

Production of Bioflocculant by *Chryseomonas Luteola* and Its Application in Dye Wastewater Treatment

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Abstract

Inorganic aluminum salts as one of coagulation and flocculation reagent have been generally used for removing the impurities in water and waste water technology. In last a few years, was found that aluminium compounds have been reported as disease carrier for human being. In general, dyes wastewaters with concentrations in the range of 10 - 200 mg/L will be appearing at highly coloured. There are many reports which are mention about the toxic effects of dyes and metals in the form of carcinogenic, genetic effects. Furthermore, direct release of dyes wastewater into the environment is not encouraged due to the high values of un-degradable and degradable organics substances. In this study a bioflocculant was produced from palm oil mill effluent (POME) isolated and found as *Chryseomonas luteola*. The experiments conduct at optimized culture conditions (at temperature 50°C, duration 1 day), a flocculating activity of 96.15% was demonstrated using kaolin suspension. The result was also showed to be better for flocculation of a kaolin suspension over pH (5-7) and cations (Ca²⁺) will enhance the flocculating activity. The bioflocculant can be used for treating dye wastewater, and the maximal removal efficiency of turbidity and chemical oxygen demand (COD) were 38.22% and 33.25%, respectively at pH 7.0 with dosage of culture broth bacteria about 0.2 mL. Besides that, the reduction of turbidity and removal of COD of dye wastewater were conducted using aluminium sulphate (alum). A maximum reduction of turbidity was 97.51% while the removal of COD was 99.64%, were observed with a concentration of 600 mg/L at pH 4.0.

Keywords: bioflocculant, chryseomonas lutela, flocculation, coagulation, dye wastewater

1. Introduction

Flocculants have been widely used in wastewater treatment, food and fermentation industries, drinking-water treatment, and industrial downstream processing (Wu & Ye, 2007). Flocculating agents can be classified into three groups: (1) inorganic flocculants, such as aluminum compounds (aluminum sulphate, alum and polyaluminium chloride, PAC); (2) organic synthetic flocculants, such as polyacrylamide derivatives and polyethylene imine; (3) naturally occurring flocculants, such as chitosan, sodium alginate and bioflocculant (Salehizadeh & Shojaosadati, 2001; Zhang et al., 2007). Although the synthetic organic flocculants are the most frequently used because of their cost-effectiveness, they are not readily biodegradable and some of their degraded monomers such as acrylamide are neurotoxic and even show strong human carcinogenic potential effects (Shih et al., 2001).

Textile factories used to apply large amounts of water and chemicals for finishing and dying processes. The chemical structures of dyes vary enormously, and some have complicated compounds of aromatic structures which are difficult to degrade in nature and conventional wastewater treatment processes because of their stability to sunlight, oxidizing agents, and microorganisms (Chu, 2001). The removal of dyes from industrial wastewater effluent is a major concern in the textile industry, with the increase in stringent legislation. Dye wastewater usually content many types of contaminants, including acids, bases, dissolved organic and inorganic materials, toxic compounds, and colour. Colour is esthetically the most concern pollutant even at very low

concentrations, and it needs to be removed or decolorized before the wastewater can be released to the nature stream.

Moreover, direct discharge of POME into the environment is not allowed due to the high concentrations of un-degradable and degradable organic (COD and BOD). Furthermore, with the introduction of effluent discharge standards imposed by the Department of Environment in Malaysia, POME has to be treated before being released into the environment (MDC, 2006). Thus, the reuse of POME was important to reduce the discharge of POME. Previously, research has been carried out to use palm oil mill effluent (POME) sludge as an inoculum. However, the cultivation of a single strain using POME as a substrate has not yet been studied in detail. In this study, the isolation of a microorganism producing a new biopolymer flocculant from POME was accomplished and characteristics of the biopolymer flocculant in terms of flocculating activity were investigated and the flocculation properties of this polymer was studied for further application.

