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Effectivness of sludge microbial consortia in the bioremediation of detergent-containing launderette wastes

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ABSTRACT: This project investigated the potential of microbial consortia obtained from several sources to reduce or eliminate detergent concentration in the wastes of launderette businesses found in the capital city of Bali. Before being used as active starters, the consortium of microbiota contained in the 3 waste sources was enriched in a medium containing (g/l); 1 glucose, 0.05 K_2HPO_4, 0.05 KH_2PO_4, 0.05 $(NH_4)_2[Fe(SO_4)_2].6H_2O$, 0.01 $MgSO_4$, 0.01 yeast extract, 1800 mL distilled water, and 200 mL of wastewater of launderette industries. An important parameter used to indicate microbial growth was the level of the volatile solid substrate (VSS) in the enrichment medium. The microbial culture that showed the highest VSS only was further investigated in the main experiments by observing its effectiveness in reducing detergent linear alkyl-benzenesulfonates (LAS) and the chemical oxygen demand (COD) of launderette wastes. In this project, microbial consortia developed from detergent-containing launderette wastes were the most active starter to decrease the COD and LAS detergent content following 7 days of exposure. This starter (microbial consortia developed from this launderette waste) showed the highest value of VSS when compared to those collected from other sources, and this was reached on day 4. In the main experiment, 85.5% and 91.9% reduction of LAS and the COD of the wastes, respectively, were observed following exposure of the launderette waste with this culture starter. This indicated that these microbial consortia have the possibility to be developed as a potential starter in larger scales of detergent bioremediation.

Keywords*:* Bali, Bioremediation, COD, detergent, laundry, microbial consortia.

INTRODUCTION

Denpasar, located in the south part of Bali is the capital city of the island. The city is populated by 4,317,404 people (Statistical Bureau of Bali Province, 2021), and its number steadily increases as a function of time due to the rapid development of tourist-related industries. Being the main gate of the island and being one of the most popular tourist destinations, the population of Denpasar has increased

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significantly in the last two decades which is in line with the number of visitors (foreign and domestic tourists). According to the statistical bureau of Bali, the population growth rate of the city in the last 2 decades is 2.14% per year (Statistical bureau of Bali Province, 2021). This phenomenon has attracted people from other islands around Bali, such as Java and Lombok to migrate to Bali and seek for jobs as any types of businesses (including launderette business) rapidly grow here.

In recent years, the launderette business in particular has become booming in Denpasar city. Their services become more significantly important for people with high daily activities (millennial modern lifestyle) as they do not have sufficient time to wash their clothes. In addition, tourism-related businesses, such as hotels, villa, small home stays, restaurants, or caterings need their services. It is undeniable that their existence in Denpasar provides some positive impacts as they can assist the local government in increasing job opportunities for low educated people and hence reduce the numbers of unemployment. Therefore, an increase in the economic status of the area is a logical consequence of launderette business development.

However, the development of launderette businesses, also has adverse effects on the environment as they produce detergent-containing wastes. Detergent is a xenobiotic that is recalcitrant or tends to be non-biodegradable, and therefore, their residues will remain stable for years in the waterways or soils (Waluyo, 2009; Embrandiri et al., 2016). Until recently, no appropriate handling method was available to reduce or eliminate such detergent-containing wastes in Bali. In the long-term operation, launderette businesses will significantly pollute our environment if their wastes are not properly handled resulting in a decrease in environmental quality. Launderette or laundry is a very complex process involving the interaction of several aspects, such as water, soiled laundry, energy, and detergent (Zavala and Estrada, 2016). During the process, clean water used becomes contaminated with soil and detergents. This waste may endanger the soil or water ecosystemif directly released as untreated waste. In Bali, laundry businesses are privately run as small businesses, and none has management of wastewater treatment. All wastes produced are directly disposed of to the environment and therefore contribute to the pollution of water bodies, such as rivers, lakes, and oceans.

