



Faculty of Resource Science and Technology

Utilizing polyglutamic acid in treatment of sago effluent

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Declaration

I, Peter Alphonso Anak Anthony Nyoel, hereby declare that this final year project report is done by me. I had put 80% effort in finishing the final year project and report writing, with 10% contributed by my supervisor in checking and correcting my experiment and writing. The remaining 10% contributions are from the postgraduate students and my fellow course mates who had help me throughout the project.

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(PETER ALPHONSO ANAK ANTHONY NYOEL)

Table of Contents

Acknowledgement	I
Declaration	II
Table of Contents	III
List of Abbreviations	V
List of Tables and Figures	VI
Abstract	1
Introduction	2
Literature review	3
Flocculating agents	3
Application of PGA	4
Efficiency of PGA.....	5
Material and Methods	6
Material	6
Filtration of sago effluent	6
Jar testing method	6
Bioreactor test	7
Optical density	7
Treatment of flocculates	7
Results and Discussion.....	8
Effect of pH on flocculating activity.....	8
Effect of temperature on flocculating activity	12
Effect of doses of PGA used on flocculating activity	13
Effect of agitation on flocculating activity	15

Conclusion.....	17
References	18

List of Abbreviations

PGA – γ -Polyglutamic acid

PAC – Polyaluminium chloride

PAM - Polyacrylamide

COD – Chemical Oxygen Demand

BOD – Biochemical Oxygen Demand

rpm – revolution per minute

ppm – parts per million

mg – milligram

mg/l – milligram per litre

OD – optical density

nm - nanometer

List of Tables and Figures

Figure 1: the structure of γ -polyglutamic acid (source: Nippon Poly-Glu Co., Ltd).....	2
Figure 2: Differences in colour caused by pH before the addition of PGA (from left to right: pH 1 until pH 10, with control at fifth left)	8
Figure 3: Flocculation activity after the addition of PGA (from left to right: pH 1 - pH 10).....	9
Figure 4: Effect of pH on flocculating activity.....	10
Table 1: pH and absorbance values before and after the addition of PGA	10
Table 2: Effect of temperature on absorbance value after the addition of PGA	12
Figure 5: Effect of PGA doses on flocculating activity	13
Figure 6: 2L B. Braun bioreactor was used to flocculate the solids of the effluent which consist of sago fibre and starch.....	16

UTILIZING POLYGLUTAMIC ACID IN TREATMENT OF SAGO EFFLUENT

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ABSTRACT

Sago effluent gives negative effect to the environment. The effluent which is rich in starch and fibre increase the nutrient level in the water. These nutrients will be used by the algae which will proliferate and will lead to eutrophication. This will eventually increase the BOD and COD level which violate the minimum level set in the Environmental Act 1974. Various methods are used to treat this effluent, which include the use of rotary vacuum filters; synthetic flocculants (e.g. PAC, PAM) and bio-flocculants (e.g. PGA). Flocculation/coagulation method using polyglutamic acid (PGA) was employed to remove the solids from the sago effluent. From the experiments, it is found that flocculation of sago starch and fibre with PGA work at its best with the temperature of 30°C, pH 4 and PGA concentration at 0.1%.

Key words: Sago effluent, polyglutamic acid (PGA), solid flocculation

ABSTRAK

Sisa buangan sago memberi kesan negatif kepada alam sekitar. Sisa tersebut mengandungi banyak kanji dan serat yang akan meningkatkan paras nutrien dalam air. Nutrien ini akan digunakan oleh alga yang akan berkembang dengan pesat dan seterusnya akan menyebabkan eutrofikasi. Pelbagai cara digunakan untuk merawat sisa tersebut. Antaranya ialah penggunaan penapis vakum berputar, penggumpal sintetik (contohnya PAC dan PAM) dan penggumpal bio (contohnya PGA). Hasil daripada eksperimen yang dijalankan, didapati penggumpalan kanji dan serat sago dengan asid poliglutamik berfungsi secara berkesan pada suhu 30°C, pH 4 dan kepekatan PGA pada 0.1%.

