

Influence of different treatment condition on biopolymer yield production for coagulation-flocculation process

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Abstract. Two different agro wastes (banana pseudostem and rice straw) were utilized in order to extract biopolymer (pectin) known as coagulant aid in water and wastewater treatment. Factors such as pH, temperature and time were chosen due to the critical role in hot acid extraction process. The yield of biopolymer extraction from banana pseudostem was found to be higher at 28 % meanwhile only 18 % from rice straw was manage to produce from the dry weight 10 g, respectively. It was found that extraction temperature and extraction time were the most important factors influencing the biopolymer yield which increased with temperature and time or decreasing pH. Based on two level factorial design, the same condition of pH 1.5, temperature 90 °C and 4 hours extraction time can produce high amount of extracted biopolymer. Fourier Transform Infrared Spectroscopy (FTIR) was used to detect the existence of functional group which helps in the coagulation-flocculation process. Result indicates a similar functional group of biopolymer were detected for both difference agro wastes.

1. Introduction

In 2012, Americans produced about 251 million tons of garbage, from which 87 million tons were recycled and composted [13]. Garbage should not only be reduced by recycling the trash but also by minimizing the waste generated globally. Likewise, agricultural waste production too has the potential to be recycled and produce new material. In conjunction, banana pseudostem and rice straw are agricultural wastes that have the capability to treat effluent from wastewater through extracted biopolymer. Annually, about 17 million tons of bananas are produced worldwide whilst global paddy production is more than 700 million tons [4]. It has been estimated that a few tons per hectare of banana pseudo-stem are produced every year [2]. Waste generated from the banana plantation was disposed on land and the effluent flows into water bodies, which result in serious ecological hazard. Similarly, a report by the International Rice Research Institute (IRRI) stated that in 2008 approximately 620 million tons of rice straws were produced in Asia alone. These agro wastes are disposed off in various ways and have been reported to be increasing every year. Thus, it can be concluded that inefficient solid waste disposal methods will create series of problems, namely air and water resource pollution, accident hazard, increase of rodents and insect vector disease which leads to serious risk towards the communities [3].



Nowadays, agro wastes are found to be interesting organic material due to the availability value in the market which can be used in wastewater treatment as biopolymer. Many researchers studied the effect of pH, temperature, extracted time, salting out time and solvent to solid ratio in order to get the optimal extracting biopolymer. However, only three of the parameters seem to influence considerably on the quantity of pectin; which is pH, temperature and extracted time [14]. The extraction rate of biopolymer increases at higher temperature of the extraction process [12]. While previous study discovered that pH and time is the most significant parameter on the biopolymer yield [13]. Biopolymer known as pectin, has an important functional group to react as bioflocculating agent, which is efficient in wastewater treatment process. It proves to be the best biopolymer since it contain a huge number of carboxyl group (COOH) and able to supply more effective sites for ferric cations to form the bridge [10]. The electrolyte group can be exposed from the longer chain of biopolymer [6]. The functional group of carboxyl and hydroxyl with the negative charges are able to stretch out the biopolymeric chain due to the electrostatic repulsion [15].

The first part of this study is to identify the highest biopolymer yield from banana pseudostem and rice straw at various treatment conditions (pH, temperature and time) by using 2^k factorial design in triplicate. The influence factors that affecting the percentage of biopolymer yield (%) was investigated. The functional group for both extracted biopolymer also been analysed.

2. Methodology

The laboratory work of this study involves two phases which is extraction of biopolymer from banana pseudostem and rice straw. In order to study the effects of factors involved towards the yield, three effects were chosen include pH, temperature and time using two level factorial designs. Design Expert 6.0.2 software was used to evaluate normal probabilities plot in order to see the influent factors. The Analysis of Variance (ANOVA) was carried out with regards to validate the model with p-value at 95 % confidence interval.

2.1 Sampling of banana pseudostem and rice straw

The agro wastes from banana plantation site and paddy fields were obtained after they were harvested. Samples of banana pseudostem and rice straw were then clean, cut into smaller pieces and then the samples were washed with Sodium Metabisulphate 0.02% for preservation purpose before they were heated at 90°C in water bath for 15 minutes [8]. Later the samples were dried at 60°C for 48 hours before being ground into the size 500 µm.

