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# Performance of immobilized-selected microorganisms in the biodegradation of textile wastewater

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ABSTRACT

Wastewater from textile industry contains of variation of pollutants within certain concentrations. To protect the environment and water bodies, polluted wastewater must be treated before it can be discharged into the environment. Anaerobic biological treatment has been used as technology in textile wastewater treatment. Several factors that affect the performance of conventional anaerobic treatment need to be addressed in order to improve the efficiency of this technology, including the utilization of a consortium consists of selected microorganisms acts as inoculums. These inoculums are expected to improve the textile wastewater biodegradation performance. In this study selected microorganisms in the form of immobilized and free cells were used. The performance of selected microorganisms was conducted by comparing the pollutants removal efficiency of immobilized, free cells and conventional sludge. Results show that selected-immobilized microorganisms achieved the best performance due to its stability and its highest efficiency in removing pollutants. Mean while microorganisms in the form of free cells had the lowest performance due to its sensitivity towards environmental conditions and having low mechanical strength of biomass. Immobilized cells succesfully treated wastewater from textile industry, with removal of suspended solid reached to 93.78%. In addition, for parameter oil & grease, $BOD_5$ and COD, the removal efficiency was 99.13%, 81.,54% and 64.94% respectively. However, the system could not sufficiently remove ammonia due to the anaerobic condition instead of aerobic condition in the reactor.

## 1. INTRODUCTION

Water is abundantly required in the production process of textile industries. Residue of materials in the production process is generally discharged in the form of wastewater. Having negative impact to environment, wastewater must be treated. Anaerobic wastewater treatment is one of the biological treatment methods for treating wastewater containing of high organic pollutant. Goel (2010) had performed anaerobic condition to treat wastewater from staining process in the textile industry.

Factor affecting pollutant degradation in anaerobic condition is the availability of appropriate microorganisms. In addition, other external factors such as pH, alkalinity, temperature and nutrients are also influencing anaerobic degradation process. In conventional anaerobic treatment, sludge was used as source of inoculum to treat textile wastewater. Acclimatization process of conventional

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anaerobic treatment takes a long time and the low efficiency of pollutant removal needs to be improved. In order to increase the performance of wastewater treatment, a selected microorganisms can become one of alternatives. Selected microorganisms in the form of isolates found via isolation stage and have passed the ability test to degrade the pollutants in textile industry wastewater that contains amylum, cellulose, oil, and dye (Handayani et al., 2016).

Some researchers reported that performance of biological wastewater treatment systems can be upgraded by using inoculum in the form of immobilized cells. Han, T. & Shim (2016) used immobilized cells to improve the performance of biological system reactors in degradation of textile waste water from staining process. Immobilized cells was also used to degrade the azo type of staining waste water (Tan, L.N, & Xu, 2014). In addition Wang et al. (2007) successfully treated carbazole, a toxic pollutant with an aromatic polycyclic structure with immobilized cells

Immobilization is a technique of maintaining microorganisms to stay in the matrix in order to increase the efficiency of the desired microorganism performance (Thirumarimurugan, 2016). There are several methods of Immobilization cells, one of them is adsorption. Adsorption is the simplest and the most passive immobilization method, in which the cells are adsorbed into the inert pores of media (Cláudia et al., 2013). According to Górecka. E (2011) the used media for immobilization should be adapted to local availability, having a stable and non-toxic matrix and does not affect the structure and activities of cell.

Matrixs commonly used for passive surface immobilization include soil, peat, activated carbon, coke, plastic media, celite and glass beads (J.Wilson, 2017). In this study, peat soil was selected as cell immobilization media due to its availability and affordability. Lee et al. (2010) had used peat soil as cell immobilization media to degrade petroleum waste, and proved that it was the best matrix because of its highest cellular adsorption capacity.

This research aimed to study the performance of selected microorganisms in the form of immobilized and free cells to degrade pollutant in textile industry waste water compared with conventional sludge.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The inoculum sources used in this research were selected isolates bacteria (Handayani et al., 2016) and sludge derived from textile wastewater equalization tank. Wastewater used in the experiment derived from Dan Liris textile industry in Pekalongan Central Java. Dried peat soil derived from the bottom of Rawapening Lake was used as matrix of immobilization. Aquadest and nutrient broth were used as media of free cells. Nutrient broth and chemical compounds used for analysis were p.a grade (Oxoid and Merck). pH meter used was Krisbow (KW0600744) Reactor used in the experiment was made of acrylic, with volume of 5 L and run with upflow system by using peristaltic pump (Cole-Parmer Masterflex Brand, easy load L/S type, model 7518-62).

