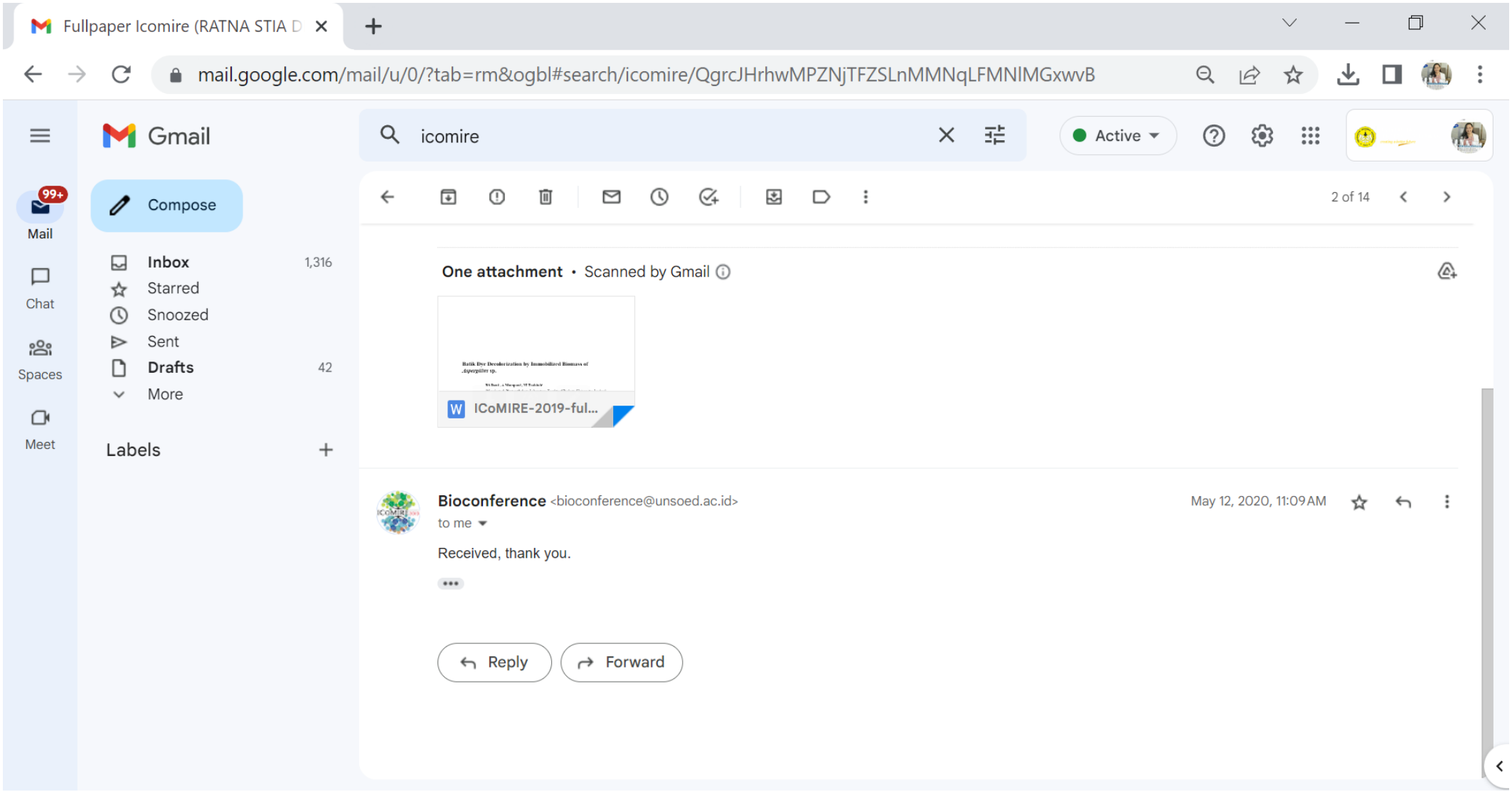
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Batik Dye Decolorization by Immobilized Biomass of *Aspergillus* sp.