2. Methodologies

2.1 Palm Oil Mill Effluent (POME) Sampling and Characterization

POME sample was taken from Malpom Industries Sdn. Bhd. located in Sungai Bakap, Malaysia. Sample was collected from the direct discharge after the production line. Sample collection is performed using the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA & WEF, 2005). The collected sample was stored at 4°C. The sample was immediately right after the sample arrived in the laboratory. Table 1 shows the characteristics of sample determined based on the Standard Methods (APHA, AWWA, & WEF, 2005).

Table 1. Characteristics of POME from Malpom Industries Sdn. Bhd.

Parameter	Range	Mean
pH	4.10 - 4.20	4.16
Turbidity (1:100 dilutions) (NTU)	95.3 - 96.2	95.8
TSS (mg/L)	13640 - 13675	13657
COD (mg/L)	67550 - 76050	72780

2.2 Media and Culture Conditions

The culture medium - Polyglutamic acid (PGA) consists of (g/L): glucose, 20; L-glutamic acid, 50; yeast extract, 0.5; MgSO₄·7H₂O, 0.5; bacteriological agar, 15. The initial pH of media was adjusted to 7.0-7.2 using 1.0 M NaOH and 1.0 M HCl. Cultures from a slant were inoculated into 100 mL flasks containing 50 mL culture medium and incubated at 50 °C on an orbital shaker (DAIKI, model KBLee 1001) at 160 rpm.

2.3 Screening for Bioflocculant-Producing Bacteria and Identification

Bioflocculant-producing strains were screened from POME. They were cultivated in PGA medium for 1 to 4 day(s), and the resultant fully grown cultures were examined for their flocculating rate for a kaolin suspension. Bacterial strains which showed considerable flocculating rate were regarded as the bioflocculant-producing bacteria. For taxonomical studies, morphological characterization is carried out according to the Gram's staining procedures (Bacteriological Analytical Manual, 2003). The API 20 NE bacterial identification kit (BioMerieux S. A., Marcy l'Etoile, France) was also used for preliminary identification (Fujita et al., 2000; Yetkin et al., 2005).

2.4 Determination of Flocculating Rate

The flocculating rate was measured according to the method of Kurane et al. (1986) using a suspension of kaolin clay as a test material with minor modifications. Kaolin clay was suspended in distilled water at the concentration of 5000 mg/L. 4.50 mL of 1% CaCl₂ and 0.5 mL of culture broth were added to 45 mL Kaolin suspended solution in 100 mL beaker in turn. The mixture was vigorously stirred and was allowed to stand for 5 minutes. The optical density (OD) of the clarifying solution (A) was measured with a spectrophotometer (MERCK, model Spectroquant NOVA 60) at 550 nm. A control experiment was prepared using the same method, but the culture broth was replaced by distilled water (B). The flocculating activity was calculated according to the equation:

$$\text{Flocculating rate (\%)} = \frac{B - A}{B} \times 100 \quad (1)$$

where A and B are the optical density of the sample and control, respectively.

2.5 Flocculation of Synthetic Dye Wastewater

To get the knowledge of flocculation characteristics of bioflocculant, the effects of dosage of culture broth bacteria and pH of solution were examined. The dosage of culture broth bacteria varies from 0.1 to 0.6 mL (Gong et al., 2008) per 150 mL synthetic dye wastewater (0.2 g/L terasil yellow dye W-4G). Solution of CaCl_2 was used as cations source and the concentration and dosage were same as that of CaCl_2 described above. A dose of 0.2 mL of culture broth bacteria and 1 ml 1% CaCl_2 solution was added to the 150 ml wastewaters at pH 7.0. After the addition of culture broth bacteria, the compound in the beaker was mixed using jar tester at 200 rpm for 1 min, and then at 40 rpm for another 3 min (Gong et al., 2008). The wastewater was left to settle for 10 min, and then the supernatant was taken for analysis. To be compared with chemically synthesized flocculants, the culture broth bacteria were replaced by aluminium sulphate.

