mycoprotein is produced according to the best condition. Finally the rheological properties of filtrated biomass are determined under steady and dynamic shear condition.

MATERIALS AND METHODS

Microorganism, Media, Inoculum and Culture Condition

Fusarium venenatum ATCC 20334 was used throughout this investtigation. Strain was maintained at 4 ˚C on agar-solidified Vogel slants. The components of modified Vogel medium (Vogel, 1956) is described elsewhere (Hosseini et al., 2009).

Inoculum was prepared in 250 conical flasks containing 50 mL Vogel medium. Flasks were inoculated and incubated on a rotary shaker at 25 ˚C, 200 rpm and 48 or 72 h (depend to trials combination of 8- run PBD). Date syrup which was purchased from Dombaz Company (Iran) contained 76.28 % solids (fructose 37.4%, glucose 34.1%, sucrose 0.08% and ash 7%) and 23 % water. Production medium was contained of date syrup as a carbon source; other medium components were the same as in seed medium described earlier.

Fermentation was conducted in 500 mL flasks each containing 100 mL of production medium. The flasks were inoculated with fungal suspension (5 or 10 % v/v), and incubated for surface culture at 25 and 30 ˚C (depend to trials combination of 8- run PBD).

Determination of Cell Dry Weight

After fermentation processes, biomass was harvested by filtration of 100 mL cultivation medium through pre-dried whatman No.1 filter papers. In the next step, clarified suspension passed throws a 0.45 µm membrane. Then the biomass was washed twice with cold distilled water and dried using an oven at 60°C to a constant weight. The cell dry weight was quantified gravimetrically.

Experimental design

The aim of present research is evaluation of important medium components by Plackett-Burman design. PBD is one of the highly fractional designs which allows for the study of $k = (N\text{-}1)/\ (L\text{-}1)$ factors, each with L levels with N experimental trials (Plackett and Burman, 1946). Choice of these factors was based on previous experience (unpublished data) for growing mycoprotein producing fungi, and selection of settings reflects a wide but reasonable numerical range. Also some changes in the response (cell dry weight) were expected for each factor over the selected range. Table 1 shows the factors include some medium components (i.e., carbon, nitrogen, and phosphorous sources concentration), environmental factors (i.e., temperature and time), and inoculums condition (seed age and seed size).

Rheological measurements

Filtrated biomass that contains about 2% water was used for rheometrical determinations. Rheological measurements were carried out using a Controlled Shear rate rheometer Physica MCR 301 (Anton paar GmbH, Graz, Austria), with a vane geometry. The temperature control was performed with a peltier system equipped with fluid circulator. In order to ensure that samples had identical shear histories, the solution pre-sheared at a shear rate of 20(1/s) for 60 s and left standing for 15-minutes to allow structure recovery and temperature equilibrum.

Flow curves were obtained at shear rates of 0.1-400(1/s). The experiments were performed at temperatures of 3, 25 and 45°C. In order to minimize water evaporation, the samples were covered with a solvent trap. The Power Low and Herschel-Bulkley models were used to describe the rheological properties of filtrated biomass of *Fusarium venenatum* and the determination coefficient (R^2) used as a parameter to verify the goodness of fit.

The Herschel-Bulkley model:

$$\sigma = k\,\gamma^{n} + \sigma_0$$

And the power law model:

$$\sigma = k\,\gamma^{n}$$

Where σ is the shear stress (Pa), k is the consistency coefficient ($Pa.s^n$), γ is shear rate (1/s), σ_0 is yield stress (Pa) and n is flow behaviour index (dimensionless) (Steffe, 1996).

Oscillatory strain sweep between 1 and 3500 % were performed to determine the LVE range at a frequency of 1 Hz. Frequency sweep tests were carried out to determine the mechanical spectra of the filtrated biomass at a fix strain value within the LVE range. The frequency sweep measurements were performed at 25°C from 1.5 to 40 Hz.

The experimental data on the frequency sweep tests were correlated according to the following power-law:

$$G' = A\omega^{1/z}$$

Where G′ is storage modulus, ω is angular frequency and according to Bohlins theory A and z are the interaction strength and coordination degree between rheological units respectively (Peressini et al., 1998).