### *2.2. Methods*

*Preparation of inoculums*

Inoculum in this research were divided into three types namely immobilized cells, free cells, and conventional sludge. Immobilization in peat soil media was carried out by inserting a starter (consortium bacteria in liquid form of nutrient broth media) into peat soil media in a ratio of 1: 1 (1 kg of peat soil mixed with 1 liter starter). The mixture was incubated in room temperature and monitored in storage up to four weeks. During incubation process, the samples of mixture were taken five times to analyze the number of active bacteria in total plate count and pH measurements. Free cells was a consortium of bacteria inoculated in nutrient broth media only, while conventional sludge was derived from waste water equalization tank of textile industry.

*Performance test of inoculums*

The Performance of three types inoculums were observed in laboratory scale. The experiment was carried out in upflow anaerobic reactor with volume of 5 L. 20% of Inoculum was added in reactor then fed with wastewater from textile industry in upflow system using peristaltic pump.

The experiment was divided in two phases, the first was acclimatization phase and the second was continuous phase. In the acclimatization phase, wastewater with COD concentration range of 500-1300 mg/L was fed into reactor in batch system, and then circulated up to get optimum level of pollutant degradation. Pollutant degradation was observed based on COD reduction.

During continuous phase, COD concentration range was 500-1900 mg/L, with hydraulic retention time (HRT) was determined in 24 hours. Inlet and outlet samples of reactors were taken regularly to be analyzed. In steady state condition, when the highest pollutant degradation was achieved, then sample was taken to be analyzed in complete parameters such as: COD, Total suspended solid, $BOD_5$, Phenol, Chrom, Ammonia, Oil and grease. Furthermore, microbial identification was also carried out in order to know the species of microorganisms used as inoculum.

## 3. RESULT AND DISCUSSION

During incubation process of inoculum preparation, both immobilized and free cells, were analyzed on total plate count (TPC) and pH. TPC method is the nearest estimation method to determine whether bacteria in both immobilized and free cell inoculum live and grow or not.

It can be seen from table 1 that, up to 28 days of storage time, the living cells in both immobilized and free cells are more than $1.0 \times 10^7$ CFU/ml, then it can be concluded that both inoculums are ready to be applied in the wastewater treatment process. After 28 days, TPC number of free cells is more stable than immobilized cells because during incubation period, free cells were incubated in refrigerator with temperature of 4-6$^o$C, while immobilized cells were incubated a room temperature. But in general, TPC number of immobilized cells is not different significantly compared to free cells.

Visual observation on the 28th day of immobilized cells shows biofilm formation on the surface of peat soil, which might be indication of inoculum adsorption in to the surface of peat soil matrix. As explained by Kilonzo & Bergougnou (2012), cell immobilization by adsorption is influenced by several factors including age and cell surface structure, composition and pH of the media, as well as the structure and size of the media pores. Furthermore, although strength level of cell immobilization with adsorption process is relatively weak, it is still able to bind the cell efficiently.

pH during incubation process was observed and the value can be seen in figure 1.

**Table 1**. Result of total plate count analysis

| Time of incubation (days) | TPC of immobilized cells (CFU/mL) | TPC of free celsl (CFU/mL) |
|---|---|---|
| 1 | $7.6 \times 10^8$ | $5.1 \times 10^8$ |
| 5 | $4.0 \times 10^8$ | $8.3 \times 10^8$ |
| 14 | $4.8 \times 10^7$ | $2.4 \times 10^7$ |
| 21 | $2.6 \times 10^7$ | $2.3 \times 10^7$ |
| 28 | $1.1 \times 10^7$ | $6.2 \times 10^7$ |

