Development and Application of Hydrolytic Bacterial Microcapsules as Bioremediation Agent of Hospital Wastewater — A Case Report in Semarang

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***Abstract*—We developed and applied a prototype of a bioremediation agent, alginate-based microcapsules containing living indigenous, hydrolytic bacteria, to treat hospital wastewater in Central Java. The use of microcapsules containing living indigenous, hydrolytic bacteria *Bacillus* spp. to improve quality parameters of hospital wastewater, as a case in Semarang City, is yet to be reported. The bacteria worked synergically as a consortium consisting of *Bacillus velezensis* R1.3, *B. amyloliquefaciens* R1.6, *B. amyloliquefaciens* R1.14, *B. velezensis* R1.16, *B. licheniformis* R2.5, *B. amyloliquefaciens* R2.9. Microencapsulation of these bacteria was made using Arabic gum, alginate, carrageenan, and maltodextrin at varied ratios of the microcarrier to bacterial biomass by freeze-drying. The best-formed microcapsules resulted in an alginate-based type at a final ratio of 1:1, which, after a year of storage at room temperature, could show cell viability as signs of successful microencapsulation of living bacteria. By aeration, the bacterial microcapsules improved values of NH_3 (77%), BOD (Biological Oxygen Demand, 57%), COD (Chemical Oxygen Demand, 44%), and PO_4 (55%). In conclusion, applying our prototype to untreated hospital wastewater improved pollution parameters and supported bacterial long-term storage.**

***Keywords*—biomedical waste, bioremediation agent, hydrolytic bacteria, microencapsulation, microcarrier**

I. Introduction

The increasing amount of infectious hospital wastewater that may contaminate other wastes threatens public health. Hospital wastewater treatment is essential for both the public and the environment's health. Hospital wastewater contains toxic and infectious substances and compounds that can be dangerous for the environment's health, employees of these centers, and the entire community [1, 2]. Hospital effluents are usually discharged into the sewer systems before they are treated with municipal sewage treatment plants [3]. Suppose hospital wastewater enters the wastewater collection network without knowing its characteristics or with incomplete treatment and finally enters the municipal wastewater treatment plant. In that case, it can cause many problems, including disturbing the balance of the biological system of the treatment plant [4, 5]. Several studies showed that wastewater from hospitals and medical centers can significantly pollute soil and aquatic environments and spread infectious diseases [2, 5]. Recent studies emphasize the significant challenges with waste management, mainly due to rapid urbanization, poor waste management technologies, and low environmental awareness. Alao et al. (2024) reported the impact of landfill leachate on nearby water bodies in Lagos, Nigeria. The elevated levels of organic compounds and heavy metals in the water samples have likely caused significant alterations to the regional surface and groundwater chemistry, posing severe risks to human health. Addressing BOD and COD contamination in water systems through improved waste management and novel testing methods is crucial for reducing environmental and health hazards. By measuring BOD and COD levels, the researchers found significant pollution levels exceeding WHO standards, emphasizing the need for improved waste management practices. Overall, proper waste disposal units at hospitals are necessary to provide safe health and environment to the public [6–8].

Hospital wastewater treatment can significantly reduce the spread of diseases caused by hazardous components of biomedical waste, such as blood, urine, bacteria, fungi, viruses, and traces of drugs. The degradation and proper disposal of hospital wastewater are considered vital actions based on environmental standards [2, 5–9]. However, current methods to treat hospital wastewater using WWTPs (Waste Water Treatment Plants), autoclaves, membranes, or UV irradiation are expensive. In contrast, those using disinfectants and incinerators are not environmentally friendly [10]. Developing a low-cost yet environmentally friendly bioremediation agent for hospital wastewater is required in low-income countries [11]. A consortium of indigenous, hydrolytic, non-pathogenic bacteria can be used as bioremediation agents for hospital wastewater. Such a group of synergic bacteria could degrade organic matter as the main component of hospital wastewater and simultaneously press the proliferation of pathogenic bacteria [12]. They are environmentally friendly as their presence is not foreign to the environment [13, 14].

Several studies have reported the isolation and selection of

indigenous bacteria for bioremediation of hospital wastewater. A study reported the isolation of two bacterial strains (*Pseudomonas aeruginosa* and *Bacillus cereus*) from municipal sludge. It tested their nutrient removal ability in a moving bed biofilm reactor from wastewater [15]. Two other studies successfully isolated bacterial strains from wastewater samples collected from different sites in Lahore, Pakistan, and Semarang, Indonesia. Both found that indigenous *Bacillus paramycoides* spp. and *Alcaligenes faecalis* could be a sustainable solution for the bioremediation of hospital wastewater [16, 17]. Another study aimed to identify possible microorganisms in wastewater as potential bioremediation agents of pesticide residues. The study isolated bacteria and fungi species from wastewater samples collected from the El-Khairy agricultural drainage, which receives agricultural and domestic wastes. This study found that some indigenous species of bacteria and fungi could be used for bioremediation of chlorpyrifos residues in wastewater [18]. These show that indigenous bacteria can be isolated and selected for bioremediation of hospital wastewater. Such bacteria can help remove nutrients, pesticides, and other toxic substances from hospital wastewater, which can be dangerous for the environment's health, employees of these centers, and the entire community.

