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THE PERFORMANCE OF BACTERIAL CONSORTIUM IN VARIOUS CARRIERS ON THE BIOREMEDIATION OF RIVER WATER POLLUTED BY DOMESTIC SEWAGE

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Abstract

This study aims to obtain raw materials as an effective carrier for starter inoculum consortium of Bacillus coagulans B. licheniformis, B. subtilis, and Pseudomonas sp. and contact time for bioremediation of polluted water from the river Cimuka. The study was conducted by using the experimental method with completely randomized design (CRD) with two-factor factorial 9x9. The first factor is the carrier material (P) consisting of 9 level and the second factor is the contact time (W) consisting of 9 level. Parameters measured were BOD, COD, TSS, and ammonia. The results showed that bioremediation by a consortium of bacteria Bacillus coagulans, B. licheniformis, B. subtilis, and Pseudomonas sp. the carrier material alginate-starch with a concentration of 1% at the time bioremediation 14 days can reduce levels of BOD by 81.49%, 81.44% COD, TSS 75%, and 35.82% ammonia by bacteria populations reach 2,89x10^{13} CFU/ml. The results also show that the encapsulation can maintain the viability of bacteria and is able to improve the bioremediation of organic matter compared to treatment without encapsulation.

Key words: *Encapsulation, bioremediation, Alginate, Bacillus, Starch, Nitrosomonas, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand).*

INTRODUCTION

Currently the waste disposal in the waters increased, which resulted in pollution of water bodies such as rivers by organic matter and chemicals from industry and households. One is Cimuka River in West Java, river pollution levels are high enough, where industrial dispose of waste waste into waters without being treated and become domestic waste effluent streams. One of the efforts to minimize the volume of waste that the bioremediation technique that utilizes living organisms to break down harmful substances into harmless (Sheehan, 1997 in Wignyanto, et al., 2009).

Bioremediation can be done by adding certain microbes in the water that will be remediated (Munir, 2006). According Ishartanto (2009), the success of bioremediation depends on the amount of organic pollutant load and duration of contact (retention).

The longer the time degradation and the higher the degrading bacteria population, the more effective is bioremediation.

In this study, bioremediation in rivers Cimuka using indigenous bacteria isolated from the river Cimuka, namely Bacillus coagulants and B. subtilis. In addition, the bacteria Bacillus licheniformis and Pseudomonas sp. added to support the process of bioremediation. Bacillus coagulants are able to decompose lipid known as produce lipase that can reduce levels of BOD and COD simultaneously (Hidayat, et al., 2010). Bacillus subtilis is known to produce the enzyme but it also can form endospores that are able to tolerate extreme circumstances (Amrah, 2002). Bacillus licheniformis, produce amylase enzymes that are tolerant to alkaline pH and high temperature (Jamilah, 2011). Whereas Pseudomonas sp. known to degrade aromatic hydrocarbons (Munir, 2006). According Sastrawidana (2008) Bioremediation in waters by a consortium of bacteria indigenous and non-indigenous is very effective due to decomposition of organic matter such as cellulose, lipids, and proteins according to their ability.

Viability of bacteria in bioremediation processes can be improved by adding a consortium of bacteria in nutrient broth and mix of bacteria that have grown into the carrier material. Nutrient broth chosen because it can provide a source of nutrients and energy such as carbon, nitrogen, minerals, and vitamins that are appropriate for testing bacterial metabolic activity. In addition, the mixing of bacteria in the carrier material can stabilize emulsions and maximize the active material protection against environmental conditions (Mosilhey, 2003). Materials commonly used carrier is alginate and starch, talcum powder and gelatin, as well as rice flour, skim milk, corn starch, and dextrose. Bacteria in the carrier material alginate and starch through the encapsulation process can produce good quality capsules, with a high number of bacteria, consistent, strong and not easily destroyable (Wijayanti, 2010). Carrier material such as talcum powder and gelatin contains elements rich in protein and minerals, capable both in forming the membrane, is biocompatibility and non-toxic (Li, et al., 2009). Arifurrahman (2012) using rice flour carrier is added skim milk and glucose are capable of producing endurance during storage because rice flour is a source of carbohydrates, skim milk as a source of protein, while glucose is functioning as a protective nutrients and microbial cells. This study aims to obtain raw materials as an effective carrier for starter inoculum consortium of *Bacillus coagulans, B.licheniformis B. subtilis* and *Pseudomonas* sp. and contact time for bioremediation of polluted water from the river Cimuka.

