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The development method of bioremediation of hospital biomedical waste using hydrolytic bacteria

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Abstract

Issuance of regulations aiming at encouraging hospital business investment and health services has led to significantly increased number of hospitals in Indonesia including Central Java Province. Primary concern is greater amount of biomedical waste exposing health risks to the environment. The search of inexpensive and environmentally friendly methods to handle biomedical waste is critical since the ability of hospitals in Indonesia to manage its waste is generally inadequate. Worldwide, investigation on biomedical waste treatment has still been going to date because the preexisting methods are considered costly and not environmentally friendly. Hydrolytic bacteria have been known for their ability in reducing water pollution parameter values of organic waste. The non-pathogenic ones play key role in accelerating degradation of biomedical wastes by limiting available nutrients suppressing growth of pathogenic microorganisms. Meanwhile, liquid biomedical waste reservoirs containing debris of organic matters are rich source of these bacteria. Hence, use of hydrolytic bacteria as bio-remediation agents is quite promising to resolve liquid biomedical waste problem. This paper justifies the need of bioremediation and contests the development method of bioremediation to handle hospital wastewater using hydrolytic bacteria as bioremediation agent. It hopefully could lead to a more economical and environmentally friendly alternative of hospital wastewater treatment in Central Java Indonesia, and possibly different areas in developing countries. As promotion of non-incineration method is among main recommendations of World Health Organization for Indonesia to handle biomedical waste, the development method of bioremediation as described in this review could be a breakthrough to improve the situation.

Keywords Hydrolytic bacterial bioremediation · Hospital waste · Liquid biomedical waste · Central Java Province

1 Introduction

As a health-care center, hospital plays critical role to provide prevention, treatment, rehabilitation, and promotion for maintaining and improving public health, [1]. Today, number of hospitals in Indonesia is significantly growing due to increased demand of health facilities coming from increased

number of population. The increase is supported by the issuance of government regulations aiming at supporting business investment and hospital services [2]. In 2013, the number of hospitals in Indonesia reached 2228 units where in particular total number of hospitals in Central Java Province increased by 16 units only from 2012 to 2013 [3]. Unfortunately, such increase in number is not in line with the increase in hospital service quality. For example, quality of hospital service in particular in the province at the same year was low reflected from General Mortality Number of Patients Treated in Hospital (GMNPTH). With a tolerance limit of 45.0, in 2012, the average GMNPTH in Central Java was 59.4 in 2012, which exceeded the permitted limit [4].

The increased number of hospitals means there are more health facilities for Indonesian people. However, it consequently means that there is increased volume of generated biomedical wastes. Indonesia is facing environmental health problems since majority of healthcare institutions, especially hospitals and major health centers, are not managing their

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wastes properly [5]. A number of cases of wastewater effluent from hospitals containing pollutants exceeding the limits of tolerance according to the East Java Governor Regulations were found [6, 7]. This indicated that the handling of hospital wastewater in Indonesia is still inadequate.

Hospital biomedical waste is dangerous for containing pathogenic bacteria, viruses, mold, toxic chemicals, and radioactive materials [8]. In addition, this kind of waste could contaminate other waste and is also infectious. The presence of these risks as well as its negative impact to public health, each hospital in Indonesia is obliged to treat their waste according to Indonesian regulation. Incineration, which is preferably used in hospitals of developing countries including Indonesia to treat solid biomedical waste is not environmentally friendly for emitting high levels of various metal contaminants [5, 9, 10].

In fact, studies on degradation of biomedical waste are much rarer than those on other types of waste, most likely due to high risk of danger in handling samples of biomedical waste. However, research on biomedical waste bioremediation should be continuously done because the increasing number of hospitals requires more effective and efficient biomedical waste treatment. Today, in many countries, the investigation on the handling of biomedical waste is still being conducted because of the preexisting methods to treat biomedical waste are still considered expensive and not environmentally friendly [5, 9–11].

Hospital waste water generally is accumulation of liquid domestic and biomedical wastes containing high levels of organic pollutants, so it could be treated biologically [12, 13]. One of the promising biological wastewater treatments is by bioremediation [13, 14]. To investigate the microbial population in the area requiring bioremediation, various advanced modern molecular facilities are available today [14]. It is therefore important to develop a research strategy with the basis of bioremediation leading to an alternative of inexpensive yet effective solutions to treat liquid hospital biomedical waste.

The objective of this paper is to justify the need of bioremediation and propose a development method of bioremediation for handling hospital liquid biomedical waste. The proposed development method is believed to be an alternative of economical and environmentally friendly way to overcome the continuously increased amount of liquid biomedical wastes in Indonesia, particularly in Central Java, Indonesia and possibly other areas in other developing countries.