With the exact purpose of bioremediation of hospital wastewater, our previous studies reported the isolation and selection of an indigenous bacterial consortium that produced multiple hydrolases with low to no pathogenicity. The consortium consisted of *Bacillus velezensis* R1.3, *B. amyloliquefaciens* R1.6, *B. amyloliquefaciens* R1.14, *B. velezensis* R1.16, *B. licheniformis* R2.5, *B. amyloliquefaciens* R2.9 (R refers to Roemani, name of hospital where the bacteria come from) [14]. Microencapsulation of these bacteria cells is needed for practical use to allow them to be stored for at least a year and applied directly to the hospital wastewater without continuous and tedious bacterial subculture.

We report on developing and applying a prototype of microcapsules functioning as a bioremediation agent for hospital wastewater. The microcapsules contain a consortium of indigenous, hydrolytic bacteria with low pathogenicity, which synergize to degrade organic matter, the main component of hospital wastewater [14]. The aim was to provide a bioremediation agent as an alternative to hospital wastewater management aside from using expensive WWTPs (Waste Water Treatment Plants commonly used in health centers in Indonesia and the lower income countries.

II. Literature Review

Microencapsulation means coating or entrapping a core material with a polymeric material to produce microspheres in the size range of 1–1000 μm. Microencapsulation of microbial cells offers many advantages over other techniques, including increased cell loading capacity, improved cell survival, and higher production rate of the expected microbial products [19, 20]. The application of microencapsulation technology to bacterial cell immobilization for bioremediation is scarce compared to that for probiotics [20]. The selection of microcarrier materials and methods for generating microcapsules is critical to ascertain minimum damage, allowing more viability of the encapsulated bacterial cells. The encapsulation efficiency of the microparticle, microsphere, or microcapsule depends upon different factors like the concentration of the polymer, the solubility of the polymer in a solvent, the rate of solvent removal, the solubility of organic solvent in water, etc. [21, 22].

Bioremediation agents from groups of bacteria are commonly used to treat petroleum or heavy metal pollution. Application of bioremediation agent products in the form of microcapsules containing consortium cells of non-pathogenic indigenous bacteria that can be used directly to degrade biomedical waste is still limited [23–25].

The use of alginate-based microcapsules containing living indigenous, hydrolytic bacteria *Bacillus* spp. to improve quality parameters of hospital wastewater in Central Java has yet to be reported. Our work aims to develop microcontainer products containing a consortium of local hydrolytic indigenous bacteria for bioremediation of hospital liquid biomedical waste through the degradation of the main component of waste, namely organic matter. Biomedical waste bioremediation agent in the forms of dry microcapsules is essential in handling biomedical waste because it can degrade organic matter as the primary material of liquid biomedical waste that can be applied directly with no laborious bacterial subculture, which is also environmentally friendly and affordable for the community.

III. Materials and Methods

A bacterial consortium producing multiple hydrolases with low to no pathogenicity consisting of *Bacillus velezensis* R1.3, *B. amyloliquefaciens* R1.6, *B. amyloliquefaciens* R1.14, *B. velezensis* R1.16, *B. licheniformis* R2.5, *B. amyloliquefaciens* R2.9 was used as the core ingredient of the developed microcapsule. All bacterial strains were obtained from our previous study, and their degradation performance was already tested *in vitro* based on a selection scheme displayed in Fig. 1 [14].

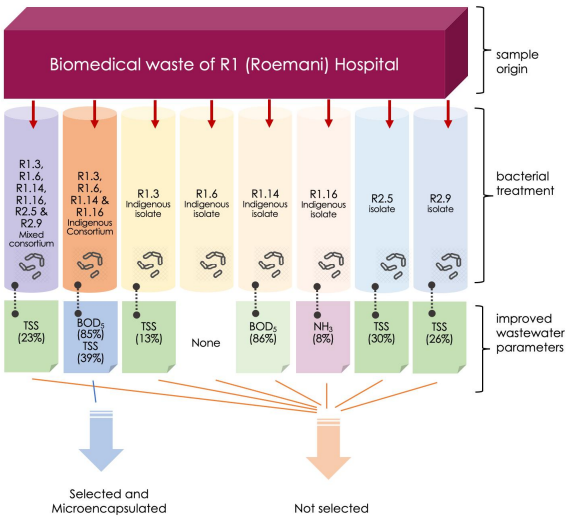

Fig. 1. Summarized results of bacterial selection as single and consortium with their outcomes in improving wastewater quality parameters, including BOD (Biological Oxygen Demand, COD (Chemical Oxygen Demand), TSS (Total Soluble Solid), NH_3, and PO_4 from the previous study [12].

The bacterial consortium was intended as the active ingredient of our bioremediation agent. Four types of materials, Arabic gum, alginate, carrageenan, and maltodextrin, at varied ratios to bacterial biomass, were used

as microcarriers or encapsulants, and their results were later compared. The primary reservoir of WWTP of Roemani Muhammadiyah Hospital provided untreated hospital wastewater.

A. Microencapsulation Process

The microencapsulation of living bacterial cells of *B. velezensis* R1.3, B. *amyloliquefaciens* R1.6, *B. amyloliquefaciens* R1.14, B. *velezensis* R1.16, *B. licheniformis* R2.5, *B. amyloliquefaciens* R2.9 strains was carried out using the Freeze-drying method. The selected bacterial strains used in this report were based on the summarized results of the previous study, and their degradation performance was tested in vitro based on a selection scheme displayed in Fig. 1 [14]. The bacterial consortium was intended as the active ingredient of our bioremediation agent.