MATERIALS AND METHODS

MATERIALS: Strain *Bacillus coagulans B. licheniformis, B. subtilis,* and *Pseudomonas* sp. alginate, calcium chloride, starch, talc powder, gelatin, rice flour, skim milk, corn starch, dextrose, nutrient agar, Cimuka river water.

Method

Preparation of inoculum consortium of strains of bacteria encapsulated in alginate.

Alginate 2 grams dissolved in 90 ml of distilled water was stirred slowly, and added 1 starch flour until is well blended and sterilized. Suspension alginate-starch which has been sterilized and inoculated by as much as 10% bacterial consortium consortium inoculum aseptically. Furthermore, the consortium inoculum in alginate-starch carrier material is inserted into the dropper tool to shed a solution of 0.1 M $CaCl_2.2H_2O$ formed Ca-alginate capsules. Furthermore capsules filtered using Whatman filter paper. Ca-alginate capsules are then put into a nutrient broth medium (Bashan et al., 2002 in Wijayanti, 2010) and incubated for 24-30 h in a rotary shaker speed 150 rpm at room temperature, to regrow consortium of bacteria contained in the capsule alginate-starch.

Preparation of inoculum in Gelatin and talc

Gelatin as much as 1% (1g) was dissolved in 100 ml of distilled water until well blended and sterilized in an autoclave at a temperature of 121^{o}C for 15 minutes at a pressure of 2 atm, after cold Gelatin-talc is inoculated by inoculum of consortium to be used with density cell is proportional to the turbidity of Mc Farland 3 Furthermore, a consortium of bacteria inoculated on the carrier material, by mixing 10% inoculum consortium to 100% (100 grams) of sterile talc powder until blended aseptically. The starter then incubated for 72 hours.

Preparation of Inoculum Starter

Medium starter consisted of 80% (80 grams) of rice flour, 10% (10 grams) of skim milk, 5% (5 grams) of corn flour and 5% (5 grams) of dextrose for the manufacture of inoculum (starter) solid as much as 100 grams. Rice flour, skim milk, corn starch, and dextrose put in clear plastic heat resistant size 15 x 25 cm. Then added to water at a ratio of 1: 1 and steamed for 1 hour, then cooled. A total of 10 ml of a bacterial suspension with a cell density comparable to McFarland 3 was inoculated into 100 grams of medium starter that has been sterilized. Plastic tied, given the holes and wrapped in aluminum foil, and then incubated at 30 ° C For 3 days and counted the population every 6 hours until the population reaches exponential phase. Upon reaching the phase of exponentially growing. The next starter dried and milled to form a powder.

Stages of Bioremediation

Starter inoculum bacterial consortium in the carrier material was inoculated into the waste water as much as 1% of the volume of waste,

while the starter inoculum in nutrient broth was inoculated as much as 10% of the volume of waste water. Then, the test parameters were measured on day 0, day 1, day 2, day 4, day 6, day 8, day 10, day 12, and day 14. Observed parameter is BOD, COD, TSS, and the concentration of ammonia.

Data Analysis.

Research using experimental methods with completely randomized design (CRD) factorial design with two factors, namely 9 x 9. The first factor is the carrier material (P) consisting of 9 level and the second factor is the contact time (W) consisting of 9 level. If found significant differences, then followed by Duncan's Multiple Range Test at 5% significance level.