Practically, the development method is intended to obtain pure isolates or consortium of indigenous hydrolytic bacteria isolated from liquid biomedical waste reservoir of hospitals potential for bioremediation of the liquid biomedical waste. As people become more aware about the effects of increased amount of liquid biomedical waste on public health due to increased number of hospital in Indonesia, the main problem

of this paper is how to set steps to obtain bioremediation agent from hydrolytic group of bacteria, which could also be considered as a breakthrough, to overcome the risk of untreated biomedical waste to public health in Central Java.

2 Research method

The present systematic review was carried to find the significance and to recommend development method of bioremediation using hydrolytic bacteria. It is expected that the method could be used as an alternative way to handle poorly treated liquid biomedical wastes in Central Java, Indonesia, which could also be applied in different areas of other countries facing similar issues. To reach this aim, first, the descriptive qualitative method will be used to obtain detailed and accurate knowledge reckoning on relevant papers for literature review. Next, a development method will be formulated from the most relevant and practical approaches in terms of their significance on potential of hydrolytic bacteria isolated from liquid biomedical waste reservoir of hospitals to be used in the bioremediation process of such waste.

2.1 Eligibility criteria for the studies

First, the types of treatments of biomedical wastes that have already done all over the world were summarized including their types of pollutant removals. Reported treatments were focused on those from biomedical or clinical types of wastewater generated from hospitals. Study selection was based on the following inclusion criteria: [i] studies with subjects limited to treatment or bioremediation applied to liquid biomedical waste type or wastewater of hospitals; [ii] published in English language; [iii] studies evaluating the knowledge and practice of hospital biomedical waste management worldwide as outcome measures to know how widespread the use of bacteria as bioremediation; [iv] observational studies. Publication date limited to the last 11 years was considered in the search strategy.

Next, summary of the use of hydrolytic bacteria in bioremediation of wastes in Indonesia was carried out which could signify the potential of using hydrolytic bacteria as agent to treat hospital wastewater, while at the same time justify novelty of this application. For this purpose, study selection was done based on the following inclusion criteria: [i] studies evaluating the knowledge and practice of the use of hydrolytic bacteria in waste management as outcome measures in Indonesia. [ii] subjects related with treatment or bioremediation using hydrolytic bacteria in Indonesia applied to all waste types including hospital wastes; [iii] published in both Indonesian and English language; [iv] observational studies. As part of the search strategy, publication date also limited to the last 11 years.

2.1.1 Identification of relevant studies

The present review of literature was performed both electronically and manually. It was conducted based on the protocol and guidelines have been used for its preparation [15]. Relevant literature search was carried for two categories: 1. Hospital liquid waste treatment worldwide. 2. Bioremediation of various wastes in Indonesia using hydrolytic bacteria as bioremediation agent.

For the first category, initial electronic and manual literature searches through MedLine and ScienceDirect Databases as well as manual search irrespective of publication date were done using MESH terms- 'hospital biomedical waste', and 'treatment'. This for "hospital wastewater (liquid waste) treatment" or "hospital wastewater bioremediation" studies worldwide yielded 550 references in total and only 72 were retained. Full texts of all the articles were extracted by electronic and manual search from both MedLine and ScienceDirect. The studies that were excluded from the present review were: [i] studies conducted before 2008 [ii] studies of bioremediation of wastes bacteria other than hydrolytic group in Indonesia; [iii] reviews; [iv] studies on treatment other than for hospital liquid biomedical waste or wastewater [v] studies on characterization of pollutant removals without emphasizing particular treatment methods of hospital wastewater.

For the second category, computerized literature searches through Google Scholar and Portal Garuda and manual search irrespective of the date of publication were carried out using MESH terms for "wastewater (liquid waste) microbial treatment" or "hydrolytic bacteria bioremediation". We identified 12 papers with this method. Various key words utilized in search strategy included- biomedical wastewater, treatment, hospital wastewater, microbial bioremediation, and clinical sewage. Various combinations of key words were made using 'and', 'or' as Boolean operators. Experts in the concerned field and authors of selected studies were also contacted for obtaining missing or unclear data whenever deemed essential. The studies that were excluded from the present review were: [i] studies conducted before 2008 [ii] studies of bioremediation of wastes bacteria other than hydrolytic group in Indonesia; [iii] reviews; [iv] studies on treatment other than for hospital liquid biomedical waste or wastewater [v] studies on characterization of pollutant removals without emphasizing particular treatment methods of hospital wastewater.