In the microencapsulation process using the Freeze-drying method, four types of materials, Arabic gum, alginate, carrageenan, and maltodextrin, at varied ratios to bacterial biomass, were used as microcarriers or encapsulants, and their results were later compared. The primary WWTP reservoir of Roemani Muhammadiyah Hospital in Semarang provided untreated hospital wastewater. In the freeze-drying process, the spore suspension in 2% (w/v) each of 4 types of microcarriers (Arabic gum, alginate, carrageenan, and maltodextrin) solution was frozen at -46 °C in liquid ethanol before sublimation. Then, the mixed solution was freeze-dried for 24 h in a laboratory-scale freeze dryer (Christ Alpha 2–4/LD Plus, Germany) at a condenser temperature below 0 °C and a chamber pressure of 0.05 mbar (5 Pa). As previously reported, the dried products in sheet form were spun into small pieces and stored in a desiccator [18].

B. SEM Analysis

To examine the external morphology of the obtained microcapsule particle by SEM (Scanning Electron Microscope) of the particles [22]. All specimens were prepared by mounting to the carbon conductive adhesive tape on an SEM stub holder and then sputtered with gold coating in a Hummer IV sputter coater. SEM photographs were taken by the scanning electron microscope (JEOL, JSM-IT300 In Touch ScopeTM, USA) at magnification 100x to 10,000x, at room temperature, equipped with an X-ray detector model with an operating of 10 kV.

C. Bacterial Viability Test

A qualitative viability test on the best-shaped bacterial microcapsules obtained was done after being stored for a year. The storage condition was at room temperature, packed in plastic clips and in a dark room. The test was intended to determine if the selected encapsulating materials have encapsulated the living bacterial cells well to protect them from environmental factors and survive storage. The test was conducted by spreading 1% b/b of microcapsules in 30 mL of Nutrient Agar (NA) solid media. After 24-h incubations, signs of bacterial growth were observed [23–26].

D. Application of Bacterial Microcapsules and Bioremediation Performance Evaluation

The bioremediation ability of our prototype bioremediation agent microcapsules containing living bacterial consortium was tested, particularly in reducing waste quality parameters of untreated hospital wastewater. Experiments with untreated wastewater were conducted at the sanitation unit of Roemani Hospital at Semarang, Central Java. The bioremediation agent operated for three days. The operating conditions of the bioreactor when treating hospital wastewater were at 25 °C, without light at night, yet obtained direct sun exposure during the day. We measured pH at the beginning and the end of the process, which means before the treatment of the bioremediation agent and three days after the treatment. The bioreactor was treated in 2 different ways: unaerated and aerated (see Table 1).

Table 1. Water pollution parameter test results of hospital wastewater samples treated with alginate-based microcapsules containing living cells of indigenous, hydrolytic bacteria

Parameter value*	Sample code			Limit
	A (control)	B (unaerated)	C (aerated)	
NH_3 (mg/L)	1.77 ± 0.07	3.85 ± 0.06	0.41 ± 0.02	1.00
BOD (mg/L)	21 ± 0.3	11 ± 0.2	9 ± 0.4	10
pH	7.2 ± 0.2	8.3 ± 0.5	8.1 ± 0.8	7.0-8.5
TSS (mg/L)	3 ± 0.2	6 ± 0.5	3 ± 0.4	20
COD (mg/L)	50 ± 1.1	34 ± 1.3	28 ± 1.2	50
PO_4 (mg/L)	1.67 ± 0.20	0.80 ± 0.12	0.75 ± 0.09	5.00

* Tests were conducted in triplicates

The dose of bioremediation agent microcapsules is 1 g per 4-L of waste fed into a bioreactor-sized 5-L. Meanwhile, the standard liquid pollution parameters, including NH_3, BOD (Biological Oxygen Demand), pH, TSS (Total Soluble Solid), COD (Chemical Oxygen Demand, and PO_4, were tested before and after treatment using bacterial microcapsules using standard methods in triplicates. These tests of wastewater parameters of pH, BOD_5, COD, TSS, and PO_4 adhered to the Standard Nasional Indonesia (SNI), which aligns with the International Standard Methods for the Examination of Water and Wastewater (BOD5 and COD: SNI 6989.72:2009 and SNI 06-6989.15-2004, TSS: SNI 06-6989.3-2004, NH3 and PO4: SNI 19-7119.1-2005 and SNI 06-6989.31-2005) as previously reported [14].

IV. Results and Discussion

The increasing amount of infectious biomedical waste contaminating other debris threatens public health. Meanwhile, handling biomedical waste, whose main component is organic matter, is still a problem because it is expensive and partly not environmentally friendly [12, 15]. Handling biomedical waste through autoclaves, Wastewater Treatment Plants (WWTP), and UV light requires high costs, while incineration and disinfectants will produce other pollutants.

In this case study, we reported the development of microencapsulated indigenous living hydrolytic bacterial cells. Next, we evaluated their application as a bioremediation agent prototype to raw wastewater of Roemani Muhammadiyah Hospital at Semarang, Central Java, which was also the origin of these bacteria. Fig. 1. summarizes the results of bacterial selection as single and consortium with their outcomes in improving wastewater quality parameters, including BOD (Biological Oxygen Demand, COD (Chemical Oxygen Demand), TSS (Total Soluble Solid), NH_3, and PO_4 from the previous study [13,

14].