Kata kunci: Sisa buangan sago, asid poliglutamik (PGA), penggumpalan pepejal

1. Introduction

Polyglutamic acid (PGA) was water soluble, non toxic substances that had been used in the food industry, cosmetics, medicines and wastewater treatment. Polyglutamic acid was produced by the *Bacillus subtilis*, which was the product of fermentation of “natto” or the fermented soya bean in Japan (Richard & Margaritis, 2003; Shih & Van, 2001).

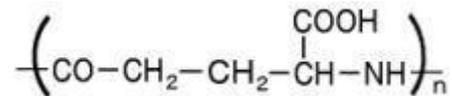


Figure 1: the structure of γ -polyglutamic acid (source: Nippon Poly-Glu Co., Ltd)

Wastewater especially the sago effluent contributed to serious problem to the environment. According to Adeni *et al.*, (2010), sago effluent contained high level of organic material, chemical oxygen demand (COD) and biochemical oxygen demand (BOD), which contravened to the standard limit stipulated in the Environmental Quality Act, 1974 (sewage and industrial effluents regulation, 1979). The microbial in the environment rapidly degraded the starch contained in the effluent. The acidity of the effluent made it difficult to be used as fertilizer for the crops. The objective of this research was to study the effectiveness of PGA in cleaning the wastewater, especially the sago effluent. To demonstrate the effectiveness of the PGA as flocculants, the effects of pH, temperature, PGA dosage and agitation on the flocculating activity of PGA was studied. The second objective was to find the optimum condition for the PGA in order to fully utilise the PGA for the removal of solids from the water, to be used as raw material for other process as the production of animal feedstock in further studies.

2. Literature review

In the field of water treatment, flocculants were important in improving the efficiency of solid removal and reducing the processing time in solid-liquid separation for both water purification and wastewater treatment.

2.1 Flocculating agents

At present there were already established methods using some synthetic flocculants and natural biodegradable polymers to treat wastewater. At present, aluminium derived flocculants such as aluminium sulphate; polyaluminium chloride (PAC) and synthetic polymer such as polyacrylamide (PAM) were widely used in coagulation treatment. The treatment by using PAM and PAC was relatively cheaper than using biodegradable polymers such as polyglutamic acid in terms of cost. The main disadvantage of using PAM was the effect to the environment and health. PAM itself was non-toxic but the end product of the procedure may produce acrylamide, which was neurotoxin to human and dangerous to the environment. PAC was also used to clean water, but there were some risks associated with its usage, such as the accumulation of the aluminium in the body will lead to Alzheimer's disease and breast cancer (Exley *et al.*, 2007; Graves *et al.*, 1990; Pan *et al.*, 2009). The suspended solid from both PAM and PAC were non-biodegradable and were deemed unsafe to the environment.

Reports were available on the production and application of bio-flocculants such as polysaccharide flocculants from *P. mirabilis* (Zhang & Zhang, 2010), and *Bacillus mucilaginosus* (Lian *et al.*, 2008), polyamide flocculants from *Bacillus licheniformis* (Shih & Van, 2001), *B. subtilis* DYU1 (Wu & Ye, 2007) and protein flocculants from *Bacillus sp.* DP-152 (Suh *et al.*, 1997). Among these bio-flocculants, poly- γ -glutamic acid (PGA) was

considered as the best because of its high yield, high flocculating activity and ability to flocculate a wide range of organic and inorganic compounds (Shih & Van, 2001). PGA was an unusual anionic and water-soluble substance, which was biodegradable, edible and non-toxic towards human and environment. The naturally-occurring homo-polyamide was consisting of D- and L-glutamic acid units which were connected by amide linkages between α -amino and γ -carboxyl groups. (Shih & Van, 2001)

2.2 Application of PGA

PGA had been used in various fields, such as biopolymer flocculants, heavy metal removal and drug carrier in medical field (Shih & Van, 2001). However, PGA was mostly used as the biopolymer flocculants, as it was more environmental-friendly and biodegradable. Reports had shown that poly- γ -glutamic acid produced by *Bacillus* sp. (e.g. *Bacillus subtilis* and *Bacillus licheniformis*) had high flocculating activity (Shih & Van, 2001). γ -PGA achieved the highest flocculating activity at the concentration of 20 mg/l in the kaolin suspension. The addition of metal cations, such as Ca^+ , Mg^{2+} , Fe^{2+} etc. increased the flocculating activity. Moreover, the high flocculating activity was high in the acidic pH range of 3.0-5.0, but decreased upon heating at 100°C (Shih & Van, 2001). These bio-flocculants could flocculate various inorganic (active carbon, acid clay, solid soil, calcium and magnesium compounds etc.), and organic (cellulose, yeast etc.) suspensions (Shih & Van, 2001). PGA was not only suitable for wastewater treatment, but also could be utilized in the processing of drinking water, food and fermentation industry (Shih & Van, 2001).