RESULTS AND DISCUSSIONS

Effect of inoculum Starter In carrier material, Against BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) during the bioremediation process Wastewater from Cimuka River.

Biochemical Oxygen Demand (BOD) is a measure of the amount of oxygen required microorganisms to decompose organic matter contained in waste water. Results Analysis of Variance (ANOVA) showed levels of BOD and COD carrier material effect on the percentage removal of BOD and COD (Table 1 and Table 2).

Table 1. Duncan's Multiple Range Test Percentage decrease in BOD (Biochemical Oxygen demand) In The remediation of waste water by a consortium inoculum of bacteria on various carrier materials.

Contact Time (H)	Material Carrier (P)			
	Nutrient Broth	Alginate -Starch	Talc-Gelatin	Rice Skim Milk-Corn Flour-Dextrose
Day 0 (mg/l)	1000	1582	1374	2430
Day -8(mg/l)	5243	4672	4741	5000
Day- 14 (mg/l)	500	335	434	538
Persentage of Reduction (%)				
Day 0-14	50,00 cd	**78,90 a**	66,26 abc	75,86 ab
Day 8-14	90,45 bc	**92,82 a**	90,86 bc	89,25 c

From Duncan's Multiple Range test results, it can be seen that the inoculum carrier material effect on the reduction of BOD and COD (Table 1 and Table 2). The percentage decrease in time from day 8 to 14 in order to be higher than decrease from the first day until day 14, this was due to the inoculum starter requires adaptation to the origin of the waste water stream before entering the phase exponential ie on days 8-14 and at the same time able to decompose organic matter and pollutants in wastewater resulting in lower levels of BOD and COD.

Table 2. Duncan's Multiple Range Test Percentage decrease in COD (Chemical Oxygen demand) in the remediation of waste water by a consortium inoculum of bacteria on various carrier materials.

Contact Time (H)	Material Carrier (P)			
	Nutrient Broth	Alginate-Starch	Talc-Gelatin	Rice Skim Milk-Corn Flour-Dextrose
Day 0 (mg/l)	1583	2476	2122	3834
Day -8(mg/l)	8171	7445	7435	7871
Day- 14 (mg/l)	807	525	686	847
Persentage of Reduction (%)				
Day 0-14	48,97 cd	**78,83 a**	65,4 abc	75,76 ab
Day 8-14	90,10 bc	**92,94 a**	90,7 bc	89,24 c

Inoculum starter in the carrier material alginate-starch resulted in removal of BOD and COD highest on days 8-14 in the amount of 92.94% with a bacterial population reaches 2.89 x10^{13} CFU/ml, indicating that the carrier material alginate and tapioca can maintain viability and activity of bacteria.

In microencapsulated probiotics by using calcium alginate and corn starch as a prebiotic improve the encapsulation of living bacteria than encapsulation without starch (Sultana, etal. 2000. Dried culture bacterial consortium in carrier-skimmed milk powder bares- corn starch and dextrose may also decrease BOD and COD by 89.25% 89.24%. Dried cultures are easily dispersed in water, the viability of bacteria in skim-milk-rice powder-starch-dextrose can be maintained, and however, the results lower than alginate-starch. In probiotics alginate-starch mixture showed effective encapsulation, also in various other bacteria (Mortazavian et al. 2007).

Encapsulation is trapping or closing the cells of microorganisms by means of a coating or

wrapping the bacteria with hydrocolloid right, and separates the cell from the surrounding environment, such as changes in pH, heat, enzyme activity, chemical compounds. Encapsulation is an efficient way to maintain the viability of bacterial cells because they have permeable membrane that keeps the bacteria that gets nutrients from prebiotic mixed. Permeable membrane that allows the bacteria to be able to come out slowly and minimize the contamination in the capsule (Kitamikado, et al., 1990 in Wijayanti, 2010). In this case, the bacteria in the encapsulation will be higher viability than the viability of bacteria in Nutrient Broth. Encapsulation produce beads containing cells are metabolically active bacteria.