There are several reasons why microencapsulation was needed. First, microencapsulation provides a protective barrier around the bacteria, which helps to prevent the degradation of the bacteria due to external environmental factors. Microencapsulation allows for the controlled release of bacteria, which can help improve the effectiveness of bioremediation processes [16]. Microencapsulation can also help improve bacteria's viability during processing and application, which is essential for the success of microencapsulated bacteria [27]. Microencapsulation is a sustainable solution for preserving bacteria with bioremediation ability, as it is a cost-effective and eco-friendly approach [28]. Eventually, microencapsulation allows for the long-term storage of bacteria, which is essential for the availability of bacteria for bioremediation processes [27]. All of these make microencapsulation a necessary tool for the success of bioremediation processes.

Fig. 2 shows the microscopic analysis results of the obtained microcapsules using four types of natural microcarrier materials (Arabic gum, alginate, carrageenan, and maltodextrin) with varied ratios between microcarrier materials and bacterial biomass of 1:1 and 1:2 from freeze-dryer. Fig. 2 shows the different morphologies of the microcapsules captured by SEM in varied magnifications. The extruded capsules were all nearly spherical, but the surface was rough with wrinkles. Their size ranged from 600–800 μm in diameter.

All freeze-dried singular microcapsules were varied in diameter between 1–10 μm, and most of them had a smooth spherical shape with some dimples. The body, however, was a sheet form that looked like a scaffold foam with multi-cavities distributed throughout it. On the other hand, the spray-dried capsules were irregular in shape and morphology, so a definite size could not be specified (data not shown).

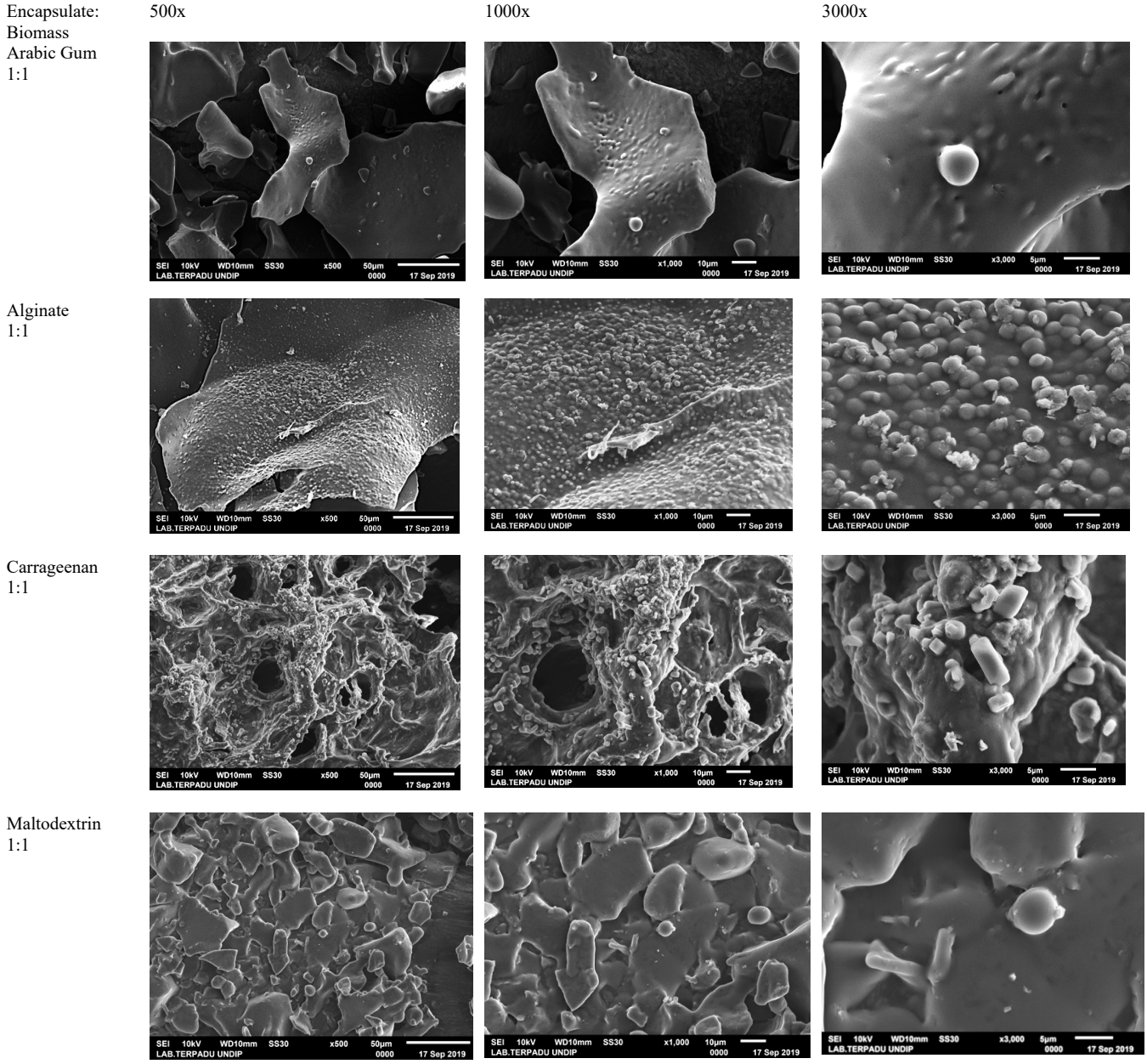

Fig. 2. Representative SEM (scanning electron microscope) micrographs of the encapsulated living indigenous hydrolytic bacterial cells showing the freeze-dried microcapsules at 600x, 1000x, 3,000x, and 5000x magnification with microcarrier material to bacterial biomass ratios of 1:1, where A. Arabic gum-based, B. Alginate-based, C. Carrageenan-based, and D. Maltodextrin-based microcapsule products.