2.6 Coagulation of Synthetic Dye Wastewater

In this study, aluminium sulphate (alum) was applied. The alum used was in powder form with the formula $\text{Al}_2\text{SO}_4 \cdot 16\text{H}_2\text{O}$ and supplied by System, Malaysia. Coagulation experiments were carried out using a conventional jar tester (VELP-Scientifica, Model: JLT6, Italy) with impellers equipped with 2.5 cm $\times$ 7.5 cm rectangular blades. The time and speed for rapid and slow mixing were set with an automatic controller. The operating parameters were adopted as rapid agitation at 80 rpm for 3 minutes, followed by a period of 10 mins of slow agitation at 30 rpm (Tan et al., 2000).

The residual COD and turbidity were determined after treatment using culture broth bacteria and alum, and the removal efficiency can be calculated as follows:

$$\text{Removal efficiency (\%)} = \frac{C_0 - C}{C_0} \times 100 \quad (2)$$

where C_0 is the initial value and C is the value after the jar test treatment.

3. Results and Discussion

3.1 Screening and Identification of Bioflocculant-Producing Bacteria

The bacterial strain incubated for one day at 50°C showed the highest flocculating rate against kaolin, and is therefore used for further study. The colony of this strain was irregular, flat, and undulate. The strain was gram negative, rod shape, and oxidase-negative. The identification using the API 20NE kit classified this strain as *Chryseomonas luteola* with a 99.6% probability (identification code No. 1477741).

3.2 Bioflocculant Production by *Chryseomonas Luteola* Strain

The effect of temperature and incubation periods on the flocculating rate was investigated to optimize the culture conditions for the bioflocculant production. The bioflocculant production was found to considerably depend on the culture temperature, and the optimum temperature was found to be 50°C (Figure 1). Besides that, the optimum incubation for most bacteria with highest flocculation rate was shown in two days, except bacteria incubated at 50°C.

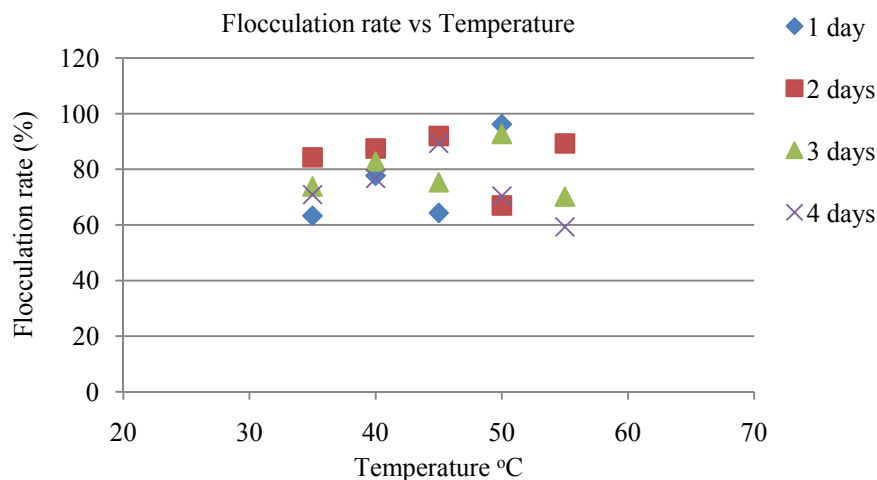


Figure 1. Flocculation rate (%) against Temperature °C for period of incubation (days)

3.3 Time Course of the Production of *Chryseomonas Luteola*

Figure 2 shows how biofloculant production varied during a growth curve of *Chryseomonas luteola*. The flocculating activity reached its maximum flocculating activity in early phase (at 24 hours). Due to cell autolysis and enzymatic activity decreasing the flocculating activity started to decrease after 24 hrs. The flocculation rate for bacteria incubated for 48 hours shows deviation. This phenomenon may due to a portion of PGA medium been dried up after 24 hours and thus nutrients and food provided were insufficient for cell growth. The maximum flocculating activity was 96.15% which is a little lower than 97% of *Bacillus* sp. DYU1 (Wu & Ye, 2007), 98% of *Citrobacter* sp. TKF04 (Fujita et al., 2000), 98.1% of *Aspergillus parasiticus* (Deng et al., 2005) and 99% of *Bacillus mucilaginosus* (Deng et al., 2003). However, considering the difference of experimental methods and the utilization of low-cost medium, the potential of *Chryseomonas luteola* was in consideration.

