

DOMESTIC WASTE WATER BIOREMEDIATION BY CONSORTIUM OF BACTERIA

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Abstract

Research has been carried out to obtain an effective bacterial consortium in the biodegradation of domestic wastewater WWTP Bojongsoang. The method used in this study is an experimental method to completely randomized design (CRD) consisting of 2 factors and repeated 3 times. The first factor is the type of bacterial consortia, these were (k1) Consortium of *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans*, (k2) Consortium of *Paenibacillus amylolyticus*, *Bacillus Nitrosomonas circulans*, and *Bacillus pumilus*; (k3) Consortium of *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus*; (k4) of domestic wastewater was heated; (k5) of domestic wastewater was not heated. The second factor is the time of bioremediation (T) of consisting of 8 levels, namely (t0) initial day, (t1) day 4, (t2) day 8, (t3) Day 12, (t4) Day 16 (t5) Day 20, (t6) Day 25. The results showed that the bioremediation by the consortium of *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus* (k3) are effective in lowering the levels of domestic wastewater BOD 67.4 % of BOD, COD 66.3 %, and 77.1% TSS.

Key words: Bioremediation, Domestic waste water, BOD, COD, TSS

INTRODUCTION

Water pollution found in rivers in the cities is the result of domestic waste disposal. Domestic wastewater contains high organic matter. Organic matter in the wastewater is composed of 65% protein, 25% carbohydrate and 10% fat, while the inorganic material is salt and metal (15). There is also ammonia from urine and feces produced by human waste. Efforts to control water pollution due to domestic waste needs to be done in order not to degrade the quality of water.

In Indonesia, especially in West Java have been there several installations Wastewater Treatment Plant (WWTP) to treat domestic waste such as wastewater Bojongsoang. Waste Water Treatment Plant (WWTP) located in Bojongsoang is household waste water treatment plant that contains a lot of organic material distributed through the channels. Installation is to process effluent from the domestic household area of Bandung and

Bandung East South Central with a service capacity of 400,000 inhabitants. WWTP was constructed to reduce the level of water pollution that enters Citarum River by biological treatment system with stabilization ponds (Siregar, 2004). Biological treatment of domestic sewage in wastewater Bojongsoang require 14 stabilization ponds consisting of two sets is the set A and set B. Before entering into the waste stabilization ponds, domestic wastewater collected in the pond inlet that has a level of BOD 91 mg/l, COD 159 mg/l, DO of 3.15 mg/l pH 6, and TSS concentration of 200 mg.

Biodegradation of domestic wastewater using a consortium of bacteria indigenous and non-indigenous is a biological wastewater treatment method that effectively and efficiently. Bacteria that are used must have the ability to decompose organic matter, such as being able to degrade cellulose, lipids, and proteins. An indigenous microbe normally present in domestic waste is the genus *Bacillus*, *Aerobacter*, *Nitrobacter*, *Nitrosomonas* and *Saccharomyces* (5). Bacteria of the genus *Bacillus* is a group of

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bacteria commonly cellulolytic, proteolytic, and lipolytic, aerobic or facultative anaerobic. *Nitrosomonas* is able to reduce ammonia. The genus *Bacillus* is widely used in wastewater treatment population (4)

While *Nitrosomonas* convert ammonia to nitrite, and *Nitrobacteria* convert nitrite to nitrate this is harmless and required by plants.

In this study, a consortium of *B. subtilis*, *B. circulans*, *B. pumilus* and *Paenibacillus amylolyticus* of indigenous domestic sewage WWTP Bojongoang used for domestic waste biodegradation Bojongoang WWTP. The use of bacterial consortium is expected to reduce levels of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and (TSS) of domestic wastewater. The purpose of this study was to determine the ability of some strains of bacteria indigenous consortium WWTP Bojongoang for biodegradation of domestic wastewater.

METHODS

This research method using experimental methods with completely randomized design (CRD) consisting of 2 factors and 3 replication. The first factor is the type of bacterial consortium (K) and the second factor is time (T). The number of replications is determined by the formula Federer, the $(n-1)(t-1) \geq 30$, with n = number of treatments; t = number of replicates, to obtain the number of repetitions (t) three times, earned total of 40 experimental trials as presented in Table 1.