2.3 Efficiency of PGA

Water cleaned with PGA had a lower COD and BOD which made it safe to be released back to the environment or even for human consumption (Taniguchi *et al.*, 2005). According to Pan *et al.*, (2009), the flocculation efficiency of PGA was dosage-dependent. PGA also had a high flocculating activity, as PGA could absorb and bind to water 5000 times more than its own weight (Nippon Poly-Glu Co., 2005). Research had shown that PGA had the flocculation rate of 95.8% in the kaolin suspension, under the condition of which the amount of PGA used was 300 mg/L, pH 7, temperature at 25°C and the agitation at 350 rpm (Pan *et al.*, 2009).

Due to the sago effluent containing mainly fibre and starch, it was reasoned that these biomaterial could be flocculated by using PGA. It was financially challenging for small industries to invest in new and expensive technologies, therefore an effort to propose a simple method for the treatment of this wastewater by using the PGA as an alternative to the synthetic polymers to have the same results in terms of decreasing the COD and BOD.

3. Material and Methods

3.1 Material

The sago effluent was obtained from a sago mill in Pusa, Betong Division of Sarawak. The polyglutamic acid (PGA), commercially known as PG α 21Ca™, was obtained from Nippon Poly-Glu Co., Ltd, Osaka, Japan.

3.2 Filtration of sago effluent

The sago effluent was put in a 25 litre black jerry can and was stored in the cold room at 4°C before being processed. The sago effluent was filtered by using the U.S Standard Sieve Series, which can filter particles up to 63 microns. This was to remove the coarse sago starch and fibres. Then the filtered sago effluent was stored in a 5 litre bottle and stored in the cold room at 4°C before being used.

3.3 Jar testing method

The optimum working condition for the sago effluent was tested with jar test procedure, as described by Satterfield, (2005). Eleven 250 ml volumetric flasks were used and each flask was filled with 100 ml of sago effluent. The pH of the sago effluent was adjusted, ranging from pH 1 to pH 10, with one control. 100 mg of PGA was added to each of the samples and the samples were shook well. The samples were then allowed to flocculate for 15 minutes, before the samples were being filtered by using Advantec No. 1 filter paper. The filtered water was then tested for the OD. The results were recorded.

3.4 Bioreactor test

After the optimum pH was found, the next test involved the use of bioreactor where conditions such as pH, temperature, agitation and volume of sago effluent could be controlled and measured. Biostat B bioreactor was used in this experiment. The volume of sago effluent was fixed at 1.5 litres. The temperature was adjusted to 25-35°C, with an increase of 5°C. As for the pH, pH 4-pH 6 was used with the increase of 1 unit. Agitation of sago effluent was done at 200 rpm for 15 minutes to homogenize the content before the addition of PGA. Various amount of PGA was added to the sago effluent, where the mixture was agitated at 200 rpm for 1 minute, followed by continuous agitation of 80 rpm for 15 minutes. At the end of agitation, the content was allowed to flocculate and sedimentation happened for 15 minutes.

3.5 Optical density

Some supernatant were taken after flocculation and tested for the optical density (OD). Libra S12 spectrophotometer was used to determine the OD of the cleaned sample. Distilled water was used as the blank, with the wavelength set at 420 nm.

3.6 Treatment of flocculates

Treated sago effluent was then put in 500 ml centrifuge bottle and was centrifuged with Hitachi himac CR22GIII refrigerated centrifuge at 8000 rpm for about 15 minutes. Then the supernatant was discarded and the precipitate was removed and dried for further study.