The temperature dependency of consistency coefficient was assessed by fitting the Arrhenius model (Dak et al., 2006) to the apparent viscosity data at constant shear rate of 100 1/s and during a temperature ramp between 20-50°C. In order to ensure about the temperature equlibrum, the data were collected during 60 minutes (10 min per point).

Arrhenius model:

Table 2. Experimental Plackett-Burman matrix and cell dry weight obtained

Run	A	B	C	D	E	F	G	Cell dry weight (g/l)
1	1	-1	-1	1	-1	1	1	5.460 ± 0.237
2	1	1	-1	-1	1	-1	1	4.867 ± 0.832
3	1	1	1	-1	-1	1	-1	4.257 ± 0.198
4	-1	1	1	1	-1	-1	1	4.840 ± 0.096
5	1	-1	1	1	1	-1	-1	4.520 ± 0.144
6	-1	1	-1	1	1	1	-1	4.230 ± 0.254
7	-1	-1	1	-1	1	1	1	3.448 ± 0.102
8	-1	-1	-1	-1	-1	-1	-1	3.066 ± 0.071

All data are the average of three replications with standard deviations of the means

Table 3. Statistical results for PBD

Factors	Effect	Coefficient	*t*-value	P-value
A	0.6932	0.4816	12.15	<0.01
B	0.5082	0.2541	6.41	<0.01
C	-0.2228	-0.1114	2.81	0.013
D	0.7697	0.3848	9.71	<0.01
E	-0.0563	-0.0282	0.71	0.487
F	-0.0578	-0.0289	0.73	0.476
G	0.7187	0.3593	9.07	<0.01

Tabulated *t*-value for degree of freedom 7 and α = 0.05 is 2.365.

$$\eta = A \exp^{[Ea/RT]}$$

Where A is the proportionality constant (or consistency coefficient at a reference temperature, $Pa.s^n$), Ea the activation energy (J/mol), R the universal gas constant (8.314J/mol K), and T the absolute temperature (°K).

RESULTS AND DISCUSSION

Screening of Variables by PBD

Experiment was conducted in 8 runs to study the effect of the seven variables. Table 2 represents the results of the experimental design for the screening of the variables. The response signal was the cell dry weight. Statistical analyses were used to identify the significance of the variables. Here, only confidence levels above 95% (p value<0.05) were accepted as significant variables. Table 3 shows the statistical results of the different variables; the results show that the carbon, nitrogen and phosphorus source concentration, seed size, and temperature are significant, as their p-value were less than 0.05. These factors had positive effects on cell dry weight. Table 3 shows that higher initial date syrup concentration (14 g/l) and lower phosphorus source content (1.6 g/l) causes increased biomass production. This result is similar to report of Jin et al.,1998, whose results indicated that further supplementation of phosphorus source did not significantly improve in biomass yield and protein content of *Aspergillus oryzae* (Jin et al.,1998).This observation is not interpreted clearly but may be due to high buffer property of KH_2PO_4 or interaction with other components of solution specially date syrup as a complex medium.

Maximum biomass production was achieved by adding 3.5 g/l ammonium dihydrogen phosphate as a source of nitrogen. This result is in agreement to Ahangi et al, who reported that an ammonium dihydrogen phosphate level of 3.5 g/l resulted in higher biomass production.

Results of table 3 show that, temperature has a profound influence on the biomass production in 30°C. This result is in agree to the work of Wiebe (2002) who reported the suitable range of 28-30°C for *Fusarium venenatum* growth. Normally high temperature may cause inactivation of enzymes of the metabolic pathway and low temperature cannot allow flow of nutrient across cell membrane (Rajoka et al., 2006). Table 3 also shows that inoculation of 10 % v/v increases cell dry weight. The lag phase was significantly short in higher inoculums size (Morales et al., 2008). The selected ranges of seed age and time did not show any significant variation in biomass production.

