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Optimizing bioconversion of deproteinated cheese whey to mycelia of *Ganoderma lucidum*

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Abstract

A novel approach to utilize deproteinated cheese whey by cultivating mycelia of the edible mushroom *Ganoderma lucidum* is described. A central composite in cube design for the experiments was used to develop empirical models providing a quantitative interpretation of the relationships between the two variables studied, which were pH and temperature. The designed intervals were $3.3 < \text{pH} < 4.7$, $23\ ^\circ\text{C} < \text{temperature} < 37\ ^\circ\text{C}$, respectively. Response surface methodology was successfully applied to determine the optimum condition where the maximum mycelial production occurred, which was pH 4.2 at 28.3 °C. The soluble chemical oxygen demand (SCOD) removal ranged from 80.7 to 93.1% within the design boundary, where the condition for maximum SCOD removal was pH 4.6 and 27.1 °C soluble chemical oxygen demand (COD). The condition for a maximum mycelial yield, 0.35 mg mycelial weight per mg $\text{SCOD}_{\text{removed}}$, was calculated as pH 4.2, and 28.5 °C soluble COD; which was almost the same as optimum condition for mycelial production. The high extract ratio of 10.7% (i.e. 1820 mg extract/17 057 mg mycelium) as well as high content of polysaccharide (i.e. 1.12 g/l) indicated that the deproteinated whey could be an alternative substrate for mycelial production. Therefore, cultivation of *G. lucidum* mycelia can offer a potential cost-effective solution for an alternative utilization of the deproteinated cheese whey.

Keywords: *Ganoderma lucidum*; Mycelium; Deproteinated cheese whey; Response surface method; Bioconversion; Organic wastewater

1. Introduction

1.1. Problems in utilizing deproteinated cheese whey

Cheese whey is a by-product of cheese production that remains when casein and butter fat are separated as curd from milk. Depending on the type of cheese being made, up to 9 l of whey is generated for every kilogram of cheese produced. The organic matter in cheese whey causes a high chemical oxygen demand (COD) in the range of 40–70 g/l, due to the high content of organics including lactose and protein [1,2]. Cheese production and the resultant whey by-product in the USA, which was approximately 260 million tonne in 1999 [3], has become one of the largest single visible sources of potential pollution. Whey has traditionally been considered a wastewater to be disposed of. However, the continued growth of the cheese industry, the necessity for reduction of pollutant in the effluent, and the need to maximize returns on raw material have encouraged producers to seek new ways of using cheese whey.

Many processes including drying, protein precipitation, and ultrafiltration have been developed to recover the valuable parts of whey, mostly protein [4–6]. Due to the low protein content in raw whey (i.e. 1% wt./vol), however, significant quantities of the deproteinated whey remain after the protein recovery processes. This deproteinated whey also causes a serious wastewater disposal problem because it contains almost 100% of the lactose from whey.

1.2. Ganoderma lucidum-mycelium of edible mushroom

Among various mushrooms, *G. lucidum* has gained wide popularity as an ingredient in many health foods and therapeutic medicines because of its perceived

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health benefits. The wild type of *G. lucidum* grows on old logs and stumps; especially, maple and oak. For centuries, this mushroom has been regarded in the Northern Asia as a popular folk medicine used to treat various human diseases such as hypertension, arthritis, and bronchitis. Recent studies of this fungus have postulated that the polysaccharide 1,3-β-D-glucan inhibits a variety of cancers by enhancing the hosts' immune functions [7–9]. It has also been suggested that this mushroom has anti-inflammatory effects and cytotoxicity to hepatoma cells [10,11].

Fruiting bodies of mushrooms including *G. lucidum* have traditionally been produced in solid cultures using substrates such as grain, sawdust or wood. However, it usually takes several months to complete a fruiting body culture, and it is often difficult to control the product quality. Therefore, submerged fermentation for mycelial culture has recently received great interest as a promising alternative for efficient production of cellular materials and valuable metabolites such as polysaccharide mainly due to its short period of mycelial cultivation (i.e. usually less than 2 weeks) [9,12,13].

Dairy wastes should be viewed as an inexpensive potential source of raw material from which valuable products can be produced. Deproteinated whey, for example, includes approximately half of the original nutrients of milk; containing 4% lactose, nitrogenous compounds, trace minerals and vitamins, which makes it nutritionally valuable [14]. In this experiment, we hypothesized that the deproteinated whey could be used as an alternative substrate for cultivating mycelium of *G. lucidum*, thus providing a unique solution to the wastewater management. The control of environmental conditions as well as the modification of media composition has been vital in order to enhance the production efficiency in mycelial culture [9,15]. Despite this effort, little information is available regarding the optimization of environmental factors affecting the growth of *G. lucidum* for various substrates in submerged culture.