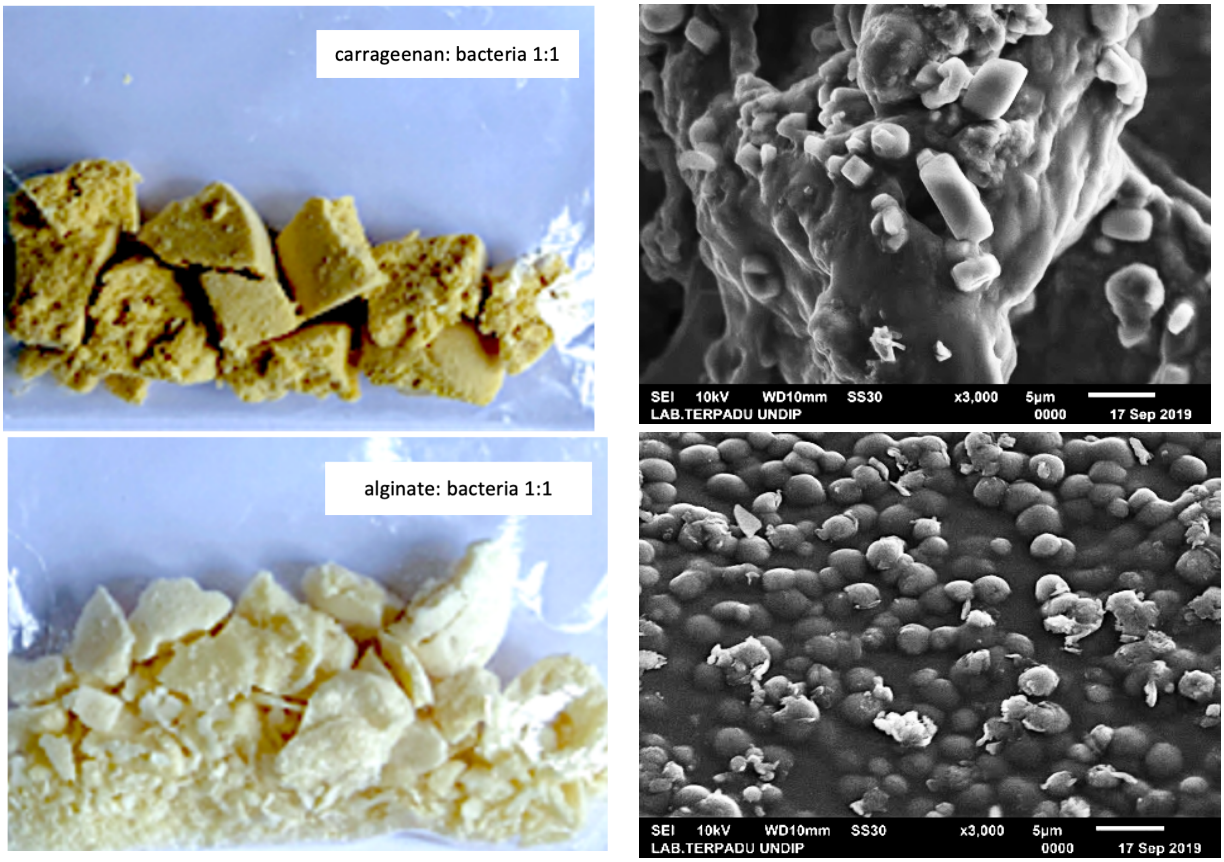

Fig. 3. Macroscopic appearance of microencapsulated living bacterial cells by freeze-drying with A. Alginate B. Carrageenan microcarriers. The microcapsules that appeared as powder can be considered dry culture, which allows more practical uses than wet culture of bacteria, as it does not require regular subculture.

The SEM image of the freeze-dried capsules in Fig. 3 showed that the spores bulged within the sodium alginate coat, meaning that these spores were probably entrapped more successfully. Moreover, indirect investigation by a viability test confirmed and ensured that the spores were protected and successfully encapsulated. As all particles were bulged, no particles were observed to comprise only coating materials.

The macroscopic appearance of alginate- and carrageenan-based microcapsules of living bacterial cells is shown in Fig. 3. As seen in Fig. 3, the microcapsules appeared to be homogenous and dry. This indicates that microcapsules are in the right shape with a low level of water content, supporting their function to prevent degradation of the bacteria due to external environmental factors such as moisture [29].

Based on microscopic analysis results presented in Fig. 3, alginate- and carrageenan-based microcapsules appeared to have the most regular and consistent shapes where bacterial cells were fully entrapped in the microcarriers. Such microscopic morphology characteristics are essential because microencapsulation was intended to protect bacterial cells so they can survive long-term storage.

Alginate hydrogel has a high water-absorption capacity and can prolong a significant amount of water inside its network without dissolving [30]. This absorbed water would then be slowly discharged to the surrounding zone. Thus, these alginate traits make it suitable as a carrier agent for protecting bacterial spores from the cement mixing, casting, and hydration processes. Moreover, its swollen feature can sustain internal water storage for spore germination, initiate bacterial activity, and likely help the $CaCO_3$ precipitation when cracking in the concrete occurs.

