Isolation and Optimization of Cellulose Crystals (CCs) from Substrate Mushroom Spent

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ABSTRACT

Spent mushroom substrate (SMS) is the co-product of mushroom industries. SMS is also called spent mushroom compost. It grows and composts during the mushroom growing process. It is agro- residues and fungal mycelium left after harvesting of mushrooms. The aim of the study is to isolate the cellulose crystals (CCs) and to optimize the yield of CCs from SMS. Three different operating parameters has been studied; solid to liquid ratio, reaction time and solvent concentration. Two methods which are screening and optimization were conducted in order to investigate the highest number of CCs produced. For optimization, Box-Behnken design under Response Surface Methodology (RSM) was used to optimize the operating parameters. The condition of 1g: 30ml in solid to liquid ratio, 45 minutes in reaction time and 55% H2SO4 showed the highest yield of CCs which was 77%.

INTRODUCTION

Spent mushroom substrate (SMS) is residual result from the mushroom harvesting. It forms during the growth of mushroom. It is also by-product of mushroom industry [1]. The word "spent" is used because of their productivity of the mushroom substrate will reduce after the mushroom are collected [2]. SMS provide organic source and general nutrients to the soil especially for agriculture land. Soil with SMS spread on it has high nutrients content and very suitable for the growing of crops. Hence, it can be used to improve the condition of the soil. SMS is lignocellulosic substance. They have high content of lignin, hemicellulose and cellulose. Thus, it has potential as raw material for synthesis the cellulose crystals (CCs). However, before it is used in the production, a process called pre-treatment process is need in order to remove impurities. There are several kinds of pre-treatment which can categories as physical, chemical and biological. For example, physical pre-treatment involved milling and grinding whereas chemical pre- treatment involved used of chemical substances. A successful pre-treatment is very important. This is because it will affect the downstream process.

During the pre-treatment process, lignin and part of hemicellulose are removed by chemical pre-treatment. After pre-treatment process, the biomass is undergone bleaching process. The purpose of bleaching process is to whiten the raw material in order to produce pure CCs which is white in colour. After bleaching process, isolation process is conducted in order to synthesize CCs. Isolation process is very important because it increases the amount of cellulose and thus produce more CCs. Normally, acid solution is used in the isolation process and known as acid hydrolysis. Acid hydrolysis is commonly used for large-scale in the biomass industry. The acid used in the acid hydrolysis has the ability to extract desired compound from the raw material. Mostly, the acid hydrolysis is applied in lignocellulosic substance. Two type of acid can be used in acid hydrolysis which are dilute acid and concentrated acid. The acid used is depended on the raw material used. Acid hydrolysis can destroy cellulose bond by breaking the disordered and

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amorphous parts of the cellulose. The cellulose is the main component in the biomass. Many products such as cellulose nanocrystals, cellulose nanofiber, paper and cotton are synthesized from cellulose. The principle of this research is to study the relationship between operating parameters and the production of cellulose crystals (CCs) from SMS. SMS is one of the potential raw materials to synthesize CCs as it is lignocellulosic material. Pre-treatment of the SMS is to remove lignin and hemicellulose. After pre-treatment, bleaching process is conducted and the screening process are applied in this experiment. By this method, a smaller range can be classified for each operating parameter. The parameters that used in this study are solid to liquid ratio, reaction time and solvent concentration. Box-Behnken Design is applied to optimize the result from operating parameters obtained.

MATERIALS

Chemicals

Sodium hydroxide (NaOH), glacial acetic acid, sodium chlorite ($NaClO_2$) and sulphuric acid (H_2SO_4) were supplied from Sigma Aldrich.

Plant materials

Spent mushroom substrate (SMS) was collected from Institute of Sustainable Agrotechnology, Sungai Chuchuh, Perlis, Malaysia. The SMS was dried at 60 °C for 24 hours in oven. The dried SMS was ground by using grinder to reduce their size and pass through sieving size of 125μm.

PROCEDURES

Pre-treatment of SMS

SMS was undergone alkaline pre-treatment after the sieving process. The alkaline solution used was sodium hydroxide (NaOH). The SMS was immersed into 4% NaOH at 80°Cfor 2 hours with the ratio of 1g: 50ml. The pre-treatment process was conducted on the hot plate. The temperature of the hot plate was set to 80 °C and being agitated continuously during the experiment. After 2 hours, the pre- treated SMS was taken out and washed with distilled water until the pH become 7. Following the alkaline treatment, the alkaline treated SMS was undergoing bleaching treatment with 5% sodium chlorite ($NaClO_2$) reflux at 80 °C for 18 minutes.