2.1.2 Selection of studies

Two of the authors [RS & SIM] independently identified studies that met inclusion criteria in the present review. Initially, both titles and abstracts of the records resulted by the search were evaluated to determine inappropriate studies that should be excluded based on exclusion criteria. Review articles were not included although their reference lists were searched in

turn for any studies not recovered by the electronic search. Full text articles of the remaining studies were retrieved that met the inclusion criteria. For observational studies, the selected studies were screened using checklist of STROBE [16].

2.1.3 Control of bias assessment

Following issues were included in the risk of bias or quality assessment in the present systematic review: (i) completeness of reporting information regarding hospital liquid biomedical waste management, (ii) selective outcome reporting, (iii) selection of outcome measures [bioremediation practices using hydrolytic bacteria application during management of biomedical waste in Indonesia], (iv) study design, and (v) conflict of interest in the conduct of the study. When overall criteria were met, the overall plausible risk of bias was considered as low [17].

2.1.4 Collection and extraction of data

The present review was performed based on the guidelines set forth by Preferred Reporting Items for Systematic Reviews and Meta-Analyses [PRISMA] [18]. Two authors [SNE & AS] were responsible for extracting data from the studies. From each of the studies, pre-specified data was extracted including the study design, sample size, hospital wastewater treatment practices among the study subjects all over the world, and knowledge related with the use of microbial bioremediation to handle the disposal of various wastes in Indonesia. Any kind of emerged disputes regarding with the screening and extraction of articles were solved through discussion.

3 Literature review

3.1 Current treatment on hospital liquid waste worldwide

Based on 72 literature reviewed, all treatments on hospital waste reported from worldwide in the last decade mostly focused on pharmaceuticals as removal (more than 30%) and mostly used non-bioremediation system (82%) (Table 1). In terms of bioremediation treatments, only microbial type of agents, which were mostly fungi used, and none of the treatment belongs to phytoremediation category. Microbial bioremediation applied on hospital wastewater mostly used fungi from species *Tremetes versicolor*. Only a study specifically reported the use of a bacterium species, *Alcaligenes faecalis*, for the removal of pharmaceutical substance [67]. Another one mentioned Pseudomonads in terms of bacteria used as bioremediation agent of hospital wastewater for the removal of antibiotics [74]. Most organic pollutants of hospital wastewater were treated using non-bioremediation system. Data showed that microbial bioremediation treatments of hospital

Table 1 Studies on hospital wastewater treatment worldwide in the last decade (2007–2018)

No	Pollutant removal	Treatment		Country	Author
		Bioremediation	Non-bioremediation		
		Microbial			
		Fungal	Bacterial	Algal	
1	Pharmaceuticals & endocrine disruptor compounds	<i>Trametes versicolor</i>	–	Spain	Cruz-Morató et al. [19]
2	Carbamazepine	–	–	Australia	Chong and Jin [20]
3	Pharmaceuticals	–	–	Luxemburg	Köhler et al. [21]
4	Pharmaceuticals	Unspecifi-ed	–	Spain	Lucas et al. [22]
5	Organic pollutants	–	–	Spain	Munoz et al. [23]
6	Atenolol, metoprolol & propranolol beta-blockers	–	–	Brazil	Wilde et al. [24]
7	Antibiotics	–	–	France	Tuc et al. [25]
8	Pharmaceuticals	<i>Trametes versicolor</i>	–	Spain	Mir-Tutusaus et al. [26]
9	Organic pollutants	–	–	Vietnam	Nguyen et al. [27]
10	Pharmaceuticals & organic micro-pollutants	–	–	Denmark	Casas et al. [28]
11	Total coliform & organic pollutants	–	–	Brazil	Berto et al. [29]
12	Pharmaceuticals & organic pollutants	–	–	France	Moussab et al. [30]
13	Antiretroviral drug	–	–	South Africa	Schoeman et al. [31]
14	Iopromide & antibiotic ofloxacin	<i>Trametes versicolor</i>	–	Australia	Gros et al. [32]
15	Organic pollutants	–	–	Turkey	Arsilan et al. [33]
16	Pharmaceuticals	–	Unspecifi-ed in hybrid biofilm & activated sludge	Denmark	Casas et al. [34]
17	Genetic materials	–	–	Brazil	Somens et al. [35]
18	Pharmaceuticals	–	–	France	Hamon et al. [36]
19	Toxins and Genotoxins	–	–	Brazil	Kern et al. [37]
20	Antibiotic resistance genes	–	–	Tunisia	Rafraf et al. [38]
21	Antimicrobial-resistant bacteria	–	–	France	Hocquet et al. [39]
22	Enteric viruses	–	–	Brazil	Prado et al. [40]
23	Pharmaceuticals	–	–	Belgium	Loos et al. [41]

Table 1 (continued)