4. Results and Discussion

4.1 Effect of pH on flocculating activity

From the jar testing method, the effective working range of PGA was pH 4-pH 7. The original pH of the sago effluent ranges from pH 3.5-pH 4.5. After the addition of PGA into the sago effluent of pH 4, there was slight increase in pH, where the pH 5 was detected.

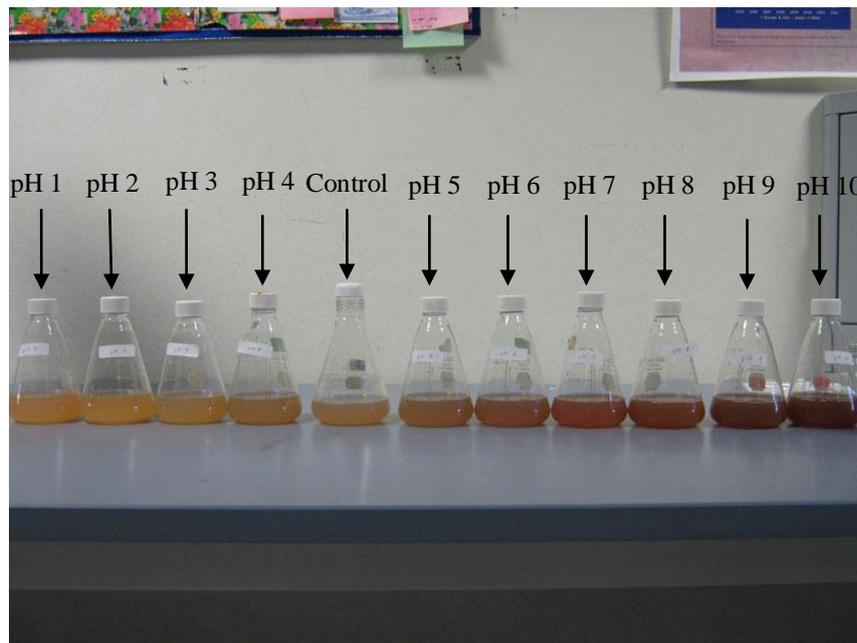


Figure 2: Differences in colour caused by pH before the addition of PGA (from left to right: pH 1 until pH 10, with control at fifth left)

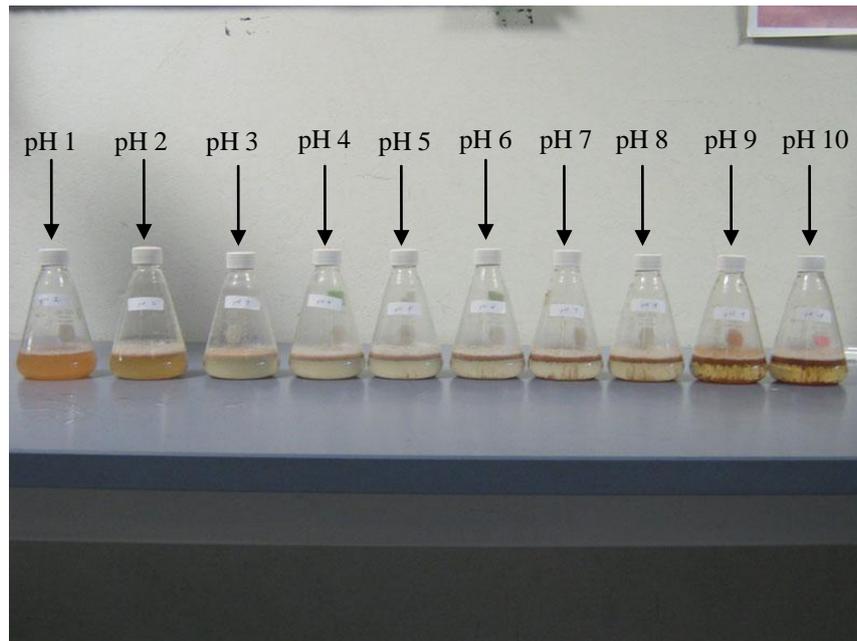


Figure 3: Flocculation activity after the addition of PGA (from left to right: pH 1- pH 10)

Figure 3 showed the changes in flocculation activity, which could be seen clearly starting from pH 3 onwards. Colour removal of the sago effluent could also be visually seen as the water became clear with the separation of the clear water from the flocculates. The colour of the effluent was caused by the residual compounds from the debarking process, where these compounds consist of lignin and cellulose. PGA particles bind with the colour compounds in the effluent and formed complex molecules, which were easily removed from the water. The sago palm possessed around 1 % of phenolic compounds (Pei-Lang *et al.*, 2006). During the transportation and processing of the samples, the effluent of the sago mills started to develop a brownish colour due to the oxidation of these compounds. Somehow, the presence and removal of these coloured compounds by PGA could be observed by visual inspection and probed by spectrophotometer by measuring OD. When the components of the effluent became oxidized it was easier to flocculate them by PGA.