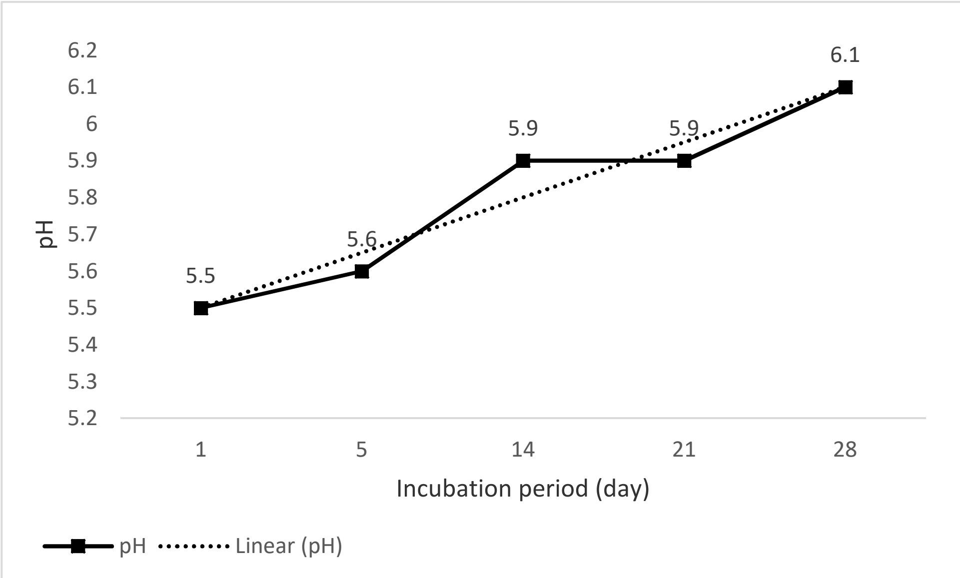


**Figure 1**. pH of immobilized cell during incubation process

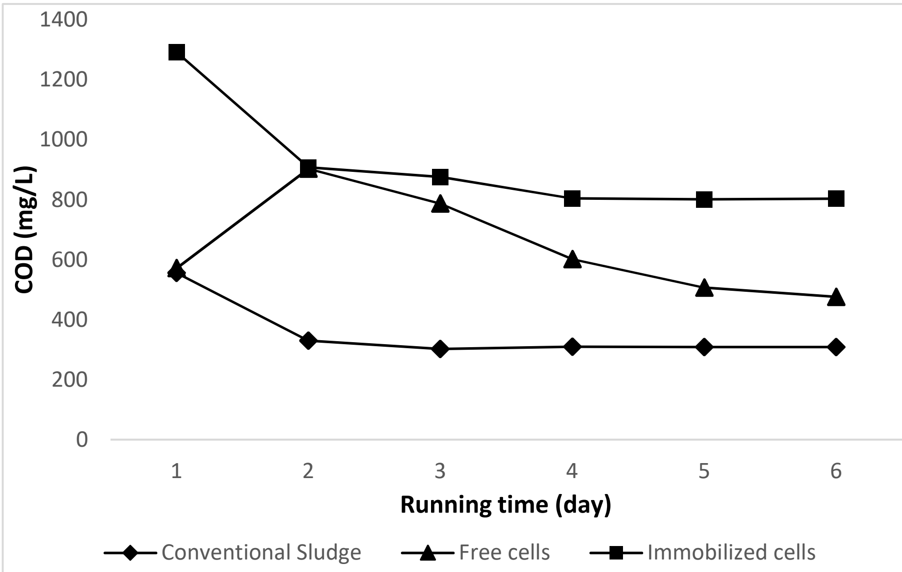


**Figure 2**. Pollutant degradation at acclimatization phase

Figure 1 shows that, pH of immobilized cells tend to increase during the incubation period, and has reached neutral range on the 28th day. Contrast with TPC analysis results (table 1) that shows a tendency of the longer incubation period of immobilized cell the lower number of TPC.

Some researchers reported that immobilized cells are able to tolerate the change in pH of medium than free cells (Linardi, 2001; Nigam, 2000). Chen et al.(2007) reported pH would have a significat on microbial activities and a neutral environment is suggested to obtain higher pollutant degradation rate with immobilized cells. Thus, in this research the immobilized cells were incubated untill pH reached on the neutral range to anticipate stress of cells because wastewater pH used in the experiment were higher than 6.5.

The results of wastewater treatment experiment during the acclimatization period can be seen in Figure 2.