Table 1 Treatment Combination Table

Time (T)	Consortia (C)				
	k ₁	k ₂	k ₃	k ₄	k ₅
t ₀	t ₀ k ₁	t ₀ k ₂	t ₀ k ₃	t ₀ k ₄	t ₀ k ₅
t ₁	t ₁ k ₁	t ₁ k ₂	t ₁ k ₃	t ₁ k ₄	t ₁ k ₅
t ₂	t ₂ k ₁	t ₂ k ₂	t ₂ k ₃	t ₂ k ₄	t ₂ k ₅
t ₃	t ₃ k ₁	t ₃ k ₂	t ₃ k ₃	t ₃ k ₄	t ₃ k ₅
t ₄	t ₄ k ₁	t ₄ k ₂	t ₄ k ₃	t ₄ k ₄	t ₄ k ₅
t ₅	t ₅ k ₁	t ₅ k ₂	t ₅ k ₃	t ₅ k ₄	t ₅ k ₅
t ₆	t ₆ k ₁	t ₆ k ₂	t ₆ k ₃	t ₆ k ₄	t ₆ k ₅

Data obtained were statistically tested using analysis of variance test (ANOVA) to determine the effect of treatment of the parameters tested, followed by Duncan test. This study was conducted to obtain the type of bacterial consortium and the optimal time for the lower levels of BOD and COD in wastewater biodegradation of domestic wastewater Bojongoang with the following steps:

- a) **Biochemical Oxygen Demand (BOD)**
The method is based on reference SNI M-69-1990-03 (1990)
- b) **Chemical Oxygen Demand (COD)**
The method is based on the reference APHA (2),
- c) **Total Suspended Solid (TSS)**
ISO reference method based on 06-6989.3-(2004)

RESULTS AND DISCUSSIONS

Levels of BOD (Biochemical Oxygen Demand) During Domestic Wastewater Biodegradation Process

BOD (Biochemical Oxygen Demand) is a characteristic that indicates the amount of dissolved oxygen required by microorganisms to break down or decompose organic material under aerobic conditions (11). This means a measure of the amount of oxygen used by the microbial population contained in the waters in response to the influx of organic material that can be parsed. According to Boyd (3), that decomposes organic material in BOD is ready to decompose organic matter (readily decomposable organic matter). Thus the BOD values can be interpreted as illustration of the amount of organic matter easily explained (Biodegradable organics) in the waters. Based on the analysis of variance (ANOVA) and Duncan’s Multiple Range Test performed as an Advanced Test is known that there is interaction between bacterial consortium and time that provide different effects on levels of BOD.



Table 2 Interaction Consortium Test against Bacteria and Time Percentage Decreased Levels of BOD (%) Domestic Wastewater

Treatment (T)	Duration (Time)					
	(t ₁) 0-4	(t ₂) 0-8	(t ₃) 0-12	(t ₄) 0-16	(t ₅) 0-20	(t ₆) 0-25
(k ₁) <i>B. subtilis</i> , <i>P. amylolyticus</i> , <i>Nitrosomonas</i> sp, dan <i>B. circulans</i>	47.52 b AB	52.57 ab A	63.15 ab A	68.93 a A	68.87 a A	70.53 a A
(k ₂) <i>P. amylolyticus</i> , <i>Nitrosomonas</i> sp, <i>B. circulans</i> , dan <i>B. pumilus</i>	51.11 d AB	60.85 c A	67.88 ab A	69.59 a A	63.43 bc A	69.59 a A
(k ₃) <i>B. subtilis</i> , <i>Nitrosomonas</i> sp, <i>B. circulans</i> dan <i>B. pumilus</i>	62.55 b A	68.57 a A	63.92 ab A	65.52 ab A	67.71 ab A	67.40 ab A
(k ₄) Wastewater is heated	38.13 a B	8.50 b B	1.83 b C	5.17 b C	25.83 a B	8.83 b C
(k ₅) Wastewater is not heated	2.96 c C	10.92 bc B	31.96 ab B	38.70 a B	34.60 ab B	38.70 a B

- The same letters in all directions showed no significant differences
- Towards small letters are letters that indicate horizontal consortium
Large font vertical direction is the letter that shows the time