No	Pollutant removal	Treatment		Country	Author
		Bioremediation	Non-bioremediation		
		Microbial			
		Fungal	Bacterial	Algal	
24	Controlled substances	–	–	Taiwan	Lin and Tsai [42]
25	Pharmaceuticals	–	–	Malaysia	Al-Qaim et al. [43]
26	Antibiotics	–	–	Australia	Watkinson et al. [44]
27	Tetracycline	–	–	Japan	Saitoh et al. [45]
28	Fluoroquinolone antibacterial agents	–	–	Vietnam	Duong et al. [46]
29	Recalcitrant anticancer drugs	<i>Trametes versicolor</i>	–	Spain	Ferrando-Climent et al. [47]
30	Coliform	–	–	Iran	Mohseni-Bandpei et al. [48]
31	Antibiotics	–	–	Brazil	Penni et al. [49]
32	Pharmaceuticals	–	–	China	Yuan et al. [50]
33	Antipsychotic drugs	–	–	Portugal	Logarinho et al. [51]
34	Penicillins	–	–	Colombia	Sema-Galvis et al. [52]
35	Cytostatic anticancer drugs	–	–	Spain	Negreira et al. [53]
36	Pharmaceutical & personal care products	–	–	Spain	Suarez [54]
37	Pharmaceuticals	–	–	Belgium	Auvinen et al. [55]
38	Nosocomial infection	–	–	Ireland	Deasy et al. [56]
39	Pharmaceutically active compounds	<i>Trametes versicolor</i>	–	Spain	Torán et al. [57]
40	Pharmaceuticals & cosmetic ingredients	–	–	Spain	Reif et al. [58]
41	Pharmaceuticals & personal care products	–	–	Greece	Kosma et al. [59]
42	Ketamines	–	–	Taiwan	Lin et al. [60]
43	Ciprofloxacin	–	–	Belgium	De Witte et al. [34]
44	Polar metamizole (dipyrone) residues	–	–	Germany	Feldmann et al. [62]
45	Adsorbable organic halides	–	–	China	Sun et al. [63]
46	Pharmaceuticals	–	–	Thailand	Prasertkulsak et al. [64]
47	Antibiotics	–	–	China	Chang et al. [65]

Table 1 (continued)

No	Pollutant removal	Treatment		Country	Author
		Bioremediation	Non-bioremediation		
		Microbial			
		Fungal	Bacterial	Algal	
48	Bacterial pathogens	–	–	Germany	Gusbeth et al. [66]
49	Sulfamethoxazole	–	<i>Alcalige-nes faecalis</i>	China	Zhang et al. [67]
50	Radioactivity & pathogens	–	–	Spain	Sancho et al. [68]
51	Antibiotics & antibiotic resistance genes	–	–	Romania	Szekeres et al. [69]
52	Dexamethasone	–	–	Brazil	Arsand et al. [70]
53	Genotoxicity	–	–	India	Gupta et al. [71]
54	Pharmaceuticals & phytochemicals	–	–	Japan	Azuma et al. [72]
55	Endocrine disruptive potentials	–	–	Germany	Maletz et al. [73]
56	Pharmaceuticals	–	–	Thailand	Prasertkulsak et al. [64]
57	Pharmaceuticals	–	–	Australia	Chong and Jin [20]
58	Xenobiotics	–	Pseudomonads	Brazil	Sanfiro et al. [74]
59	Lopromide & antibiotic ofloxacin	<i>Trametes versicolor</i>	–	Australia	Gros et al. [32]
60	Atenolol, metoprolol & propranolol beta-blockers	–	–	Brazil	Wilde et al. [24]
61	Coliforms & organic pollutants	–	–	Brazil	Berto et al. [29]
62	Pharmaceuticals	–	Unspecified in activated sludge	Netherlands	Langenhoff et al. [75]
63	Pharmaceuticals	–	–	Slovenia	Kosjek et al. [76]
64	Pharmaceuticals	–	–	Spain	García-Muñoz et al. [77]
65	Anti-estrogene micropollutants	–	–	Germany	Itzel et al. [78]
66	Pharmaceuticals	–	–	Canada	Ouarda et al. [79]
67	Pharmaceuticals	–	–	Spain	Álvarez-Torrellas et al. [80]
68	Cytostatic compounds	–	–	Spain	Ferre-Aracil et al. [81]
69	Antibiotics	Unspecified in bioreactor	–	Spain	Lucas et al. [82]
71	Pharmaceuticals	–	–	Belgium	De Corte et al. [83]
72	Pharmaceuticals	–	–	Slovenia	Kosjek et al. [84]

Table 1 (continued)

No	Pollutant removal	Treatment		Country	Author	
		Bioremediation				Non-bioremediation
		Fungal	Microbial			
73	Pharmaceuticals	–	–	TiO ₂ nanofiber	Chong and Jin [20]	
Σ	8	5	1	63	Australia	
Percentage	11%	7%	1%	84%		

MBR Membrane bioreactor, MBBR Moving bed biofilm reactor, WWTW Wastewater treatment plant, STP Sewage treatment plant, CWPO Catalytic wet peroxide oxidation

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wastewater were intended or applied for organic pollutant removal.