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Abstract. The rapidity of the batik industry in the Former Residency of Banyumas raises problems. Wastewater that discharged into upstream causes accumulation in downstream up to empties into the sea, so does not only threatens the river ecosystem but also has the potential to cause problems in the mangrove ecosystem in Segara Anakan (the downstream area of the Citanduy River which is very close to the downstream area of the Serayu River Basin). The strategy of batik wastewater management in an effort not to pollute the environment is offered. This study tried indigosol blue batik dye which are often used in the batik industry by using immobilization of the pure fungi biomass compared to biomass which was induced with tanic acid. Combination between incubation time and shaking treatment in immobilization of pure *Aspergillus* sp. biomass that capable to decolorize indigosol blue batik dye were also done. Data of degradation percentage was analyzed using spectrophotometry. The decolorization result of comparison between biomass of *Aspergillus* sp. with or without the addition of tannic acid under agitated treatment conditions at 24, 48, 72 hours were 54.7, 75.8, 77.4% and 78.8, 84, 80.1%, respectively. While the result of treatment under static conditions were 65.4, 86.3, 73.7% and 79.2, 78.6, 68.2, respectively. The next experiment is showed that 48th hour on static treatment have the highest decolorization percentage up to 67.1%. pH was measured before and after treatment. pH after treatment were decrease both in static and shaking treatment, and also in control treatment both in static and saker treatment. Based on the results of the research, the fact is the use of immobilization biomass of *Aspergillus* sp. in the form of alginate beads can be use for decolorization application in the environment.

1. Introduction

Indigo dye has a specific blue color and known as one of the oldest dyestuffs [1]. At several textile industries include batik industry, the intensive use of indigo blue dyes and production is a serious challenge of water treatment. At those locations, the deep blue water color from dye is a strongly indicating sign of water pollution and has to be removed in order to avoid the common strong public rejection. Although usually ignored, the presence of small amounts of any dye in water is later causes serious problems to both local fauna and flora. Color removal from textile effluents is often considered to be more urgent than any other contaminant of the same effluent.

There are several data that showing a correlation between the decolorization role by *Aspergillus* with the removal of dyes and a consequent improvement in water quality. According to Jin *et al*. (2007), degradation of dyes is difficult due to their complex structure. Currently, various chemical, physical and biological treatment methods are used for decolorization process. The high cost and disposal problems cause most of the chemical and physical methods for water treatment were not widely applied in the textile industries. As stated by Kang *et al.* (2018), dyes could be removed efficiently by *Aspergillus* sp. using the biodegradation properties of enzymes and the biosorption capacity of mycelia. Biosorption may dominate the decolorization system.

Determine optimum condition before obtaining the efficiency of degradation by fungus in large quantities is necessary. As stated by Ali & Muhammad (2008), the degradation of dyestuff using fungi is influenced by factors such as pH, mold concentration, incubation time and ambient temperature. The incubation time also influences the degradation process of textile waste such as dye due to the influence of the duration of contact time of the fungus with the dye.

The longer incubation time, the absorbed dyestuff will also be greater. This happens because the longer incubation time, then the contact between the sorbent with the dyestuff will also last long and the probability to absorb the dye is bigger. In the other words the dye that absorbed will be much more, but at any given time the adsorption process will be stop or absorption rate will be decreases if the sorbent is at the critical point of its absorption. According to Purnama & Setiati (2004), variation of incubation time greatly affect the process of decolorization, the longer incubation time so the process of absorption will be more effective. The incubation time has an effect on the adsorption in the process of decolorization to the dye solution.

Only nontoxic end products of dye degradation which are considered to be acceptable and nonharmful to the environment. For successful detoxification, the native biomass needs to be immobilized to improve its mechanical strength and resistance to the various chemical constituents. As stated by Goksungup *et al.* (2002), immobilization of biomass can increase particle strength, density, porosity and chemical resistance of biomass. Immobilization of biomass can also be extracted and can increase the ability of biomass adsorption.