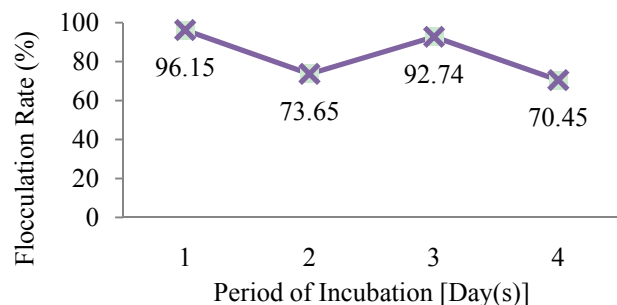


Figure 2. Flocculation rate (%) against period of incubation at 50 °C

3.4 Flocculating of Synthetic Dye Wastewater

3.4.1 Effect of pH on Turbidity Reduction and COD Removal

The effectiveness of coagulant in decolorization was influenced by specific pH range. By varying the solution pH at a constant dosage, the optimal pH corresponding to the highest percentage turbidity reduction and COD removal can be determined by plotting the turbidity reduction efficiency and COD removal efficiency against the pH of the solution. It can be seen from Figure 3 using culture broth bacteria. The best pH for culture broth bacteria was studied on the ranges of pH 6.0 to pH 8.5.

The effective pH on turbidity reduction and COD removal using culture broth bacteria was at pH 7.0. According to Poh et al. (2009) most microbial growth is between 6.8 and 7.2 while pH that is lower than 4.0 and higher than 9.5 are not tolerable. Thus, turbidity reduction efficiency and COD removal efficiency were highest at pH 7.0, which is 38.35% and 33.65% respectively. Followed by pH 6.5, where the turbidity reduction efficiency and COD removal efficiency was 37.82% and 32.87% respectively.

Among all studied pH, the lowest turbidity reduction efficiency was 34.26% obtained at pH 8.5. Meanwhile, the lowest COD removal efficiency was 30.17% obtained at pH 8.5 too. Besides that, turbidity reduction and COD removal efficiency have the same trend, which the reduction or removal efficiency were increasing from pH 6.0 to 7.0; whereas the trend decreasing after pH 7.0. The turbidity reduction efficiency was increasing from 35.14% at pH 6.0 to 38.35% at pH 7.0. Likewise, the COD removal efficiency was increasing from 30.64% at pH 6.0 to 33.65% at pH 7.0. The turbidity reduction and COD removal efficiency was decreasing from 38.35% at pH 7.0 to 34.26% at pH 8.5 and 33.65% at pH 7.0 to 30.17% at pH 8.5 respectively.

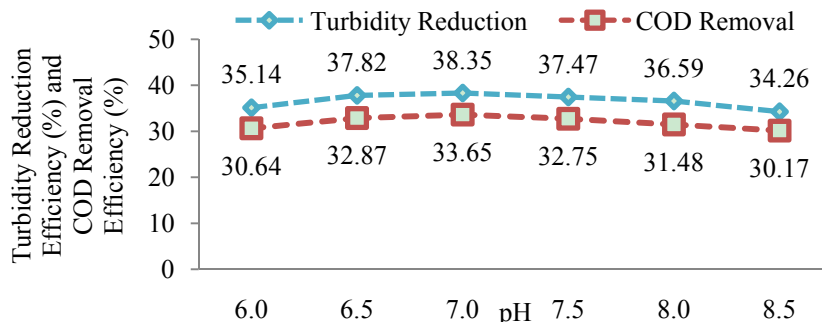


Figure 3. Turbidity reduction efficiency and COD removal efficiency using culture broth bacteria at different pH

3.4.2 Effect of Dosage on Turbidity Reduction and COD Removal

Several dosages of culture broth bacteria were studied to determine the turbidity reduction and COD removal efficiency. The dosages used for this study were 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, and 0.6 mL. The turbidity reduction and COD removal efficiency can be shown in Figure 4. From Figure 4, the best dosage for the turbidity reduction and COD removal efficiency was 0.2 mL of culture broth bacteria. The turbidity reduction efficiency was 38.22% whereas the COD removal efficiency was 33.25%. According to Gong et al. (2008) the effect of culture broth bacteria dosage showed that flocculating activity was over 90% in the range of 0.2 - 0.4 mL. The lowest turbidity reduction and COD removal efficiency was 28.36% and 19.42% respectively at the dosage of 0.6 mL.