In Table 2 it can be seen that the process of biodegradation of organic matter in the Wastewater from day 8 to the liver to 25. Consortium *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus* (k3) can reduce levels of domestic wastewater BOD highest at 68, 57%. Decreased of BOD levels also take place during the process of biodegradation in the wastewater produced by a consortium of bacteria *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans* (k1). Biodegradation of organic materials for 8 days has reduced more than 50% BOD levels. Decreased levels of rapid BOD indicate the presence of synergy interactions in the consortium. According to Valbuzzi (17) *B. subtilis* is a bacterium that can be found in soil, water, air, and plants, contribute to nutrient cycling because it produces a variety of enzymes. Furthermore, many members of *Bacillus* produce extra - and intracellular proteases cellular and has been used intensively to produce amylase and protease on an industrial scale (7). Several *Bacillus* species are also known to be produced commercially for use as a fertilizer and soil nitrifier nitrification in aquaculture systems due to a breakdown of protein and organic matter into ammonia and subsequent nitrification process. Biodegradation process only reached 70 percent during the 25 days, it is thought to be caused reduced the amount of organic matter and begin accumulating products of

metabolism that can be toxic and inhibit microbial growth rate and decrease the rate of reduction of BOD

Here is a chart to decreased levels of BOD results biodegradation of domestic wastewater (fig. 1). In Figure 1. Known that bacterial consortium *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans* (k1) resulted in a decrease in the BOD levels high at 70.5%, but did not differ with the resulting decrease in bacterial consortium *Paenibacillus amylolyticus*, *Nitrosomonas*, *B. circulans*, and *B. pumilus*. (k2) and a consortium of *B. subtilis* *Nitrosomonas*, *B. circulans* and *B. Pumilus* (k3). It can be said that the biodegradation of organic matter by bacteria of the three consortium equally effective in lowering domestic effluent BOD. *Bacillus* microorganisms classified into decomposers (12). *B. subtilis* enzyme is a cellulase producing bacteria that decompose cellulose in domestic waste. into simpler molecules such as Cellobiose, cellotriose, glucose monomers, CO₂ and water (9). *Bacillus subtilis* is the most widely used for the production of the enzyme amylase, and protease. While *B. pumilus* is a protease enzyme producing bacteria that can break down proteins contained in the waste. *Nitrosomonas* sp. is chemolithotroph bacteria that obtain energy from the oxidation of ammonia compounds and using CO₂ as the main source of carbon in biomass synthesis (8).

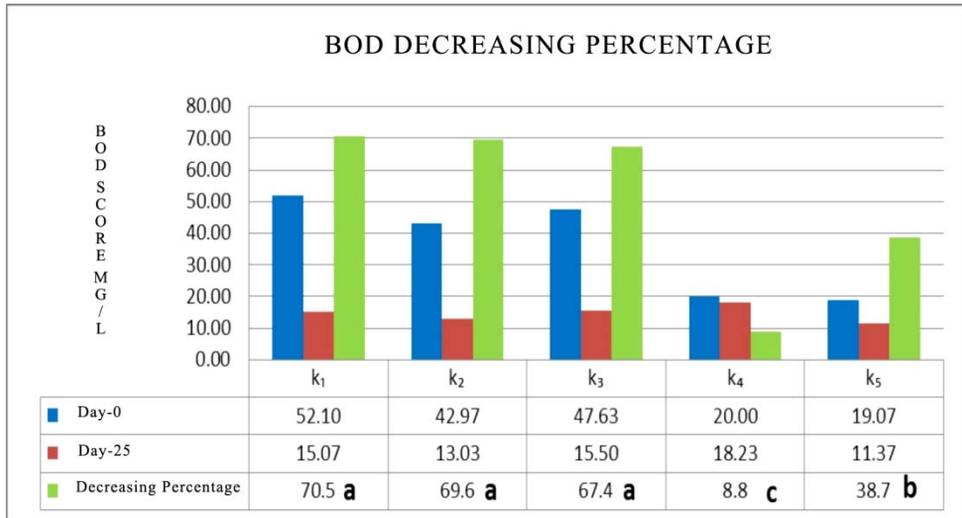


Figure 1 Percentage Decrease Levels Charts of BOD (%)
During the process of Biodegradation of Domestic Waste Water

Description:

k1: *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans*

k2: *Paenibacillus amylolyticus*, *Nitrosomonas*, *Bacillus circulans*, and *Bacillus pumilus*.

k3: *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus*.

k4: domestic wastewater sterile

k5: domestic wastewater that is not in the sterile

Therefore decompose organic material in domestic sewage, *Nitrosomonas* sp using oxygen resulting in decreased levels of BOD. The survey results revealed that the average BOD levels were obtained at 15.1 mg/l BOD includes quality standards of domestic wastewater is 100 mg/l. Thus the decrease in BOD levels in the addition of bacterial consortium showed that the bacteria used plays a role in lowering levels of BOD. The study found that the average BOD levels were obtained at 15.1 mg/l BOD includes quality standards of domestic wastewater is 100 mg/l. Thus the decrease in BOD levels in the addition of bacterial consortium showed that the bacteria used plays a role in lowering level of BOD.