The wastes of laundry contain energy, soil from laundry, lint, dyes, finishing agents, and detergents (Zavala and Estrada, 2016) which have a major contributor to water and soil pollution. These constituents make laundry wastes have high chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), total organic carbon (TOC), linear alkyl-benzene-sulfo-nates (LAS's), or more ionic of surfactant. The latest decreases the surface tension of water (Yunusa et al., 2017). High values of these parameters are of great concern in any waste before being discharged into the environment. Their values must be reduced below the threshold values as specified by the local authority. Waste of laundry also contains a high level of phosphate that leads to eutrophication when it pollutes water bodies (Kunduet al., 2015). Attempts to reduce phosphate either using bacterial consortia or chemicals approach from the waste of laundry have been conducted by researchers, such as Zairinayati and Shatriadi (2019) and Adesoye et al.

(2014). The chemical-based approach appeared to be much more effective than the biological-based approach in reducing physical and chemical parameters, particularly from laundry waste.

A small scale biological-based treatment was conducted on laundry wastes using microbial consortia residing in active sludge as active starters. The objectives of this research were: (1) to compare the growth of microbial consortia, residing in the three sites of sludge collection, in the production of active starter cultures (VSS determination); (2) to investigate the effectiveness of microbial consortia (of the most active starter) to reduce COD and LAS detergent of laundry wastes.

MATERIALS AND METHODS

Collection of Sludge with Microbial Consortia

Samples of sludge were collected from three sites; mangrove, ponds in Denpasar city contaminated with launderette waste, and rain forest in Bali. Three samples (each amounted at 100g) were randomly and aseptically collected from each site to 3 different spots ($\pm$10 cm from the soil surface), composited in plastic bags, and transported to the laboratory in a cool box for further analysis.

Sampling of Launderette wastes

Samples (three samples from each collection site) of launderette wastes to be treated with microbial consortia of sediments above were collected in 25 L capacity of plastic containers from the disposal outlet of a laundry service (a laundry service with at least 200 costumers per week) located at *Pamogan* village, south Denpasar city. The containers were rinsed with those laundry wastes prior to use in sample collection. A portion of those wastes was used as a component of the seeding medium in the starter production. The rests were later treated with a starter prepared in this experiment. The samples were first composited prior to treatment.

Preparation of medium for seeding

The enrichment medium was prepared by dissolving the following compounds in 1.8 liters of distilled water; 2 g glucose, 0.1 g K_2HPO_4, 0.1 g KH_2PO_4, 0.1 g $(NH_4)_2[Fe(SO_4)_2].6H_2O$, 0.02 g $MgSO_4$, and 0.02 g yeast extract. This solution was then added with launderette waste to a final volume of 2000 mL, homogenized, autoclaved at 121°C for 15 minutes, and stored until required.

Enrichment of microbial consortia (Seeding)

A volume of 600 mL of enrichment (seeding) medium prepared above was inoculated with 1 g of sediment sample, aerated for 1 hour, placed at room temperature for 15 minutes, and observed for microbial growth by measuring the value of volatile solid substrate (VSS). This VSS value was measured at one daytime interval until it reach peak. Sediment sample with microbial consortia in it that show the best growth rate was used in the subsequent experiment. Triplicate measurements were conducted and the results were averaged.

Determination of *Volatile Suspended Solid (VSS) values*

Volatile Suspended Solid (VSS) indicates the mass of organic suspended matter in the waste (Clesceri et al., 1998). This parameter was determined gravimetrically according to the method specified in Wisjnuprapto (1995). A volume of 10 mL sample suspensions

was added into porcelain containers previously dried in a furnace (*C.T. Moloney PTY.UD Sidney*) at 600°C for 1 hour and placed in a desiccator for 15 minutes. These sample suspensions were put in an oven at 105°C until their water content totally evaporated, cooled down in a desiccation chamber for 15 minutes, and weighted (determination of the initial weight of residues). These residues were then burnt in a furnace at 600°C for 1 hour, cooled down in a desiccation chamber for 15 minutes, and weighed (final weight determination of ashes). This procedure was repeated at one day time intervals (triplicates) until the peak of exponential phase was reached. The value of VSS was calculated using the following formula:

$$VSS = \frac{(a - b)}{c} \times 10^6 \text{ mg/L}$$

Where:

a = Initial weights of porcelain containers plus residues prior to burning at 600°C (g)

b = Final weights of porcelain containers plus residuesafter burning at 600°C (g)

c = Volumes of sample suspensions (mL.)

