OPTIMIZATION OF FLOCCULATION PROCESS BY MICROBIAL COAGULANT FOR REMOVAL OF TURBIDITY IN RIVER WATER

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ABSTRACT: The existing process of coagulation and flocculation is using chemicals that known as the cationic coagulant such as alum, ferric sulfate, calcium oxide, and organic polymers. Thus, this study focuses on optimizing of the flocculation process by microbial coagulant in river water. Turbidity and suspended solids are the main constraints of river water quality in Malaysia since they may reduce the dissolved oxygen in the water and affects the aquatic life. Hence, a study is conducted to produce microbial coagulants isolated locally for river water treatment. The chosen microbe used as the bioflocculant producer is *Aspergillus niger*. The parameters optimized in the flocculation process were pH, bioflocculant dosage and effluent concentration. The research was done in the jar test process and the process parameters for maximum turbidity removal was validated. The highest flocculating activity was obtained on day seven of cultivation in the supernatant. The optimum pH and bioflocculant dosage for an effective flocculation process were between 4-5 and 2-3 ml for 0.3 g/l of effluent concentration, respectively. The model was validated by using a river water sample from Sungai Pusu (Pusu river) and the result showed that the model was acceptable to evaluate the bioflocculation process.

ABSTRAK: Proses sedia ada pada pembekuan dan pemberbukuan adalah menguna pakai bahan kimia yang dikenali sebagai kationik kogulan seperti alum, sulfat ferum, kalsium oksida dan polimer organik. Oleh itu, kajian ini memberi tumpuan kepada mengoptimumkan proses pembekuan daripada mikrob kogulan dalam air sungai. Kekeruhan dan pepejal terampai adalah punca utama pada kualiti air sungai di Malaysia kerana ia mungkin mengurangkan oksigen larut dalam air dan menjejaskan kehidupan akuatik. Oleh itu, satu kajian telah dijalankan untuk menghasilkan mikrob kogulan terasing bagi merawat air sungai. Pemilihan mikrob yang digunakan sebagai penghasil bio-pembekuan adalah *Aspergillus niger*. Parameter yang dioptimumkan dalam proses pembekuan ini adalah pH, sukatan bio-pembekuan dan sisa kepekatan. Kajian telah dijalankan dalam proses ujian jar dan proses parameter untuk membuang kekeruhan secara maksimum telah disahkan. Aktiviti pembekuan yang paling tinggi telah didapati pada hari ke-tujuh dalam supernatan. pH optimum dan sukatan bio-pembekuan untuk menghasilkan proses efektif pembekuan adalah antara 4-5 dan 2-3 ml bagi 0.3 g/l sisa kepekatan, masing-masing. Model telah disahkan dengan menggunakan sampel air sungai dari Sungai Pusu dan keputusan menunjukkan model yang boleh diterima untuk penilaian proses bio-pemberbukuan.

KEYWORDS: *bioflocculant; river water; microbial*

1. INTRODUCTION

One of the main constraints of river water quality in Malaysia is turbidity that caused by suspended solids. Currently, no technology has been established in managing the river basin. However, many purification processes are practiced for water and wastewater treatment using coagulation/flocculation processes. In water refinement, usually, there are a few processes that will be followed in order to remove impurities which involve screening, coagulation and flocculation, sedimentation, pH correction, filtration, disinfection and floridation [1]. There are many water treatment plants that have been established to clean the river water such as Puncak Niaga Holdings Berhad. Most of Puncak Niaga's treatment plants basically utilize conventional water treatment. Unfortunately, these conventional water clarification treatment systems,at times, are unable to eliminate all those contaminants that polluted the river water, thereby, they might enter the distribution and supply network [2]. The existing process of coagulation and flocculation are using chemicals known as cationic coagulants such as alum, ferric sulfate, calcium oxide, and organic polymers [3]. Chemical coagulation is usually accomplished by adding metallic salts like aluminum sulfate (alum) or ferric chloride. Until now there are no organic materials as good as chemicals that can be used in coagulation and flocculation [4]. Organic materials are safer than chemicals for water treatment and they are cheaper as well. Basically, microbial coagulant which is sort of organic materials is relatively cheap, non-toxic, new efficient and would not make secondary pollution since the water treatment factor getting from the fermentation of microbiology and separation by biological procedure, which has biology resolvability and security [5].These chemicals are not safe for the environment as well as human health [6]. Therefore microbial coagulants are necessary to overcome these limitations for water refinement towards maintaining river water quality. The distillation of river water by integration of coagulation and flocculation process is a good way of water treatment since its required simple equipment, convenient management and occupying small place [7]. Although many researchers have been done to improve the flocculating activity of polysaccharides based or develop new-type bioflocculants [8], somehow, the results were not good enough for commercial applications due to high production cost [9]. Basically, microorganisms that having good characteristics to be a bioflocculant and has been a worldwide researching focus is metabolites of microorganisms which comprise of polysaccharide, protein, nucleic acid and lipid [10]. The polysaccharides-based bioflocculants (PSBs) having the most attention because of their tendency in disposing of various type of pollutants that involving heavy metal ion, dyeing pigment and other suspended impurities [11]. A bioflocculant candidate with a wide range of pH and high flocculating activity capability would be significant to replace the chemical flocculant. Bioflocculant has been reported that it has the ability to remove organic and inorganic colloids by their flocculating activity. The effectiveness of natural coagulant to eliminate suspended solids, heavy metals, microorganisms and turbidity of different types of industrial wastewater effluents as well had been stated [12-14]. Adebami and Tayo [15] have been reported that fermentative conditions and nutritive compounds have an effect on bioflocculant production. Besides that, bioflocculant production by microbial isolated from wastewater samples has been reported as a comparative effect of the medium composition.