Levels of COD (Chemical Oxygen Demand) During Domestic Wastewater Biodegradation Process

Chemical Oxygen Demand is the amount of oxygen needed to decompose the entire

organic matter contained in water (3). This is because the organic material that is chemically decomposed deliberately using strong oxidizing agents in acidic potassium bichromate and heated using silver sulfate catalyst (3); (11). Thus all forms of organic matter, both easy and difficult to unravel, will be oxidized. The difference between COD and BOD gives an overview of the magnitude of organic matter decomposition in difficult waters. COD depicts the total amount of organic matter content. Based on the results of ANOVA and Duncan's Multiple Range Test performed is known that there is interaction between the bacteria and the long biodegradation consortium to decreased levels of COD.

Table 3 Test Interaction Consortium Against Bacteria and Time Percentage Decrease Levels of COD (%) Domestic Liquid Waste

Treatment (K)	Time (T)					
	(t ₁) 0-4	(t ₂) 0-8	(t ₃) 0-12	(t ₄) 0-16	(t ₅) 0-20	(t ₆) 0-25
(k ₁) <i>B. subtilis</i> , <i>P. amylolyticus</i> , <i>Nitrosomonas</i> sp, dan <i>B. circulans</i>	5.66 c B	42.71 b A	57.45 ab AB	62.28 a A	69.39 a A	70.20 a A
(k ₂) <i>P. amylolyticus</i> , <i>Nitrosomonas</i> sp, <i>B. circulans</i> , dan <i>B. pumilus</i>	10.68 c B	40.89 b AB	65.07 a A	67.60 a A	66.59 a A	71.09 a A
(k ₃) <i>B. subtilis</i> , <i>Nitrosomonas</i> sp, <i>B. circulans</i> dan <i>B. pumilus</i>	34.01 d A	46.46 c A	59.67 b AB	64.28 ab A	67.05 a A	66.26 a A
(k ₄) Sterile Waste	12.96 a B	14.41 a C	4.35 a C	6.74 a C	10.04 a C	17.76 a B
(k ₅) Non-Sterile Waste	3.55 d B	21.62 c BC	44.41 a B	38.61 ab B	31.68 bc B	29.13 bc B

Note: Different letters indicate there is a very real difference
 The same letters in all directions showed no significant differences
 Towards small letters are letters that indicate horizontal consortium
 Large font vertical direction is the letter that shows the time

In Table 3 known that within 12 days the consortium of *Paenibacillus amylolyticus*, *Nitrosomonas*, *Bacillus circulans*, and *Bacillus pumilus* (k₂) the best in lowering domestic wastewater COD is equal to 65.07%, and this continued until the day 25. The result of the reduction in COD levels did not differ with decreased levels of COD by a consortium of *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans* (k₁) and a consortium of *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus* (k₃). But the consortium k₃ slower than the other two consortia. However, it can be said that the reduction in COD levels within 12 days already provide more than 50% decline. Decreased levels of COD quickly showed the presence of

synergism between species member in the consortium. According Roheim (13), and *B. subtilis*, *B.circulans*, *B.pumilus* belong to the genus *Bacillus* are capable to decompose cellulose, but it is also the genus *Bacillus* lipid compounds break down into glycerol and fatty acids to be used as a source of nutrients. On the other hand, *Nitrosomonas* sp. is also able to transform the ammonia into nitrite. *Paenibacillus amylolyticus* also produce the enzyme cellulase. Thus it can be said that the more organic materials are broken down by bacteria, the greater the amount of COD reduction in wastewater. Here is a chart to decreased levels of COD results biodegradation of domestic wastewater.