Measurement of chemical oxygen demand (COD)

The COD value was determined using the closed reflux titrimetric method as specified by the Department of Health of Republic of Indonesia (1993). Some 3 mL of sample were added into a test tube in the COD reactor, added with 2.5 mL of a mix of $AgSO_4$ and H_2SO_4 as a catalyst, and added with 2 mL of 0.0167 M $K_2Cr_2O_7$ to give a final volume of 7.5 mL. This mix was then gently shaken, heated at 150 °C in the COD reactor for 2 hours, diluted twice by adding the same distilled water volume, and added with 1-2 drops of ferro indicator. After heating in the COD reactor, the excessive dichromate was titrated with a standard solution of Ferro-ammonium-sulfate until color change was observed. The same procedure was also applied to blank solution. The value of COD (triplicates) was calculated using the following formula:

$$\text{COD value (mg/L)} = \frac{(a-b) \times M \times 8000}{mLsample} \text{ x df}$$

Where:

a = volume of $Fe(NH_4)_2(SO_4)_2$ needed to titrate the blank solution

b = volume of $Fe(NH_4)_2(SO_4)_2$ needed to titrate the sample solution

M = Molarity of $Fe(NH_4)_2(SO4)_2$

df = dilution factor

Determination of linear alkylbenzene-sulfonates (LAS) detergent concentration

The method of methylene blue was applied in this analysis (triplicate experiment). This method was specified by Department of Health of Republic of Indonesia (1993). Some 50 mL of sample were poured into a separating funnel, added with several drops of 1 N NaOH to increase the pH of the sample (slightly alkaline), added with 10 mL of $CHCl_3$ and 25 mL methylene blue, shaken for 30 seconds, added with 10 mL isopropyl alcohol to avoid emulsification, and let it settle to form 2 layers. The upper layer was then removed from the funnel. The residue ($CHCL_3$) was extracted twice with 50 mL of isopropyl alcohol solution, shaken for 30 seconds, and added with 50 mL of chloroform. The absorbance of this solution was subsequently measured at 652 nm with a spectrophotometer, where $CHCl_3$ was used as the blank solution. The detergent concentration was finally determined following the establishment of a

standard curve (Department of Health of Republic of Indonesia, 1993).

Data analysis

Data obtained in our experiment was analyzed qualitatively or descripttively by comparing it with pollution standard quality values (threshold values) as specified by the Environmental Minister of the Republic of Indonesia (Kep.MenLH No.51/ MENLH/10/1995 and PP RI No.82 year 2001).

RESULTS AND DISCUSSION

Growth of microbial consortia in an enrichment medium for starter develpment

The conditions of the three sites where sludge samples were collected are shown in Figure 1. Sludge samples taken from those sites were activated in an enrichment medium, and the results are shown in figure 2. Microbial consortia developed from sludge containing laundrette waste (site C) were investigated for further study as it showed the best growth rate in this experiment.

A significant increase in the VSS value of starters to be used in the main experiment was observed in the first 4 days of incubation. In the first 4 days, the nutrient contents of the medium were sufficient to support the growth of indigenous microbial consortia in the enrichment medium. These values appeared to reach a peak following 4 days of incubation and began to plateau in the prolonged incubation (Figure 2). This phenomenon indicated that the medium components needed to support the growth of microbial consortia have depleted (Himeoka and Kaneko, 2017; Krishnamurthi, et al., 2021) after 4 days of incubation. The starter inocula seeded from detergent-contaminated ponds (site C) to be used in the subsequent experiments showed the best growth rate. It reached the highest value of 2010 mg/L following this four-day incubation time. According to Rinawati et al. (2016), the VSS value is positively correlated with bacterial bio-mass in the sample. Our finding was in line with that reported by Ibayati (2003) who reported that microbial consortia continuously exposed with detergent containing wastes tend to show better growth response in medium supplemented with detergent. These findings indicated that the bacterial consortia sampled from extreme conditions would grow better in such conditions than those collected from other sources, as they are already well-adapted to such conditions. Based on this analysis, bacterial consortia collected from detergent-contaminated ponds were applied to the bioremediation detergent from water bodies in the subsequent experiments.