2.2 Hot acid extraction process

Approximately 10 g of banana pseudostem and rice straw powders were mixed with distilled water at 1:10 ratio. Extraction of biopolymer known as pectin was carried out at different pH, temperature and time (Table 1). The extraction process was slightly modified from previous researcher [5]. The pH of the mixture was adjusted to the desired pH using sulphuric acid, H₂SO₄. The mixture was then extracted in a water bath stirrer at 80°C/ 90°C for 0.5/ 4 h, while being stirred. The mixture was left to cool to room temperature before the pH of mixture was increased to pH 3.5 by adding KOH 0.2M to promote pectin extraction [11]. Next it was filtered through 125 mm Whatman No.1 filter paper. 70% of Isopropyl Alcohol (IPA) was added for 4 hours to precipitate the biopolymer and freeze dried for 2 days. The collected biopolymer was ground to powder form and weighed for yield assessment. Finally, the homogenous biopolymer powder was stored at room temperature. Yield of biopolymer extracted was calculated as follow:

$$\text{Yield of biopolymer(\%)} = \frac{w_o}{w} \times 100 \quad (1)$$

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Where:

W_0 (g): the dried product weight after freeze dried

W (g): the dried raw material weight.

The factors were analyzed through two level factorial designs in order to get the analysis of variance and determine the interaction of the factors involved. Biopolymer yield from banana pseudostem and rice straw went through the various treatment conditions (pH, temperature and time). The comparison of biopolymer yield (%) was studied between banana pseudostem and rice straw. The condition of biopolymer extraction for both agro wastes was tabulated in Table 1 as followed:

Table 1. Condition for biopolymer extraction

Experimental Runs	A: pH	B: Temperature/ °C	C: Time/ h
1	1.5	90	4.0
2	2.0	80	4.0
3	1.5	80	0.5
4	2.0	90	0.5
5	1.5	80	4.0
6	1.5	90	0.5
7	2.0	80	0.5
8	2.0	90	4.0

2.3 Determination of biopolymer functional group

Fourier Transform Infrared (FTIR) Spectroscopy Attenuated Total Reflectance (ATR) was used to determine the functional group of biopolymer at mid infrared region ($4000-600\text{cm}^{-1}$). Biopolymer powder was placed in the spectrophotometer and scanned in the FTIR. Ethanol was used intentionally for all the cleaning part in the FTIR.

3. Result and Discussion

3.1 Yield of biopolymer extracted from banana pseudostem and rice straw

As shown Figure 1, biopolymer powder was observed in the beaker after the extraction process. The colour of the extracted biopolymer obtained was light brown powder. There are many factors that contribute to the colour discrepancy, such as surface contamination, amount of isopropyl alcohol used during precipitation or environmental factors [7].



Figure 1. Biopolymer in powder form after the extraction process.

The result for both biopolymer yields derived from banana pseudostem and rice straw after the extraction process is illustrated in Figure 2. Three selected factors were involved: pH (1.5 and 2.0); Temperature (80°C and 90°C) and Time (0.5 and 4.0) as shown in Table 1.

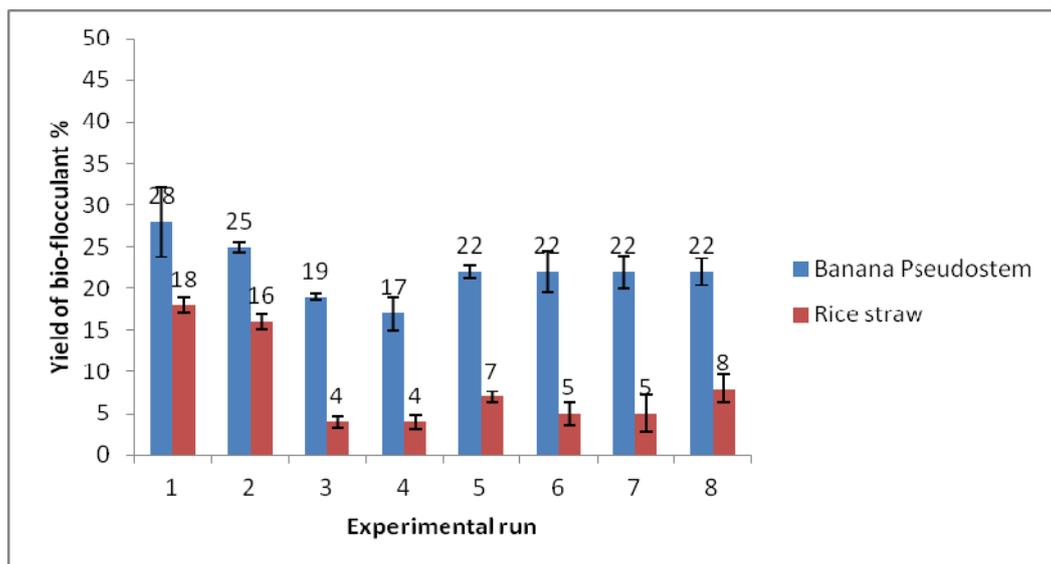


Figure 2. Yield of Extracted Biopolymer from banana pseudostem and rice straw.