In fact, wastewater treatment still remains a grey area in many countries. Even though countries claim to have a National Rule or Act on water management, there is no specific regulation related with the management of liquid waste in health-care settings [85].

In reality, the construction of STP itself to treat liquid biomedical wastes is still considered costly for many hospitals in developing country such as Indonesia [11, 86]. Indonesia lacks adequate waste treatment facilities and the biological process for treatment of wastewater is recommended for Indonesia [85]. Based on this recommendation, the search of inexpensive bioremediation agents to handle liquid biomedical waste in an effective, efficient and environmentally friendly way particularly in Indonesian hospital setting becomes urgent.

3.2 Hospital biomedical waste and its treatment in Indonesia

Hospitals in Indonesia usually consist of various units including clinics, surgery or operating rooms, laboratories, administrative office, kitchens, as well as waste treatment area [1]. Various activities of each unit in the hospital produce by products in the form of waste which amount will increase as consequence of the increase in the number of hospitals in Indonesia.

Based on data from World Health Organization (2017), in Indonesia the average use of water in a hospital is ~1.120 KLD/bed. The total quantity of medical waste generated in Indonesia is – 225 tons per year with an average of 0.68 kg/bed/day [85]. Along with Sri Lanka, Indonesia reported that about 60% of the health-care facilities in the country have infrastructure for health-care waste management. The health-care facilities usually discharge their wastewater after treating it in sewage treatment plants. The Province/ District Environment Agency monitors the sewage output in accordance with the Ministry of Environment Regulation No. 5 year 2014, but it does not regulate the requirement and specification of STPs (Sewage Treatment Plants) [5, 85].

In Indonesia, proper waste management have been a major challenge in waste problem [87]. Treatment of biomedical waste in various hospitals including those in Indonesia mostly involves incineration, though the non-incineration technologies are also used [85, 88]. In addition, for onsite treatment of waste, health care facilities majorly also use incineration. Studies indicate that some facilities openly burn their medical waste due to lack of awareness. The country has only six offsite treatment facilities that are insufficient to treat such a large quantity of waste [85].

It has been known that there are associated risks of fire, injury, as well as the effects of waste or inhaling ash generated incinerator by the operator, which could endanger safety. In addition, reduction in the use of incinerator is proven to reduce

the amount of pollution significantly. For example, in Tanzania autoclave for replacement of incinerator has reduced the production of 3.8 g ITEQ of dioxins and furans annually [89]. In Philippines, autoclave and microwave have largely replaced incinerator at hospitals [88].

Biomedical waste is biological type medical waste [90]. Approximately 75–90% of the biomedical wastes are classified as harmless while the remaining approximately 10–25% are considered as dangerous because they can damage health and the environment. However, if both groups are mixed, then all of them would become dangerous [91]. In terms of liquid biomedical waste, the establishment of WWTP in hospitals is responsibility from hospital management team to protect its surrounding public health. WWTP functions to restore the quality of waste water to meet the standards according to decree of Indonesian Ministry of Environment [92].

Among the remaining problems are the primary survey indicates that hospitals dispose of their infectious waste in municipal dumps without pretreating it. None of the facilities applies any pretreatment of health-care infectious type of waste. Most facilities (80%) send the waste either for incineration or landfill [85, 88, 89]. In addition, Indonesian government regulation issued in 2013 requires all hospitals to be assessed with a rating system ranging from black, red, blue, green and gold. Black means no efforts taken to manage health-care waste; red means environmental management efforts are not implemented as set by the law; blue means that the implementation of environmental management efforts is in compliance with the law; and green and gold is beyond criteria/ or compliance is more than what is required. In a report by WHO (2017), totally 121 hospitals were monitored against the criteria and the grades were as: black – 8; red – 70; and blue – 43, which means that the majority of hospitals were not implementing the government rules and regulations [85].

Among strategies for managing wastes in Indonesia are: Reduce and recycle the waste, obligate all health-care facilities to manage waste properly in accordance with standards, improve monitoring and evaluation, and explore alternative treatment other than incineration [85]. In 2017 report, the World Health Organization has recommended that the use of non-incinerator technologies for the disposal of its medical waste. Hence bioremediation could be seen as a breakthrough method should be developed to meet the current Indonesian need of biomedical waste treatment. The method could be more environmentally friendly alternative than incinerator, which is also more affordable than autoclave and WWTP.

