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ABILITY OF BACTERIAL CONSORTIUM: *Bacillus coagulans, Bacilus licheniformis, Bacillus pumilus, Bacillus subtilis, Nitrosomonas* sp. and *Pseudomonas putida* IN BIOREMEDIATION OF WASTE WATER IN CISIRUNG WASTE WATER TREATMENT PLANT

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Abstract

This study was conducted in order to determine the ability of bacterial consortium: Bacillus coagulans, Bacilus licheniformis, Bacillus pumilus, Bacillus subtilis, Nitrosomonas sp., and Pseudomonas putida in bioremediation of wastewater origin Cisirung WWTP. This study uses an experimental method completely randomized design (CRD), which consists of two treatment factors (8x8 factorial design). The first factor is a consortium of bacteria (K), consisting of 8 level factors (k1, k2, k3, k4, k5, k6, k7, and k8). The second factor is the time (T), consisting of a 7 level factors (t0, t1, t2, t3, t4, t5, t6, and t7). Test parameters consist of BOD (Biochemical Oxygen Demand), COD (Chemical Oxygen Demand), TSS (Total Suspended Solid), Ammonia and Population of Microbes during bioremediation. Data were analyzed by ANOVA, followed by Duncan test. The results of this study showed that the consortium of Bacillus pumilus, Bacillus subtilis, Bacillus coagulans, Nitrosomonas sp., and Pseudomonas putida with inoculum concentration of 5% (k6) is a consortium of the most effective in reducing BOD 71.93%, 64.30% COD, TSS 94.85%, and 88.58% of ammonia.

Key words*: Bioremediadtion, Bacillus, Consortium, BOD, COD, TSS.*

INTRODUCTION

Water river pollution, especially in Citarum River, West Java, has achieved the status of heavily polluted due to the amount of liquid waste discharged into the water body without being processed. Therefore, the government built the Waste Water Treatment Plant (WWTP) Cisirung for treatment of industrial wastewater surrounding South Bandung area before it is discharged into water bodies. Industrial wastewater generally have concentration of organic matter so high that need microorganisms as bioremediation agents, especially bacteria. Bacteria are microorganisms that are easy to find because of the wide spread.

Genera Bacillus have interesting physiological properties because each type has different abilities, including: capability of degrading organic compounds such as proteins, starch, cellulose, hydrocarbons, and order; ability to produce antibiotics; role in nitrification and denitrification; nitrogen; oxidizing selenium; oxidizing and reducing manganese (Mn); is chemolithotroph, aerobic or facultative anaerobes, acidophilic or alkaliphilic, or thermophilic (Clous and Berkeley, 1986).

Bioremediation is defined as the use of microorganisms that have been selected to be grown on certain pollutants in an effort to reduce levels of these pollutants.

During the bioremediation process, enzymes produced by microorganisms modify the structure of toxic pollutants into less complex structure so that it becomes harmless metabolites (Priadie, 2012). In Indonesia, the bioremediation of waste water treatment has not been carried out effectively. Therefore, any research on bioremediation of waste using bacteria is expected to help solving the problem of industrial waste in Indonesia.

The purpose of this study is to obtain bacterial consortium and the most effective concentration of inoculum to remediate WWTP (Wastewater Treatment Plant) effluent in Cisirung.