The yield of extracted biopolymer from banana pseudostem ranged from 17 % to 28 % of the dry weight. The extraction for 4 hours at 90 °C, with pH 1.5, produced the highest yield among others. The variance analysis from Table 2 showed small interactive effects between pH and temperature with p-value at 0.0348. As for individual effects, temperature and time were the most influential factors at p-value 0.0246 and 0.0001 respectively. It is believed that by increasing the temperature in a harsh condition, it could increase the extraction rate of the extracted biopolymer [12]. In addition, favourable acidic conditions contribute to increase of biopolymer recovery by hydrolysing the insoluble biopolymer constituents into soluble biopolymer. Besides, more galacturonic acid content can be achieved at pH 1.5 rather than pH 2 [14]. However, galacturonic acid content did not influence the extraction rate of biopolymer.

Normal probabilities plot was used to analyze the factors influenced in the study. Figure 3 shows a normal probabilities plot for biopolymer extraction from banana pseudostem. At Point A, B, C, AB and BC, the distance from the red line due to the significance effects with P value < 0.05. In contrary, point AC and ABC were on the red line which means the effect was not significant in the treatment. It is seen that the larger significant effect showed a great distance from the line compare to non significant effects.

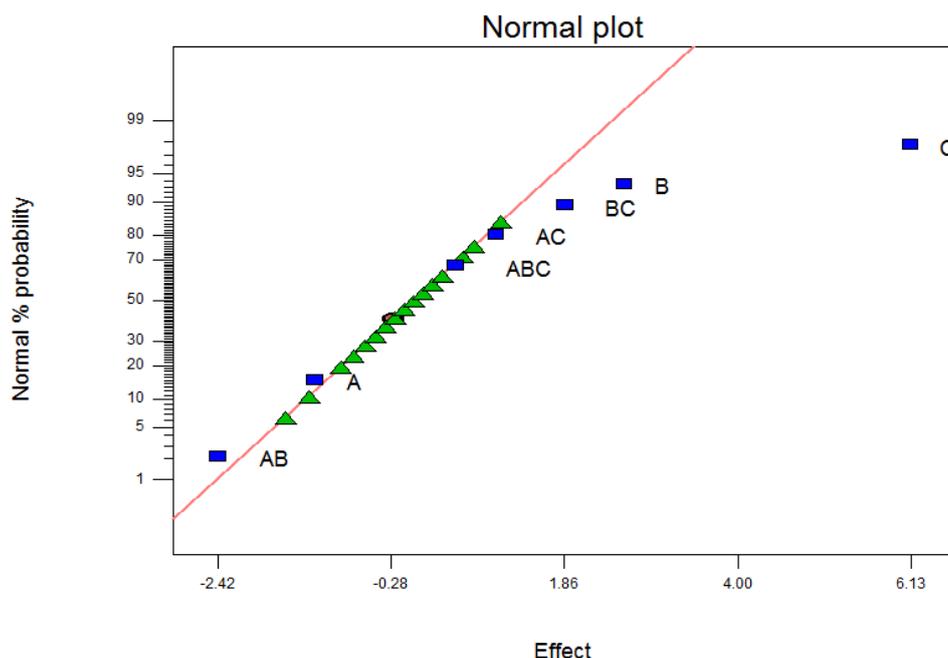


Figure 3. Percentage of normal probabilities plot of extracted biopolymer from banana pseudostem

Table 2. Analysis of variance (ANOVA) for extracted biopolymer from banana pseudostem

Extraction of Pectin from Banana Pseudostem					
Source of variance	Sum of Squares	DF	Mean square	F-value	p-value
Model	338.9	7	48.41	7.35	0.0005
A	8.88	1	8.88	1.35	0.2627
B	40.56	1	40.56	6.15	0.0246
C	225.71	1	225.71	34.25	0.0001
AB	35.04	1	35.04	5.32	0.0348
AC	6.20	1	6.20	0.94	0.3464
BC	20.91	1	20.91	3.17	0.0939
ABC	1.60	1	1.60	0.24	0.6287
Pure error	105.44	16	6.59		
Cor total	444.34	23			