Based on Figure 1 and 2 can be seen, removal of BOD and COD Cimuka river water polluted industrial and domestic waste is also generated by the consortium strain encapsulated bacteria by alginate and starch in the amount of 81.49% with a decrease of 1 810 mg/l to 335 mg/l and 81.44% with a COD content of 2829.7 mg/l to 525 mg/l for 14 days, and the result is higher than strain consortium of bacteria in nutrient broth, gelatin and mix talcum powder bares-skim- milk powder corn - dextrose. This means, the use of a carrier material with the encapsulation process is more effective in reducing the COD and BOD levels of polluted waste water.

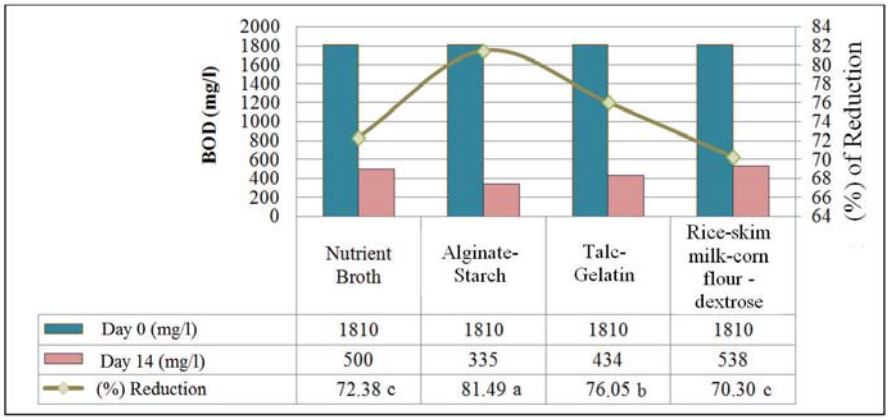

Figure 1. Graph of BOD (Biological Oxygen Demand) of water from the river Cimuka were inoculated by a consortium of bacteria in a variety of carrier materials.

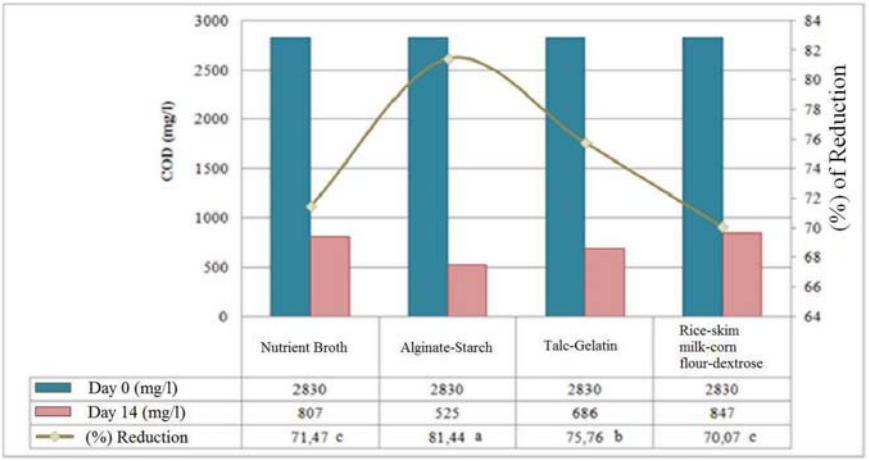

Figure 2. Graph of COD (Chemical Oxygen Demand) of water from the river Cimuka were inoculated by a consortium of bacteria in a variety of carrier materials