On the basis of the results of this research, the optimum conditions for biomass production are date syrup 14 g/l (10 g/l glucose), $(NH_4)\ H_2PO_4$ 3.5 g/l, KH_2PO_4 1.6 g/l, temperature 30 ˚C, time 72 h, seed age 48 h, and seed size 10 % v/v.The dry biomass weight was obtained

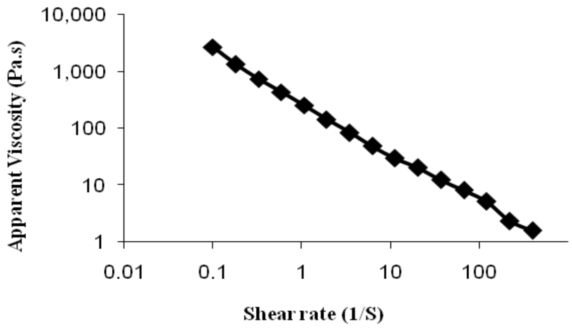

Figure1. Effect of shear rate on apparent viscosity at 25˚C.

Table 4. Values for Power low and Herschel–Bulkley model parameters.

T(˚C)	3	25	45
Power low model			
k (Pa.s^n)	264.3	283.31	331.07
n	0.0851	0.1255	0.0993
R^2	0.919	0.897	0.920
Herschel-Bulkely model			
σ_0	227.25	196.61	285.69
k (Pa.s^n)	29.017	75.985	32.730
n	0.3740	0.3026	0.4310
R^2	0.960	0.946	0.971

5.46 g/l under these conditions.

Rheological Measurements

Steady Shear Flow Experiments

Shear rate dependence of the apparent viscosity for filtrated biomass at 25°C is given in Figure 1. Decrease in viscosity with increase shear stress can be explained by the bulk of the fluid beginning to flow. The Power law and Herschel-Bulkley models were used to describe flow curves. The fitting parameters are presented in Table 4. High values of determination coefficient (R^2) indicate that Herschel-Bulkley is the proper model for describing the flow behaviour of the filtrated biomass. These parameters and the decrease of apparent viscosity values versus increase shear rate values confirmed a shear-thinning behaviour with yield stress. Such behaviour is typical of most of the fluids and purees prepared from legumes and fruits that consists high value of fiber (Tonon et al., 2009).

Dynamic shear flow experiments

Figure 2 shows the amplitude sweep of the filtrated biomass at 25°C. The limiting value of strain (the strain where G′ begins to fall) is about 8.53% the structural strength character of the food material which usually expressed by G′ value in LVE range was calculated about 657 Pa for our sample. The rheological behaviour of filtrated biomass is similar to raw, Meat Batters with 30% fat, 10.4% protein and 57.5% moisture content (Steffe, 1996). The limiting value of the LVE range in terms of shear stress σ is the yield point (σ_y) and it was calculated about 57.5 Pa for filtrated biomass. At the cross over point G′= G″ in the strain sweep data the gel character with G′> G″ changes to liquid character with G″> G′ (Mezger, 2006). This point is also called yield point (or flow point) and it was about 203 Pa for our sample which is in good agreement with the yield value calculated from Herschel-Bulkley model.

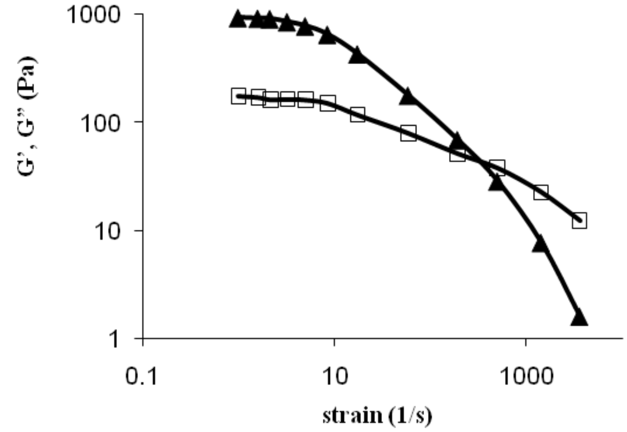

Figure 2. Oscillatory strain sweep results for filtrated biomass at 25 ˚C, the storage (▲) and loss (□) modulus.

Steady shear flow measurements are in nature destructive techniques. So they are not able to provide sufficient information with regard to relationship between the rheological behaviour and the microstructure of a material. In order to gain an insight into the microstructure of food materials from its rheological properties, the measurements should be made under small deformations (Steffe, 1996).Therefore small amplitude oscillatory test usually perform to determine viscoelastic behaviour of food materials. The dynamic behaviour of the filtrated biomass illustrated in Figure 3. The sample exhibited viscoelastic behaviour with a storage modulus (G′) greater than the loss modulus (G″).The data given in Figure 3 were fit to a power low model resulting in following equations $G' = 727\ \omega^{0.0653.}$ The fitting parameters were similar to the values which have been reported for mayonnaise (Peressini et al.,1998) and indicate a gel like behaviour for the filtrated biomass.

