Recycle of Semarang City Liquid Waste With "Reuse" Consortium of Mangrove Probiotic Bacteria Treatment

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Abstract
Statement of the Problem: Reuse bioactivator liquid composter had been produced for liquid waste treatment consist of four probiotic bacteria function as pathogenic antibacteria-agent. Whereas the use of four consortium probiotic bacteria will have a better pathogenic antibacterial activity than only one type. The purpose of the research is to process liquid waste and produce clean water by means of eliminating the number of pathogenic bacteria using a "Reuse" probiotic consortium. Further step is pathogenic bacteria count after treatment and phyto-chemical analysis of the "reuse" product. Methodology & Theoretical Orientation: Reused liquid is added to each waste water sample in 10 and 20 ppm concentration. Observation includes: resulting odor, microbial development, absorbance rate, identification of pathogenic bacteria by morphology, and phytochemical screening of the resulting compounds. The results show that sample with 20 ppm reused water is odorless, with lowered density of pathogenic bacteria population from 12 x 10^8 cell/mL to 3 x 10^8 cell/mL, and lowered absorbance rate from 0.625 nm to 0.225 nm. Staphylococcus Aureus is found to be the most prevalent pathogenic bacteria in the waste water. The microbial consortium is found to produce alkaloid, tannin, and steroid. The formation of inhibition zones is the result of microbial consortium activity against the pathogenic bacteria. Conclusion & Significance: Applied technology of "Reuse" mangrove probiotic bacteria consortium had significantly be able in recycling Semarang city liquid waste to produce clean water.

Keywords: mangrove waste, water waste, bacterial consortium, anti bakterial, identification

Introduction

The existence of rivers in Semarang city is important for the livelihood of its citizens. As such, the development of buiding, housing and settlements is supposed to fully use the land in ways that do not damage riverbank areas. Based on the data from Statistics Indonesia (BPS) in 2001, the biggest portion of land use in Semarang City is settlemet (33.12%). This shows that the dominant function of land in the city is domestic service. The land use for settlement is spread along the main route, especially near the city center [1].

Healthy environment is a vital part of good healthcare. Unsanitary environment will both indirectly and indirectly impact the health of the people living in it. Clean water is one of the most important means of attaining good health and sanitation. It is difficult, if not impossible altogether, to be healthy and hygienic when the water consumed daily is contaminated by microbes and is turbid, with strong odor and will cause various disease. Consumption of unclean, contaminated water will have adverse effect on the body, such as diarrhea, parasitic infestation, dysentery and cholera. Yet, many people in Semarang still seemingly ignorant toward the impact of these diseases, which can lead to death.

This issue is caused by an uncontrolled population growth and environmental deteriaoration, creating unpleasant smell and a squatter area in the middle of the city. Contamination of water sources in urban areas is a very harful situation, since the use of water from the said source can cause disease, yet the community often have limited, with great difficulty if any, access to other clean water supplies. Disease prevention depends highly on individual behavior supported by environmaental condition, the availability of infrastructure and support from regulations promoting a healthy lifestyle.

The more people come in, less land become available and the more demand there are for clean water. In addition, rainy season with heavy precipitation causes many areas of Semarang city to flood, further limiting access to clean water. In such condition, the possibility of disease outbreak caused by poor sanitation and hygiene is high. Although there are a number of chemical compounds that can be used in water purification, such as chlorine (Cl_2) or potassium aluminium sulfate ($KAl(SO_4)_2 \cdot 12H_2O$), excessive and prolonged use of the compounds may pose health risk. Among the identified risk caused by water purification chemicals are skin irritation, sunburn, and compromised immune system.

In site observation at Sekayu urban village showed that in addition to contaminated water from flood, most people in the community had unhealthy sanitation practice by defecating in the river although access to sanitation facilities such as public toilet is widely available. In addition to causing health issues, this unhealthy practice also damages the aesthetics of the area. The fact that the water in Pekunden river often dried up added to the inconvenience in that the feces is not flushed away by the water. The existence of squatter eateries surrounding the river also exacerbate the possibility of outbreak of diseases such as cholera, dysentery, typhoid, parasitic infestation, disgetion system diseases, dermal irritation and bacterial poisoning. That Sekayu urban village is located near the city center of Semarang proves that even in the center of urban area there are still citizens who have unsanitary practices of defecating in the river [2].