3.3 Method development of bioremediation for treatment of hospital wastewater

40 Bioremediation is a biological remediation involving the use of living organisms including bacteria to reduce or

to eliminate pollutants in the contaminated area, which resulted in the restoration to its previous natural state without further disruption to the local environment [93–95]. Bioremediation is economical, environmentally friendly and inexpensive. It is an alternative way help in degrading the waste. It is eco-friendly and much cost effective as compared to other traditional technique such as incineration. However, its implementation is often limited by a lack of information about the factors controlling the growth and metabolism of microorganisms in the contaminated environments. In practice, the right bacteria are to be used in appropriate places considering proper environmental factors [96, 97].

Regarding with the hospital biomedical waste problem in Central Java, hydrolytic bacterial bioremediation appeared to be a potential way to handle the increased amount of biomedical wastes due to increased number of hospitals. Outlines of steps for acquiring new bioremediation agent from group of non-pathogenic hydrolytic bacteria isolated from hospital biomedical waste reservoir could be designed to hamper the proliferation of pathogens as well as to remove other pollutants contained in hospital liquid biomedical wastes, which are harmful to human beings.

The quality of wastewater effluent is determined by parameters such as COD, BOD, nitrate, TSS, TDS, and more, which could describe the quality of wastewater [98]. It means that tests on bioremediation ability of bacteria to treat liquid waste could be done by measuring the ability of these bacteria to reduce the values of those parameters, which represent better quality measurement of waste water.

3.4 Diversity studies of Bacteria isolated from liquid biomedical waste

Diversity study of bacteria isolated from biomedical waste has been carried out in many countries although the frequency is not as often as that isolated from other waste. For example, a study of changes in the bacterial population of the hospital waste treatment facilities had been conducted in India where results showed that the isolated bacteria from biomedical waste with potential degradation consist of *E.coli*, *B.subtilis*, *S.aureus*, and *K.pneumonia*, with an abundance of 15%, 12%, 9%, and 6%, respectively [99, 100]. Meanwhile, in Tanzania a molecular analysis of bioremediation potential of bacteria isolated from biomedical waste was successfully done by studying the genetic diversity based on bacterial 16S rRNA genes [8]. The diversity of bacteria in biomedical waste brings possibility to find groups of hydrolytic bacteria producing various enzymes capable of degrading biomedical wastes from hospitals. It is expected that the group of bacteria could selected, tested and later utilized as new bioremediation agents as local isolates capable of producing hydrolytic enzymes.

3.5 Hydrolytic bacteria and their role in bioremediation

Hydrolytic bacteria are bacteria capable of secreting hydrolytic enzymes to catabolize major components of biomass such as polysaccharides, proteins and fats [101]. Examples of hydrolytic enzymes are amylase, protease, lipase, DNase, and xylanase [102]. Screening of bacteria capable of producing extracellular hydrolytic enzymes could be performed by plate tests, while the identification could be done based on Bergey's Manual of Systematic Bacteriology and analysis of the 16S rRNA genes [103–106].

Hydrolytic bacteria, particularly the non-pathogenic ones, which could metabolize organic wastes play important role in accelerating biomedical waste degradation process, as it will reduce the chance of pathogenic microorganisms to proliferate, also to reduce the danger of infection and contamination they may cause [104]. Ethica & Raharjo [105] succeeded the characterization of bacterial isolates producing hydrolytic lipase, *Alcaligenes* sp. JG3, which is capable of degrading fat as well as glycerol and thereby potential to become effective agents of biodegradation of organic waste (especially fat). Unfortunately, the obtained strain is known to be pathogenic [107].

Bacteria are considered suitable as biodegrader for being able to use a variety of carbon sources or electron acceptors [104]. Bacterial hydrolytic enzymes are important degraders of organic pollutants because they could break the main chemical bonds of toxic molecules in wastes, thus playing key role in bioremediation [108, 109]. Hydrolytic bacteria have been known for their ability to improve water pollution parameters such as COD, BOD, NH₄, and PO₄ of organic waste. They also play key role in accelerating degradation of organic wastes by limiting available nutrients required for the proliferation of pathogenic microorganisms [11, 110]. Therefore, it is important to study the profile of hydrolytic bacteria isolated from liquid biomedical waste reservoir of hospitals in Central Java, a province located in the

most populous island in Indonesia, as well as to study the ability of these bacteria to lower waste water pollution parameter.

Nevertheless, in Indonesia, studies about the role of hydrolytic bacterial group associated with biomedical waste bioremediation are barely found. Even in the last decade, among all studies related with bioremediation by hydrolytic bacteria such as protease, lipase or amylase producers in Indonesia, none of them related with biomedical waste (see tabulation in Table 2). Figure 1 showed summarized factors contributing to the importance of evaluation on the potential of hydrolytic bacteria to treat hospital liquid biomedical waste.