Immobilization is a modification to imitate its original state in nature which is believed to be in a state bound to membranes or particles in cells. cell immobilization has the advantage of being able to be reused (Zaborsky, 1973). Alginate beads as immobilization technique is mostly used due to its simplicity, permeable, and nontoxic, so it can simplify the decolorization process (Shuler & Kargi, 1992).

Shaking the flask can cause the liquid to wash up the sides of the flask, which, when it done at the proper speed, it will creates more liquid surface area. In increasing the surface area exposed to the air, it increase the oxygen transfer to the liquid. Mentioned by Bates *et al.* (2016), aeration is one of the character which controlled by the shaker. In order to provide oxygen, when shaking bacteria in a flask it is mostly concerned with the surface area of the liquid because the interface is where the oxygen dissolves.

The objectives of this study are to determine that combination between incubation time and shaking treatment in immobilization of *Aspergillus* sp. capable to decolorize indigosol blue dye and to determine which combination that more optimum for decolorization of indigosol blue dye.

2. Methods

The isolate that used was *Aspergillus* sp. which isolated from batik waste disposal sites in the Sokaraja batik home industry (Dewi et al., 2018, 2019), Potato Dextrose Agar medium, Potato Dextrose Broth medium, tween 80, Natrium alginate 4%, $CaCl_2$ 0,2M, alcohol 70%, indigosol blue dye, and distilled water.

Tools made of glass material was sterilized first. Sterilization was done using autoclave at 121°C with 2 atm pressure for 20 minutes. Tools made of metal was sterilized using 70% alcohol and bunsen burner.

Potato Dextrose Agar (PDA) medium was made by 200g potatoes, 15g agar, and 20g dextrose. Distilled water as much as 500 ml was added by 200g of sliced potatoes, then it was boiled until half cooked. The potato extract was added with dextrose and agar, and mixed well. Distilled water was added up to 1000 ml. Sterilization was performed with autoclave at 121°C with 2 atm for 15 minutes.

2.1. Rejuvenation of Fungus Isolate

The fungus isolates that use was rejuvenated into petri dish that contain PDA medium, then it was incubated for 7 days at room temperature.

2.2. Preparation of Spore Suspension

Slanted medium in reaction tube was prepared as much as 10ml. One plug (5 mm) of isolate was inoculated and incubated for 7 days. At the end of incubation, sterile distilled water added with tween 80 1% was added into reaction tube to moult the spore. Spore was counted with haemocytometer under microscope until it get 10^8 spore suspension.

2.3. Production of Biomass

Erlenmeyers flask which contain Potato Dextrose Broth (PDB) medium was prepared as much as 100 ml each flask and added with spore suspension as much as 100µl, then it was incubated for 7 days at room temperature on shaker. As a comparison, spores are inoculated in PDB medium added with tannic acid. At the end of incubation time, biomass was separated from medium by filtration with filtration paper, then biomass was the result.

2.4. Immobilization of Biomass (Mishra et al., 2015)

Biomass that growed on the PDB medium were harvested separately from the medium then immobilized. Natrium alginate 4% was added into 100ml boiled sterile distilled water then biomass isolate was added and mixed. The making of beads was done with pipete by dripping the mixture little by little into CaCl2 solution. Beads was soak in CaCl2 solution for 30 minutes, then washed with sterile distilled water as much as 2 times. Control was made by dripping the mixture of boiled distilled water and natrium alginate 4% into CaCl2 solution.

2.5. Preparation of Indigosol Blue Dye (Suparno, 2010)

Indigosol blue dye solution was made by mesuring 0,1g indigosol blue dye, then added with 1L sterile distilled water. Indigosol blue dye solution was dried uder the sunlight for 30 minutes. This solution called indigosol blue dye solution 100 ppm.

2.6. Decolorization Test

The first stage of decolorization was to compare the biomass of *Aspergillus* sp. on PDB medium with or without the addition of tannic acid (carried out at 24, 48, 72 hours) under agitated conditions. The second stage of decolorization was to compare the biomass of immobilized *Aspergillus* sp. under agitated and static conditions at different incubations without the addition of tannic acid (at 48, 72, and 96 hours).