The trend of turbidity reduction efficiency was increasing from 33.74% to 38.22% that is from 0.1 mL to 0.2 mL. The COD removal efficiency also increasing from 0.1 mL to 0.2 mL, the percentage of removal was from 28.74% to 33.25%. Likewise, the decreasing trend of turbidity reduction and COD removal efficiency occurred after 0.2 mL. The turbidity removal efficiency was decreasing from 38.22% at the dose of 0.2 mL to 28.36% at the dose of 0.6 mL. The COD removal efficiency was decreasing from 0.2 mL to 0.6 mL, where the percentage removal was decreasing from 33.25% to 19.42%.

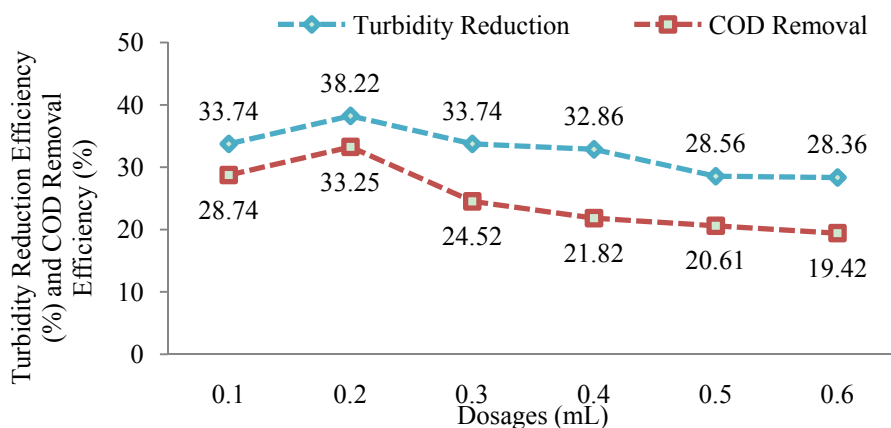


Figure 4. Turbidity reduction efficiency and COD removal efficiency using culture broth bacteria

3.5 Coagulation of Synthetic Dye Wastewater

3.5.1 Effect of pH on Turbidity Reduction and COD Removal

The pH ranges from pH 2.0 to pH 14.0 were studied using aluminium sulphate (alum) as coagulant. The turbidity reduction and COD removal efficiency can be shown in Figure 5. The highest turbidity reduction and COD removal efficiency for alum was 83.65% and 99.24% respectively at pH 4. Followed by pH 6 where the turbidity reduction and COD removal efficiency was 81.29% and 95.91% respectively. From these result, the optimal range of pH was between pH 4 to 6. According to Tan et al. (2000), the effective range of the pH for alum, PAC and $MgCl_2$ are 4.0 - 6.0, 6.0 - 9.0 and 10.5 - 11.0 respectively.

The lowest turbidity reduction and COD removal efficiency was 47.31% and 57.34% respectively at pH 14. From the observation, changes of colour on yellow dye during pH adjustment showed that pH 12 and 14 were not suitable for the turbidity reduction and COD removal. Furthermore, turbidity reduction and COD removal efficiency have the same trend, which the reduction or removal efficiency were increasing from pH 2.0 to 4.0; whereas the trend decreasing after pH 4.0.

The turbidity reduction efficiency was increasing from 80.56% at pH 2.0 to 83.65% at pH 4.0. On the other hand, the COD removal efficiency was increasing from 97.79% at pH 2.0 to 99.24% at pH 4.0. The turbidity reduction and COD removal efficiency was decreasing from 83.65% at pH 4.0 to 47.31% at pH 14.0 and 99.24% at pH 4.0 to 57.34% at pH 14.0 respectively. The turbidity reduction and COD removal efficiency of pH 6.0 was slightly more than the turbidity reduction and COD removal efficiency of pH 2.0.