The present study was to achieve the optimization process for bioflocculation in a jar test for removal of turbidity of river water and to validate the process parameters for maximum turbidity removal. Hence, the study was proposed to produce microbial coagulants for river water treatment.

2. MATERIALS AND METHOD

2.1 Sample Collection

Three liters of river water sample were collected from Sungai Pusu (Pusu river), Gombak, Malaysia. The sample was stored in the container during the sample collection and then kept in the refrigerator at 4 ^oC. The time obf sampling the river water was done on November 2015 at evening right after rain during the rainy season in order to have the high concentration of colloidal in river water. The parameters involved in the river water analysis were turbidity, total suspended solids, chemical oxygen demand and pH. All the data presented were the average of duplicate.

2.2 Bioflocculant Production

The strain, *Aspergillus niger* was collected from a microbial stock in the Environmental Engineering Laboratory, International Islamic University Malaysia which was isolated from river water sediment. The *A.niger* was subcultured in sterilized PDA medium for 4-5 days. After 7 days, the inoculum was prepared by wash it with sterilized distilled water and incubate it on a rotary shaker for 1 day at 150 rpm. By using sterilized filter paper, the other substances were separated with the spores suspension. The fermentation of spores by using malt extract as the medium was carried out in 7 days with 150 rpm.

For bioflocculant production the cultivation was carried out on nutrient broth (bile peptone broth, BPB) containing (g/l): glucose 20, urea 0.5, yeast extract 0.5, $(NH_4)_2SO_4$ 0.2, K_2HPO_4 5.0, KH_2PO_4 2.0, NaCl 0.1 and $MgSO_4.7H_2O$ 0.2. The initial pH of the medium was adjusted to 6.0-7.0. The in the sterilized media (2-4 ml) and incubated on a rotary shaker at 150-200 rpm and 32±2 ^oC for 3 days. The biomass and supernatant were separated by filtration. Biomass was dried using the oven at 105 ^oC.

2.3 Determination of Bioflocculant Activity and Turbidity

According to Kurane et al. [12], the mixture containing 9 ml kaolin clay suspension (5g/l, pH 7.0), 0.1 ml sample and 0.9 ml $CaCl_2$ solution (1%, pH 7.0) was stirred vigorously and left standing for 5 minutes. The samples were taken every 24 hours in order to measure for flocculating activity. The supernatant was measured for absorbance at 550 nm. A control was prepared by replacing the sample with distilled water. The flocculating activity has been done by following the equation shows below [6]

$$\text{Flocculating activity} = 1/\ (A550) - 1/\ (A550)c \quad (1)$$

Where, A550 = absorbance of the sample (A550)c = absorbance of control.

All experiments were done following the Standard Jar Test procedure [16].

2.4 Optimization of Flocculation Process

The optimization of flocculation process was investigated by manipulating some parameters such as pH, and bioflocculant dosage and effluent concentration. The agitation and settlement time were fixed variables with value 150 rpm and 40 min and 30 min respectively. The pH range was 4-7, bioflocculant dosage of 2%v/v, 4%v/v and 6%v/v, and effluent concentration range was between 0.3 mg/l - 0.5 mg/l.

The design of experiment was prepared to know the number of the experiment should be conducted. The response surface method (RSM) was applied to identify the total experimental runs in the design of experiment. By using the Design-Expert 6.0.8 software, the design of experiment for the optimization of sedimentation process was Face Centered

Composite Design, level 3 [low (-1), medium (0), and high (+1)]. The 3 factors were pH, bioflocculant dosage and effluent concentration.

The statistical analysis was conducted with ANOVA, P-and t-test, regression coefficient (R^2), 2D and 3D surface plots. The optimum parameter was determined by using numerical solution in Design Expert Software.