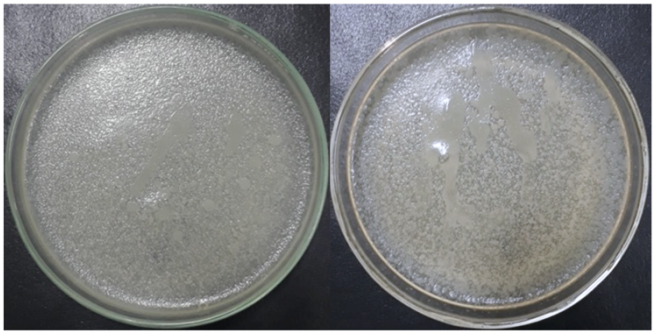

Fig. 4. Viability test results on living bacterial cells entrapped in A. Alginate-based B. Carrageenan-based microcapsules developed as prototypes of bioremediation agents.

Viability test results on NA solid media (Fig. 4) showed that the microcapsule prototype developed in this study has reasonably good storage stability for at least a year. In contrast, the bacterial cells entrapped in the microcapsule can show good viability after being kept for a year at room temperature. These viability test results proved that the microencapsulation process provides stability and, thus, a longer shelf life to bacterial culture, which is essential for the availability of bacteria for bioremediation processes [23, 31, 32].

Both alginate and carrageenan are promising microcarriers for beneficial microbes [29, 33]. Alginate is a natural polysaccharide derived from brown seaweed, while carrageenan is derived from red seaweed. Alginate is known

for its biocompatibility, biodegradability, and ability to protect bacteria from harsh environmental conditions. In contrast, carrageenan is known for its ability to resist acidity and control and sustain the release of encapsulated bacteria [34]. The choice between the two may depend on the specific needs of the bioremediation process, where degradation is the primary process. Thus, alginate appeared to be compatible with our purpose. Based on this consideration and SEM results, we focused on alginate-based microcapsules to undergo further bioremediation tests using untreated hospital wastewater on the site.

The implementation of the process of testing bioremediation agent microcapsules with untreated wastewater at its reservoir in Roemani Hospital Semarang is shown in Fig. 5. The fig. shows the sampling process performed with adequate PPE (Personal Protective Equipment) for microbiological sampling. Samples were taken from 8 points in the primary body hole using a 2.5 L plastic pump in new, clean plastic jerry cans.

Fig. 5. Untreated hospital's wastewater sampling (in 8 spots) was used in the implementation trials of microcapsules of living bacterial cells developed as a bioremediation agent prototype.

The amount of hospital wastewater, including that at Roemani Hospital, which contains a mix of organic components originating from human body fluids, pathogens, chemicals, pharmaceuticals, and cleaning agents, is usually small. However, this type of waste should be treated quickly as it can contaminate other wastes if left untreated [16]. This implies that the quantity of bioremediation agents in microcapsules containing living, waste-degrading bacterial cells does not need to be significant to deal with such a small amount of waste. Another consideration is that good bacteria derived from bioremediation agent microcapsules will be able to multiply and grow in waste environments that are their habitat to compete with unwanted pathogenic bacteria.

The ability of microcapsules to reduce the value of hospital biomedical waste has been tested in this study, either with or without aeration in bioreactors. Accompanied by the development of bacterial microcapsules in our research, a bioreactor is also designed and constructed to support the growth of our waste-degrading bacteria (Fig. 6). This is a simple 5-L bioreactor with a low-speed stirrer (10-30 rpm), so much so that it can homogenize and give aeration to the treated wastewater.

The results of the bioremediation tests conducted in triplicates on untreated Roemani hospital wastewater showed that the bacterial consortium microcapsules processed with bioreactors were able to reduce four values of pollution parameters, namely NH_3 (77%), BOD (57%), COD (44%), and PO_4 (55%) (see Table 1). This is likely due to the bacterial degradation activity of the microcapsule supported by the aeration process in the bioreactor. Application of the unencapsulated living bacterial cell in the previous report could only decrease values of 2 pollution parameters, i.e., BOD (Biological Oxygen Demand) and TSS (Total Suspended Solid) by 85% and 39%, respectively [14].

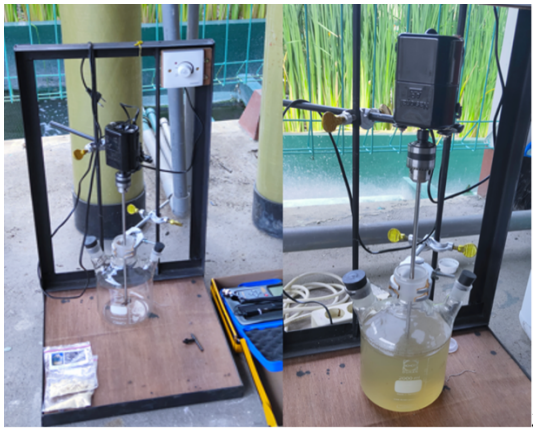

Fig. 6. A simple bioreactor was designed and created in this study to provide aeration (5 rpm) to bioremediation agents (living bacterial microcapsules) directly applied to untreated hospital wastewater. The bioreactor is equipped with a pH meter and thermometer (documentation by Ethica *et al*., 2024).