3.6 Steps required to obtain bioremediation agent from groups of hydrolytic bacteria

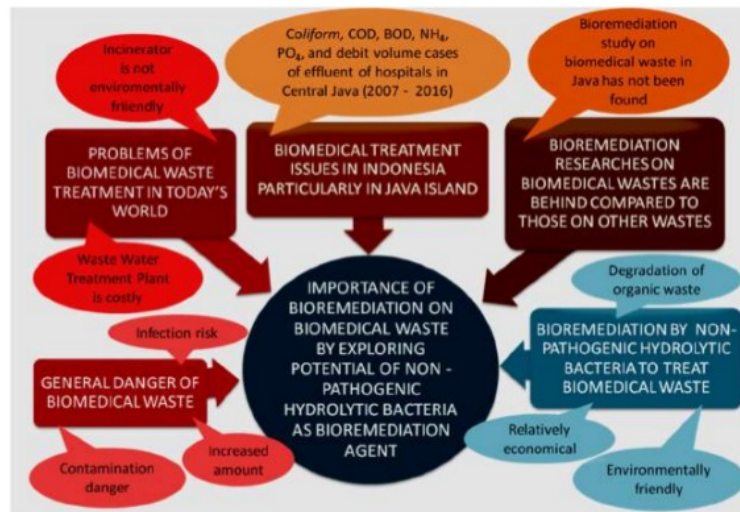
Bioremediation study aiming to obtain bioremediation agent could be started by sampling bacteria from primary reservoir of liquid biomedical waste in one or several hospitals in the cities of Central Java Province [123]. This step shall be followed by purification of bacterial colonies. After being selected by groups of hydrolytic enzymes produced and the nature of their pathogenic properties using selective media (tributyryn, skim milk, starch, McConkey, blood agar, etc.) various isolates could be identified morphologically by Scanning Electron Microscopy (SEM), biochemically with BD-Phoenix Microbiology System and genetically by analysis of their RNA genes. After being morphologically, biochemically and genotypically identified, finally all hydrolytic pathogenic bacterial isolates obtained will be tested for their ability to decrease values of pollution parameters such as COD, BOD, TSS, and phosphate on samples of liquid biomedical waste.

In sampling step, liquid biomedical waste samples were taken from primary reservoir of biomedical waste of several hospitals located in various regions of Central Java. Eight samples from 8 sampling points containing 40 ml of liquid

Table 2 Studies related with bioremediation by hydrolytic bacteria in Indonesia (2007–2018)

Hydrolytic bacterial type	Type of waste	Reference
Proteolytic, amylolytic, lipolytic, & cellulolytic	brackishwater	Setyati et al. [111]
Hydrolytic, not specified	liquid pineapple	Sutanto [112]
Amylolytic, proteolytic & cellulolytic	heavy metal	Anna and Evy [113]
Lipolytic, amylolytic & proteolytic	organic	Zahidah et al. [114]
Proteolytic & amylolytic	shrimp feed	Jamilah [115]
Lipolytic	tempeh production	Wignyanto and Ariningrum [116]
Lipolytic & cellulolytic	not specified	Pulungan [117]
Lipolytic	spent bleaching earth	Elyza et al. [118]
Proteolytic	liquid tofu	Lestari [119]
Hydrolytic, not specified	oil	Komarawidjaya [120]
Lipolytic	diesel oil	Abram et al. [121]
Amylolytic	domestic sewage	Safitri et al. [122]

Fig. 1 Factors contributing to the importance of evaluation on potential of hydrolytic bacteria as bioremediation agent for hospital liquid biomedical waste



waste of each from every primary waste reservoir were stored in sterile bottle covered with zip plastics prepared. In principle, isolation of bacteria could be done using microbiology tests according to *Standar Nasional Indonesia (SNI) 6887-1:2012*. Dilution of samples, cultivation, counting, purification and morphological observation of the growing colony could be done according to methods previously reported [95].

Characterization of colonies and bacterial cells could be performed according to *Bergey's Manual of Determinative Bacteriology* [100]. The process of bacterial selection is based on the type of hydrolytic enzyme produced could be done using selective media [104]. Various bacterial enzymes which could be obtained from this step also have the potential to become subjects of research regarding with their activities.

The initial screening of bacterial isolates for bioremediation studies could be conducted with medium containing the hospital wastewater, while the wastewater characterization test should be done before and after bacterial administration. Wastewater parameters to be measured ideally encompass all water pollutant parameters such as pH, BOD, COD, TSS, and phosphate, based on *Standard Methods for the Examination of Water and Wastewater* [124].