MATERIALS AND METHODS

The experimental method is applied to the bioremediation of wastewater WWTP Cisirung with completely randomized design (CRD). 8x8 factorial design with three replications consisting of two level factors. The first factor is the type of consortium (K), consists of 8 levels, the second factor is the time (T), consisting of 8 levels sampling time, namely: Factor (t0): 0, (t1) 60, (t2) 120, (t3) 180, (t4) 240, (t5) 300, (t6) 360, (t7) 420 hours. The whole treatment consists in: 1. (k1): Waste heated inoculated by consortium of *Bacillus coagulans*, *B. licheniformis B. pumilus Pseudomonas putida* and *Nitrosomonas* sp. Inoculated by concentration of inoculum 10% of the total volume 2. (k2): Waste heated inoculated by consortium of *B. licheniformis*, *B. pumilus, B. subtilis Pseudomonas putida*, *Nitrosomonas sp.* Inoculated by concentration of inoculum 10% of the total volume. 3. (k3): Waste heated inoculated by consortium of *B. pumilus, B. subtilis B. coagulans, Pseudomonas putida* a *Nitrosomonas* sp. Inoculated by concentration of inoculum 10% of the total volume 4. (k4): Waste heated and inoculated by consortium of *Bacillus coagulans, B. licheniformis, B. pumilus Pseudomonas putida, Nitrosomonas* sp. Inoculated by concentration of inoculum 5% of the total volume. 5. (K5): Waste heated and inoculated by consortium *B. licheniformis, B. pumilus, B. subtilis, Pseudomonas putida* and *Nitrosomonas* sp. Inoculated by concentration of inoculum 5%. 6. (k6): Waste heated and inoculated by consortium *B. pumilus, B. subtilis, B. coagulans, Pseudomonas putida, Nitrosomonas* sp. Inoculated by concentration of inoculum 5% of the total volume. 7. (k7): Wastewater WWTP heated Cisirung no additional indigenous bacteria and other bacteria. 8. (k8): WWTP Cisirung industrial wastewater, not heated.

Parameters measured consists of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), acidity (pH), Dissolved Oxygen (DO), the number of bacteria, temperature, and Total Suspended Solid (TSS). The experimental data obtained and analyzed by using analysis of variance (ANOVA). If there is a real effect then continued with Multiple Range Test (Duncan) with a significance level of 5% (Gomez and Gomez, 1995).

RESULTS AND DISCUSSIONS

Growth of Bacteria Consortium During Bioremediation

The ability of microorganisms to degrade wastewater contained in Cisirung WWTP can be assessed from the calculation of the number of microbial cells during the process of bioremediation. The number of bacteria that grow during the biodegradation process wastewater was measured by the method of Total Plate Count (TPC). The number of bacteria was counted every 60 hours for 420 hours. In Table 1 it is known that the consortium (k2) which consists of a strain of *Bacillus licheniformis, B. pumilus, B. subtilis, P. putida,* and Nitrosomonas sp. inoculated as inoculum concentration of 10% is a consortium that has the most number of cells which reached 3:13 x1012 CFU/mL at 240 hours. This shows that the bacterial strains Consortium (k2) work synergistically so as to decompose the organic content of the waste, or it can be said that the biodegradation process can increase the number of microorganisms and metabolism activity. The more the number of microorganisms, the more enzymes is produced by these microorganisms. Enzymatic reactions were carried out by bacteria which is the key to the process of gradual change of wastewater containing complex compounds into simpler components and stable so that the levels of pollutants in the effluent to be down (Sutanto, 2012). Microbes use these chemical compounds for growth and reproduction through various oxidation processes in the degradation process.

Based on Table 1 it is known that the bacterial population in the consortium (k2) in all 240 hours were not significantly different with a consortium of *B. coagulans, B. licheniformis, B. pumilus, P. putida,* and *Nitrosomonas* sp. the inoculum concentration of 5% (k4) in all 180 hours. The presence of bacteria in the control, the waste heated (k7) and waste that are not heated (k8) indicates the existence of indigenous bacteria in the wastewater that were not killed in the heated.

Tabel 1. Duncan's multiple range test of bacterial population during bioremediation process (log10 CFU/ mL)

Treatmnet	Time of Bioremediation (Hours)							
	0	**60**	**120**	**180**	**240**	**300**	**360**	**420**
(k_1)	11.42 c B	11.57 bc B	11.73 bc AB	11.85 b BC	11.73 bc BC	12.33 a AB	11.86 b A	11.47 ab AB
(k_2)	11.44 d B	11.54 cd B	11.77 bc AB	12.42 a A	**12.56 a A**	12.3 a AB	11.88 b A	11.68 bcd A
(k_3)	11.59 cd A	11.56 d B	11.80 bc AB	12.09 a AB	12.03 a ABC	11.88 ab AB	11.42 d BC	11.19 e B
(k_4)	11.31 d BC	11.91 bc A	12.00 bc A	12.46 a A	12.21 ab AB	12.15 ab AB	11.91 bc A	11.65 cd A
(k_5)	11.25 b B	11.45 ab B	11.66 ab AB	11.85 a BC	11.57 ab C	11.78 a B	11.72 ab AB	11.68 ab A
(k_6)	11.31 b BC	11.56 b B	11.46 b B	11.67 b C	12.48 a A	12.51 a A	11.3 b C	11.33 b AB
(k_7)	2.34 bc E	2.43 abc D	2.33 c D	2.58 a E	2.60 a E	2.55 ab D	2.49 abc E	2.51 abc D
(k_8)	9.63 ab D	9.73 a C	9.47 abc C	9.34 abcd D	9.44 abcd D	8.90 bcd C	8.81 cd D	8.67 d C

