Optimization of Protein Extraction from the Low Organic Residual Municipal Sludge Waste

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Abstract. In this study, municipal sludge waste was used for protein extraction. Two pretreatment ways such as acid and alkaline hydrolysis were compared and the alkaline hydrolysis method was selected as the better way for protein extraction from the residual sludge. In order to obtain the optimal condition for protein extraction, the factors such as NaOH concentration, NaOH solution volume and pretreatment period were selected and optimized by response surface methodology, and the optimal condition for protein extraction was obtained: NaOH concentration was 3.85 mol/l, 24.0 g wet municipal sludge waste was added in 1 L of water, and the pretreatment period was of 12.16 h at 50 °C. Under the optimal extraction condition, the protein extraction yield could reach 5923±107 μg/g sludge, which were 2.07 folds than the initial production pretreated by NaOH.

1. Introduction
The increasing production of municipal sludge waste has brought a worldwide environmental problem in these years. It was reported that the sludge could be treated by dumping, burning, discharge into the sea and composting [1]. The cost of treatment and disposal of the residual sludge was 25%-60% of the total cost of the whole sewage treatment plant [2]. The components of residual sludge was very complex, containing of many kinds of heavy metals, pathogens and other toxic materials such as PAHs, phenols, and etc. [3]. So the residual sludge was harm for the environment if treated improperly. The sludge was mainly formed by microorganisms, protozoa, metazoan and algae, which containing a lot of protein. And the protein in the residual sludge was expensive and could be used as foam extinguishing agent, so higher value application and resource utilization of the residual sludge was realized. In order to obtain high protein extraction efficiency, many ways for cell wall breakdown were conducted including physical methods (high-pressure injection [4], thermal hydrolysis [5], ultrasonic wave [6], chemical methods (alkaline process, acid process [7] and ozone treatment [8] and biology methods. There were many factors could affect the extraction ratio of protein in the sludge.
In this paper, HCl heat and NaOH pretreatment were investigated and compared for protein extraction, and it was found that the NaOH pretreatment was the best way in this study. And then the pretreatment factors for protein extraction from the residual sludge was further optimized the optimal condition was obtained by response surface methodology.

2. Materials and methods

2.1. Characteristics of the municipal sludge waste
The raw municipal residual sewage sludge was collected from Jinan Sewage Treatment Plant (Jinan, China), and the waste characteristics are listed in Table 1.

| Moisture content | Ash | Volatile matter | Fixed carbon | Total carbon | Total nitrogen | pH | Organic matter |
|------------------|-----|-----------------|--------------|--------------|---------------|----|----------------|
| 83.2%            | 42.1% | 7.6%            | 0.6%         | 3.8%         | 0.5%          | 7.3 | 7.4%           |

2.2. Protein extraction from municipal sludge waste
In this study, 20 g of wet residue sludge was sampled each for the follow experiments if there was no special explanation. The sample was placed into a 250 ml glass breaker, and then a certain amount volume of HCl or NaOH solution (4 mol/l) was used for protein extraction. The hydrolysis reaction was conducted under a certain temperature. When the reaction was ended, the samples were centrifuged (10,000 g, 15 min) at 4 °C, and the supernatant was used for protein determination. HCl pretreatment (HP): 4 mol/l HCl solution was added to the samples and treated for 60 min at 50 °C. NaOH pretreatment (NP): 4 mol/l of NaOH solution was added to the samples and treated for 60 min at 50 °C. Folin-Phenol reagent (Sigma-aldrich, USA) was used for protein concentration determination as Lowry et al. reported [9].

2.3. Optimization of the protein extraction from residue sludge
In the former experiments, in order to find the optimal condition for the protein extraction from residue sludge, the selected factors such as temperature, NaOH concentration, treatment time were investigated and further optimized response methodology by Box-behnken design [10], and a quadratic model was used for fitting the data obtained from the tests, and then analysis of variance (ANOVA) was performed and the parameters including $R^2$ value, pure error, residue error, and the lack-of-fit were calculated to evaluate the model in the experiment. The software Design Expert (version 7.0, STATEASE Inc., Minneapolis, USA) was used for the experimental design and regression analysis.