Table 1: pH and absorbance values before and after the addition of PGA

	pH	OD ₄₂₀
Before addition of PGA	5.65	1.983
	Control	0.044
	1	0.232
	2	0.230
	3	0.174
After addition of PGA	4	0.251
	5	0.095
	6	0.023
	7	0.043
	8	0.034
	9	0.100
	10	0.097

Table 1 showed the results of the jar testing method. The addition of PGA decreased the absorbance value of the effluent. The OD decreased in the acidic pH range of 1-6 with the exception of pH 4, but increased in the alkaline pH range of 7-10, with the exception of pH 8.

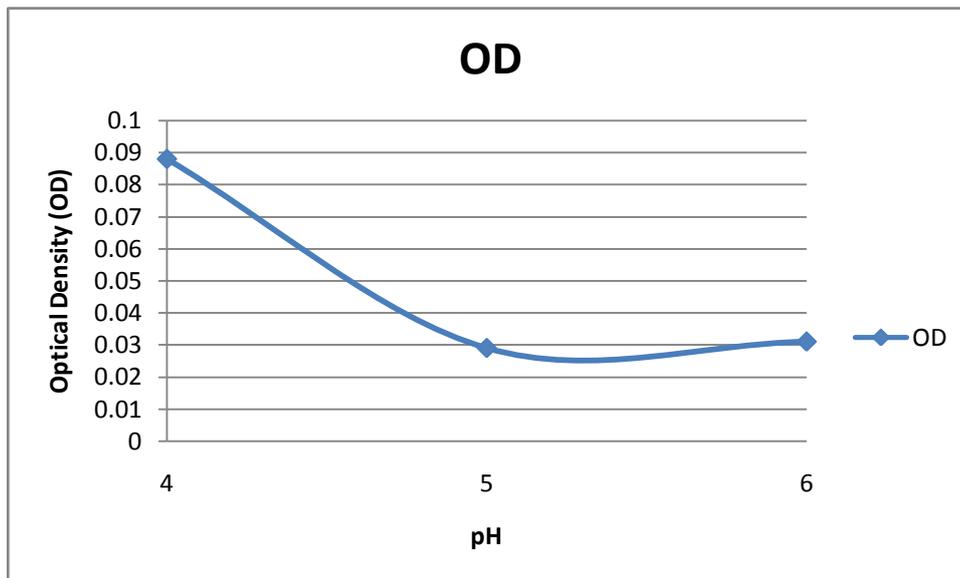


Figure 4: Effect of pH on flocculating activity

In **Figure 4**, the tested parameter of the pH showed that higher pH lowers the turbidity of the flocculates. The possible explanation was PGA showed different electric states at different pH, which in turn affects the surface electric property of sago effluent. PGA was easily absorbed into the effluent when the electrostatic repulsion was lower than the electrostatic attraction between the PGA particles and the effluent particles (Pan *et al.*, 2009). The destabilization of the effluent particles by the PGA caused the reduction of surface charges of the effluent, which was responsible for the particles repulsion. This reaction in turn caused the flocculation of the effluent particles. This flocculation made it easier for the solids to be removed by sedimentation, filtration and centrifugation.