Different capital letters vertical direction there is a real difference
k1: *B. coagulans, B. licheniformis, B. pumilus, P. putida* and *Nitrosomonas* sp. inoculum 10%
k2: *B. licheniformis, B. pumilus, B. subtilis, P. putida* and *Nitrosomonas* sp. inoculum 10%
k3: *B. pumilus, B. subtilis, B. coagulans, P. putida* and *Nitrosomonas* sp. inoculum 10%
k4: *B. coagulans, B. licheniformis, B. pumilus, P. putida* and *Nitrosomonas* sp. inoculum 5%
K5: *B. licheniformis, B. pumilus, B. subtilis, P. putida* and *Nitrosomonas* sp. inoculum 5%
k6: *B. pumilus, B. subtilis, B. coagulans, P. putida* and *Nitrosomonas* sp. inoculum 5%
K7: Liquid waste Cisirung heated
k8: Cisirung WWTP waste water, not heated, contain indigenous bacteria Cisirung

Effect of type Consortium Against Bacteria Bioremediation of Industrial Wastewater

Biodegradation is the decomposition of complex organic materials into simple organic materials which take place biologically. Biodegradation process occurs observed with changes in parameters that support the process of biodegradation. The parameters used in this study is the level of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), ammonia, and total suspended solid (TSS).Biochemical Oxygen Demand (BOD) or biological oxygen demand is the amount of oxygen needed by microorganisms in the water environment to degrade organic waste contained in the water. BOD parameters are commonly used to determine the level of contamination of waste water. Initial BOD value of industrial waste before it is added medium starter medium was 260 mg/L. This value exceeds the quality standards that have been set, which is equal to 85 mg/L. Once added to the medium in the effluent starter on hour-0, BOD increased varying. This is due to the medium containing Nutrient Broth starter (NB) add organic content in the waste. The high value of BOD in the effluent showed a high oxygen demand required to perform the decomposition of the organic materials contained in the waste. Based on the results of the analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (Table 2). From Table 2 it is known that bacterial consortium of *B. pumilus, B. subtilis, B. coagulans, P. putida,* and *Nitrosomonas* sp. inoculum 5% (k6) is able to reduce the BOD levels high at 71.93%. Based on Table 2 known that bacterial consortium of *B. pumilus, B. subtilis, B. coagulans, P. putida,* and *Nitrosomonas* sp. inoculum 5% (k6) is able to reduce the BOD levels high at 71.93%.

This shows that there is a synergistic interaction between bacteria in the use of organic materials as nutrients.

Table 2. Duncan's Multiple Range Test on the influence of the consortium on the percentage decrease in the value of BOD wastewater

Treatment by Consortium	Percentage decline in the value of BOD **(%)**
B. pumilus, B. subtilis, B. coagulans, P. putida, Nitrosomonas sp. inoculum 5% (k6)	**71.93 a**
B. coagulans, B. licheniformis, B. pumilus, P. putida, Nitrosomonas sp. inoculum 5% (k_4)	69.7 b
B. licheniformis, B. pumilus, B. subtilis, P. putida, *Nitrosomonas* sp. inoculum 5% (k_5)	69.7 b
B. pumilus, B. subtilis, B. coagulans, P. putida, *Nitrosomonas* sp. inoculum 10% (k_3)	57.3 c
B. licheniformis, B. pumilus, B. subtilis, P. putida, Nitrosomonas sp. inoculum 10% (k_2)	57.3d
B. coagulans, B. licheniformis, B. pumilus, P. putida, Nitrosomonas sp. inoculum 10% (k_1)	49.8 e
Heated liquid wastes no indigenous bacteria and other bacteria (k7)	30.8 f
Liquid waste is not heated, there are indigenous bacteria (k8)	29.1 g

Note: Different letters indicate there are significant differences

Based on Table 2 known that bacterial consortium of *B. pumilus, B. subtilis, B. coagulans, P. putida,* and *Nitrosomonas* sp. inoculum 5% (k6) is able to reduce the BOD levels high at 71.93%.

