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Journal of Physics: Conference Series **1567** (2020) 042070 doi:10.1088/1742-6596/1567/4/042070

Morphology characterization of bioactivator microorganisms in product of septick tank phosphate degradator

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Abstract. Septic Tank is a shelter for solid materials of human waste (faeces) which will quickly fill up when there is no decomposition process by bacteria decomposers. The amount of bacterial decomposers in a septic tank is generally less compared to the accumulation rate, so it is necessary to supply bacteria decomposers. The addition of these microorganisms is very cheap when compared to the cost of desludging or drying beside being practical, healthy and environmentally friendly. The decomposing bacteria (microorganisms) will decompose the solid materials in the septic tank into water (H_2O) and some gas (CO_2).Microorganisms that can decompose or degrade human faeces are PAOs (polyphosphate accumulating organisms) by degrading the polyphosphate into phosphate. Microorganisms that play a role in the decrease of phosphates are bacteria and fungi. Aerobic bacteria such as Pseudomonas sp. is one of the phosphate-degrading microorganisms. This research used the Pour Plate and gram staining method for microscopic observation to find the morphology of *Pseudomonas sp*.

1. Introduction

A septic tank is a building to dispose of and collect dirt to prevent disease [1-2]. Septic Tank which is a reservoir for solid materials of human waste (faeces) will quickly fill up if there is no decomposition process by decomposing bacteria. The number of bacterial decomposers in septic tanks is generally less compared to the speed of accumulation. Thus, it is necessary to supply decomposer bacteria. The addition of these microorganisms is very cheap when compared to the cost of desludging or drying beside being practical, healthy and environmentally friendly. The decomposing bacteria (microorganisms) will decompose the solid materials in the septic tank into water (H_2O) and some gas (CO_2) [3].

Human faeces contain nitrogen (from dry weight) 5.0-7.0%, Phosphorus (as P_2O_5) (from dry weight) 1.0-2.5%, carbon (from dry weight) 40-55%, calcium (as CaO) (from dry weight) 4-5%, C / N (from dry weight) 5-10% [4-5]. Microorganisms that can decompose or degrade septic tanks containing human faeces are *Polyphosphate Accumulating Organisms* (PAO) [6-7]. The PAO is meant to degrade polyphosphate to phosphate, and microorganisms that can play a role in reducing phosphate are bacteria and fungi [8].

Septic tank products are products beneficial for solving septic tank problems. Septic tank products are more likely to refer to full septic tanks causing clogged toilet (water closet). These septic tank products are often found on the market, but they do not list the microorganisms used. The emerging problem is that it is not precisely known what types of microorganisms found in septic tanks and those needed to be added to it. Besides knowing the types of microorganisms, it is essential to understand the characteristics of microorganisms to accelerate the decomposition process [9-10].

This research intended to isolate and explore local microorganisms as well as identify the microorganism morphology contained in phosphate-degrading septic tank products.

2. Methods

This research run from April to December 2018 and took place in Biology Laboratory of Universitas Negeri Semarang. The tools employed for this study were clean plastic clips, label paper, freezer, alumunium foil or cotton, autoclaves, micropipettes, pipette tips, BSC, test tubes, vortex, Petri dish, osseous needles, incubators, digital balance, alcohol sprayers, Bunsen lamps , microscope, deglass, glass object, colony counter, gloves, and masks.

The materials used in this study were NA Medium, PCA Medium, Aquadest, alcohol, methylation, Gram colouring (crystal violet Hucker, iodine Lugol, safranin acetone alcohol), immersion oil, and xylol. Gram staining and pour plate method were used in this research. The steps we did are as follows:

2.1. Media Making

The first step in making media was by weighing the media using a digital balance. The media used were NA and PCA. NA of 3.5 grams and PCA 4.2 grams.

2.2. Tools and Media Sterilization

Sterilisation of tools and media from microorganisms is exceedingly essential. Physical sterilisation was done by autoclaving for 20 minutes.

2.3. Microorganism Isolation

The samples from the septic tank were diluted in multilevel from 10^{-2} to 10^{-5}. Furthermore, a 1000 µl was taken from the dilution using a micropipette and dropping it onto the agar [11]. The Pour plate method was done in 2 ways, namely by mixing bacterial suspensions with the agar medium at 50ºC and spraying the suspension on a Petri dish base, then pour the agar medium to the top and stir it [12]. After the agar has hardened, the bacteria appeared in the petri dish. Therefore, the petri dish must be reversed to avoid the presence of water droplets that may be attached to the lid wall of the petri dish [13] and then incubated at 37 ° C for 24-48 hours. After the incubation period, macroscopic and microscopic growth of bacterial colonies was observed. Subcultures were carried out until a single colony was obtained. Then purification was done on bacteria to get maximum results.

2.4. Biochemical tests

The biochemical tests named catalase was performed using Pikovskaya media or by dropping phosphate in bacteria with NA and PCA media.

2.5. Identification of bacterial morphology

The identification of bacterial morphology was carried out referring to the existing literature review [14-15].

3. Results and Discussion

The isolated bacteria came from three different samples. The first one was Degrasept liquid sample (T1), the second was A solid sample (T2), and the third was B solid sample (T3). The medium employed was NA and PCA. The microscopic isolation results showed different bacterial colonies in the three samples namely punctiform, irregular, filamentous, rhizoid, and spindle. The growth of bacterial colonies based on oxygen conditions generally resulted in aerobic bacteria except on spindles. Different colonies produce distinct cell forms.