The objectives of this research were to (1) find the optimum condition with respect to the simultaneous effects of pH and temperature where the mycelial production is maximized using deproteinated whey, and (2) develop continuous response surfaces of mycelial production, waste stabilization as soluble chemical oxygen demand (SCOD), and yield of mycelial growth using mathematical and statistical techniques.

2. Materials and methods

2.1. Deproteinated cheese whey and microbial strain

Dried whey powder from SamIk Co., Korea, was dissolved in distilled water in appropriate proportion (57.1 g dry powder per l) to obtain the lactose concentration of typical cheese whey (i.e. 40 g lactose per l), since lactose is the major carbonaceous compound in whey. Whey protein was initially removed using isoelectric precipitation by adjusting the pH of the whey solution to 4.6, followed by centrifugation at $8000 \times g$ for 15 min to remove aggregates [4]. The supernatant was filtered through Whatman No. 2 filters to remove fine protein particles. The characteristics of the deproteinated whey are presented in Table 1.

The resulting deproteinated whey was used as a substrate for cultivating mycelia of *G. lucimum*. Because cheese whey contains most of the essential nutrients for microbial growth [14], and in order to obtain information about treatment of raw deproteinated whey, no additional nutrients were added. However, the content of ammonium nitrogen and phosphorus in the form of orthophosphate were carefully monitored in every trial in order to ensure these two critical nutrients were not limiting.

G. lucidum (KCTC 6283) was obtained from the Korean Collection for Type Cultures (KCTC; Seoul, Korea) and was maintained in a potato dextrose agar (PDA) slant at 4 °C. The seed culture of *G. lucidum* was transferred to a Petri-dish containing PDA medium and incubated at 25 °C for 4 days. Mycelial agar discs (5 mm) were obtained by a round cutter and were used as inocula for subsequent experiments.

2.2. Submerged fermentation of G. lucidum

Three identical fermentors with working volumes of 4 l (BioG-M, BioTron), equipped with temperature, pH, foam, and dissolved oxygen (DO) controllers, were used to incubate the mycelia of *G. lucidum* in batch mode. Each bioreactor was inoculated with 15 agar discs with *G. lucidum* that were grown on the surface of PDA medium. Purified air using air filters was supplied to the bioreactors at a rate of 1 vol/vol per min to maintain a minimum DO concentration of 2 mg/l. Buffers of 2 N

Table 1
Characteristics of the deproteinated whey used in this research

Organics	Concentration (mg/l)	Inorganics	Concentration (mg/l)
COD	54 137 (298)[a]	Ammonium	148 (4)
SCOD	52 993 (534)	Calcium	275 (2)
TOC	16 369 (664)	Magnesium	60 (6)
Lactose	40 000 (340)	Iron	3.1 (1)
Lactic acid	397 (99)	Nitrate	30 (5)
Acetic acid	427 (90)	Phosphate	638 (5)
Propionic acid	151 (16)	Potassium	998 (10)
Butyric acid	18 (0.3)	Sodium	327 (4)
Crude Protein	755 (26)	Sulfate	62 (1)
Fat	1.6 (0.2)	Zinc	6 (1)

[a] S.D. are in parentheses.

sodium hydroxide and 2 N sulfuric acid were separately used to adjust the pH to the desired levels. Foam was controlled using 10% antifoaming agent (A5758, Sigma).

2.3. Optimization using response surface methodology

The mycelial growth of *G. lucidum* associated with simultaneous changes in pH and temperature were examined because these two parameters have been key variables to maximize the mycelial production of various edible mushrooms in submerged cultures. The initial starting points of pH and temperature were selected as close as possible to the literature values of similar condition [9,12,13,16].

Response surface methodology (RSM), a collection of mathematical and statistical techniques for building empirical models [17–19], was applied to locate optimum condition for mycelial production of *G. lucidum*. In this experiment, 'optimum condition' meant the operating condition for maximizing the mycelial production within the investigated space of the independent variables. A sequential procedure of collecting data, estimating polynomials, and checking the adequacy of the model was used. The method of least squares was used to estimate the parameters in the approximating polynomials.