In the other hand, the use of marine produce in health products is a viable yet not yet sufficiently explored in Indonesia. The country, of which 80% of its area is covered with bodies of water, is located in the longitude and possess a wealth of flora and fauna; one of which is mangrove. After the Aceh tsunami disaster, coastal mangrove forest rehabilitation has been a prominent agenda of the Indonesian government. In mangrove ecosystem management, mangrove waste has become an issue in need of a solution. A recent study suggests that mangrove waste possess secondary metabolites with active chemical compound which shows viability in health products applications. The active compounds are produced by symbiote microbes which are similar to its host [3].

Based on the narratives mention above, this study is undertaken to see the viability of the application of symbiont microbes found in mangrove waste in water treatment in the area of Semarang city and its immediate vicinity. The basic idea is to obtain purified water which is treated with materials taken from nature, of which side effect is negligible and has compounds that organically breaks down.

Coastal mangrove forest rehabilitation program in Indonesia often face the challenge of mangrove waste management, since many mangrove forest rehabilitation sites do not have a designated waste management site or facilities. The existence of microorganism which is associated with mangrove waste has shown secondary metabolite synthesis activities which is a potential source for the exploration of new chemical compounds. Symbiont microbes of mangrove plants are a colony of microbe which thrives and associates with mangrove waste. These symbiont microbes also contribute in the nutrition cycle for the sustenance of their host and are also useful as waste degradation materials. Chemical compounds produced by symbiont microbes have the potency to be used as percusors for biosynthesis metabolism against pathogenic and other predatory microbes [4]. Isolated microbes from plants with bioactive compounds has been known to exhibit more activity, even more intense than that of their host [5]. Based on the finding, it is assumed that the *Reuse* product in the form of compost bio activator can also be used to purify dirty water to obtain clean, more sanitary water. Four microbe species have been identified in such *Reuse* products, namely *Pseudomonas* sp., *Flavobacterium* sp., *Acinetobacter* sp., *Bacillus subtilis*, all of which are potent antimicrobial agents. When used together in an environment, the microbes reinforce each other and form a microbial consortium which increase the efficiency of breaking down harmful materials during processing. Synergical interaction among microbes in the consortium and with the immediate environment results in an organic break down of pollutants in the water. Based on the narrative, this study aims to provide an organic water purification solution by eliminating harmful microbes in the water, to turn dirty water into water safe for consumption, and to identify pathogenic microbes which causes repugnant odor in the water waste.

Materials and Methods

Samples were collected from the site and isolation of microbe within the sample was performed.

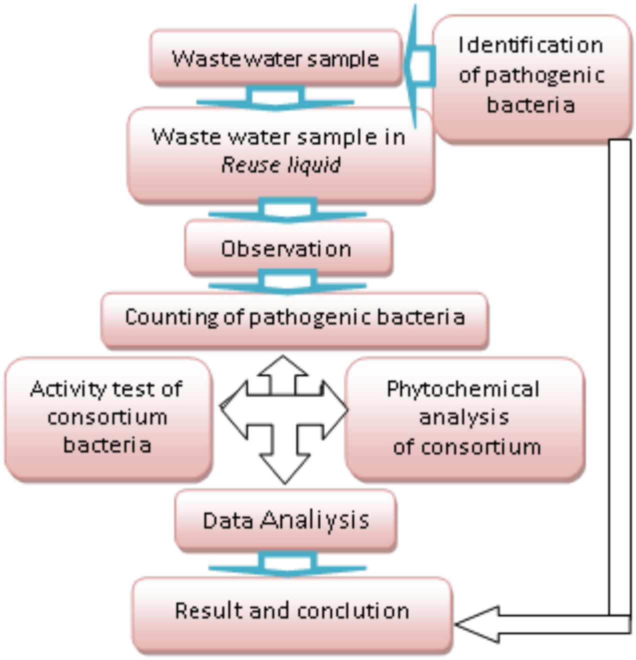

Figure 1: Research procedure in the production of purified water using mangrove waste by the use of consortium of symbiont microbes.

Bioactivity screening of consortium microbes, pathogenic microbe cell density measurement, pathogenic microbe identification, and phytochemical screening of consortium microbes were then carried out. Antimicrobial activity of consortium microbe against waterborne pathogenic microbe was carried out according to the method employed in [1], which can be seen in the flow chart above..

Results and Discussion

Odor Test Result

The results of *reuse* microbial test showed significant results, as shown in Table 1, that water sample from Pekunden possessed a very strong odor when taken during sampling. *Reuse* microbe from mangrove waste in 20 ppm concentration was added and incubated for one night. This results in elimination of odor in the sample after incubation. Another sample, added with 10 ppm of *reuse* microbe, did not show significant result.

Table 1: Results for odor observation of the water samples from all four research sites.