Aeration of the starter culture during the incubation period improved the growth rate of the microbial consortia. Under the aerobic condition, the degradation of complex carbon compounds will produce significantly higher cellular energy in the form of ATP (Halling, 2020). The excessive ATPs produced in the metabolic pathways of carbon compounds will be used by cells of microbial consortia to produce their cell biomass (Greiner and Glonek, 2021). This phenomenon was probably involved in the significant increment (exponential growth) of VSS values in the three starter cultures prepared in our experiment (Figure 2).

A B C

Figure 1. The conditions of sites where the sludges were collected for starter culture in this experiment. A: Rain forest; B: Mangrove area; C: Outlet of laundrette business.

Figure 2. Values of volatile suspended solid of starter cultures generated from 3 different sites of samples. Values±standard deviations are averages of triplicate measurements.

Effectiveness of active inocula to reduce LAS detergent and COD

Application of active inocula (starter inoculum developed from site C) in the bioremediation of detergent-contaminated water in the subsequent experiment was found to be very effective. More than 85% reduction in LAS detergent content was observed following 7 days of contact time (Figure 3). However, the final value reached in this experiment was still slightly higher than the threshold value specified by the Indonesian regula-ion (*Peraturan Pemerintah Republik Indonesia Nomor 82* year 2001). The allowable threshold value for LAS detergent residue in the water wastes before being released in Indonesia is 0.2 mg/L (ppm). Prolonged incubation time was probably required in this experiment in order to reduce the LAS detergent content so that its residue in the water body meets the value specified by the Indonesian Government, before being released into the environment. However, these results clearly demonstrated that the bacterial consortia actively used the LAS detergent as a source of energy in their metabolic pathways during this incubation period. The highest rate of detergent biodegradation occurred in the first five days, when the amount of the LAS detergent used as the main source of energy by the bacterial consortia was optimum. Optimum available energy

sources in the media, supported by other optimum environmental conditions, such as temperature, pH, etc., will be favorable for the indigenous microbial consortia to grow (Stanaszek-Tomal, 2020). The mechanisms by which the microbes involved in this process to attack the LAS detergent compounds were not investigated. Therefore, this process needs to be addressed in further experiment in order to elucidate the types of enzymes involved in the process.

A significant reduction in the COD parameter was also observed in this experiment. Similar to the detergent degradation, a significant reduction was observed in the first five days (Figure 4). COD is an important parameter to assess the degree of effectiveness of the detergent biodegradation (Osadebe et al., 2018; Toledo et al., 2021). As shown in figure 4, the process successfully reduced the value of COD by about 92% from its original value in 5 days of incubation. This indicated that LAS detergent degradation took place in the system, as the LAS detergent residue contributes significant value to the COD in the water body (Wulandari and Soedjono, 2017). According to Ginting (2007), the rate of COD reduction may be increased by aeration because the metabolic rate of the microbial consortia to degrade LAS detergent in the system increases under aerobic conditions.

A slight decrease in the COD value was also observed in the control treatment. This indicated that the significant decrease of the COD value in the launderette waste treated with active starter must be due to the activity of the microbial consortia contained in the starter to degrade the LAS detergent which subsequently resulted in a decrease in the COD value. Although the value of the COD significantly decreased in this experiment, the value reached after 7 days of incubation was still higher than that specified by the regulation released by the ministry of environment (Kep.MenLH No.51/ MENLH/10/1995), where the maximum allowable COD value is 300 mg/L, and therefore longer contact time is probably needed, so that the waste is safe to be released to the environment.

Figure 3. Reduction of LAS detergent residue in the water body as a result of microbial consortia activity. Values in the graph ± standard deviation are average of triplicate measurements.

Figure 4. Reduction on the COD value as an indication of LAS detergent degradation by microbial consortia in the experiment. Values in the graph ± standard deviation are averages of triplicates measurement.

CONCLUSION

Microbial consortia developed from detergent-contaminated water bodies (site C) were very effective in reducing the LAS detergent and the COD values. Application of this active starter in the detergent-contaminated water successfully reduced the LAS detergent residue and the COD value by 85.5% and 91.9%, respectively, indicating that this starter has the potential to be used in the larger scales of detergent bioremediation.

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