Once the optimum region was found, a second-degree model (Eq. (1)) or higher was used to approximate the response because of the curvature in the surface. An analysis was then performed to locate the optimum, which was the set of independent variables such that the partial derivatives of the model response, Y, equaled zero (Eq. (2)).

$$Y = c_o + \sum_{i=1}^{n} \alpha_i x_i + \sum_{i=1}^{n} \alpha_{ii} x_i^2 + \sum_{\substack{i \\ i<j}} \sum_{j} \alpha_{ij} x_i x_j \quad (1)$$

$$\frac{\partial Y}{x_1} = \frac{\partial Y}{x_2} = \ldots = \frac{\partial Y}{x_n} = 0 \quad (2)$$

where Y is model response; x_i is independent variable i; ε is the random error; c_o is regression constant; α_i is regression coefficients of the independent variable i.

The optimum response is called the stationary point and it could represent a point of maximum response, a point of minimum response, or a saddle point [17].

The central composite in cube (CCC) design [20], which consisted of an orthogonal 2^2 factorial design augmented by a center and 2×2 axial points (Table 2), was employed in this research. The orthogonal design is a unique class of experimental design techniques that minimize the variance of the regression coefficients [21]. This design can be built from the first-order design by adding the axial points and/or center point.

2.4. Analytical methods

The COD of the deproteinated whey and mixed liquor of the fermentors were measured by the closed reflux colorimetric method, and the amount of protein was measured according to the Kjeldahl method [22]. An automated extraction unit (Soxtech 2050, FOSS) was used to measure fat content. A Hewlett–Packard gas chromatograph (6890 plus) equipped with an Innowax capillary column and flame ionization detector was used to measure short-chain organic acids and helium was used as the carrier gas at a flow rate of 2.5 ml/min. Heavy metals were analyzed using an inductively coupled plasma (ICP) spectrophotometer (IRS/AP, Thermo Jarell Ash). Total organic carbon (TOC) was quantified using a TOC analyzer (TOC-V_{CHP}, Shimadzu). Two identical ion-exchange chromatographs (790 Personal IC, Metrohom) were used to quantify the cations and anions in the samples. A high performance liquid chromatography (HPLC 1100 series, Agilent) equipped with a refractive index detector was used to quantify lactose.

Samples taken at each stationary growth phase were filtered using Whatman No. 2 filters and dried at 103 °C for 2 h to measure concentration of mycelial mass in freely suspended culture. Extraction of polysaccharides was followed by Yang et al. (1998). Total polysaccharide in the culture medium was determined by phenol-sulfuric acid assay [11].

3. Results and discussion

3.1. Characterization of the deproteinated whey

The COD of 54.1 g/l indicated the amount of potentially biodegradable substances in the deproteinated whey wastewater, and a high ratio of SCOD to COD, 98%, meant that most of organic materials were soluble (Table 1). Efforts were made to estimate the contribution of each organic to COD in the deproteinated whey. CODs of organic components listed in Table 1, except fat, were stoichiometrically calculated [23]. Because of the low fat content, milk cream close to the composition of the fat in the cheese whey was used to estimate the empirical COD concentration due to the presence of the fat.

Lactose was the major organic in the deproteinated cheese whey. The COD due to the presence of lactose was 44.8 g/l, which was 83% of the COD. Not all whey protein could be removed (91.3% removal), but the protein concentration in the deproteinated whey was so low as to be an insignificant growth factor compared with the lactose. In addition to the high organic content, the deproteinated whey was rich in various inorganics,

Table 2
Experimental conditions and results of the central composite design

Trials	Conditions of variables			Responses	
	pH	Temperature (°C)	Mycelial dry weight (mg/l)	Residual SCOD concentration (mg/l)	Mycelial yield (mg mycelia per mg SCOD)
1	3.5	25	14 269	6751	0.31
2	4.5	25	16 520	4049	0.34
3	3.5	35	13 365	10254	0.31
4	4.5	35	15 123	7758	0.33
5[a]	4.0	30	16 945 (475)	4454 (784)	0.35 (0.1)
6	4.0	37.1	13 985	8452	0.31
7	4.0	22.9	15 789	6227	0.34
8	4.7	30.0	16 324	4632	0.34
9	3.3	30.0	14 320	8871	0.32

[a] Experiment was replicated five times and the response presented average values (S.D.).

which could support the mycelial growth of *G. lucidum* [13,24].