No	Water Sample	Odor
1	Control	Tidak bau
2	Pekunden without treatment	Very strong and pungent
3	Pekunden, 10 ppm treatment	Strong (similar to gutter waste)
4	Bugangan, 10 ppm treatment	Strong
5	Rejosari, 10 ppm treatment	Strong
6	Kaligawe, 10 ppm treatment	Strong
7	Pekunden, 20 ppm treatment	Odorless

Cell Density and Absorbance Rate Test Results

Water sample from Pekunden before treatment showed 12 x 10^8 cell/ml density with 0.625 absorbance rate. After the the addition of consortium microbe at 20 ppm, a significant drop in cell density to 3 x 10^8 cell/ml with absorbance rate of 0.225 was obtained, as shown in Table 2, Table 3, Table 4, Image 2, and Image 3.

Table 2: Cell (microbe) density measurements results of all samples with the addition of consortium microbe from mangrove waste.

No	Sample	Cell density (x 10^8 sel/ml)
1	Control	0
2	Pekunden without treatment	12 x 10^8
3	Pekunden, 10 ppm treatment	6 x 10^8
4	Bugangan, 10 ppm treatment	3 x 10^8
5	Rejosari, 10 ppm treatment	9 x 10^8
6	Kaligawe, 10 ppm treatment	6 x 10^8
7	Pekunden, 20 ppm treatment	3 x 10^8

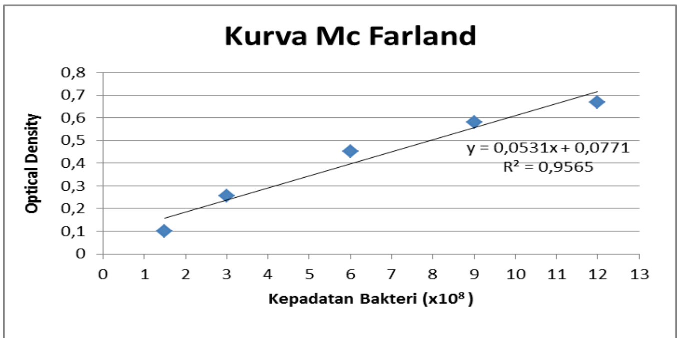

Figure 2: Graphic of microbe cell density (x 10^8 cell/ml)

Table 3: Absorbance rate test results of all the samples with the addition of consortium microbe from mangrove waste.

No	Sample	Absorbance Rate (λ = 600 nm)
1	Control	0
2	Pekunden without treatment	0.625
3	Pekunden, 10 ppm treatment	0.483
4	Bugangan, 10 ppm treatment	0.307
5	Rejosari, 10 ppm treatment	0.503
6	Kaligawe, 10 ppm treatment	0.436
7	Pekunden, 20 ppm treatment	0.225

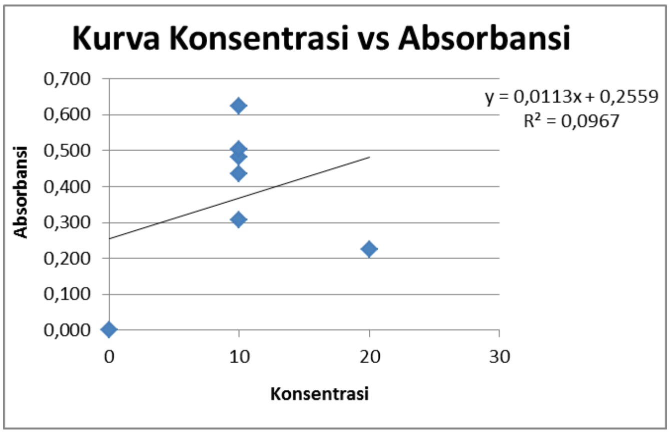

Figure 3: Graph representing probiotic concentration relative to absorbance rate.

Table 4: Microbe cell density ($x10^8$ cell/ml)

Kepadatan Sel (x 10^8 sel/ml)	OD
1.5	0.100
3	0.257
6	0.451
9	0.582
12	0.669
y = 0,053x + 0,077	
0.737	12.453

Identification of Pathogenic Microbe from Waste Water Sample

By observation, water sample from Pekunden produced the strongest odor of all the samples. The Pekunden sample was then measured for cell density which showed a

very high density of 12 x 10^8 cell/ml. Microbe isolation of the sample identified 16 isolates, of which morphologies can be seen in Table 5.
Gram staining on all three microbe isolates showed purple coloration, which can be concluded that the isolates contained gram positive bacteria from the species *Staphylococcus aureus*.