Isolation of CCs

The SMS (1 g) was treated with sulfuric acid at acid concentrations of 40, 50, 55, 60, and 70 %, solid to liquid ratio of 1g:10ml, 1g:20ml, 1g:30ml, 1g:40ml and 1g:50ml and reaction time of 25, 35, 45, 55 and 65 minutes, respectively. When reaching a predetermined time, each reaction was terminated by adding 10-fold volume of distilled water.

Purification of CCs

The sample was centrifuge at 8000 rpm for 15 minutes. Then, it was washed repeatedly with distilled water to neutralize the pH. The CCs obtained was dried in an oven at 100 C until constant weight was achieved. The percentage yield was calculated by using Equation 1.

$$Percentage\ yield = \frac{w_1}{w_0} x\ 100\% \quad (1)$$

where w_1 refer to dry weight of product after drying process and w_0 as the initial weight of the sample that used in the isolation process

Optimization Process

Box-Behnken design was used in optimization process. Box-Behnken design is one of the components under response surface methodology (RSM). RSM is a design experiment model use to investigate the relation between parameters and evaluate the significant of the parameters on the responses. It can optimize the operating parameters and figure out the responses. Box-Behnken design is a 3-level incomplete factorial design. It used for investigate second-order response surface for input variable. It made up of three different levels which are high level, medium level and low level. In this study, three factors which are solid to liquid ratio (1:10 ml to 1:50 ml); reaction time (25-65 minutes); and solvent concentration (40-70%) were studied.

RESULTS AND DISCUSSION

Effect of Solid to Liquid Ratio

The effect of solid to liquid ratio during isolation process in production of CCs was inspected by conduct different ratio of solid to liquid. However, other parameters were constant. The other parameters were temperature, reaction time and concentration of H_2SO_4 which were set to 45 ºC, 45 min and 60% respectively. Figure 1 shows the effect of solid to liquid ratio in weight of CCs produced.

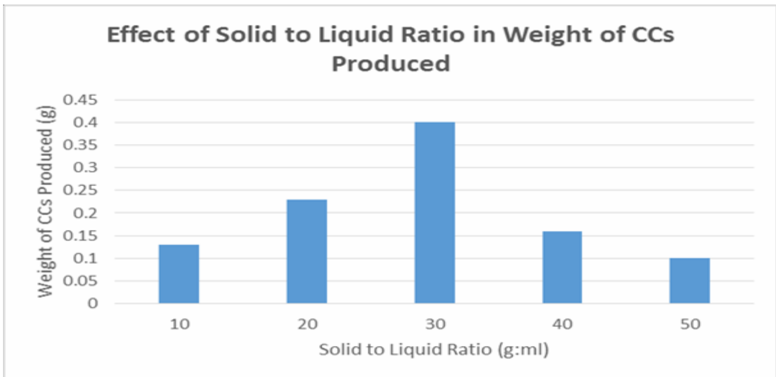

Figure 1. Effect of solid to liquid ratio on weight of CCs produced.

Amount of water play an important role in ratio of solid to liquid. The higher the volume of solid to liquid ratio, the more water content and the acidity of the mixture become lower. Different in solid to liquid ratio will result in the colour of solution which is milky in colour [3]. According to Chen et al., 2017, the higher the ratio of solid to liquid, the faster the rate in isolation process. This is due to more hydronium ion (H_3O^+) is produced and shift the reaction to the product side which also known as Le Chatelier's principle. The formation of H3O+ is caused by a proton which is positive hydrogen ion from the H_2SO_4 dissolve and react in the water. From the graph, the CCs produced increased as ratio of solid to liquid increase. However, after solid to liquid ratio reach 1g: 30ml, the products, CCs started to decrease. This phenomenon occurs because of the cellulosic material and isolation catalyst are not mix well and only partially react with each other [4]. Hence, some of the cellulose is not reacted with the solution and only a few of CCs produced during the solid to liquid ratio of 1g: 40ml and 1g: 50ml in isolation process. Therefore, the preferred solid to liquid ratio was 1g: 30ml.

Effect of Reaction Time

Another factor affecting the yield of CCs formation was reaction time. Different length of duration in producing CCs were varied in order to study the number of CCs produced. The experiment was carried out in different reaction time from 25 min to 65 min. The other conditions such as solid to liquid ratio, temperature, concentration of H2SO4 were fixed. Figure 2 shows the effect of reaction time in weight of CCs produced.