3.2. Optimization for maximal production of G. lucidum mycelia

Thirteen trials were run to locate optimal condition for the mycelial production of *G. lucidum*. The initial substrate concentration was 52 993 mg SCOD per l. For all trials, the presence of nitrogen, with a minimum value observed of 40 mg NH_4^+ per l, and phosphorus, which was 250 mg PO_4^{3-} per l, indicated that no additional nutrients were required for the mycelial production of *G. lucidum* using whey.

The first region of exploration for the first-order model was decided as {3.5, 4.5} pH and {25, 35} °C because conditions for culturing mycelia of *G. lucimum* on synthetic media were acidic and mesophilic [9,13,24]. The orthogonal design, a 2^2 factorial augmented by five center point runs (from trials 1 to 5 in Table 2), was used to collect data. Repeat observations at the center point (i.e. pH 4.0 and 30 °C) were used to estimate the experimental error.

Nine trials were tested first to investigate the vicinity of the optimum condition. A first-order and an interaction-model, $MDW = c_o + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_1 x_2$, were separately tried to fit the data by least squares and the following models were obtained.

$$MDW_1 = 11434 + 2005x_1 - 115x_2 \quad (3)$$

$$MDW_2 = 5518 + 3484x_1 + 82x_2 - 49x_1x_2 \quad (4)$$

where MDW_i is mycelial dry weight at stationary growth phase with model i (mg/l) (i, first-order and interaction models in order); α_j is the coefficient values of jth term (mg mycelial dry weight per l per x_k, where c_o is constant); x_k is the corresponding variable term (k, pH and temperature (°C) in order).

The P-values for regression coefficients and for lack of fit of the equations were used to validate the models. The regression coefficients of both models were below 0.5, an indication of low linearity between dependant and independent variables, and the P-values of the regression coefficients were not significant at the 5% α level. The P-values of the lack of fit for first-order- and interaction-models were 0.007 and 0.003, respectively, which were significant at a 1% α level. Therefore, it was concluded that both first-order and interaction-models were not adequate approximations for the response surface of mycelial weight of *G. lucidum*. The curvature in the response indicated that the optimum condition might be inside the investigated regions of pH and temperature, and nonlinear models should be used to locate the optimum condition. Quadratic or higher order model, however, could not be fit using the data from trials 1 to 5 in Table 2 due to the lack of axial data points. Therefore, additional four trials were conducted (from 6 to 9 in Table 2) to augmented with the previous experiment to make a central composite design.

In order to find a maximum in the response, various models from linear to partial cubic were tested with these augmented trials. Eqs. (5)–(8) represent first-order, interaction, quadratic, and partial cubic models, respectively.

$$MDW_1 = 12392 + 1833x_1 - 130x_2 \quad (5)$$

$$MDW_2 = 4700 + 3754x_1 + 126x_2 - 64x_1x_2 \quad (6)$$

$$MDW_3 = -91075 + 32649x_1 + 2741x_2 - 52x_1x_2 - 3667x_1^2 - 44x_2^2 \quad (7)$$

$$MDW_4 = -195409 + 65936x_1 + 8815x_2 - 1809x_1x_2 - 5458x_1^2 - 130x_2^2 + 21x_1x_2^2 + 59x_1^2x_2 \quad (8)$$

where MDW_i is mycelial dry weight with model i (mg/l) (i, first, interaction, quadratic, and partial cubic models in order).

P-values of regression, lack of fit, and corresponding coefficients were tested for each model. Regressions were significant at 1% α level and lack of fit was not significant at 5% α level only for quadratic and partial cubic models. The regression coefficients and residual standard deviations (S.D.) of quadratic and partial

cubic models were 0.95 and 0.96, and 401 and 420, respectively. This indicated that curvature existed in the response surface of mycelial dry weight within the augmented experimental region. Because of an equal level of statistical significance for the quadratic and partial cubic models, both models were separately used to fit the response.

The condition that maximized the mycelial production of *G. lucidum*, defined as the optimum condition earlier, was calculated by setting the partial derivatives of the functions to zero with respect to the corresponding independent variables. The optimum condition for the mycelial production was identical using the quadratic and partial cubic models, which was pH 4.2 and 28.3 °C. The calculated model outputs at the optimum condition were slightly different as $17\,289 \pm 903$ and $17\,258 \pm 982$ mg dry weight per l for second and partial cubic models, respectively. Independent *t*-tests also showed that the predicted mycelial concentrations using the two models were not different at the 1% α level.