3. Results and discussion

3.1. The effects of HCl solution and NaOH on the protein extraction from municipal sludge waste
In Table 2, it could be found that NaOH treatment was the best way for sludge wasted treatment for protein releasing, and the highest yield was of 2859.3 μg/g dry weight. so NaOH was selected for further investigation.

| Sludge | HP | NP | CK |
|--------|----|----|----|
| Protein concentration (μg/g) | 2369.4 | 2859.3 | 1061.8 |
3.2. Optimization of protein extraction

NaOH was better than HCl for protein extraction from the residual sludge which was found in the former experiments, and the conditions were further optimized by Box-behnken design (Table 3). Three factors such as NaOH concentration, sludge concentration and treatment period were used for optimization by response surface methodology.

The experimental design and the results in the Box-behnken design were listed in Table 3. The final estimated response model equation was

\[ Y = 5764.6 + 205.8X_1 + 237.4X_2 + 121.8X_3 - 281.7X_1X_2 - 160.7X_1X_3 - 308.1X_2X_3 + 295.7X_1^2 - 256.6X_3^2 \]  

(1)

where \( Y \) was the response factor (protein production, \( \mu g/g \)), \( X_1 \), \( X_2 \) and \( X_3 \) represented the three independent factors such as NaOH concentration (g/l), sludge concentration (g/l) and treatment period, respectively.

Table 3: Box-behnken design and results for protein extraction from residual sludge

| Run No. | NaOH (\( X_1 \)) (mol/l) | Sludge (\( X_2 \)) (g/l) | Treatment period(\( X_3 \)) (h) | Protein production(\( Y \)) (\( \mu g/g \)) |
|---------|------------------------|-----------------|-----------------|-----------------|
| 1       | 1(5)                  | 1(30)           | 0(12)           | 5882.7          |
| 2       | 1(5)                  | 0(20)           | -1(8)           | 5920.4          |
| 3       | 1(5)                  | -1(10)          | 0(12)           | 6192.4          |
| 4       | -1(3)                 | 1(30)           | 0(12)           | 5829.2          |
| 5       | 1(5)                  | 0(20)           | 1(16)           | 5893.4          |
| 6       | 0(4)                  | -1(10)          | -1(8)           | 4424.5          |
| 7       | -1(3)                 | 0(20)           | -1(8)           | 5392.7          |
| 8       | -1(3)                 | -1(10)          | 0(12)           | 5012.3          |
| 9       | 0(4)                  | 1(30)           | -1(8)           | 5736.6          |
| 10      | 0(4)                  | -1(10)          | 1(16)           | 5233.4          |
| 11      | -1(3)                 | 0(20)           | 1(16)           | 6008.5          |
| 12      | 0(4)                  | 1(30)           | 1(16)           | 5313.2          |
| 13      | 0(4)                  | 0(20)           | 0(12)           | 5792.3          |
| 14      | 0(4)                  | 0(20)           | 0(12)           | 5759.1          |
| 15      | 0(4)                  | 0(20)           | 0(12)           | 5742.5          |

The ANOVA analysis results were shown in Table 4. In the table, the fit of the model was checked by the coefficient of determination \( R^2 \), which was calculated to be 0.9356 indications that about 93.56% of the variability in the response could be explained by this model. The statistical significance of the model equation was evaluated by the F-test for ANOVA. The \( P \)-value was also very low \( (P < 0.0167) \), indicating the significance of the model. The lack-of-fit was insignificant at 5% level. The linear and the quadratic items were significant. It could be concluded that the statistical results showed a good fit between the model and the experimental data.