4.2 Effect of temperature on flocculating activity

The suitable temperature range for PGA was 25-40°C. Lower flocculation activity happened when the temperature was below 20°C, while higher temperature of above 50°C caused the PGA to stop flocculating. Flocculating efficiency of PGA increased with the tested temperature, which ranges from 25-35°C, but decreased when the temperature rose above 60°C. Similar results were also reported by Pan *et al.*, (2009). According to Pan *et al.*, (2009), this condition could be explained by chemical kinetics. At higher temperature, the suspended solids moved faster and the collision frequency was increased, which lead to the increase in the rate of reaction. However, when the temperature was too high, although the reaction increased, the flocculates were too small and had stronger hydrating trend. The flocculates were difficult to be separated by precipitation. As for the lower temperature, the reaction slowed down and the flocculants were susceptible to the increase of shear intensity of water, therefore made the flocculates too small to be separated by precipitation. Since the original temperature of sago effluent ranges between 28-35°C, there was no such need to adjust the temperature as this temperature was suitable for the flocculation to happen. This process was beneficial to the small and medium sago mill shareholders, as they were not required to invest in heating equipments to optimize the flocculation process. These will eventually reduce the operational cost and increase the revenue, besides helping to reduce the pollution to the waterway.

Table 2: Effect of temperature on absorbance value after the addition of PGA

Temperature (°C)	OD ₄₂₀
25	0.110
30	0.004
35	0.062

4.3 Effect of doses of PGA used on flocculating activity

The PGA used in the experiment was recommended at 1 g/l. Lower amount of PGA at 500 mg/l was possible but with little flocculation. Larger doses of PGA at more than 1 g/l flocculate better but uneconomical in terms of cost. The amount of PGA used correlates with the storage period of the sago effluent. Fresh samples required only small amount of PGA in order to flocculate the sago fibre and starch. Older samples require larger amount of PGA. Microbial activity in the older sago effluent reduces the efficiency of PGA to bind to the sago fibre and starch.

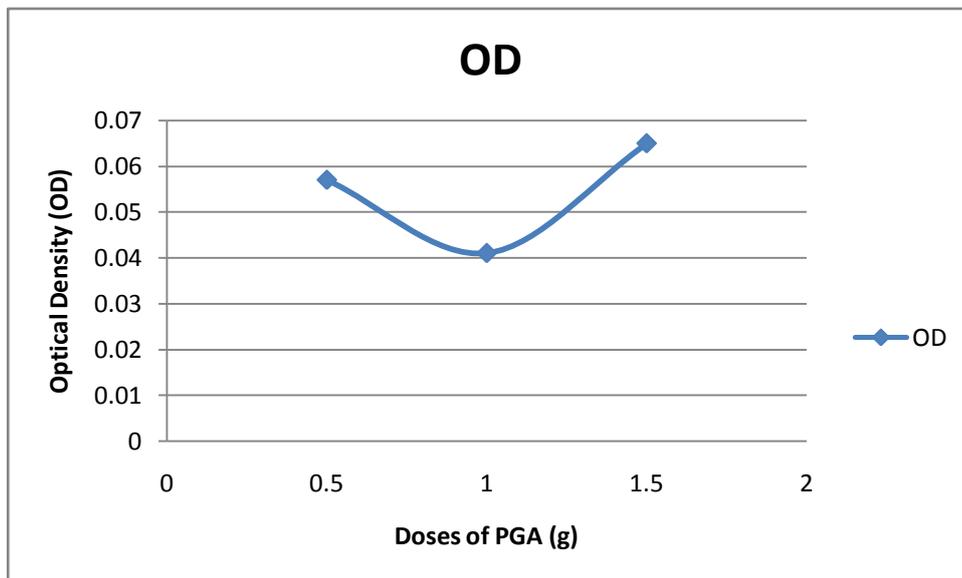


Figure 5: Effect of PGA doses on flocculating activity

Figure 5 showed the decrease of OD when the dosage was increased from 0.5 g to 1 g. However, the OD increased when the dosage was increased from 1 g to 1.5 g. The possible reason for this condition was the ability of PGA to flocculate sago fibre and starch increased when there were more effluent particles than the PGA particles, but decreased when the PGA particles were more than the effluent particles. Interaction between the

PGA and the residual organic compounds of the effluent, which consist mostly of polysaccharides (starch), was caused by the large active surface areas of the PGA. This reaction enabled PGA to absorb the polysaccharides and form complex compound, thus remove the solid compounds from the liquid during the flocculation phase.