Meanwhile, the yield obtained from the biopolymer extracted rice straw varied from 4 % to 18 % of the dry weight. As expected from the extraction process from banana pseudostem, the extraction of the

highest yield for extracted biopolymer from rice straw were also produced at the same condition of pH 1.5, temperature 90 °C and 4 hours. The variance analysis from Table 3 shows no interactive effects between pH and temperature and between pH and time at p-value 0.2178 and 0.2105, respectively except for interaction effects between temperature and time at p-value 0.0015. Whereas, all factors were significant for individual effects of pH at p-value 0.0397 and for temperature and time was at p-value 0.001. At constant pH and temperature, the yield of biopolymer produced for 4 h was higher than those at 0.5 h. The yield of biopolymer has been reported to increase with the increase of temperature and time [13, 14].

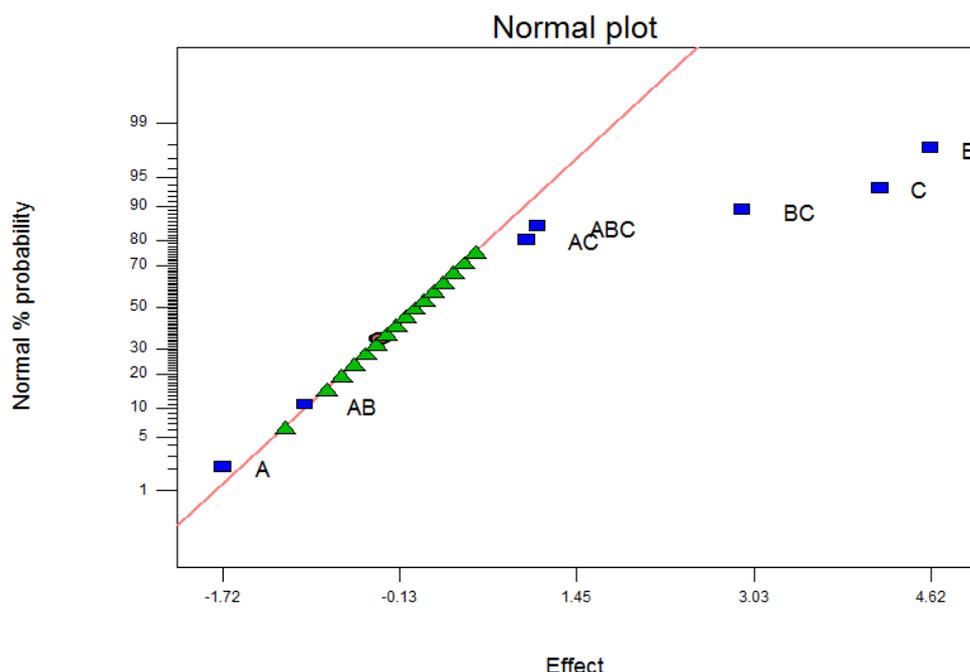


Figure 4. Percentage of normal probabilities plot of extracted biopolymer from rice straw

Normal probabilities plot was used to analyze the factors influenced in the study. Figure 4 show a normal probabilities plot for biopolymer extraction from rice straw. Point A, B, C, AC, BC and ABC showed a distance from the red line due to the significance effects with P value < 0.05. In contrary, point AB was on the red line which means the effect was not significant in the treatment. It is seen that the larger significant effect showed a great distance from the line compare to non significant effects.

Table 3. Analysis of variance (ANOVA) for extracted biopolymer from rice straw

Extraction of Biopolymer from Rice Straw					
Source of variance	Sum of Squares	DF	Mean square	F-value	p-value
Model	320.42	7	45.77	12.98	0.0001
A	17.68	1	17.68	5.02	0.0397
B	127.88	1	127.88	36.27	0.0001
C	104.17	1	104.17	29.55	0.0001
AB	5.80	1	5.80	1.65	0.2178
AC	6.00	1	6.00	1.70	0.2105
BC	51.63	1	51.63	14.64	0.0015
ABC	7.26	1	7.26	2.06	0.1705

Pure error	56.41	16	3.53
Cor total	376.82	23	

This indicated that the biopolymer was difficult to be extracted from the plant cell wall when the condition was too minimal in temperature and time. According to other study, degradation of biopolymer chain molecules occurred during longer extraction time and the higher extraction temperature which affected biopolymer extraction rate [12]. It was found that pH 1.5 with constant temperature and time can produce higher yield compared to pH 2. Similar results were obtained in previous study found that the increase amount of pectin extracted from agro wastes was affected by lower pH values [5]. In order to see the influence of the factors involved in the extraction of biopolymer, the variance analysis was analysed and is shown in Table 4. The significant effects with P value < 0.05 indicate that the factors have an impact on the results obtained. On contrary, those factors with p value more than 0.05 were not significant in the treatment.