Based on the data in Table 1, the TSS value did not change with the intervention. This is related to the need for a longer time for bacteria to break down solid flakes in liquid waste and degrade them so that the solute concentration does not decrease. However, it can still be said that in the condition of liquid biomedical waste without aeration, the provision of bioremediation agents in the form of microcapsules of the hydrolytic indigenous bacteria consortium *Bacillus* sp. produced in this study functions well to reduce the parameter value of liquid biomedical waste at Roemani Hospital This means that bioremediation agent microcapsules have been tested to perform after being applied directly in the field.

The ability of microcapsules to reduce the value of hospital biomedical waste has been tested in this study, either with or without aeration in bioreactors. However, the results obtained with the bioreactor are better. Theoretically, using bioreactors increases the ability of bacteria to reduce the value of several pollution parameters [35]. Adding bacterial microcapsules to liquid biomedical waste without aeration can only reduce the value of BOD, COD, and PO_4 pollution parameters. On the other hand, adding bacterial microcapsules to liquid biomedical waste without aeration increases the value of NH_3 and TSS pollution parameters. Furthermore, conducting field testing with larger samples and replications is necessary.

The invention product that has been produced is a microcapsule with an alginate contemplator material consortium of indigenous, hydrolytic, living bacteria that has a low pathogenicity level, can hydrolyze organic matter, and can work synergistically with each other [16]. In more detail, this invention is a microcapsule product of bioremediation agents made from alginate collectors, which contains consortium cells of hydrolytic indigenous bacteria of the genus *Bacillus* from 4 strains, namely *B. velezensis* R1.3, *B. amyloliquefaciens* R1.6, *B. amyloliquefaciens* R1.14, and *B. velezensis* R1.16 as a component of biomass, which is mixed with sterile ddH_2O with a ratio of staple material: bacterial biomass: sterile ddH_2O = 1:1:500 which is processed by a

freeze dryer, so that the composition of the final product is in powder form with the ratio of encapsulating material: bacterial biomass = 1:1.

This innovative product targets the degradation of organic matter measured from BOD (Biological Oxygen Demand) parameters and the competitive and antagonistic properties of indigenous bacteria against pathogenic bacteria in biomedical waste. Organic matter is the main component of all liquid biomedical waste, consisting of blood, urine, and other body fluids containing microorganisms such as bacteria and viruses. This bacterial consortium microcapsule product has been directly applied to liquid biomedical waste to degrade the primary waste material. The degradation ability is reflected in the power of microcapsules of degrading bacterial consortium as bioremediation agents to reduce the pollution parameters values of liquid wastes such as NH_3, BOD, COD, TSS, and PO_4 [16].

Comparative data between the performance of bioremediation agents that become innovations with liquid biomedical waste treatment practices applicable in hospitals (Roemani hospital as a model) in terms of investment/cost, the quality of degradation results (percentage of improvement in the value of liquid waste parameters) is shown in Table 2. Based on the data in Table 2, it can be seen that the improvement of hospital liquid biomedical waste parameters with bioremediation agents is quite competitive with WWTP. The cost of WWTP installation is much higher than that of bioremediation agents. This shows that the potential use of this bioremediation agent will be very suitable because it is very affordable for local health centers and laboratories that cannot afford WWTP.

As seen in Table 2, The results of tests conducted in triplicates on Roemani Hospital's wastewater directly without screening showed that the bacterial consortium microcapsules processed with bioreactors were able to reduce Values of 4 pollution parameters, namely NH_3 (77%), BOD (57%), COD (54%), and PO_4 (55%). In our previous report, without microencapsulation, the bacterial consortium could only improve BOD and TSS values of liquid waste by 85% and 43%, respectively [16]. On the other hand, values of 5 pollution parameters when treated with WWTP also decreased as follows: NH_3 (83%), BOD (63%), TSS (50%), COD (55 %), and PO_4 (50%). It means that our results were only slightly below those of WWTP operated at Roemani Hospital, with the advantage that a bioremediation agent is far more cost-effective for a smaller amount of clinical waste. However, the remaining issue is that our bioremediation agent intervention could not significantly improve TSS value compared to WWTP. This is likely related to the need for a longer time for bacteria to break down solid flakes in liquid waste and degrade them so that the solute concentration does not decrease.

Table 2. Comparison of installation costs and quality of liquid biomedical waste degradation results based on daily data from the WWTP installation of Roemani Muhammadiyah Hospital Semarang and the results of external examinations

Treatment	Installation cost (in million Rupiah)	Parameter value*					
		NH_3 (mg/L)	BOD (mg/L)	pH	TSS (mg/L)	COD (mg/L)	PO_4 (mg/L)
Control	N.A.	1,77 ± 0.07	21.0 ± 0.3	7.2 ± 0.2	3,0 ± 0.2	61 ± 1.1	1,67 ± 0.20
Bioremediation agent (microcapsules)+ bioreactor	7.	0,41 ± 0.02	9.0 ± 0.4	8.1 ± 0.8	3,0 ± 0.4	28 ± 1.2	0,75 ± 0.09
WWTP treatment	1.125	0,30 ± 0.04	7.8± 0.3	7.2± 0.4	1,5 ± 0.3	27 ± 0.8	0,84 ± 0.11
Value reduction (%) by bioremediation agent treatment	N.A.	77	57	N.A.	0	54	55
Value decrease (%) by WWTP treatment	N.A.	83	63	N.A.	50	55	50

* Tests were conducted in triplicates

The ability of microcapsules to reduce the value of hospital biomedical waste has been tested in this study, either with or without aeration in bioreactors [36]. However, the results obtained with the bioreactor are better. As previously reported, theoretically, using bioreactors increases the ability of bacteria to reduce the value of several pollution parameters [34]. Adding bacterial microcapsules to liquid biomedical waste without aeration can only reduce the value of BOD, COD, and PO_4 pollution parameters. However, adding bacterial microcapsules to liquid biomedical waste without aeration adversely increased the value of NH_3 and TSS pollution parameters.