Two- and three-dimensional response surfaces of the quadratic model for the mycelial production with estimated optimums are shown in Fig. 1. The convex hulls in the figures are the minimum *N*-dimensional volumes, which contain all the trials for which data have been collected. Contour plots are 2-D slices through these multidimensional design spaces [25,26]. The limits of the convex hull within the contour plots are shown as straight lines forming a polygon on the plots. The optimum condition was well inside the design boundary. The response surface of the mycelial production with respect to pH and temperature showed a clear peak with constant contour lines. From statistical inspection of the coefficients of the model (Eq. (7)), it was shown that the interaction term in the mycelial production was not significant at the 5% α level, while other terms were significant at a 1% confidence level. This meant that the two independent variables, pH and temperature, were not interdependent and the combined effect on the mycelial production of *G. lucidum* using deproteinated whey was not significant. Contour plots of the partial cubic polynomial (Fig. 2) show nearly identical shapes with those of the quadratic model (Fig. 1). The interaction terms in the partial cubic model (Eq. (8)) were not significant, while other terms were significant at a 5% confidence level. This similarity with the second order model could be another indication of the clearly

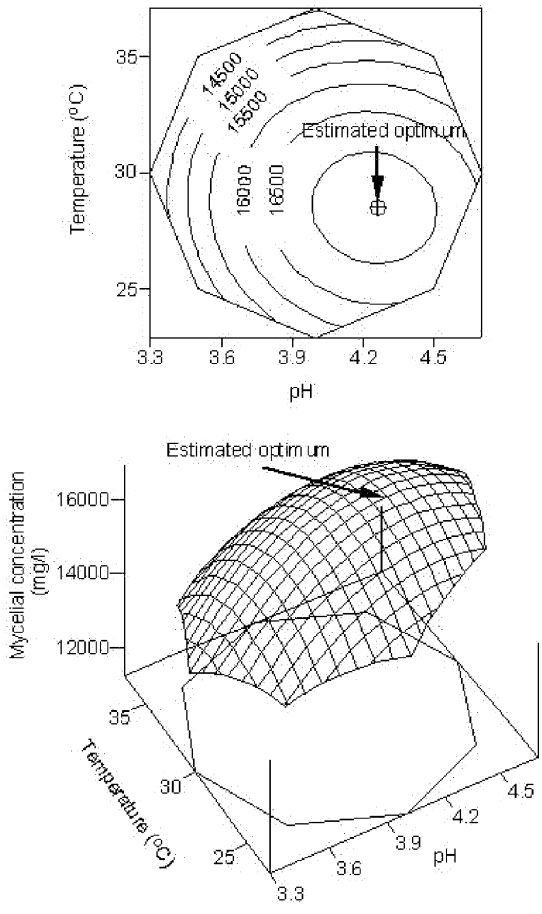

Fig. 1. Two- and three-dimensional contour plots of the quadratic model for the mycelial production with respect to pH and temperature.

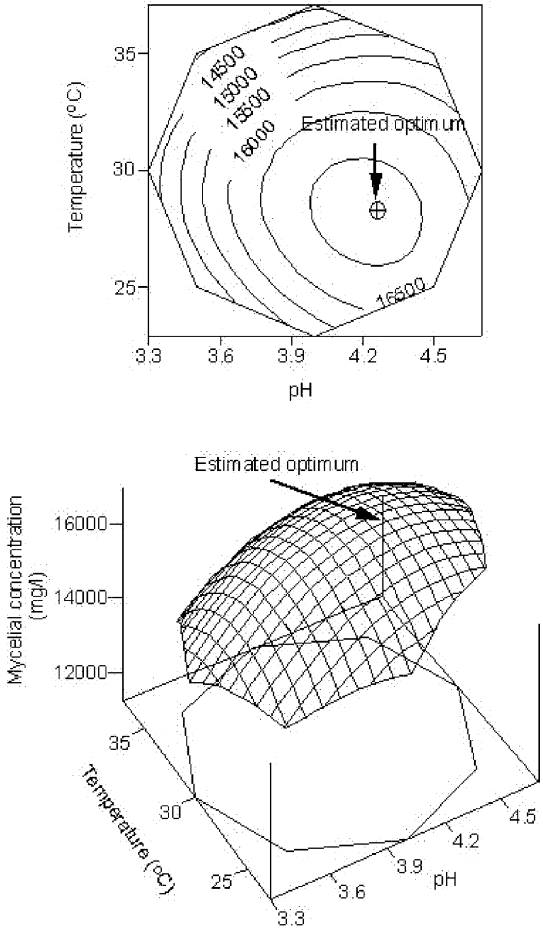

Fig. 2. Two- and three-dimensional contour plot of the partial cubic model for the mycelial production with respect to pH and temperature.

rounded shape of the response surface. This is in accordance with the previous research demonstrating that the response surface of acetic acid production had a clear peak for which a quadratic or higher order model with a different design boundary could be fit to in the general vicinity of the stationary point [19].