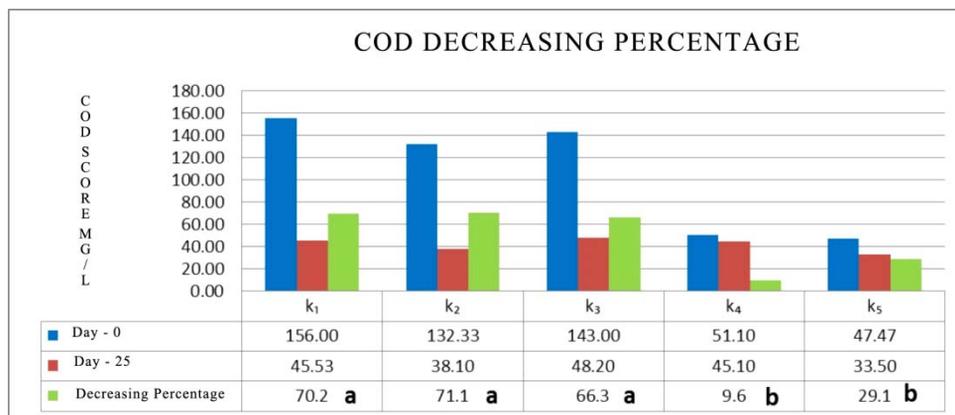


Figure 2 Graphs Percentage Decrease in the COD on Domestic Waste Biodegradation Process
 Description: k₁: *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans*
 k₂: *Paenibacillus amylolyticus*, *Nitrosomonas*, *Bacillus circulans*, and *Bacillus Pumilus*
 k₃: *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus*
 k₄: domestic wastewater is heated
 k₅: domestic wastewater that is not heated

Based on Figure 2. Known consortium of *Paenibacillus amylolyticus*, *Nitrosomonas*, *Bacillus circulans*, and *Bacillus pumilus* (k2) resulted in a decrease in the COD levels high at 71.1 %. The reduction in COD levels are not different from the reduction in COD levels produced by consortium of *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans* (k1) and a consortium of *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus* (k3). This suggests that the members of the consortium are synergistic in lowering the COD levels higher than the control and the bacterial consortium third as effective in lowering domestic wastewater COD. Decreased levels of COD occur because the bacteria are able to consume organic matter as a nutrient substrate. This is consistent with the statement Fardiaz, (6) in Thamrin (16) microorganisms in sewage continuously throughout the metabolic process energy needs are met and will produce compounds that can have an impact on the decline in the value of COD.

Levels of Total Suspended Solid (TSS) during the process of Biodegradation of Domestic Waste Water

Total Suspended Solid (TSS) or solids suspension is a set of solids consisting of particles smaller size and weight compared to the sediment, such as organic matter and microorganisms cells (10). Suspended solids in the water are polluting the environment because it is not soluble and are not directly deposited in waters that can cause water turbidity. Suspension solids levels can be reduced by microorganisms that help decompose organic compounds work becomes simpler. Based on the results of ANOVA is known that there is no interaction between bacterial consortium and TSS levels at the time of the domestic wastewater, but the consortium of bacteria and time effect on TSS levels decrease during biodegradation process in domestic wastewater. Based on these results we performed Duncan's Multiple Range Test as an advanced test.

Table 4 Effect of Duncan's Multiple Range Test Consortium Against Decrease of Level in TSS (%) Domestic Liquid Water

Consortia	Level Reduction (%)
(k ₁) <i>B. subtilis</i> , <i>P. amylolyticus</i> , <i>Nitrosomonas</i> sp, and <i>B. circulans</i>	37.1 a
(k ₂) <i>P. amylolyticus</i> , <i>Nitrosomonas</i> sp, <i>B. circulans</i> , and <i>B. pumilus</i>	3813 a
(k₃) <i>B. subtilis</i>, <i>Nitrosomonas</i> sp, <i>B. circulans</i> and <i>B. pumilus</i>	4316 a
(k ₄) Wastewater is heated	2416 b
(k ₅) Wastewater is not heated	2211 b

Note: The same letters in all directions showed no significant differences

In Table 4 it is known that a consortium of *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus* (k3) gave the highest TSS levels decrease to 43.6%. These results did not differ with decreased levels of TSS by a consortium of *Paenibacillus amylolyticus*, *Nitrosomonas*, *Bacillus circulans*, and *Bacillus pumilus* (k2), and a consortium of *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans* (k1). Thus it can be said that the three consortium equally effective to reduce TSS, which means that the bacteria in the consortium has the ability to decompose organic matter in domestic wastewater suspended substance.