Biomass of *Aspergillus* sp. / beads were added into indigosol blue dye solution. It was treated with shaker and static, then incubated for different hours. On second stage of decolorization, every treatment was added by 20g beads for 50 ml indigosol blue dye solution (Hasnan, 2014). All decolorization treatment were filtered with filter paper and was count with spectrophotometer with 604,5 nm maximum wavelength.

Decreasing of color was the sign of decolorization process. The decolorization process can be expressed by:

$$decolorization\ (\%) = \frac{OD\ 0\ hour - OD\ t\ hour}{OD\ 0\ hour} \times 100\% \qquad (3\text{-}1)$$

2.7. pH and Temperature Measurement

pH was measured using pH meter digital before and after treatment. Prior to the pH measurement, the pH meter is calibrated with a pH buffer solution (4 and 9). The electrodes was dried with a tissue then dipped in distilled water and dried back with a tissue. The pH measurement was performed by dipping the electrode into the treatment bottle until it showed a constant value.

The temperature wll be measured before and after the treatment, by dipping the thermometer into the sample for several minutes until indicating a constant number.

3. Results

Indigosol blue dye has a maximum wavelength of 604.5nm which will be used to calculate the decolorization percentage by alginate biomass beads. Percentage of decolorization indigosol blue dye using biomass of *Aspergillus* sp., with and without addition of tannic acid, can be seen in Figure 1. Tanic acid used in this study is intended to induce ligninolytic enzymes. At different incubations (24, 48, 72 hours), all treatments were shown that the addition of tannic acid had a lower decolorization (54.7, 75.8, 77.4%) than without the addition of tannic acid (78.8, 84, 80.1%).

The percentage of decolorization in indigosol blue liquid dye with 100ppm concentration using immobilized *Aspergillus* sp. under shaker and static treatment, can be seen in Figure 2. Based on Figure 2, the percentage of decolorization of indigosol blue dy by *Aspergillus* sp. and natrium alginate at incubation times of 48th hours, 72nd hours, and 96th hours on shaker, respectively are 37,2%; 65,2%; and 43,8%. The percentage of decolorization of indigosol blue dye by Aspergillus sp. and natrium alginate at incubation times of 48th hours, 72nd hours, and 96th hours on static, respectively are 66,5%; 44,9%; and 58,5%. The percentage of decolorization of idigosol blue dye by control treatment on shaker at incubation times of 48th hours, 72nd hours, and 96th hours, respectively are 44,5%; 42,3%; and 47,6%. The percentage of decolorization of idigosol blue dye by control treatment on static at incubation times of 48th hours, 72nd hours, and 96th hours, respectively are 51,5%; 54%; and 52,6%.