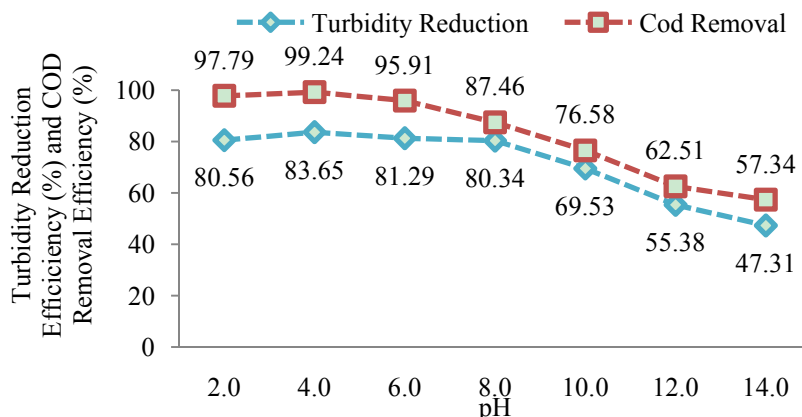


Figure 5. Turbidity reduction efficiency and COD removal efficiency using alum at different pH

3.5.2 Effect of Dosage on Turbidity Reduction and COD Removal

The dosages of alum used for this study were 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, 500 mg/L, 600 mg/L, and 700 mg/L. The turbidity reduction and COD removal efficiency can be shown in Figure 6. The best dosage for the turbidity reduction and COD removal efficiency for alum was 600 mg/L. At this dosage, the turbidity reduction efficiency and COD removal efficiency was 97.51% and 99.64% respectively. Followed by the turbidity reduction and COD removal efficiency were obtained at 500 mg/L. The turbidity reduction efficiency and COD removal efficiency were 96.55% and 97.53% respectively. The lowest turbidity reduction and COD removal efficiency were obtained at 100 mg/L, where the percentages were 82.76% and 89.21% respectively.

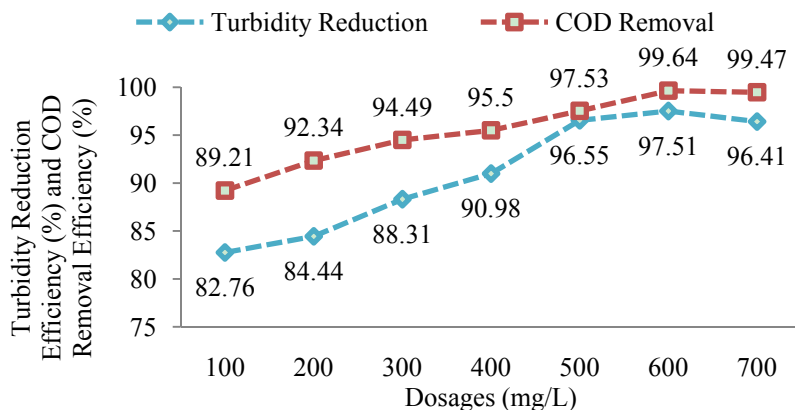


Figure 6. Turbidity removal efficiency and COD removal efficiency using alum

The trend of turbidity reduction efficiency was increasing from 100 mg/L to 600 mg/L, which is from 82.76% to 97.51%. The COD removal efficiency also increased from 100 mg/L to 600 mg/L, the percentage of removal was from 89.21% to 99.64%. In addition, the decreasing trend of turbidity reduction and COD removal efficiency occurred after 600 mg/L. The turbidity removal efficiency was decreasing from 97.51% at the dose of 600 mg/L to 96.41% at the dose of 700 mg/L. The COD removal efficiency was decreasing from 600 mg/L to 700 mg/L, where the percentage removal was decreasing from 99.64% to 99.47%.