Table 5: Morphology of the identified microbes in Pekunden river water sample

No	Code	Form	Margin	Elevation
1	PK.2.1	Filamentous	Filamentous	Low convex
2	PK.2.2	Irregular	Serreted	Low Convex
3	PK.2.3	Filamentous	Lobate	Low Convex
4	PK.3.1	Circular	Entire	Low Convex
5	PK.3.2	Irregular	Urodate	Low Convex
6	PK.3.3	Punctiform	Entire	Convex
7	PK.3.4	Circular	Entire	Raised
8	PK.3.5	Circular	Entire	Low Convex
9	PK.4.1	Rhizoid	Rhizoid	Umbonate

Discussion

The strong odor of Pekunden river water sample originated from the thriving pathogenic microbe as a result of degradation of solid and chemical household waste as well as pesticides from agricultural activities. The contaminated river water then seeped into ground water, a source of well water. Pekunden river water sample, without treatment, has been observed to produce a very strong odor with a cell density of 12 x 10^8 cell/ml with an absorbance rate of 0.625. This means that the river water posed a very significant health risk when it seeped into sources of ground water in the vicinity. An addition of *Reuse* microbe in 20 ppm concentration to the water eliminated the odor and lowered cell density in the water to 3 x 10^8 cell /ml with an absorbance rate of 0.225.

During sampling, skin contact with Pekunden river water caused burning sensation and rash (similar to skin irritation caused by direct contact with strong acid), even after washing hands. These sensations felt in the skin epidermis was one of the indicators that the water had been contaminated with pathogenic microbes. Pathogenicity is the ability of any pathogenic agents to cause disease. It covers initiation from the infection process and mechanism in developing disease [6]. This study found that *Staphylococcus aureus* was the most prominent species. *Staphylococcus aureus* is a gram positive species which produces yellow pigmentation, facultative anaerobic, does not produce spore and is not motile. The species commonly develops in pairs or in a colony, with a diameter of 0.8-1.0 µm. *Staphylococcus aureus* thrives in an environment with 37 ^oC temperature with a reproduction rate of 0.47 hour. This species is categorized as pyogenic microbe, which means that most disease caused by the species results in purulent local infection. *S. Aureus* produces catalase enzyme, which converts H_2O_2 into H_2O and O_2, and coagulase enzyme, which causes fibrin to coagulate and to clot. Coagulation is associated with pathogenicity since the clotting of fibrin caused by this enzyme is accumulated around the bacteria, forming a barrier which restrict the access of host protective agents to the bacteria which hinders phagocytosis process [7].

The activity of *Reuse* liquid microbe comes from 4 species of bacteria namely *Pseudomonas* sp., *Flavobacterium* sp., *Acinetobacter* sp., and *Bacillus subtilis*. These activities together significantly enhance the potency of the liquid against pathogenic microbe. *Bacillus subtilis* shows a specific extracellular activity through protease enzyme. Protease is an enzyme which is capable of hydrolyzing peptide bond in proteins. This enzyme is often distinguished into proteinase and peptidase. Protease catalyzes protein molecule hydrolysis into large fragments, whereas peptidase catalyzes polypeptide fragments into amino acids. Protease is vital in various biological functions, from cell to organism level, in running metabolic reactions dan regulatory functions. *Bacillus subtilisi* has also been known to mitigate the development of pathogenic microbes.

Generally, bacteria work by breaching cell wall integrity, alter cell permeability, convert protein molecules and nucleic acid, disrupt the mechanism of enzymes and hamper synthesis of nucleic acid and proteins [8]. The important targets in the work of antimicrobial agents against gram-positive and gram-negative microbes are ribosome, cytoplasmic membrane, fat biosynthesis enzymes, as well as replication and transcription of DNA. Antimicrobial agents are bacteriolysis in nature against both gram-positive and gram-negative bacteria, they eliminate threat by performing lysis on the cell and extracting its cytoplasmic components. Lysis can lower the number of cells and culture density, as seen in Table 2. Bacteriolythic compound is one of the antibiotic compounds which can prevent cell wall synthesis.

Conclusion

The study found that the application of biotechnology from consortium microbe found in mangrove waste showed potency in sterilizing pathogenic bacteria in contaminated water. Microbe identification of contaminated water showed *Staphilococus aureus* as the dominant pathogenic species. Phytochemical screening of consortium microbe showed contents of alkaloid, tannin, and steroid. Antimicrobial activity test of consortium microbe against the pathogenic microbe in the sample resulted in the formation of inhibition zone.

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