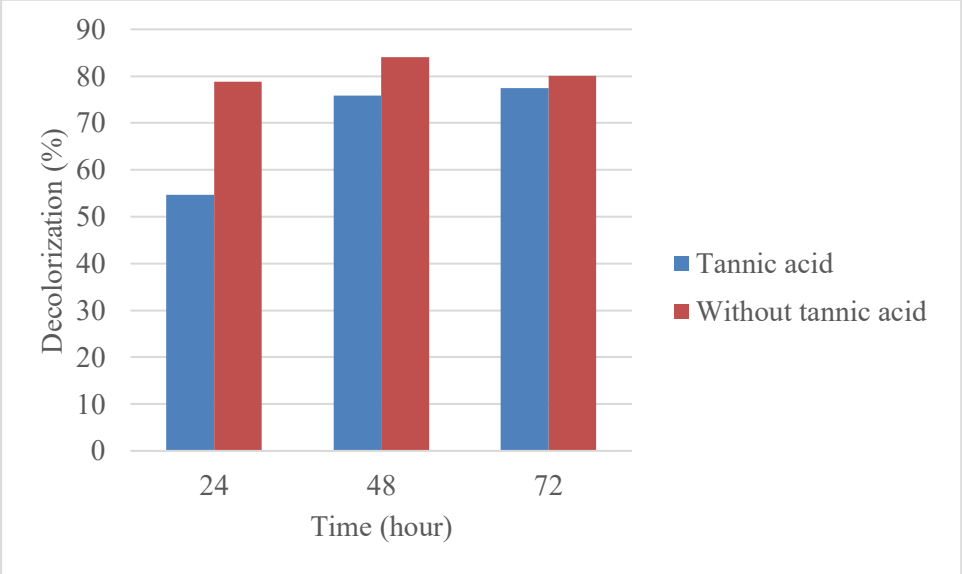

Figure 1. Percentage of decolorization indigosol blue dye using biomass of *Aspergillus* sp., with and without addition of tannic acid.

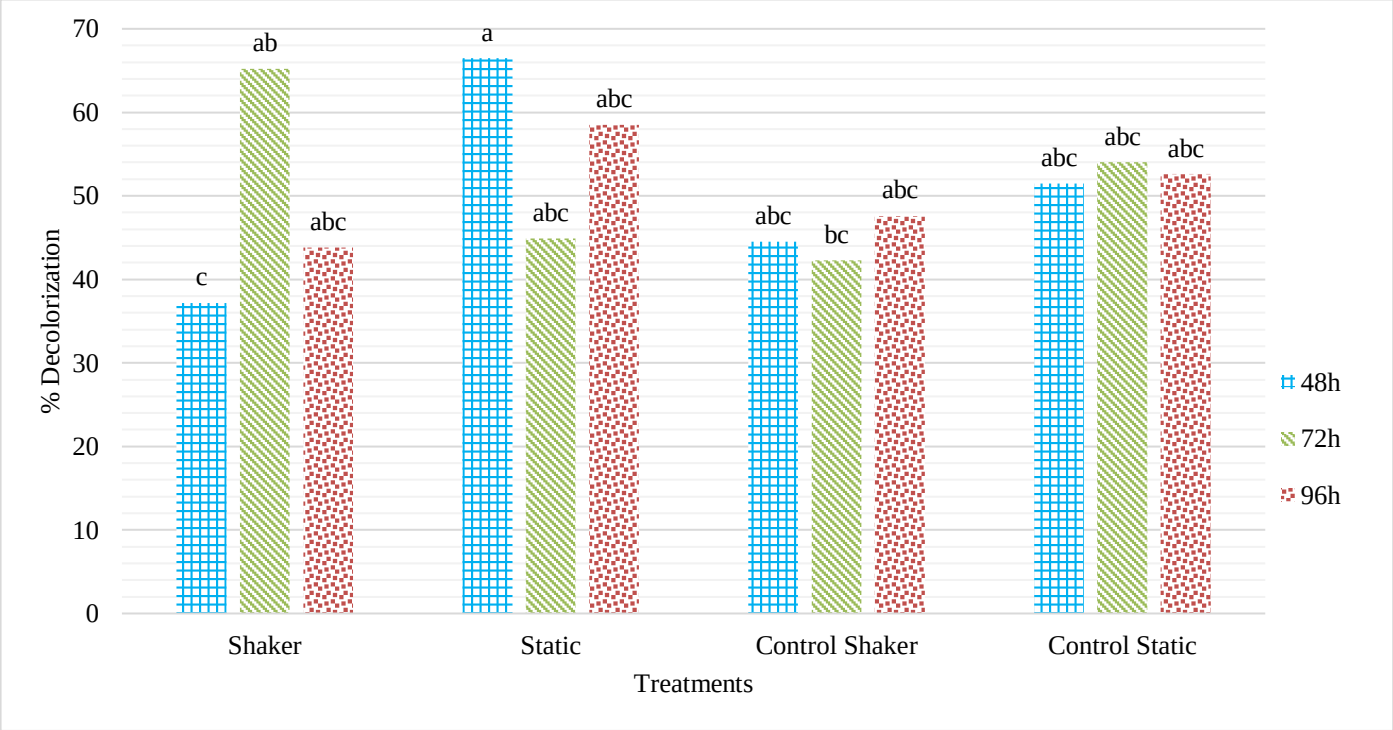

Figure 2. Percentage of decolorization indigosol blue dye using immobilized *Aspergillus* sp. under shaker and static treatment