This shows that there is a synergistic interaction between bacteria in the use of organic materials as nutrients. According Lynd et al. (2002) *Bacillus* sp. compounds can degrade lipids into glycerol and fatty acids so it is more easily digested by microbes as a source of nutrients.

Decreased levels of BOD on all treatments inoculated by genus Bacillus, showing that the genus bacillus has a high ability to decompose organic materials from industrial wastes in large quantities. *Bacillus* sp. can produce extracellular enzymes decomposing cellulose and hemicellulose, *Nitrosomonas* sp. able to decompose ammonia into nitrogen compounds that are simpler and *Pseudomonas putida* and *Bacillus* can degrade phenol (Prasanna et al., 2008).

This is consistent with the opinion of Davis and Cornwell (1991) that the bacteria have the ability to use organic nutrients as a source of energy, so that the bacteria can degrade organic matter in the wastewater into CO_2 and water. Therefore, it can be concluded that consortium of bacteria can reduce levels of BOD in industrial wastewater.

Influence of wastewater bioremediation by a consortium against the COD

Chemical Oxygen Demand (COD) is the amount of oxygen needed by microorganisms to oxidize organic compounds chemically. COD covers all compounds in a body of water, whether or not easily parsed. COD can describe the content of organic material that can be chemically oxidized, both biodegradable and non-biodegradable.

Higher COD values describe the higher level of contamination of a water body (Metcalf and Eddy, 1991). According to Boyd (1990), the difference between COD and BOD value gives an overview of the magnitude of organic materials that are difficult outlined in the water bodies.

Initial COD concentration of industrial waste before adding starter medium at 441 mg/L. The COD levels exceed the quality standards established government regulations, ie 250 mg/L. Once added to the medium in the effluent starter on hour-0, COD increase varied. Data were analyzed by ANOVA followed by Duncan's Multiple Range Test (Tabel 3).

Table 3. Duncan's Multiple Range Test the Effect of Bacterial Consortium Against COD content (%) of Industrial Waste Water

Treatment by Consortium	Percentage decline in the value of COD (%)
B. licheniformis, B. pumilus, B. subtilis, P. putida, *Nitrosomonas* sp. inoculum 5% (k_5)	65.56a
B. pumilus, B. subtilis, B. coagulans, P. putida, *Nitrosomonas* sp. inoculum 5% (k_6)	64.30b
B. pumilus, B. subtilis, B. coagulans, P. putida, *Nitrosomonas* sp. inoculum 10% (k_3)	54.82c
B. coagulans, B. licheniformis, B. pumilus, P. putida, Nitrosomonas sp. inoculum 10% (k_1)	53.23d
B. licheniformis, B. pumilus, B. subtilis, P. putida, Nitrosomonas sp. inoculum 10% (k_2)	52.00e
B. coagulans, B. licheniformis, B. pumilus, P. putida, Nitrosomonas sp. inoculum 5% (k_4)	41.26f
Heated liquid wastes no indigenous bacteria and other bacteria. (k7)	30.67g
Liquid waste is not heated, there are indigenous bacteria (k8)	26.25h

Note: Different letters indicate there are significant differences

Based on Table 3 it is known that bacterial consortium *B. licheniformis, B. pumilus, B. subtilis, P. putida*, and *Nitrosomonas* sp. inoculum 5% (k5) is able to reduce the COD levels high at 65.56% and reduce levels of

BOD by 69.7%. While the consortium (k6) consisting of *B. pumilus, B. subtilis, B. coagulans, P. putida, Nitrosomonas* sp. menurunkan highest BOD level that is equal to 71.93%.