3.5.3 The Comparison of the Application of Different Coagulants

Figure 7 shows the plot of reduction / removal efficiency of turbidity and COD for culture broth bacteria and alum. The effective pH for culture broth bacteria and alum was pH 7.0 and pH 4.0 respectively. The dosage for culture broth bacteria was 0.2 mL, whereas for alum was 600 mg/L. The best turbidity reduction efficiency for culture broth bacteria was 38.22% whereas the best COD removal efficiency for culture broth bacteria was 33.25%. In contrast, the best turbidity reduction and COD removal efficiency were 97.51% and 99.64% respectively. According to Sheng et al. (2006), the flocculating efficiency of the bioflocculant is evaluated using

measurements such as the ability to remove suspended solids and pigments and the chemical oxygen demand (COD). Thus, the turbidity reduction, decolorization and COD removal efficiency are important parameters used to determine the optimal flocculating efficiency of bioflocculant and coagulant such as alum, PAC, and MgCl_2 . From this experiment, results showed that alum was better in the treatment of dye wastewater in the reduction of turbidity and removal of COD compared to culture broth bacteria.

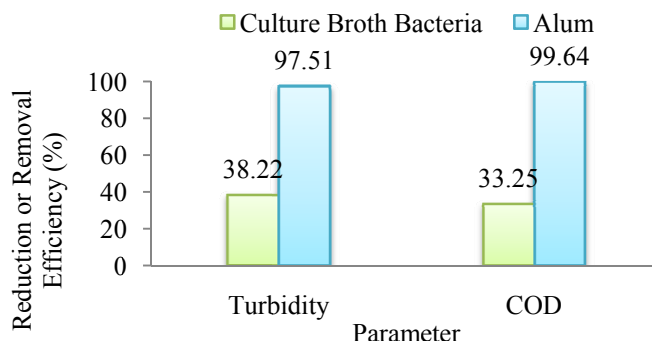


Figure 7. Comparison of reduction or removal efficiency on turbidity and COD between culture broth bacteria and alum

Decolorization was also an important parameter in determining the flocculating efficiency of a bioflocculant. In this study, the bioflocculant producing bacteria was grown in culture broth media and the media was orange-brown in colour. This phenomenon contributes colour to the wastewater. Therefore, decolorization was not successful by using culture broth bacteria. According to Deng et al. (2005), the bioflocculant was effective in flocculating some soluble anionic dyes in aqueous solution, in particular Reactive Blue 4 and Acid Yellow 25 with a decolorization efficiency of 92.4 and 92.9%, respectively as they were using crude bioflocculant.

Crude bioflocculant was produced using centrifuge and it is in powder form. The viscous culture broth was diluted with the two volumes of distilled water and then centrifuged at 5000g for 10 mins to remove cell pellets. The supernatant was poured into four volumes of cold ethanol to precipitate the bioflocculant. The resulting precipitate was collected by centrifugation at 10,000 g for 15 mins and re-dissolved in water. After two such steps, the crude bioflocculant obtained was purified by dialysis overnight at 4°C in distilled water and then lyophilized. The removal efficiency of COD and colour using crude bioflocculant are higher compared to culture broth bacteria.

4. Conclusions

A bioflocculant-producing bacterium was isolated from POME and identified as *Chryseomonas luteola*. Using optimized conditions (50°C, one day incubation) for the production of the bioflocculant, a flocculating activity of 96.15% was obtained. When treating synthetic dye wastewater, the reduction rate of turbidity and removal rate of COD were 38.22% and 33.25% respectively. On the other hand, the reduction of turbidity and removal of COD of synthetic dye wastewater using alum were up to 97.51% and 99.64% respectively. As a conclusion, alum was better coagulant used in the treatment of dye wastewater in the reduction of turbidity and removal of COD as compared to culture broth bacteria. The culture broth bacteria were insufficient in the treatment of wastewater. The ability of culture broth bacteria to decolorization, to remove COD and reduce turbidity were not efficient than crude bioflocculant. Therefore, crude bioflocculant should be produced to replace culture broth bacteria in the treatment of dye wastewater in further study.

Acknowledgements

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