During acclimatization process, a maximum point of pollutant degradation of three inoculum types are reached after day 4 of the experiment, indicated by decrease of COD concentration significantly . This result is almost similar to the report of Mostafa et al.(2015), that has the highest degradation rate after 4-6 days using similar immobilized and free cells. It can be seen in figure 2 treatment with immobilized cell and conventional sludge perform more stable condition process. Percentage of degradation pollutant by using immobilized cell is 38.07%, mean while free cells and conventional sludge reach on 16.73% and 44.49% respectively.

Figure 2 also shows that free cells get the lowest performance in degrading pollutant, even on the second day COD effluent slightly rise, which can be an indication of the occurance of death cells washed out, contribute to the increase of COD level in the effluent. Supported by some researchers, inoculum in free cells suspension having higher sensitivity to environmental conditions than immobilized cells. Reported that, pH and temperature affect on longlive of free cells suspension. In this research, pH of wastewater were 8 to 8.5 indication of alkali condition and reactors were operated at room temperature. According to Chen et al. (2007), if the ambient is higher than the optimal temperature, thermal death of the cells might occur, and removal rates would decrease. However, Murakami-nitta et al.(2003) observed that a free cell system was more sensitive to temperature change than immobilized cells, and immobilization increased the thermal stability of the cells.

The performance of inoculums in continuous phase are showed in figure 3, 4 and 5.

Conventional sludge in waste water treatment experiment perform stable condition during the experiment shows a trend of COD outlet and percentage of COD removal. Average of COD removal with conventional sludge in the experiment is 29.29%.

Running experiment period of free cells inoculum was shorter than conventional sludge and immobilized cells due to the death of cells presumption. In visual observation in the free cells outlet, some layers is found and different odor is also smell, which can be indication of washed out dead cells. Reported by Godjevargova et al.(2004), free cells on waste water treatment tend to washed out because of having low mechanical strength of biomass.