According Banu et al. (2001) encapsulated bacterial conditions will be better than the bacteria-free or without encapsulation of the efficiency of removal of harmful content in the waste. Viability of bacteria and bacterial strains in the consortium affect the removal of BOD and COD. According Eweis (1998) that the consortium of microorganisms will help the process of bioremediation quickly and more efficient or it can be said that stren synergistic bacterial consortium was instrumental in the process of bioremediation. In this study used Strain *Bacillus coagulans B. licheniformis, B. subtilis,* and *Pseudomonas* sp. Dwipayana (2010) using Bacillus sp., Pseudomonas sp. and Pseudomonas luteola in industrial wastewater treatment, and can reduce levels of COD by 57.5% within 12 days. Pseudomonas assessed as having ability to decompose pollutants and available from wastewater. However, the average BOD and COD results obtained in this study do not meet water quality criteria for maximum levels of class III in Government Regulation No. 82 of 2001, that each of 6 mg/l and 50 mg/l.

Effect of Bacterial Consortium in Various Materials Carrier to Decrease Levels TSS (Total Suspended Solid) during bioremediation Contaminated Waste Water from the River Cimuka

Total suspended solid or total suspended solids (TSS) is a residue of total solids that can block the sunlight thereby blocking photosynthesis. Results of analysis of variance, followed by Duncan test at the level of the TSS (Table 3.) shows that the inoculum consortium in a variety of carrier materials, with a bioremediation for 14 days, is able to reduce levels of TSS.

Inoculum consortium of bacteria in nutrient broth medium and alginate- starch produces TSS levels for 14 days is 133.33 mg/l with the bacterial population reaches 4.6 $x10^5$ CFU/ml and 2.89 $x10^{13}$ CFU/ml. Inoculum of the bacterial consortium in Nutrient Broth medium and in alginate and starch resulted in decreased levels of TSS for each 75% of the initial TSS 533.33 mg/l to 133.33 mg/at the end of bioremediation. Nutrient broth is a liquid medium for growing microorganisms that do not affect the levels of TSS than solid medium.

Table 3. Duncan's Multiple Range Test of carrier material containing a starter inoculum of bacterial strains consortium and Time on levels of TSS (mg/l) of water from the River Cimuka

The carrier Material (P)	Contact Time								
	0	**1**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Control	533.33 bc A	466.67 abc A	400 abc A	466.67 abc A	333.33 abc A	666.67 c A	266.67 ab A	200 ab A	**133.33 a A**
Nutrient Broth	533.33 b A	466.67 b A	333.33 b A	466.67 b A	533.33 b A	600 b A	333.33 b A	267.67 ab A	**133.33 a A**
Alginate-Starch	600 b A	533.33 b A	466.67 b A	533.33 b A	600 b A	666.67 b A	533.33 b AB	400 ab A	**133.33 a A**
Talc-Gelatin	1733.33 b B	3866.67 c B	1200 b B	4800 cd B	5866.67 d B	6133.33 d C	3266.67 bc C	3133.33 bc B	1066.67 a B
Rice-Skim Milk-Corn-Dextrose	600 bc A	666.67 bc A	666.67 bc A	600 bc A	733.33 c A	800 c A	666.67 bc B	466.67 ab A	**333.33 a A**

Note: The same letter indicates no difference

Talc is a mineral that TSS levels are higher when compared to other carrier materials. Gelatin is a protein derived from denatured collagen of the skin, connective tissue, bone and cartilage that contains Hydroxyproline, proline, and glycine were high (Li, et al., 2009), thus becoming a source of nutrients for the bacteria. Gelatin is used as a stabilizer, gelling agent, a binder, thickener, emulsifiers and adhesive. The use of alginate and starch as carrier material through encapsulation technique is still the material and the best way compared to other carrier materials.