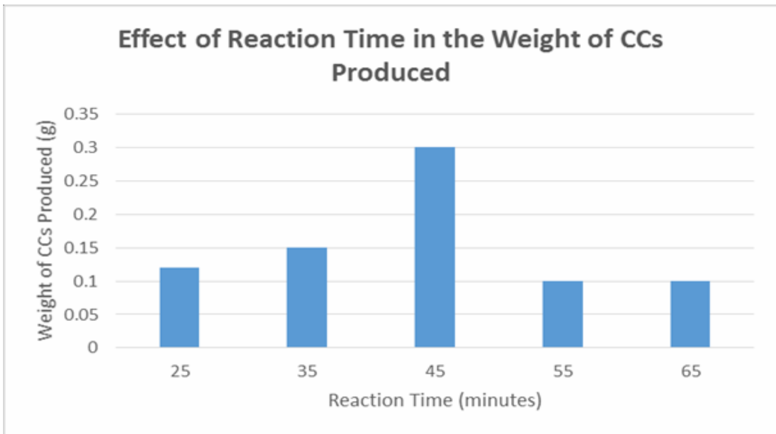

Figure 2. Effect of reaction time on weight of CCs produced.

It can be seen that the yield of the CCs was increasing as the reaction time become longer and then decreased over time. Cellulose enlarged as the isolation time increase. Sometimes, intra- and inter-fibres pore of cellulose become swollen with increasing reaction time. This is helpful in degrading the cellulose network so that crystals cellulose is easily to be produced [4]. Cellulose is a long and unbranched carbon chain that held together by Van der Waals forces and hydrogen bonds. Amorphous cellulose is very susceptible for the isolation process. Hence, high yield of CCs will be produced in that region [5]. Therefore, suitable reaction time is very important in synthesis of CCs. Lower CCs are produced when the isolation time is shortened. This may be due to insufficient time for the acid to react with the lignocellulosic substance. Acid cannot break down the strong cellulose macromolecules network in a short time [5]. On the other hand, too high reaction time can result in unfavourable isolation process in cellulose crystallization. This will be destructed the amorphous cellulose and hence damage the high order crystalline [4]. Besides, most cellulose is degrading to glucose as the duration reaction time is long and causes the yield of CCs reduce. Thus, isolation time play a significant effect in synthesis of CCs. Longer or shorter reaction time will result in producing a limited number of CCs. Hence, a suitable reaction time in formation of CCs was 45 minutes.

Effect of Solvent Concentration

Solvent concentration gives significant influence to the synthesis of CCs. The effect of solvent concentration had been investigated by varying solvent concentration from 40% to 70%. Type of solvent concentration used was sulphuric acid (H_2SO_4). H_2SO_4 is a strong acid, hence it can rupture the strong bond between the cellulose macromolecular networks. Figure 3 indicates the effect of solvent concentration in the weight of CCs produced.

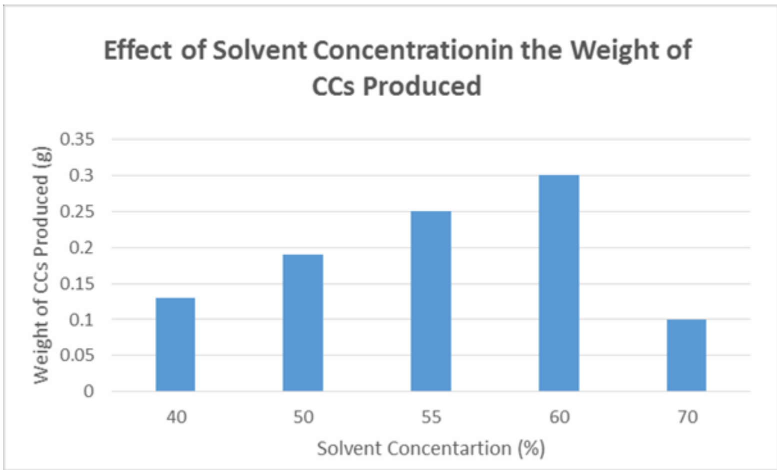

Figure 3. Effect of solvent concentration in weight of CCs produced.