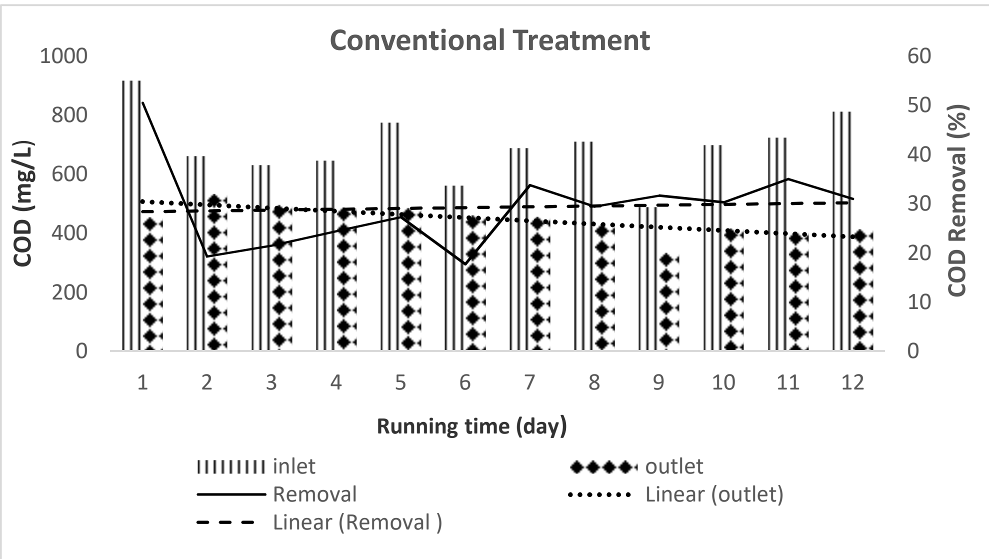


**Figure 3.** Performance of conventional sludge

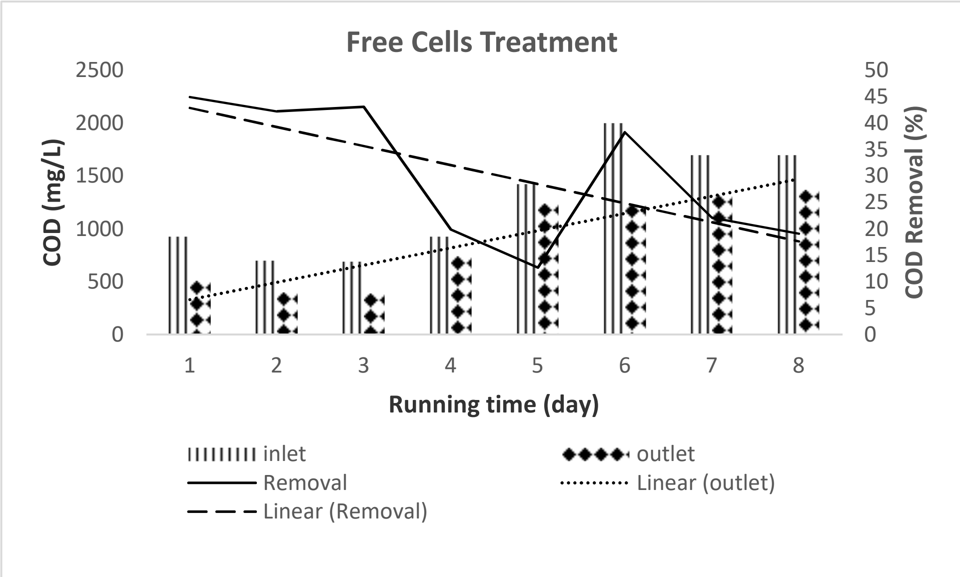


Figure 4. Performance of free cells suspension

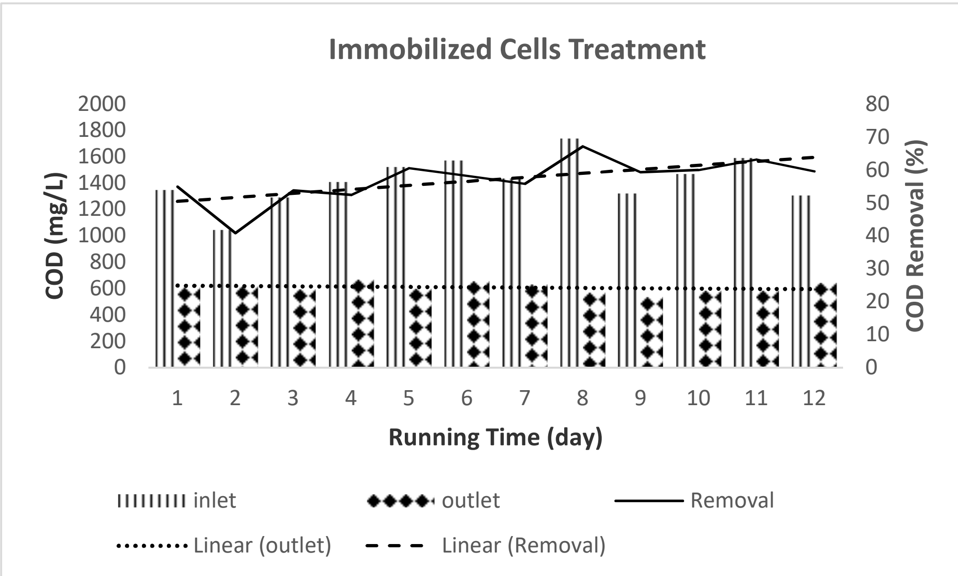


Figure 5. Performance of Immobilized Cells

In contrary with conventional sludge, COD removal of free cells treatment decline, that can be seen in figure 4. trend of COD removal percentage tend to decrease, on the contrary COD outlet sample tend to increase. As Reported by researcher, the use of native biomass (such as bacteria, yeast fungi and algae) for degradation pollutant in freely suspended state has a limitation, owing to their inherent disadvantages such as small particle size, possible clogging, and washed out potentially (Mostafa et al., 2015). Free supension cells also could not tolerate the toxicity pollutant at high levels (Chen et al., 2007; Godjevargova et al., 2004).

Performance of wastewater treatment using immobilized cells illustrates in figure 5.