In Table 5 it is known that on the 25th day of use bacterial consortium able to reduce the high levels of TSS, but not different on day 20. Thus it can be said that the highest TSS levels decrease after 20 days. Graph of reduction in TSS levels for domestic wastewater biodegradation process (Figure 3).

Table 5 Duncan's Multiple Range Test Against Time Effect Decreased Levels of TSS (%)

TIME	CONTENT DECREASING (%)
(t ₁) 0-4	16.9 c
(t ₂) 0-8	20.2 bc
(t ₃) 0-12	24.7 bc
(t ₄) 0-16	33.0 b
(t ₅) 0-20	48.3 a
(t₆) 0-25	55.7 a

Note: The same letters in all directions showed no significant differences



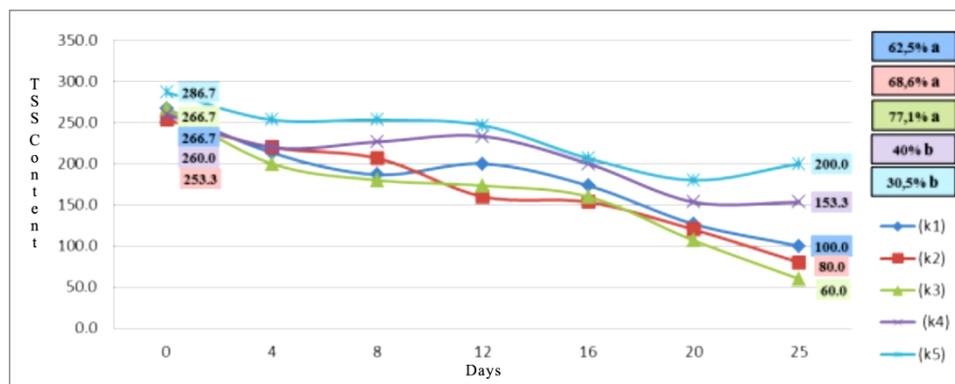


Figure 3 Reduction levels of Total Solid Suspended During Domestic Wastewater Biodegradation
Description:

k1: *Bacillus subtilis*, *Paenibacillus amylolyticus*, *Nitrosomonas*, and *Bacillus circulans*

k2: *Paenibacillus amylolyticus*, *Nitrosomonas*, *Bacillus circulans*, and *Bacillus pumilus*

k3: *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus*

k4: domestic wastewater heated

k5: domestic wastewater is not heated

Based on Figure 3 it can be seen that the consortium of *Bacillus circulans*, *Bacillus pumilus*, *Bacillus subtilis*, and *Nitrosomonas* sp (k3) gave the highest TSS levels decrease in the amount of 77.1 %. The ability to reduce levels of TSS did not differ with the ability to decrease the levels of TSS consortium *Paenibacillus amylolyticus*, *Nitrosomonas*, *Bacillus circulans*, and *Bacillus pumilus* (k2) and a consortium of *Bacillus subtilis*, *Nitrosomonas*, *Bacillus circulans* and *Bacillus pumilus* (k3). This suggests synergistic species in the consortium so as to outline the solute particles in domestic wastewater. These results also indicate that the three types of bacteria consortium is effective in lowering the levels of domestic effluent TSS. *B. subtilis* and *B. circulans* high cellulolytic ability, thus containing cellulose dissolved solids will be described. Besides *B. subtilis* and *B. Circulans*, *Bacillus* genus includes groups that have the ability to decipher crude fiber and lignin are difficult to decompose lignin and delignification process through hydrolysis of cellulose so solid that dissolved organic matter in the form of lignin, lipids, and cellulose in the waste water can be reduced (18). *B. pumilus* and *Nitrosomonas* sp. also has the ability to remodel organic materials, Adhi (2008) states that nitrifying

bacteria are bacteria found in the waste containing organic material and effectively decompose organic matter in the wastewater. The study revealed that the levels of TSS were obtained as 60 mg/l TSS has entered the standard quality of domestic wastewater is 100 mg/l, with a decrease in TSS levels in the entire addition of bacterial consortium showed that the bacterial consortium used a role in lowering the levels of TSS.

CONCLUSIONS

Based on those result of this study concluded that consortium of *Bacillus subtilis*, *Nitrosomonas*, *Bacillus pumilus* and *Bacillus circulans* effective in the biodegradation of domestic wastewater for being able to reduce level of BOD 67.4%, COD 66.3%, and TSS 77.1% TSS in the process biodegradation of domestic wastewater.

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