4.4 Effect of agitation on flocculating activity

Initial agitation of 200 rpm was used in the mixing phase for about 1 minute, before the speed was reduced to 80 rpm for 15 minutes in the reaction phase to enable complete mixture. Then the agitation was stopped to allow flocculation and sedimentation of flocculates. PGA worked by principle of the charge on the molecules. A net negative charge in the PGA molecules attracted the positively-charged sago fibre and starch molecules. Agitation of the mixture maximized the flocculation activity of the sago fibre and starch, as the agitation opened up the PGA molecules and increased the surface area of the PGA for the attachment of the sago fibre and starch. Rapid stirring of the mixture in a short time allowed the PGA to be uniformly mixed with the effluent during the mixing phase. Then slower stirring at a longer time was applied to the mixture to allow flocculation of the flocculants and the impurities, and to prevent desorption of the flocculates during the reaction phase.

$$\text{Flocculation efficiency (\%)} = \frac{\text{OD}_i - \text{OD}_f}{\text{OD}_i} \times 100$$

Where

OD_i = the initial optical density of the sample

OD_f = the optical density after the removal of solids by PGA

$$\begin{aligned} \text{Flocculation efficiency (\%)} &= \frac{\text{OD}_i - \text{OD}_f}{\text{OD}_i} \times 100 \\ &= \frac{0.389 - 0.072}{0.389} \times 100\% \\ &= 81.49\% \end{aligned}$$

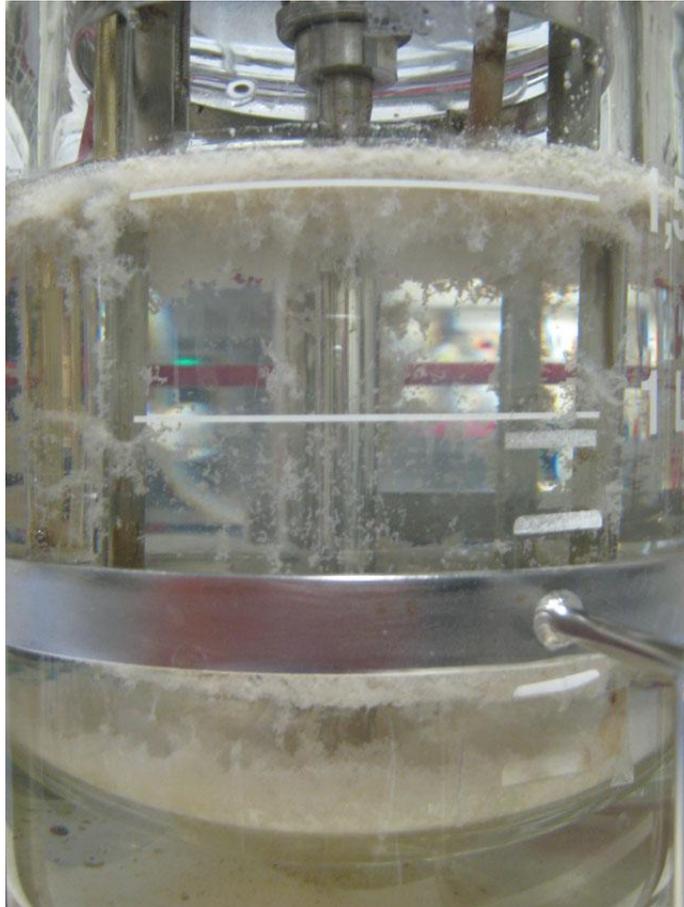


Figure 6: 2L B. Braun bioreactor was used to flocculate the solids of the effluent which consist of sago fibre and starch

Figure 6 showed the formation of three layers after the agitation. These layers consist of starch at the bottom layer, cleared water in the middle layer and the fibre at the top layer.

5. Conclusion

The recommended solution to clean the sago effluent with PGA is by using 0.1% (w/w) of PGA, pH 4, temperature at 30°C and agitation at 200 rpm for 1 minute, followed by agitation at 80 rpm for 15 minutes. The pH 4 and temperature of 30°C match the original condition of the sago effluent, by which no adjustment was necessary for the flocculation to happen. PGA would be useful for the wastewater treatment, as it was biodegradable and safe for the environment. Further study on the obtained solids flocculated by PGA could be done to determine the suitability of the solids as the animal feeds and as organic fertilizers.