Immobilized cells treatment gives a stable condition in wastewater treatment, which can be seen in figure 5 percentage of COD removal tend to slightly incline. Average of COD removal with immobilized cells in the experiment reach on 56.90%. This result agrees with some researchers, that immobilized cells has higher stability and better performance than conventional sludge and free cells, Immobilization of whole cells for the degradation pollutant in wastewater provides stability, because of high activity, yield and good operational. Moreover, the cell mass also can be separated from bulk liquid for possible reuse (Lan, G.& Jinbao, 2009). When immobilized microbial cells are used, the efficacy of biodegradation is often improved. Wang et al.(2007) reported that immobilized cells can increase biodegradation rate through a higher cell loading at high dilution rate without washed out.

Based on visual observation, the appearance of immobilized cells outlet sampel is clearer than appearance of sampel from conventional sudge and free cells treatment. As supported by a research, saying that the matrix in immobilized cells also served as barriers that may separate the microorganisms from the effluent and minimize the discharge of high molecular weight biodegraded products (Allabashi et al., 2007).

In order to evaluate the performance of three inoculums in treating waste water, percentage of COD removal has been compared and illustrated in figure 6.

At continuous phase, as can be seen on figure 6, immobilized cells and conventional sludge perform more stable condition than free cells. However, the highest ability of pollutant degradation is achieved by immobilized cells. COD removal percentage of Immobilized cells is also higher than conventional sludge, while the free cells COD removal tend to decrease.

This result is consistent with the statement of Cláudia *et al.*(2013) that immobilized cells have performance in degrading pollutants higher than conventional sludge. This is because immobilized cells have metabolic activity and stronger resistance to toxic chemical components. Immobilization through the adsorption process leads to direct contact between waste and microbes. This process brings microbial cells to the surface of porous media followed by cell adhesion and the formation of colonies on the media. This condition will expand the contact area between microbial cells and wastewater to allow for higher level of pollutant degradation (Kilonzo and Bergougnou, 2012). Kuo & Shu, (2004) also reported that the matrix as carrier of immobilized cells would provide a high specific surface area for microbial growth and also provide a shelter for bacteria that encountered chemical toxicity. The aforementioned reasons support the result of this experiment that immobilized cells perform more stable activity and higher in degrading pollutants than conventional sludge and free cells.

The other advantages of using immobilized cells reported some researchers are no obvious loss of cell activity was observed over the four consecutive uses of the immobilized cells (Chen *et al.*, 2007). Murakami-nitta *et al.*(2003) also reported that the number of viable cells increased after reuse. This suggests that, the activity of the immobilized cells can be maintained, and immobilized cells could use pollutant as carbon source throughout pollutant degradation experiments.

At steady state condition, treatment with immobilized cells perform degradation pollutant in complete parameter showed in Table 2.

Can be seen in table 2 almost all kind of pollutants succesfully treated by immobilized cells. Degradation of pollutant in the form of total suspended solid and oil & grease reached more than 90%. In wastewater treatment process, utilization of immobilized cells could improve pollutant degradability due to microbial activity enhacement. The presence of matrix inside immobilized cells also has a function as a barrier that able to minimize the discharge of suspended molecule to the effluent, so that removal of suspended solid pollutant could be improved.

Oil and grease, generally in the form of complex compounds and have a large molecular weight, was succesfully treated with immobilized cells up to 99.13%. This result agrees with the result of some researchers (Lan et al., 2009 ; Nisola et al., 2009 ; Mostafa et al., 2015) which showed that immobilized cells succesfully treated fat, oil and grease (FOG) in wastewater and was able to sustain the required FOG and COD removals even at highly fluctuating influent concentrations.