Table 1. Data of pH value of indigosol blue dye before and after treatment

Replication		pH (Before)			pH (After)		
		Treatment			Treatment		
		48h	72h	96h	48h	72h	96h
SH	1	6,80	6,80	6,80	6,21	5,88	5,79
	2	6,80	6,80	6,80	6,07	5,71	6,65
	3	6,80	6,80	6,80	5,94	5,64	5,56
SC	1	6,80	6,80	6,80	5,54	5,61	5,67
	2	6,80	6,80	6,80	5,50	5,60	5,62
	3	6,80	6,80	6,80	5,64	5,61	5,66
Control SH	1	6,80	6,80	6,80	5,63	5,66	5,65
	2	6,80	6,80	6,80	5,62	5,66	5,66
	3	6,80	6,80	6,80	5,64	5,63	5,63
Control SC	1	6,80	6,80	6,80	5,66	5,73	6,00
	2	6,80	6,80	6,80	5,67	5,70	6,10
	3	6,80	6,80	6,80	5,67	5,72	5,98

4. Discussion

All treatments were shown in Figure 1 described that the addition of tannic acid had a lower decolorization than without the addition of tannic acid at different incubations, 54.7, 75.8, 77.4% and

78.8, 84, 80.1, respectively. This showed that the addition of tannic acid did not affect the decolorization of the dye. The results informed that the addition of tannic acid did not positively correlate to decolorization.

Figure 2 showed that the largest decolorization occurred at the 48th hours observation with a static treatment which was equal to 66,5%. The use of different incubation times in this study was to determine the time that needed by *Aspergillus* sp. and natrium alginate to decolorize indigosol blue dye. The longer incubation time, then the duration of contact between fungal and the dye also longer. According to Novak et al. (2001), the duration of contact between fungal mycelium and dyestuffs affects the activity of fungi in remodelling the dyestuff.

Based on Figur 2 after further statistical analysis, showed that the best treatment occurred was in the static treatment with 48th hours incubation time. Then the worst treatment occured was in the shaker treatment with 48th hours incubation time. Decolorization potency at 48th hours experienced a very high increase, this was likely due to the absorption of the dye into the beads during the time of application. This observation is similar to that obtain by Hasnan (2014), the data was occur because at the beginning of the incubation time, the fungus was at its greatest absorption period. At the next incubation time there was a decrease in absorption ability because the fungus has been at the saturation point of absorption.

In this study, the condition of dyes in shaker became increasingly turbid over time, while the dye condition of static treatment did not experiene turbidity. It was assumed that this occurs because of the shaking treatment cause strong movement of the beads which can cause damage to the beads. In control treatment using alginate without fungi, either in shaker or static treatment the dye does not become turbid. This can be happen because the control beads doesn't contain fungi. Similar result were also obtained by Hasnan (2014) who found that turbidity of dye caused by damage of beads due to shaker treatment. The more biomass that wasn't bound in the beads matrix can increase the absorbance value at the time of observation using spectrophotometer. Damage to the beads can increase the absorbance value because the solids molecules of beads was dissolved into the dye, or the dye that has been absorbed into the beads was dissolved again.

The used of *Aspergillus* sp. and natrium alginate for decolorization was efficient both in static or shaker treatment, but the result showed that the static treatment give better result than the shaker treatment. Knapp et al. (1995) and Revankar & Lele (2007) also found that the decolorization of dye in static treatment was mainly due to the sorption of the dyes, while no adsorption of dye was seen in shaker treatment and that indicates that the dye removal was attributed to dye biosorption.

From the data showed that the use of alginate beads without fungi can be used for the decolorization process. Alginate beads which combined with both static and shaker treatment can be carried out in the decolorization process. The data also showed that static treatment had better result for decolorization. Suvachittanont & Pookingdo (2013) also reported that this was considered to be occur because beads have a considerable absorption ability. Beads alginate has a highly porous structure, the same particle size distribution, and also a high surface area which influences its adsorption ability.