The adequacy of the fit of each model (i.e. Eqs. (7) and (8)) was verified by comparing the maximum model outputs of the mycelial weight with experimental values at an optimum condition, and then residual plots for all observed values were examined for any weakness in the models [17]. The residual plots for the models and data set showed no patterns or trends (Fig. 3). A check of the constant variance assumption also could be addressed because a random plot of residuals meant homogeneous error variances across the observed values. Excellent predictions of maximum responses along with constant variance in residual plots indicated adequacy of the models, which meant the models, either quadratic or partial cubic, could fit the response surface of the mycelial weight with respect to pH and temperature. Therefore, it could be concluded that the RSM with orthogonal experimental design could be used to locate the condition that maximized the mycelial production of *G. lucidum* using deproteinated whey within the investigated experimental region.

3.3. Pollution reduction by cultivating G. lucidum

The same method was separately applied to describe the response surfaces of the residual SCOD concentration at the stationary growth phase. Models from first to partial cubic were sequentially used to fit the surface. The *P*-values of regression, lack of fit, and corresponding coefficients were also tested. Residual variance and plots [21] were simultaneously analyzed to discriminate models if multiple models were statistically significant to describe the response.

Lack of fit was not significant and regression was significant at a 0.1% α level for the quadratic and partial cubic models in the response of SCOD concentration, which indicated the two models reasonably fit the response surface. The partial cubic model,

$$\mathrm{SCOD} = -309\,752 + 169\,858x_1 + 13\,652x_2 - 7534x_1x_2 - 19\,837x_1^2 - 7x_2^2 + 16x_1x_2^2 + 821x_1^2x_2, \quad (9)$$

was selected to describe the response surface within the experimental region (Table 2) since the residual plots of this model showed constant and less variance compared with the quadratic model. The residual plots of quadratic and partial cubic models are presented in Fig. 4.

All interaction terms of independent variables in Eq. (9) were not significant at the 5% confidence level because *P* values of the terms were in the range of 0.06–0.11. However, it should be noted that the two variables (pH and temperature) were more interdependent or that there was more significant interaction among them for SCOD reduction than those for mycelial production. This interaction is clearly seen as a region of elongated ellipse in the response surface of residual SCOD concentration (Fig. 5). The rounded ridge, close to the design boundary, was running diagonally on the plot from lower left to the upper right. The plots might appear slightly different if a higher order polynomial had been chosen because of greater flexibility of the polynomial. However, as there was no lack of fit for the partial cubic model, the differences would not have been significant.

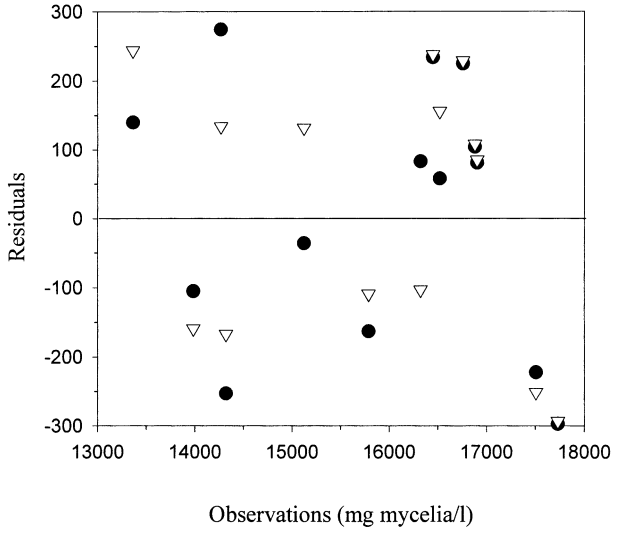

Fig. 3. Residual plots of the quadratic and partial cubic models for mycelial production at stationary growth phase (○, quadratic model; ■, partial cubic model).