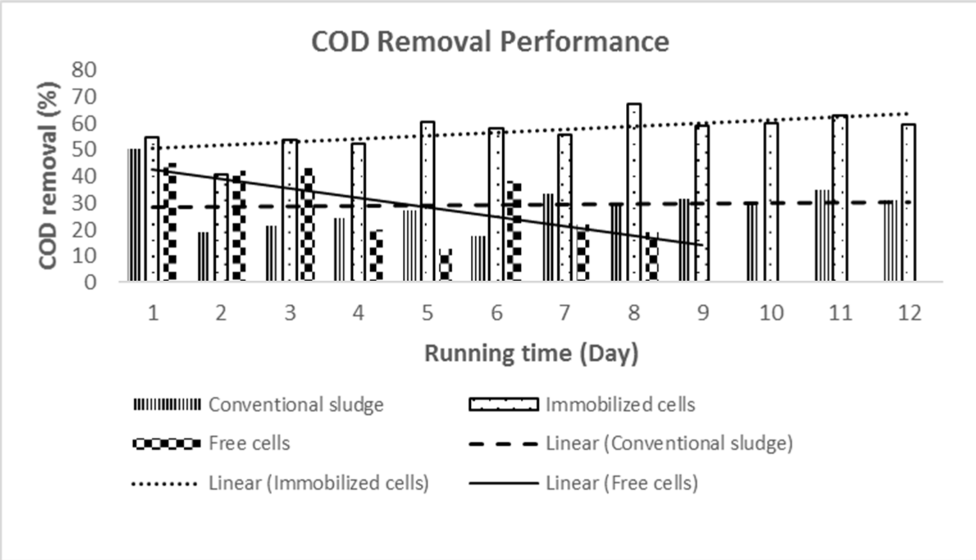


**Figure 6**. Comparison of COD removal performance

**Table 2**. Analysis result in complete parameter treated by immobilized cells

| No | Parameter | Inlet | Outlet | % Degradation |
|---|---|---|---|---|
| 1 | Total suspended solid (mg/L) | 660 | 41 | 93,78 |
| 2 | BOD5 (mg/L) | 316,1 | 58,33 | 81,54 |
| 3 | COD (mg/L) | 1510 | 529,3 | 64,94 |
| 4 | Penol total (mg/L) | <0,01 | <0,01 | # |
| 5 | Khrom total (Cr) (mg/L) | <0,01 | <0,01 | # |
| 6 | Oil and grease (mg/L) | 81,0 | 0,7 | 99,13 |
| 7 | Amonia total (NH3-N) (mg/L) | 8,60 | 7,05 | 18,02 |

$BOD_5$ is also succesfully treated, indicating that microorganisms have a highly biodegradation activity. Immobilized cells have a better metabolic activity and stronger resistance to toxic chemical components than conventional sludge and free cells, then pollutant in the waste water could be degraded in to sources of energy to support microbial growth.

Ammonia has not been succesfully treated in this experiment. This result does not mean that immobilized cells could not treat ammonia in wastewater treatment, this result because of the system used in the experiment was anaerobic condition. Understood that, ammonia only will be succesfully treated in aerobic condition (Oh, 2003 ; Show et al., 2012). Meanwhile, application of immobilized cells in waste water treatment containing ammonia pollutant also showed good results in aerobic condition (Dong et al., 2017 ; Taylor et al., 2015 ; Dong et al., 2014).

The identification of species of selected microorganisms are shown in Table 3. The species identification analysis gives 8 of dominant species microorganisms used in the experiment.

Total of eight species were combined as consortium in degrading wastewater. Both of all species having ability in degrading kind of pollutants organic carbon, oil and grease, also dye (Handayani et al., 2016). But, in the experiment of wastewater treatment, consortium in the form of immobilized cells having better performance and stability than in the form of free cells.

**Table 3**. Species of inoculums

| No | Species | No | Species |
|---|---|---|---|
| 1 | Corynebacterium *variabile* | 5 | Brevundimonas *diminuta* |
| 2 | Bacillus *cereus* | 6 | Bacillus *amyloliquefaciens* |
| 3 | Bacillus *sp* | 7 | Pseudomonas *otitidis* |
| 4 | Bacillus *subtilis* | 8 | Rhodococcus *ruber* |

## 4. CONCLUSION

Results obtained from this study indicate that, in the form of immobilized cells, inoculum perform higher efficiency and stability in degrading pollutant of waste water than in the form of free cells and conventional sludge. Free cells having the lowest performance and stability in waste water treatment, due to its sensitivity to environmental condition. Immobilized cells succesfully treat wastewater from textile industry, with removal of total suspended solid parameter reaches on 93.78% while for oil & grease, $BOD_5$ and COD, removal reach on 99.13%, 81.54% and 64.94% respectively. Pollutant in the form of amonia has not been succesfully treated with immobilized cells in the anaerobic condition. Results from this study provide us with insight into the characteristics of pollutant biodegradation in textile industry waste water treatment using immobilized cells as inoculum in the anaerobic condition.

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