The innovation target of this bioremediation strategy is to find pure bacterial isolates part of indigenous microbial diversity of Indonesia, particularly from hydrolytic group. The selected and tested isolates could later be field tested as bioremediation agent and then produced on an industrial scale for larger application. This is expected to become solutions for

Fig. 2 Steps of methods required to obtain bioremediation agent from groups of hydrolytic bacteria isolated from hospital liquid biomedical waste reservoir and targeted outcomes of each step

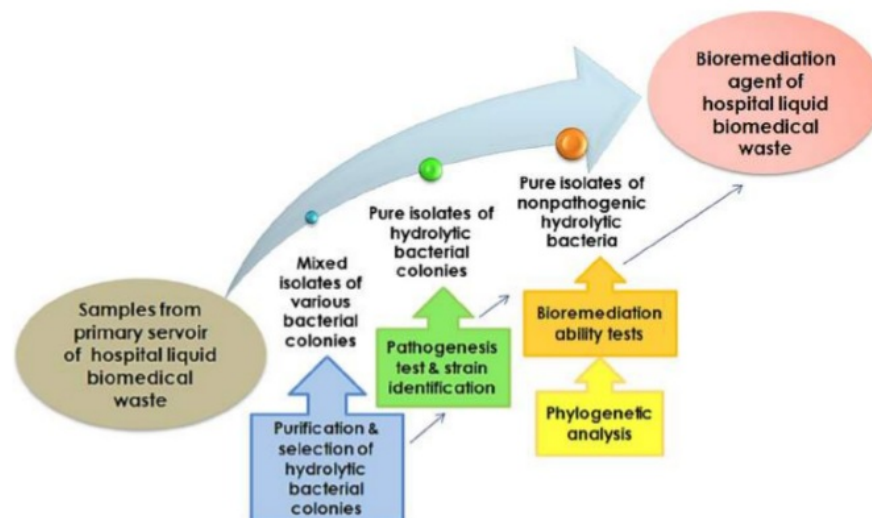




Fig. 3 More detailed scheme of steps required to obtain bioremediation agent from groups of hydrolytic bacteria isolated from hospital liquid biomedical waste reservoir and the targeted outcomes of each step

handling liquid biomedical waste effectively and efficiently in a wide range of hospitals, not only in Central Java, but also in other regions in Indonesia.

As summarized from previous studies reporting feasible facilities to develop bacterial bioremediation system, the summary of of bioremediation using hydrolytic bacteria is schemed in Fig. 2. According to Fig. 2, the initial step should include the obtaining information about the most potential isolates of hydrolytic bacteria to be used as bioremediation agent. The more detailed steps for the development method of bioremediation using hydrolytic bacteria is described in Fig. 3. Based on Fig. 3 the expected outcomes from the steps include:

1. Phylogenetic data of various non-pathogenic hydrolytic bacteria isolated from hospital liquid biomedical waste
2. The rRNA gene sequences of these potentially beneficial bacteria registered in GenBank
3. Pure isolates groups of non-pathogenic hydrolytic bacteria most potential as agents of hospital biomedical waste bioremediation.
4. Encapsulated bacterial consortia that work as synergic group of bioremediation agents of hospital liquid biomedical waste.

4 Conclusion

1. Most treatments for hospital liquid biomedical waste all over the world in the last decade used the non-bioremediation methods. Meanwhile, in Indonesia all studies reporting hydrolytic bacteria as bioremediation agents in the last 11 years had been applied for wastes other than biomedical ones. Hence, known as inexpensive and environmentally friendly, bioremediation of hospital liquid biomedical waste using hydrolytic bacteria offers both necessity, yet novelty.
2. The development method of bioremediation of hospital liquid biomedical waste using hydrolytic bacteria to obtain bioremediation agents for such waste is quite promising because:
 - a. Hospital biomedical waste consists of high amount of organic matters. Hence, the presence of hydrolytic bacteria producing lipase, amylase and protease should be abundant in such waste making it highly possible to isolate and use them as candidates of bioremediation agents.
 - b. Hydrolytic bacteria are widely known for their capability in reducing pollution parameter values of liquid

organic waste such as COD, BOD, TSS, and phosphate.

- c. Many feasible facilities are available to test extracellular enzyme production of bacteria, including techniques to test their pathogenicity and ability to decrease parameter values of liquid organic waste.
3. **Development method of bioremediation of hospital liquid biomedical waste using hydrolytic bacteria** as described in this review could be a breakthrough for Indonesian government to meet World Health Organization's recommendation on promoting non-incineration technology for handling biomedical wastes.

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Compliance with ethical standards

Conflict of interest Both authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent does not apply.

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