Table 4: ANOVA analysis of the regression model

| Source       | Sum of square | Degree of freedom | Mean square | F-value | \( P>F \) |
|--------------|---------------|------------------|------------|---------|-----------|
| Model        | 2737347.2     | 9                | 304150.0   | 8.0657  | 0.0167    |
| Error        | 188544.2      | 5                | 37709.0    |         |           |
| Lack of fit  | 187258.3      | 3                | 62419.4    | 97.0793 | 0.0102    |
| Pure error   | 1286.0        | 2                | 643.0      |         |           |
| Corrected total | 2825891.4    | 14               |            |         |           |

\( R^2=0.9356; R^2_{adj}=0.8196; \) C.V.%= 3.46
The response surface curves were plotted to explain the theoretical combination of NaOH concentration ($X_1$), sludge concentration ($X_2$) and treatment period ($X_3$) vs. protein production ($Y$) (Figure 1). The solution of $X_1$, $X_2$ and $X_3$ were $-0.15$, $0.40$ and $0.04$, and the corresponding optimum concentration of the actual value were $3.85$ mol/l NaOH, $24.0$ g wet municipal sludge waste in $80$ ml water, and the pretreatment period was of $12.16$ h, and the predicted protein production was $5799.8$ µg/g dry sludge. In order to confirm the optimized protein extraction conditions, three additional experiments were performed using the predicted condition, and the mean value of the protein production was $5923±107$ µg/g dry sludge, which agrees well with the predicted yield.

Figure 1. Three dimensional graph of the surface response plot

4. Conclusion
Residual municipal sludge waste has brought seriously pollution to the environment in these years with the rapid urbanization process in China, and it is very important to find appropriate treatment and disposal method for these solid wastes. Municipal sludge waste was found containing high content of protein, which is the valued product used in many areas such as feed additives, foam fire extinguishing materials and microbial culture media. In this paper, the waste residue sludge was used for protein extract by acid (HCl) alkaline (NaOH) hydrolysis. These two ways were evaluated, compared and alkaline hydrolysis was selected for further optimization by response surface methodology, and the optimal condition for the protein extraction was: NaOH concentration was $3.85$ mol/l, $24.0$ g wet municipal sludge waste was added in $1$ L of water, and the pretreatment period was of $12.16$ h at $50^\circ$C. Under the optimal extraction condition, the protein extraction yield could reach $5923±107$ µg/g sludge, which were $2.07$ folds than the initial production pretreated by NaOH. The parameters could provide references for the large-scale production of protein from the municipal sludge waste.

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References
[1] X. H. Liang, Y. X. Zhao, D. L. Hua, J. Zhang, X. D. Zhang, M. T. Gao, Optimization and process analysis of biodrying of low organic matter content municipal sludge, J. Biobase Material. Bioener. 9(2015) 66-73.
[2] E. W. Low, H. A. Chase, Reducing product ion of excess biomass during wastewater treatment, Water Res. 1999, 33(1999) 1119-1132.
[3] D. Q. Zhang, H. Zhang, C. L. Wu, L. M. Shao, P. J. He, Evolution of heavy metals in municipal solid waste during bio-drying and implications of their subsequent transfer during combustion, Waste Manag. 2011, 31(2011) 1790-1796.
[4] H. B. Choi, K.Y. Hwang, E. B. Shin, Effects on anaerobic digestion of sewage sludge pretreatment, Water Sci. Technol. 35(1997) 200-207.
[5] U. Kepp, I. Machenbach, M. Weisz, O. E. Solheim, Enhanced stabilisation of sewage sludge through
thermal hydrolysis-three years of experience with full scale plant, Water Sci. Technol. 42(2000) 89-96.

[6] C. P. Chu, B. V. Chang, G. S. Liao, D. S. Jean, D. J. Lee, Observation on changes in ultrasonically treated waste activated sludge, Water Res. 35(2001) 1038-1046.

[7] B. Y. Xiao, J. X. Liu. Impacts of different pretreatments on characteristics of excess sludge. Environm. Sci. 29(2008) 327-331.

[8] H. Jung, K. D. Sohn, B. Neppolian, H. Choi. Effect of soil organic matter (SOM) and soil texture on the fatality of indigenous microorganisms in entergrated ozaonation and biodegradation. J. Hazard. Material. 150 (2008) 809-817.

[9] O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 1951, 193(1951) 265-275.

[10] C. H. Dong, X. Q. Xie, X. L. Wang, Y. Zhang, Y. J. Yao, Application of Box-Behnken design in optimisation for polysaccharides extraction from cultured mycelium of Cordyceps sinensis. Food Bioprod. Proc. 87(2009) 139-144.