Reported by de-Bashan & Bashan (2010) and Mallick (2002), there are various immobilization processes that use these days, such as adsorption, capturing with semipermeable membranes, and entrapment within polymers. Godlewska-Zylkiewicz (2003) found that among other processes, the most common process of immobilization is entrapment of cells in the polymer matrix, which the cell is covered with a polymer matrix. As stated by de-Bashan & Bashan (2010), both synthetic and natural polymers can be applied to the matrix entrapment immobilization process. Along with research by Hameed & Ebrahim (2007) and Liu et al. (2009), reported that matrix entrapment is one of the most common immobilization methods that consists of trapping cells in a three dimensional gel, which made of either natural (agar, cellulose, alginate, carrageenan) or synthetic (polyacrylamide, polyurethane, polyvinyl, polypropylene) polymers. Leenen et al. (1996) also reported that natural polymers have higher diffusion rates and more environmentally friendly rather than synthetics polymers. Lee et al., (1991) found that immobilization with beads method has structure that is more like a semipermeable membrane that allows materials with low molecular weight and water-soluble materials diffused in and

out of the beads. The absorbed cell cannot get out of the beads, while the substrate can still enter the beads. As stated by Risch (1995), cells that are absorbed using alginate continue to experience growth even in limited terms. This can be seen visually by the more murky beads.

Other factor that might affect decolorization was temperature. Temperature measurements was done before and after treatment. In this study there was no change in temperature because all treatments were carried out in the same place. Knapp et al. (2001) observed that the optimum temperature was around 27°C – 30°C. The optimal temperature for enzyme reactions is usually higher, but the enzyme becomes unstable and degrades when the temperature is too high close to 50°C. Erum & Safia (2011), reported that the optimum temperature for reducing dyes was 25°C – 35°C.

Other factor that might affect decolorization was pH. Observation of pH changes was done to determine the effect of dye decolorization with changes in pH. Observations were made by calculating the pH of dye before and after incubation with the addition of beads. Lim (1998) found that generally the fungus will grow in a fairly wide pH range of 4.5-8.0 with an optimum pH between 5.5-7.5 or depending on the type of fungus. Along with Martiani et al. (2010) research which reported that decolorization was decrease along with the increasing of pH. Murad et al. (2017) also reported that the highest of immobilization percentage was at pH 6. This can be relate to the optimum pH for fungal inoculum growth in range 5 - 6. The data of pH value of indigosol blue dye before and after treatment can be seen on Table 1.

Table 1 showed that there was decrease number of pH after treatment both in static and shaker treatment, and also in control treatment both in static and saker treatment. There was no significant difference between each of the data. As mentioned by Singh (2006), changes in pH occur because of the accumulation of organic acids, namely vanillic acid and formic acid that formed by the activity of fungal biomass. According to Crawford's research (1981) which states that the decreasing in pH is caused by the decomposition of dyes into simple compounds with lower molecular weight, such as the formation of organic acids.

In this study, the application of 20g beads for 50ml dye was applied to optimize the movement of beads during shaking treatment. Shaking treatment is done to make sure that the liquid dye is always homogeneous and also for aeration during the application. According to Rohmah (2011), the aeration system in the liquid medium accelerates the degradation process, compared to solid medium culture. Giving aeration can increase the amount and transfer of oxygen between cells and media, which is optimal for extracellular oxidative enzymes in fungi. Optimal extracellular enzymes produced can increase degradation activity.

Based on the data obtained in this study indicate that the hypothesis was opposed against the results. This was happened because the shaking treatment cause damage to the beads which affects the absorbance value. Solids molecules of beads that dissolved into the dye cause increasing of absorbance value.

5. Conclusion

Based on the research result, the conclusion of this research were addition of tannic acid did not affect the dye decolorization, combination between incubation time and shaking treatment in immobilization of *Aspergillus* sp. were capable to decolorize indigosol blue dye. Combination between static and 48 hours incubation time is the most optimum combination for decolorization of indigosol blue dye.

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