2.5 Validation of the Model

The optimum values for manipulated variables such as pH, bioflocculant dosage and effluent concentration was applied to the river water sample in order to validate the values.

3. RESULT AND DISCUSSION

The water quality of Sungai Pusu was investigated in order to determine total suspended solids (TSS), turbidity, pH and chemical oxygen demand (COD). After the TSS test was done, the sample was diluted to make sure the final concentration of 0.3 g/l from the initial concentration of 0.8 g/l. After dilution, the turbidity of the river water sample changed to 128 NTU. These two parameters were important to proceed with the validation of the model. Besides that, the pH and COD of the sample water were 6.4 and 3.3 mg/l respectively.

An experiment was conducted to investigate the highest day of production bioflocculant in terms of the presence of many exopolysaccharides. From the result obtained, it can be seen that, increased in the cultivation day resulted into an increased in cell growth and the related increase in bioflocculant production. The decrease in flocculating activity observed after 168 hours and could be referred to the presence of bioflocculant-degrading enzyme produced by the microorganisms [17]. As stated by Manivasagen et al. [18], the supernatant was used throughout the research instead of the pallet. This is because the flocculant that produced by microorganisms was polysaccharides-based bioflocculant that was secreted as macromolecular substances.

Table 1: Experiment parameters designed by Design Expert software with the response

Run	pH	Bioflocculant Dosage (ml)	Effluent Conc. (g/l)	Turbidity (NTU)
1	7	6	0.5	170
2	5.5	4	0.4	106
3	7	4	0.4	93
4	4	6	0.5	4
5	5.5	4	0.4	106
6	5.5	2	0.4	90
7	5.5	4	0.5	223
8	5.5	4	0.4	106
9	7	2	0.5	87
10	5.5	4	0.3	101
11	7	2	0.3	6
12	4	6	0.3	98
13	4	2	0.5	119
14	4	2	0.3	27
15	5.5	4	0.4	106
16	7	6	0.3	168
17	5.5	6	0.4	136
18	5.5	4	0.4	106
19	5.5	4	0.4	106
20	4	4	0.4	86

The model formulated by DOE with the response of turbidity is as follows:

$$Turbidity = 10.84 + 1.01 * A + 1.22 * B + 0.97 * C - 2.19 * A * A - 1.07 * B * B + 0.85 * C * C + 2.30 * A * B + 1.14 * A * C - 2.55 * B * C \quad (2)$$

According to Table 1 the lowest turbidity obtained was 4 NTU at pH 4, bioflocculant dosage of 6 ml at maximum concentration 0.5 g/l. In contrast, the highest reading of turbidity after the experiment was 223 NTU which was in pH 5.5, 4 ml of bioflocculant dosage and at the maximum effluent concentration as well. The lowest turbidity obtained lies into the class I of National Water Quality Standard for Malaysia which is 5 NTU was the maximum value [19].

The analysis demonstrated that the model was highly significant with a model F-value of 9.94 and Prob>F less than 0.0006. in general, Prob > F values of less than 0.05 indicate the model was significant, while values greater than 0.10 indicate the model terms were not significant. In this case, A, B, A^2, AB, AC and BC were significants where A,B and C were pH, bioflocculant dosage and effluent concentration respectively. The values of coefficient of determination, R^2 and adjusted R^2 were in fair agreement 0.8995 and 0.8090 respectively and quite close to 1, which indicated a reasonable correlation between the experimental values and predicted values.

Table 2: Analysis of variance (ANOVA) of response surface quadratic model for the turbidity response of the sedimentation by bioflocculant

Source	**Sum of Squares**	**DF**	**Mean Square**	**F value**	**Prob.F**	
Model	170.17	9	18.91	9.94	0.0006	Significant
A	10.29	1	10.29	5.41	0.0424	
B	14.87	1	14.87	7.82	0.0189	
C	9.31	1	9.31	4.90	0.0513	
A^2	13.14	1	13.14	6.91	0.0252	
B^2	3.15	1	3.15	1.66	0.2270	
C^2	1.97	1	1.97	1.04	0.3327	
AB	42.45	1	42.45	22.32	0.0008	
AC	10.45	1	10.45	5.49	0.0411	
BC	52.09	1	52.09	27.39	0.0004	
Residual	19.26	10	1.90			
Lack of Fit	19.13	5	3.83	150.21	<0.0001	Insignificant
Pure Error	0.000	5	0.000			