Table 4. Values of p-value for biopolymer yield from banana pseudostem and rice straw

Source of variance	Biopolymer Yield from Banana Pseudostem	Biopolymer Yield from Rice Straw
	P value (95% Confidence Level)	
Model	0.0005	0.0001
A	0.2627	0.0397
B	0.0246	0.0001
C	0.0001	0.0001
AB	0.0348	0.2178
AC	0.3464	0.2105
BC	0.0939	0.0015
ABC	0.6287	0.1705

3.2 Functional group of biopolymers using FTIR

FTIR was analysed for both biopolymer extracted from banana pseudo-stem and rice straw with regards to see the presence of effective functional group for flocculation. The FTIR spectrum of biopolymer was taken in the frequency range of 4,000–400 cm^{-1} . The functional group of extracted biopolymer from banana pseudo-stem and rice straw can be observed in Table 5.

Table 5. Intensity (cm^{-1}) of FTIR extracted biopolymer from banana pseudo-stem.

Biopolymer from banana pseudo-stem		Biopolymer from rice straw	
Intensity (cm^{-1})	Functional Groups	Intensity (cm^{-1})	Functional Groups
655.18, 1,031.43, 1,089.94, 1,120.28, 1,196 and 3,257	Hydroxyl (-OH)	656.10	Hydroxyl (-OH)
884.09	CH deformation (-CH)	753.5 – 778.25	Methylene group (-CH)
1,312	CH ₃ deformation (CH ₃)	1,090.28 – 1,194.53	Hydroxyl (-OH)
1,424	Carboxyl group (COOH)	1,137.19	Alyphatic ether (C-O-C)
2,922	Methylene group (-CH ₂)	1,313.75 – 1,609.96	Carboxyl group (COOH)

The FTIR spectra of biopolymer shows –OH stretching vibration peaks with clear absorption peaks at 655.18, 1,031.43, 1,089.94, 1,120.28, 1,196 and 3,257 cm^{-1} . The existence of symmetric C-H stretching vibrations of methylene groups at peak 2,922 cm^{-1} can be considered as the increment of C-H bond content after amidation reaction [9]. The peaks at 1,312 cm^{-1} and 884.09 could be assigned to CH_3 and CH deformation respectively. The FTIR spectra of biopolymer show that the spectrum also has an ester peak at 1196 cm^{-1} . There are carboxyl group detected at 1,424 cm^{-1} , which are strongly contributed to the flocculating mechanism [6].

Similarly, the functional group of biopolymer from rice straw is detected at the same range in FTIR spectra as observed in Table 5. Based on the result, hydroxyl group (-OH) which is one of the bio-flocculant's functional group is detected at peaks 656.10, 1,090.28, 1,122.01 and 1,194.53 cm^{-1} in an IR spectrum with the broad O-H absorption of alcohols. The peak at 753.85 and 778.25 cm^{-1} indicates C-H stretching vibration. The peak at 1090.28, 1,122.01 and 1194.53 cm^{-1} suggests –OH bending point out as the presence of secondary alcohol. The peak at 1137.19 and 1313.75 cm^{-1} could be assigned to aliphatic ethers C-O-C and carboxylic acid (-COOH) respectively. The peak at 1,609.96 also indicated the presence of carboxylate stretching. Nevertheless, the presences of hydroxyl and carboxyl group have a potential to increase electrostatic attraction in order to provide a strong bridging mechanism [1].

4. Conclusion

Three different factors were investigated to see their influence on biopolymer yield after hot acid extraction process. Two level factorial design indicates that, extraction temperature and extraction time were the major effects that contribute to higher amount of biopolymer. The range values of yield obtained from banana pseudostem were between 16 % up to 28 % whereas 4 % until 18 % were collected from rice straw. Thus, the functional group for both agro waste were similar which contained of hydroxyl group, methylene group and carboxyl group.

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