In the environmental field, the development and application of bacterial microcapsules as bioremediation agents of hospital wastewater is still behind that of other wastes, mainly petroleum waste [37]. Therefore, the use of hydrolytic bacterial microcapsules to treat biomedical waste is also scarce. Our results inferred that the provision of bioremediation agents in the form of microcapsules of hydrolytic bacterial consortium *Bacillus* sp. produced in this study greatly reduces the parameter values of liquid biomedical waste at Roemani Hospital in the field. However, conducting field testing with a more significant number of samples and repetitions is necessary to obtain more valid data by minimizing errors.

The advantage of hydrolytic bacterial microcapsules as a bioremediation agent is that they do not require particular installation in their application, so they can be used directly to degrade liquid biomedical waste [11]. This means that it can be used for waste management on a small and large scale, from local health centers and laboratories to hospitals. Our microcapsules are also environmentally friendly because they are 100% made from natural materials that are easily degraded. In addition, this innovative product is also economical because of the use of local isolate bacteria that are easily renewable and the availability of abundant alginate ingredients at affordable prices in Indonesia.

Above all, the potential applications of this innovative product are extensive, especially in liquid biomedical waste management in Indonesia and other developing countries. In

developing countries, biomedical waste management, such as autoclaves and Wastewater Treatment Plants (WWTP), is expensive. UV light is still limited to higher classes of hospitals due to high cost. On the other hand, using less costly methods to treat hospital wastewater, such as disinfectants, is not environmentally friendly because using both products will produce other pollutants [8]. Hydrolytic bacterial microcapsules, which function as biomedical waste bioremediation agents, can be a more economical and environmentally friendly alternative to liquid biomedical waste management.

Bioremediation of hospital wastewater is intricately connected to various Sustainable Development Goals (SDGs) due to its significant impact on environmental health, public safety, and sustainable resource management. This process enhances health and well-being by reducing contaminants in wastewater, minimizing the risk of disease transmission and toxic exposure. By improving wastewater treatment and supporting water reuse initiatives, bioremediation contributes to clean water and sanitation, ensuring discharged water meets safety standards [38]. Additionally, it promotes responsible consumption and production by encouraging sustainable practices that reduce the environmental footprint of healthcare facilities. Furthermore, bioremediation protects aquatic ecosystems and terrestrial biodiversity by preventing the release of harmful substances into water bodies and soil. It also represents an innovative approach to healthcare infrastructure, fostering the development and adoption of environmentally friendly technologies [39]. These actions are crucial in promoting a healthier environment and sustainable practices, benefiting both people and the planet.

Despite the positive results obtained in this study, some limitations should be addressed to optimize the application of this bioremediation technology in real-world settings. First, this study focused on a specific consortium of Bacillus strains. While these strains have shown efficacy, the diversity of bacterial species is limited. Broader microbial diversity might enhance bioremediation by targeting a more comprehensive range of pollutants. Second, this study might have been conducted under controlled laboratory conditions, which may not fully replicate the complexities of real-world hospital wastewater. Factors such as fluctuating pollutant levels, varying temperatures, and the presence of other microorganisms could affect the performance of the microcapsules in actual wastewater treatment plants.

Although the study reported successful storage of the microcapsules for up to a year at room temperature, the long-term viability and effectiveness of the bacteria beyond this period should be discussed. Over time, the viability of the encapsulated bacteria might decline, potentially reducing the efficacy of the treatment. Lastly, this study reported improvements in NH_3, BOD, COD, and PO_4 levels. Still, it does not address the full spectrum of pollutants in hospital wastewater, such as pharmaceuticals, heavy metals, and pathogens. The effectiveness of the microcapsules against these other contaminants remains unknown.

V. Conclusion

The experiences gained from our case study showed that sodium alginate-based microcapsules containing a consortium of living hydrolytic bacterial cells can function as a low-cost bioremediation agent for hospital wastewater. These microcapsules can effectively degrade organic matter in the waste, significantly reducing parameters, mainly the NH_3, BOD, COD, and PO_4, with quality results just slightly below those of the Roemani Hospital's current WWTP. However, they are environmentally friendly, not foreign to the environment, economical, and practical as they can be applied directly to liquid biomedical waste without complex installations. It offers a more sustainable and affordable alternative for biomedical waste management, particularly in developing countries where WWTPs are expensive and limited.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors contributed to the study. S.N.E and A.S. led and designed the experiments, H.H., T.R., A.R.S., and A.S. provided materials and data and facilitated *in situ* bioremediation test in the hospital, SNE, T.R., and A.R.E. conducted selection tests on bacteria and prepared the microcapsules, H.H., T.R., and A.R.E. analyzed waste parameters. At the same time, A.R.E. and A.R.S. performed data collection and analysis. H.H. and A.S. arranged and provided valuable feedback on method applications. S.N.E. and ARS. wrote the first draft of the manuscript, and A.S. contributed to reviewing and completing it. All authors commented on previous versions and then read and approved the final manuscript.

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