R^2 = 0.8995 and Adjusted R^2 = 0.8090

It can be summarized from the contour and 3-D plots as shown in Fig. 1. The pH and bioflocculant dosage have the significant effect on the turbidity results that represent the sedimentation process was at optimum. The lowest of turbidity reading was at pH value 4, the interaction between pH and bioflocculant dosage was significance since the shape of response surface was curves. The interaction between the corresponding variables is significant as it can be indicated by an elliptical or saddle nature of the contour plots [20]. According to Zainuddin [21], the pH influences the flocculating activity thus, affecting the sedimentation process. One of the ways of pH influences is by affecting the stability of suspended particles and floc formation [22]. Usually, bioflocculant had a strong activity over a wide range of pH 3-11 [23]. As for bioflocculant produced by *Aspergillus niger,* the optimum pH required to result in lowest turbidity was in the range 4-5. The black fungi were capable to produce a bioflocculant that can work in acidic up to pH 4. On the other hand, the amount of bioflocculant dosage should be used accordingly because it also may affect the turbidity reading. The optimum bioflocculant dosage for effluent concentration 0.3 g/l in the finding was not more than 3 ml. As stated in the literature review, the common amount of bioflocculant dosage needed was 1 ml. Ideally, a high dosage produces high viscosity which could prevent the settling of suspended particles by restabilization of particles. The high viscosity inhibits and blocks the adsorption site thereby reduce the flocculation process and floc formation. In contrast, a low dosage not favorable to make bridging flocculation mechanism of the bioflocculant to be effective [24]. The bridging flocculation takes place when bioflocculant extend from the particles' surface into the solution for a distance greater than the distance over which inter-particle repulsion acts and bioflocculant adsorb other particles to form flocs [25-26].

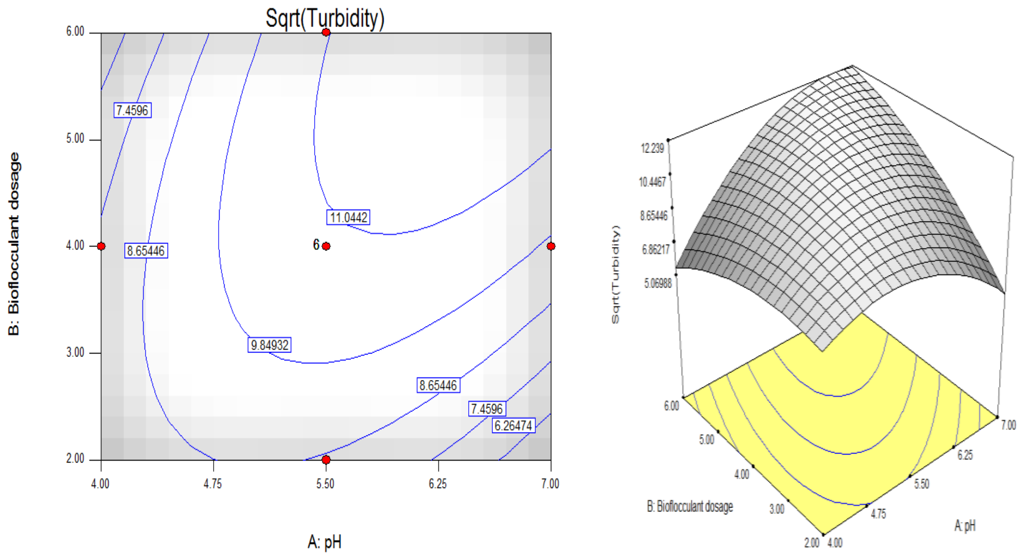

Fig. 1: Plot of contour and 3-D of interaction between pH and bioflocculant dosage.

Validation experiments are conducted in order to verify the obtained model's predictability (Table 3). Three validation experiments were designed and conducted. They have classified into three which are minimum, medium and maximum. After that, validation model has been done according to model formulated by DOE as shown in the Eq. (2), the result of turbidity experimentally do not differ too much with the turbidity theoretically. Hence, the model can be accepted as valid since it produces a parallel result between theoretical and experimental result. The error might come due to the inaccuracy during the preparation of the raw river water since it is difficult to achieve exact pH needed. But the all error is less than 10% and can be accepted.

Table 3: Result of validation model by comparing the turbidity of theoretical with experimental

	pH	Bioflocculant dosage (ml)	Effluent concentration (g/l)	Turbidity (Theoretically)	Turbidity (Experimentally)
Minimum	5	2.9	0.3	25	27
Medium	6	3.19	0.3	39.94	43
Maximum	6.18	3.29	0.3	69.99	74

4. CONCLUSION

The study shows that the *Aspergillus niger* was capable of removing turbidity in the river water with optimum process conditions and could be one of the potential bioflocculant producers. By having optimization of certain parameters like pH and bioflocculant dosage, the bioflocculant can perform very well in reducing the turbidity of the river water. The optimal pH for the bioflocculant was between 4 and 5 while the bioflocculant dosage found was 3 ml for 0.3 g/l of effluent concentration. It is recommended to have a further study on characterization and evaluation as this bioflocculant has the potential to replace the use of chemical flocculant in water industries.

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