Decreased levels of TSS, besides resulting carrier material capable of maintaining viability, also strains of bacteria in the consortium work synergistically with biodegradation properties of each strain of bacteria. Research conducted by Jamilah, et al (2009) stated that the Bacillus believed to produce proteolytic enzymes and amylolytic and capable lower levels of up to 30.4% TSS. B. coagulans is also

able to remodel lipid bacteria being able to produce the enzyme lipase (Hidayat, et al., 2010). Bacillus subtilis form endospores that can live in extreme environmental conditions and can decompose organic matter as the treatment of waste water quality (Amrah, 2002). B. licheniformis α-amylase produces enzymes that are thermostable and can hydrolyze α-1,4 bond-glycoside polysaccharides by producing oligosaccharides, dextrin, and glucose (Hasan, et al., 2006). Sandri (2011) explains that Pseudomonas sp. have the ability to degrade hydrocarbons and proteins in organic solvents. Fourth bacteria are considered to be synergistically remediate organic content in wastewater is more effective. In the carrier material alginate and starch decreased levels of TSS reached 87.31% with initial TSS concentration of 533.33 mg/l to 66.67 mg/l. The TSS levels are included in the maximum levels in the water quality criteria of class III according to the Government Regulation No. 82 of 2001 of 400 mg/l.

Effect of starter inoculum of the Consortium in the carrier material against ammonia Levels for Bioremediation of Polluted Water Treatment Origin Cimuka River

Ammonium nitrogen compounds are toxic at high levels, so as to reduce the quality of water which is the source of life for living things, especially aquatic biota. Results of analysis of variance (ANOVA), there is an interaction between the consortiums with a carrier material to reduced levels of ammonia waste from contaminated water Cimuka River. Based on this, the Duncan's Multiple Range Test performed as a follow-up test. Duncan test results in Table 4 show that the bacterial inoculum strain consortium in carrier material alginate and starch able to reduce levels of ammonia by 35, 82% with initial ammonia concentration of 6.49 mg/l to 4.17 mg/l on day 14.with the bacterial population reaches 2.89 x10^{13} CFU/ml. These results did not differ with ammonia reduction by a consortium inoculum in Nutrient Broth medium and rice flour corn-flour-milk skim-dextrose on day 12 and day 14.

Table 4. Duncan's Multiple Range Test of carrier material containing a starter inoculum of bacterial strains consortium and Time on levels of Ammonium (mg/l) of water from the River Cimuka

The carrier Material (P)	Contact Time								
	0	**1**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Control	6.50 bcd C	6.36 bc C	6.63 d B	6.41 bcd B	6.46 bcd A	6.59 cd A	6.43 bcd B	6.06 a C	6.31 b C
Nutrient Broth	5.53 bc A	5.54 bc AB	5.33 bc A	5.73 cd AB	5.96 d A	6.45 e A	5.20 b A	4.30 a A	4.20 a A
Alginate-starch	5.56 b A	5.02 b A	5.24 b A	5.10 b A	5.50 b A	7.58 c B	5.66 b AB	5.26 b B	**4.17 a A**
Talc-Gelatin	6.16 bc B	6.21 bc BC	5.46 ab A	6.42 c B	8.48 d B	8.10 d B	7.93 d C	5.16 a B	4.69 a B
Rice-Skim milk-corn-dextrose	5.60 b A	5.21 b A	4.86 b A	5.26 b AB	7.58 d B	6.30 c A	6.37 c B	4.37 a A	4.23 a A

Note: The same letter indicates no difference

The decrease ammonia indicates that the descending levels of ammonia, the lower the concentration of ammonia toxicity and improve water quality. It is also supported by the high viability and growth of bacteria in the carrier material.

According Ishartanto (2009) the success of bioremediation processes (aerobic) is highly dependent on the amount of organic pollutant load, the length of contact time (retention) between contaminants and bacteria and bacterial populations effectively.

CONCLUSIONS

Based on the results, it can be concluded as follows: Consortium Bacillus coagulans, Bacillus licheniformis Bacillus subtilis, and Pseudomonas sp encapsulated with a carrier material alginate and starch effective in bioremediation of polluted waste water of Cimuka river with lower levels of BOD by 81.49%, COD: 81.44% COD, 75%, and ammonia 35.82% with bacterial population

reaches $2.89x10^{13}$ CFU/ml in the bioremediation of contaminated waste water origin Cimuka River in 14 days

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