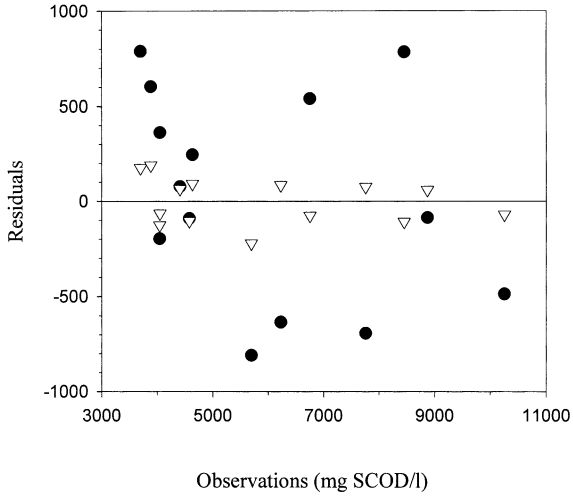

Fig. 4. Residual plots of the quadratic and partial cubic models for soluble COD concentration at stationary growth phase (○, quadratic model; ■, partial cubic model).

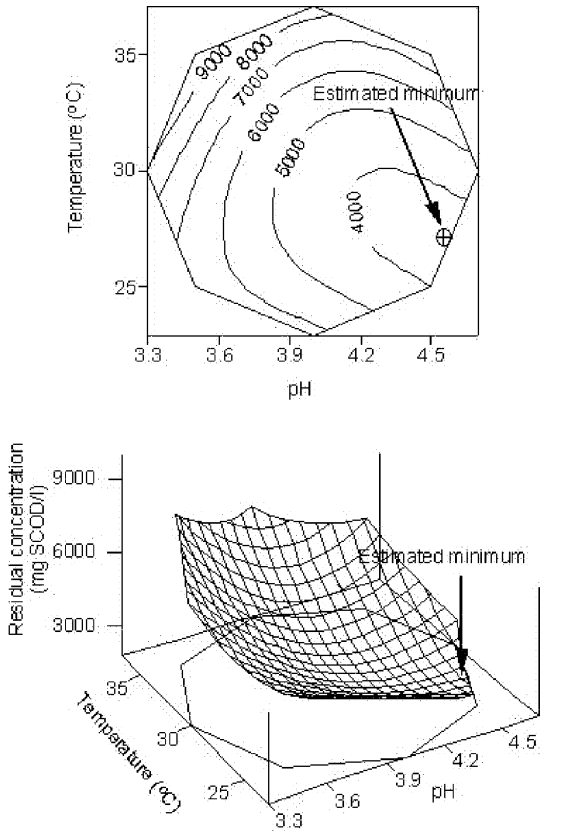

Fig. 5. Two- and three-dimensional graphs of the partial cubic model for the residual SCOD concentration within the orthogonal design.

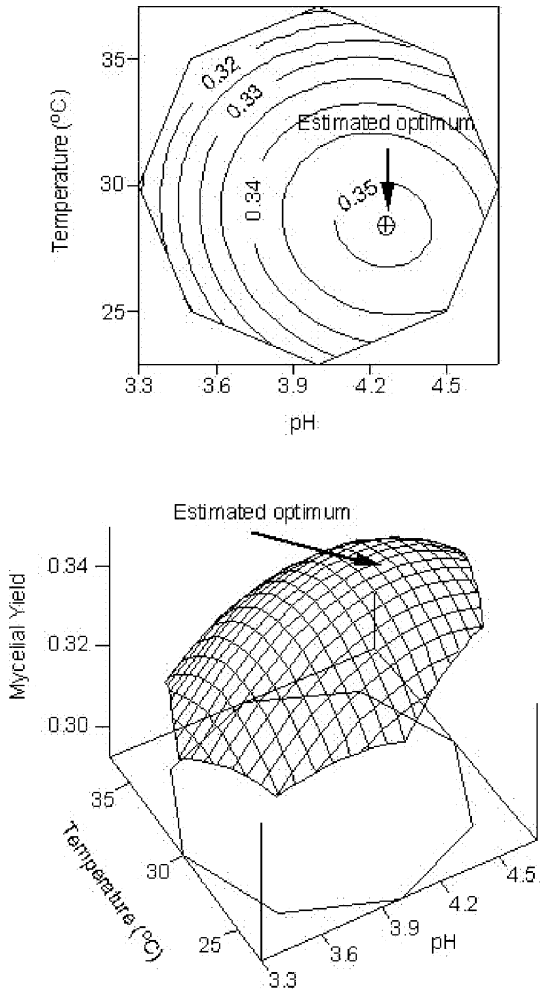

Fig. 6. Two- and three-dimensional plots of the quadratic model for the mycelial yield on the deproteinated whey within the orthogonal design.