This shows both consortium that can be used for bioremediation of industrial waste water. Industrial wastewater contains high organic matter such as carbohydrates, proteins and fats; there are also chemical pollutants such as phenol compounds.

The waste decomposition process require assistance cellulase enzymes able to break the glycosidic bond (Howard, 2003). *B. coagulans* and *Bacillus* that related *B. subtilis* is known as a group that able to produce high amounts of cellulase enzymes, while *B. licheniformis* produces amylase enzymes that is resistance to heat resistance (Anthony and Philip, 2002), so the use of both of these bacteria strongly support the decomposition of organic matter in industrial wastewater.

Furthermore, *Pseudomonas* and nitrosomas a nitrifying bacteria are widely used in bioremediation in shrimp culture systems, and lake or water that has a high content of ammonia (Anthony and Philip, 2002, Ranjan et al., 2014).

Pseudomonas is also used for the bioremediation of oil spills, and waste pulp and *Bacillus* group was used for mineralization and decomposition of protein. The genus *Pseudomonas* and *Bacillus* is used for municipal solid waste.

The genus *Bacillus* such as *Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Bacillus coagulans*, and of the genus *Paenibacillus*, such as *Paenibacillus polymyxa* is used for bioremediation of organic detritus and effective cleaning of the carbon waste of waters (Ranjan et al., 2014). Based on this study the average value obtained COD levels at 1177.7 m/l still exceed the maximum levels COD quality standards in the textile industry which is regulated by the government in the amount of 150 mg/l.

Decreased levels of COD in bioremediation by bacteria showed that bacteria play a role in the process of biodegradation in the industrial wastewater.

Effect of Bacterial Strains Consortium Against Levels TSS (Total Suspended Solid) During Bioremediation Process Liquid Waste

Total suspended solids (TSS Total Suspended Solid or) is suspended material with a diameter of more than 1 um milliophore retained on the filter with a pore diameter lm 0:45 remaining for evaporation and heating at a temperature of 103-105ºC (Saeni, 1989). TSS initial value of industrial waste by 1100 mg/L. This value exceeds the quality standards that have been set, that is equal to 60 mg/L. Once added to the medium in the effluent starter on hour-0, TSS increase varied. Addition of medium starter containing nutrient Broth (NB), causing the increase content of TSS in the effluent. TSS data for bioremediation analyzed by ANOVA followed by Duncan's Multiple Range Test.

The results of Table 4. It is known that a decrease in TSS concentration is highest in treatment by a consortium of k6, k5 and k2 are composed of bacteria *B. pumilus, B. subtilis, B. coagulans, P. putida, B. licheniformis* and *Nitrosomonas* sp. with inoculum concentration of 5%, which amounted to 94.85% respectively and and 91.82%.

This shows that the bacterial strain and the consortium are effective in letting decompose organic waste and industrial waste water pollutants.

This suggests also that the bacteria in the consortium (k6) is able to utilize substrates decompose organic substances and liquid industrial waste to energy sources.

According to research Barros (2013), the species *Bacillus* sp. has a unique ability that can be used in the process of biodegradation. *B. subtilis* is able to oxidize hydrocarbons, producing biosurfactant and has the ability to produce the form of lipopeptide antibiotics. *B. coagulans* is able to produce the enzyme cellulase and lipase. Cellulose can be broken down by the bacteria *Bacillus coagulans* and Bacillus subtilis because the bacteria have a high cellulolytic capabilities.

The genus *Bacillus* has the ability to degrade crude fiber and lignin are difficult to decompose through the process of delignification and cellulose hydrolysis so that the dissolved organic material in the

wastewater industry in form of lignin, lipids, and cellulose can be reduced.