Temperature effect

The influence of temperature on the viscosity of liquids

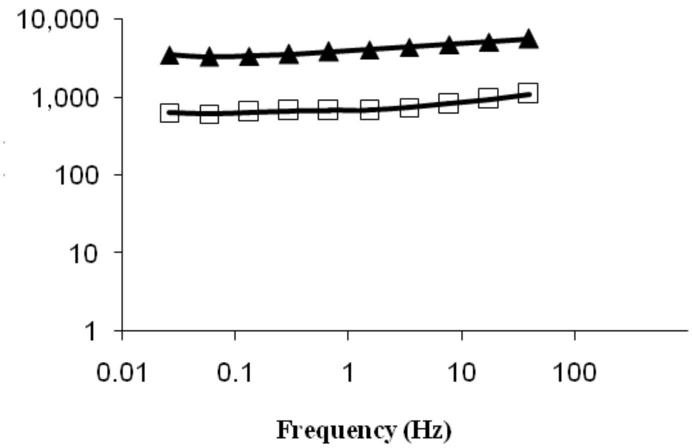

Figure 3. Mechanical spectra for filtrated biomass at 25 ˚C, the storage (▲) and loss (□) modulus.

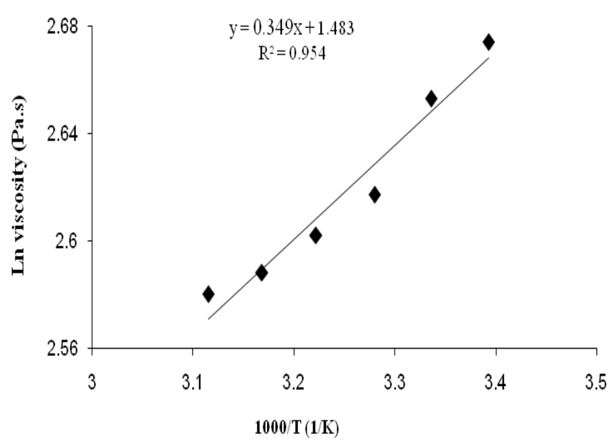

Figure 4. Arrhenius curve obtained for filtrated biomass using experimental data (symbols) and fitted model (line).

can be expressed by the Arrhenius model. According to this model the viscosity decreases with increasing temperature (Dak et al., 2006). The viscosity should be considered at a specific shear rate in order to evaluate the temperature effect. In this research, a shear rate of 100 1/s was used to describe the effect of temperature on viscosity. The viscosity data on temperature ramp fitted according to the Arrhenius model at range of 20-50 ˚C can be observed in Figure 4. The goodness of fit of the model was checked by the determination coefficient (R^2). In this case the value of the determination (R^2=0.954) indicates that only 4.6 % of the total variations are not explained by the model. The flow activation energy was calculated about 2.904 KJ/mol from slop of the Arrhenius curve in Figure 4. Usually the low values of activation energy indicate a less sensitivity viscosity to temperature (Tonon et al., 2009).

Conclusion

Mycoprotein production was conducted in surface culture using *Fusarium venenatum* from date syrup by PBD. The dry weight of biomass was obtained 5.46 g/l under optimum conditions (date syrup 14 g/l, (NH_4) H_2PO_4 3.5 g/l, KH_2PO_4 1.6 g/l, temperature 30 ˚C, time 72 h, seed age 48 h, and seed size 10% v/v). Filtrated biomass showed shear-thinning behaviour with yield stress and the data were best fitted to the Herschel–Bulkley model. With respect to the oscillatory tests the mechanical spectra of biomass showed a gel-like behaviour. The viscosity showed Arrhenius dependence on the temperature with low the activation energy.

These results provided rheological informations about fungal biomass from *Fusarium venenatum* which has a somewhat special chemical composition (rich in protein and fibers). The results could be useful to industries that produce mycoprotein due to its fibrous texture and the advantage of being devoid of animal fats and cholesterol.

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