The effect of solvent concentration played an important role in the formation of CCs. The concentration strength of solvent influenced the number of CCs produced during isolation process. In this experiment, sulphuric acid (H2SO4) was used because of its strong hydrogen ion. The strong hydrogen ion reacted with water molecules to form hydronium ion (H3O+). The formation of H3O+ then resulted in the effect of solid to liquid ratio. H2SO4 was dispersed into pre-treated SMS and led to rupture of glycosidic bond of the cellulose. Thus, the cellulose will then break into small size [6]. The strength of the acidity promotes the degree of isolation process. Therefore, cellulose is more easily break down into crystals size as stronger acid concentration is used in the isolation process [4]. Theoretically, the yield of CCs was rising as the concentration of H2SO4 increasing. The yield of CCs was low when the concentration of acid was 40%. This is because the strength of the acid is too low and not enough for isolation the biomass [3]. There was an exponential growth in the yield of CCs especially during the concentration of H_2SO_4 are 55% and 60%. According to Liu et al., (2015), all the cellulose has fully isolated by the acid. In spite of that, when 70% of solvent concentration was used, a dramatically decreased in the yield of CCs. The decline in the percentage of the CCs formed was due to the high acidity of the H_2SO_4 applied in the reaction. The biomass which contain mainly cellulose is degraded into the glucose instead of in crystals formed of cellulose [3]. Solvent concentration gives a great influence in the formation of CCs. Proper solvent concentration is very important in producing high yield of CCs. On the authority of Kargarzadeh et al., (2012), suitable concentration of acid will promote a positive isolation process. More CCs will produce as strong macromolecule networks in the amorphous part of cellulose is fully disintegrate by the acid. Therefore, a sulphuric acid concentration of about 60% was the most appropriate.

Optimization conditions of CCs Isolation

The orthogonal experiment was designed to study the effect of the three main factors (solid-liquid ratio, time and sulfuric acid concentration) on the yield of CCs. The results of yield of CCs (response) were measured according to design matrix and the measured responses are listed in Table 1.

Table 1 Results of the Box-Behnken tests

No	Solid to Liquid Ratio (g:ml)	Reaction Time (minutes)	Solvent Concentration (%)	Yield (%)
1	10	45	40	55
2	50	65	55	65
3	30	25	70	63
4	30	45	55	67
5	10	25	55	61
6	30	45	55	73
7	30	45	55	70
8	30	45	55	75
9	30	65	70	55
10	30	25	40	60
11	50	45	40	67
12	50	45	70	57
13	30	45	55	77
14	10	45	70	57
15	50	25	55	61
16	10	65	55	59
17	30	65	40	63

In order to validate the optimize conditions validation was carried out. Triplication of data was done by repeating for the optimum condition. The optimum condition was 1g: 30ml in ratio of solid to liquid, 45 min of reaction time and 55% H_2SO_4. This condition was conducted three times because the yield of CCs obtained in optimization process was the highest among other condition. Table 2 shows the results of validation process.

Table 2 Result of Validation Process

Predicted Value (%)	Actual Values (%)	Average	Percentages Error
77	Run 1 = 72 Run 2 = 79 Run 3 = 75	75.75	1.62%

Overall, the mean of CCs produced was 75.75% while the percentage error was 1.62%.

CONCLUSION

Isolation of CCs is affected by ratio of solid to liquid, reaction time and solvent concentration. Among all the variables, the maximum yield of CCs produced from different in solid to liquid ratio showed the highest which was 0.40g with the condition of 1g: 30ml in ratio of solid to liquid, 45 minutes of reaction time, 60% H_2SO_4 and temperature of 45^{0}C. For the optimization process, Box- Behnken design was used to acquire maximum yield of CCs. The result for the combination of 1g: 30ml in ratio of solid to liquid, 45mins in reaction time and 55% of solvent concentration showed the highest which was 77% of the yield.

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REFERENCES

H. J. Zhu, J. H. Liu, L. F. Sun, Z. F. Hu, J. J. Qiao, Bioresource. Technol. 136, 257–266 (2013).
C. W. Phan, Sabaratnam, V. Appl. Microbiol. Biot. 4,863–873 (2012).
Hong, B. O. Hong, F. Chen, G. Xue, Cell. Chem. Technol. 2, 225–231 (2016).
Y. W. Chen, T. H. Tan, H. V. Lee, S. B. A. Hamid, Materials. 1 (2017).
P. Krishnamachari, R. Hashaikeh, M. Chiesa, K. R. M. Gad, E. Rab, Cell. Chem. Technol. 12, 13–18 (2012).
X. Liu, H. Dong, H. Hou, Sci. Technol. 6, 466–473 (2015).
L. A. D. S. Costa, A. F. Fonseca, F. V Pereira, J. I. Druzian, Cell. Chem. Technol. 2, 127–133 (2015).
P. Selulosa, T. Kenaf, Sains. Malays. 11, 1635–1642 (2015).
Y. Tang, X. Shen, J. Zhang, D. Guo, Carbohyd. Polym. 125, 360–366 (2015).
P. Nascimento, R. Marim, G. Carvalho, S. Mali, Mater. Res. 1, 167–174 (2016).
H. Kargarzadeh, I. Ahmad, I. Abdullah, A. Dufresne, S. Y. Zainudin, R. M. Sheltami, Cellulose. 3, 855–866 (2012).