Table 4. Duncan's Multiple Range Test of Influence Consortium of Bacteria Against Percentage Decrease of TSS concentration (%)

Treatment	Percentage decline of the TSS (%)
B. pumilus, B. subtilis, B. coagulans, P. putida, Nitrosomonas sp. Inoculum 5% (k_6)	94.85 a
B. licheniformis, B. pumilus, B. subtilis, P. putida, Nitrosomonas sp. Inoculum 5% (k_5)	91.82 ab
B. licheniformis, B. pumilus, B. subtilis, P. putida, Nitrosomonas sp. Inoculum 10% (k_2)	91.82 ab
B. coagulans, B. licheniformis, B. pumilus, P. putida, Nitrosomonas sp. inoculum 5% (k_4)	88.79 bc
B. pumilus, B. subtilis, B. coagulans, P. putida, Nitrosomonas sp. Inoculum 10% (k_3)	86.36 c
B. coagulans, B. licheniformis, B. pumilus, P. putida, Nitrosomonas sp. inoculum 10% (k_1)	85.76 c
Heated liquid wastes no indigenous bacteria (k7)	27.27 d
Liquid waste is not heated, there are indigenous bacteria (k8)	17.27 e

Note: Different letters indicate there are significant differences

Effect of Bacterial Strains Consortium Against Levels Ammonia During Bioremediation

Data Ammonia during bioremediation analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (Tabel 5).

Table 5. Duncan's Multiple Range Test the Effect of Bacterial Consortium Against the reduction of Ammonia concentration (%)

Treatment	Perc. Reduction of Ammonia (%)
B. licheniformis, B. pumilus, B. subtilis, P. putida, Nitrosomonas sp. inoculum 10% (k_2)	92.647 a
B. pumilus, B. subtilis, B. coagulans, P. putida, *Nitrosomonas* sp. inoculum 5% (k_6)	88.581 ab
B. licheniformis, B. pumilus, B. subtilis, P. putida, *Nitrosomonas* sp. inoculum 5% (k_5)	87.615 abc
B. pumilus, B. subtilis, B. coagulans, P. putida, *Nitrosomonas* sp. inoculum 10% (k_3)	83.998 bc
B. coagulans, B. licheniformis, B. pumilus, P. putida, *Nitrosomonas* sp. inoculum 5% (k_4)	83.549 bc
B. coagulans, B. licheniformis, B. pumilus, P. putida, Nitrosomonas sp. inoculum 10% (k_1)	81.074 c
Heated liquid wastes no indigenous bacteria (K7)	15.326 d
Liquid waste is not heated, there are indigenous (k8)	0.3850 e

Note: Different letters indicate there are significant differences

From Table 5 it is known that the consortium *Bacillus licheniformis, B. pumilus, B. subtilis, P. putida*, and *Nitrosomonas* sp. in inoculation with inoculum concentration of 10%, a starter (k2) can reduce the ammonia levels at 92.467% and was as effective as the consortium (k6) and (k5). These results indicate that the strain of bacteria in the third consortium is effective in reducing ammonia levels. Ammonia is a compound of nitrogen and hydrogen which has a pungent aroma with a characteristic odor and has a chemical element represented as NH_3.

The smell of ammonia which is derived from the decomposition of urea, as a component of most organic material is broken down by bacteria into energy and NH_3. *Nitrosomonas* sp. a nitrifying bacteria capable of oxidizing ammonia to nitrite or nitrate, in autorof and use it as a source of carbon. Besides *Nitrosomonas*, *Bacillus* sp. also able to reduce levels of ammonia. Genus *Bacillus* can reduce levels of ammonia because of its ability to oxidize ammonia content in waste and can utilize the basis of heterotrophic ammonia as a source of nutrition (Edwards, 2011). Therefore, the addition of *Nitrosomonas* sp. and *Bacillus* on industrial waste contribute to lower ammonia levels. Organic compounds contained in waste and effluent such as proteins, carbohydrates, and fats, according to Gower (1980), used by bacteria as a food source. In this process, large molecules are broken down by enzymes into compounds of lower molecular weight. For example, the protein is broken down into amino acids and then further degraded to produce ammonia.

CONCLUSIONS

Consortium of *Bacillus pumilus, Bacillus subtilis, Bacillus coagulans, Nitrosomonas* sp., and *Pseudomonas putida* (k6) is a consortium of the most effective, resulting in 71.93% BOD, COD 64.30%, 94.85% TSS, and ammonia 88.58%.

Consortium *Bacillus coagulans, Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis, Nitrosomonas* sp., and *Pseudomonas putida* is effective for the bioremediation of industrial waste water.

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