The SCOD removal ranged from 80.7 to 93.1% within the design boundary, where the condition for maximum SCOD removal was pH 4.6 and 27.1 °C. The model response at the estimated condition was 3639 ± 560 mg SCOD per l, which was 93.1% SCOD reduction. Using the Eq. (9), the residual SCOD concentration at the optimum condition for mycelial production was 3788 mg/l, which was close to the minimum value. This indicates the grade of this elliptical region is not steep, thus the optimum condition for mycelial production was likely to be used for the overall process parameter to maximize mycelial production of *G. lucidum* using deproteinated whey with nearly maximum reduction of SCOD concentration.

In the case of response analysis for the mycelial yield, lack of fit was not significant and regression was significant at a 1% α level only for the quadratic model; therefore, the quadratic model was selected to describe the response surface, which was:

$$\text{Yield} = -1.0 + 4.1 \times 10^{-1} x_1 + 3.5 \times 10^{-2} x_2 - 1.1 \times 10^{-3} x_1 x_2 - 4.4 \times 10^{-2} x_1^2 - 5.3 \times 10^{-4} x_2^2 \quad (10)$$

Fig. 6 shows two- and three-dimensional response surfaces of the quadratic model for the mycelial yield. The condition for a maximum mycelial yield, 0.35 mg mycelial weight per mg $\text{SCOD}_{\text{removed}}$, was calculated as pH 4.2, and 28.5 °C, which was almost the same as the optimum condition for mycelial production. Therefore, it could be concluded that the overall optimum condition for utilization of the deproteinated whey for the cultivation of *G. lucidum* should be based on the condition to maximize the mycelial production.

Dry mycelia of edible mushrooms are usually processed further to produce mycelial extract for various industrial applications. Approximately, 15–20 kg dry mycelia are required to produce 1 kg commercial extract giving 5–6.7% extraction efficiency [8,9]. The polysaccharides in the extract of *G. lucidum* mycelia are glucans such as branched 1,3-β-D-glucans, which are used in variety of commercial applications including health foods and medicines [7–9]. Because the polysaccharide content as well as productivity of the extract is an important parameter to indicate process efficiency in mycelial cultivation, we investigated characteristics of

the extract from *G. lucidum*'s mycelia cultivated at the optimum condition.

A total of 1820 mg extract per l was obtained from the submerged culture. Polysaccharide, protein, phosphorus, and potassium were the major components composing 66.9% of dry weight of the mycelial extract and corresponding concentrations were 1120 ± 13, 32 ± 3, 40.2 ± 1.0, and 24.9 ± 3.8 mg/l. Although the production of *G. lucidum* polysaccharides is greatly dependent on culture conditions such as pH, temperature, and types of carbon sources, typical yield usually varies from 0.6 to 1.1 g polysaccharide per l using glucose medium [9,13]. The high extract ratio of 10.7% (i.e. 1820 mg extract per 17 057 mg mycelium), as well as high content of polysaccharide (i.e. 1.12 g/l) at optimum condition estimated in this research indicated that the deproteinated whey could be an alternative substrate for mycelial production of *G. lucidum*. Therefore, cultivation of *G. lucidum* mycelia can offer a potential cost-effective solution for the utilization of the deproteinated cheese whey.

4. Conclusion

For bioconversion of deproteinated cheese whey, a set of experiments was carried out to cultivate mycelia of *G. lucidum*. The following conclusions are based on the results of the data from batch fermentations:

1) The deproteinated whey was rich in various organics and inorganics, which could support the mycelial growth of *G. lucidum*. Lactose was the major organic component contributing to the COD of the wastewater. A total of 83% of the total COD in the wastewater was due to the presence of lactose.
2) RSM was successfully applied to determine the optimum physiological condition for the maximum production of the mycelia, which was pH 4.2 at 28.3 °C. The predicted value for mycelial weight at the optimum condition was $17\,274 \pm 943$ mg mycelia per l. The SCOD removal ranged from 80.7 to 93.1% with a minimum of 3639 ± 560 mg residual SCOD per l at pH 4.6 and 27.1 °C. The condition for a maximum mycelial yield, 0.35 mg $\text{mycelia}_{\text{produced}}$ per mg $\text{SCOD}_{\text{removed}}$, was calculated as pH 4.2, and 28.5 °C, which was almost same as optimum condition for the mycelial production.
3) The high extract ratio of 10.7% along with high content of polysaccharide of 1.12 g/l at the optimum condition indicated that cultivation of *G. lucidum* mycelia would offer a potential cost-effective solution for an alternative treatment of the deproteinated cheese whey wastewater.

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