Table 1. Macroscopic observation of bacterial colonies

Treatment	Colony Growth				
	Shape	**Edge**	**Elevation**	**Color**	**Growth**
T1	Punctiform	Entire	Flat	Milky white	Aerobic
	Irregular	Lobate	Flat	Milky white	Aerobic
	Irregular	Undulate/lobate	Flat	Transparent	Aerobic
T2	Filamentus	Filamentus	Flat	Milky white	Aerobic
	Punctiform	Entire	Flat	Milky white	Aerobic
	Irregular	Undulate/lobate	Flat	Milky white	Aerobic
	Irregular	Undulate/lobate	Flat	Transparent	Aerobic
	Rhizoid	Rhizoid	Flat	Milky white	Aerobic
T3	Irregular	Undulate	Flat	Milky white	Aerobic
	Punctiform	Entire	Flat	Milky white	Aerobic
	Rhizoid	Rhizoid	Flat	Milky white	Aerobic
	Irregular	Undulate	Flat	Transparent	Aerobic
	Spindel	Entire	Raised	Milky white	Facultative

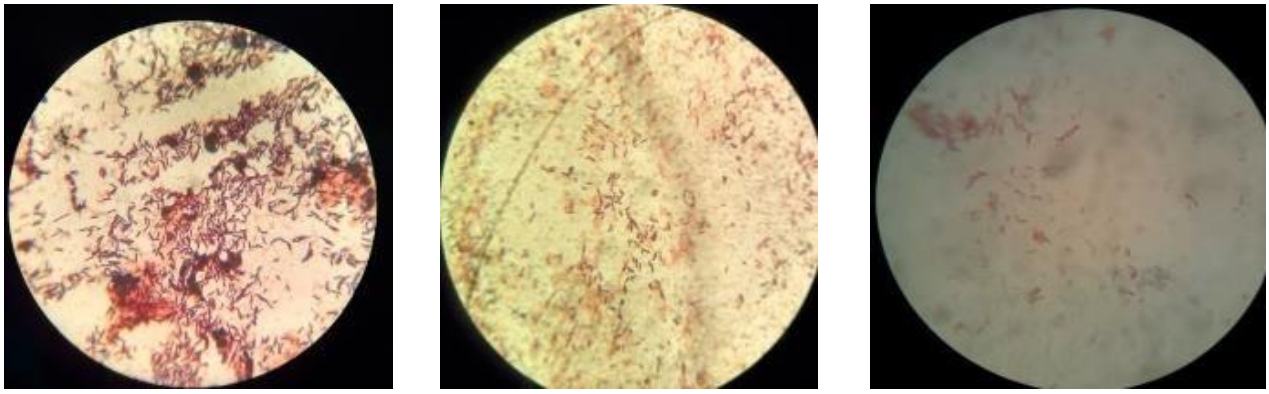
The morphological identification results with gram staining are presented in Table 2 and Figure 1. The morphology of the three samples was mostly gram-negative and generally had basil bacterial cell forms. However, there was one coccus and gram-positive. Gram-positive bacteria will have purple colour as they can withstand primary dye complexes, namely the gram A (Crystal Violet) until the end of the staining procedure. On the other hand, gram-negative bacteria will be red when observed using a microscope because they cannot maintain the Crystal violet colour complex by rinsing the gram C (acetone alcohol) and then coloured by the gram D (safranin) which will be absorbed on the cell wall [16-17].

Table 2. Microscopic Observation Results

Treatment	**Colony Form**	**Bacteria Form**	**Gram**
	Punctiform	Basil	Negative
T1	Irregular 1	Basil	Negative
	Irregular 2	Basil	Negative
	Filamentous	Coccus	Positive
	Punctiform	Basil	Negative
T2	Irregular 1	Cocobasil	Negative
	Irregular 2	Basil	Negative

	Rhizoid	Cocobasil	Negative
T3	Irregular 1	Cocobasil	Negative
	Irregular 2	Basil	Negative
	Punctiform	Basil	Negative
	Rhizoid	Cocobasil	Negative
	Spindel	basil	Negative

Based on microscopic observation results, almost all treatments contained gram-negative bacteria and only treatment one (T1) had the filamentous colony while coccus is included in gram-positive bacteria.



A B C

Figure 1. Forms of bacterial colonies. a). Forms of bacterial colonies in treatment one, b). Forms of bacterial colonies in treatment two and c). Forms of bacterial colonies in treatment three

Some colony forms are finally observable. Filamentous is a form of a colony whose edges are shaped like fine threads (filaments), and an irregular is a form of bacterial colonies that are irregular, brimmed, with an uncertain shape while rhizoid bacterial colonies shaped like roots with diffuse growth.

The identification results of the colony morphology revealed that each of them had similarities. The bacterial morphology was bacilli and gram-negative, and the punctiform morphology using the determinant key showed Pseudomonas genus. In line with this [18] and [19], Pseudomonas has punctiform colony criteria such as basil in form and has gram-negative. Moreover, [20-21] stated that the shape of Pseudomonas is round, flat edge and gram-negative. In other words, the two studies agree with this research result that basil, gram-negative bacteria, punctiform morphology, flat edge, and oxygen-resistant categorized as aerobic [22]. Moreover, Pseudomonas also includes phosphate-degrading bacteria [23].

4. Conclusion

The morphology of bacterial colonies from the three samples of septic tank products is punctiform, irregular, rhizoid, filamentous, and spindles with flat and aerobic. Furthermore, microscopic observations revealed gram-negative in basil, cocobasil, and cocus form. The morphological identification of the bacteria obtained milky white punctiform colonies with flat edges and aerobic growth while the gram-negative basil cell form was the genus *Pseudomonas sp*., a phosphate-degrading bacteria.

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