

EXPLORATION OF DOMINANT BACTERIA ON MODERATE SANITATION RISK WASTEWATER TREATMENT PLANT (WWTP) AT SLEMAN, YOGYAKARTA

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ABSTRACT

Wastewater treatment plant (WWTP) contains various kinds of microbes, primarily bacteria, because of the role of bacteria in helping the degradation process of organic matter in wastewater. Based on data from the Environmental Agency of Sleman Regency, about 163 Communal WWTPs were tested for their physical and chemical parameters. However, not many tests have been carried out for biological parameters. The study of dominant bacteria in Communal WWTPs with moderate sanitation risk is expected to add information related to these biological parameters. This study used the Direct Plating method on *Diluted Nutrient Broth* (DNB) media and the gram staining method to determine the morphological characteristic of the bacterial cells. Six of communal WWTPs namely Candi Indah, Tirta Asri, Gading Indah, Guyup Rukun, Wahana Bina Lingkungan, and Banyu Bening were used as study sites. From this study, the bacterial dominant in each Communal WWTPs were bacteria having similar morphological characteristics to the bacteria in the genus of *Staphylococcus*, *Streptococcus*, *Bifidobacterium*, *Lactobacillus*, *Micrtothrix*, and *Micrococcus*. The results of this study will contribute to a better understanding of the microbial role in the WWTP process, which is further expected to give insight into improving the WWTP performance

Keyword: Communal WWTP, Bacteria, Direct Plating, *Dilute Nutrient Broth*

1. INTRODUCTION

Biological treatment is used as the main treatment in Communal WWTPs, where in this treatment microorganisms are the main agents in helping the decomposition of organic matter in wastewater [1]. The content of microorganisms in wastewater treatment is abundant and has various types such as bacteria, fungi, viruses, and protozoa where some of these microorganisms have the possibility of being pathogenic. [2]. The final result of waste treatment at the Communal WWTP will then be discharged into water bodies, but the effluent discharged must be adjusted to the quality standards determined by the government [3]. In the discharged effluent there are various types of microbes that can interfere with human health if the amount exceeds the quality standard [4].

According to the Central Statistics Agency (BPS) of Sleman Regency, the population in Sleman Regency in 2021 is 1,088,109 people, while in 2020 the population in Sleman

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DOI: <https://doi.org/10.20885/icsbe.vol4.art46>



Regency is 1,082,754 people, this data shows that there is an increase in the number of residents in Sleman Regency. This increase can affect the quality of the wastewater that flows to the WWTP, especially the bacteria contained in the domestic wastewater. Domestic wastewater generally contains beneficial bacteria, such as bacteria that degrade organic matter, but not a few bacteria that are pathogenic [5].

Based on data obtained from the Environmental Service of Sleman Regency, the number of Communal WWTPs recorded is approximately 163 Communal WWTPs spread across all sub-districts in Sleman Regency and most of them can operate well. Then, based on data obtained from the Environmental Agency of the Special Region of Yogyakarta, 41 Communal WWTPs have been tested for effluent quality and some of them are located in Sleman Regency. However, this test is only based on physical and chemical parameters, which for biological parameters are not widely carried out. Therefore, this study was conducted to determine the dominant bacterial content contained in Communal WWTPs where bacteria are the main components in assisting wastewater treatment in biological processes used in Communal WWTPs. With the implementation of this research, it is hoped that it can help add information about the dominant bacteria in Communal WWTPs with moderate sanitation risk and the role of these bacteria in helping wastewater treatment.

2. MATERIALS AND METHODS

2.1 Comunal Wastewater Treatment Plant Classification

Communal wastewater recorded in Sleman Regency will be classified, its function to minimize wastewater area to be studied. The classification of Communal WWTPs is based on previous research where the classification is based on four criteria that were obtained after a survey and search for data were taken from practical guidebooks for implementing EHRA and WWTP processing engineering planning guidelines. The four criteria are as follows:

1. Population density more than 25 people/ha
2. WWTPs has a service coverage ratio of more than 75 families
3. WWTPs peak discharge of more than 50 m³/day
4. WWTPs is more than 8 years old

Further, the Communal WWTP will be divided into four classes level. The first level is a WWTP with a low-risk level that meets one of the four criteria mentioned above. The second level is WWTP that have met two of the four criteria above and is referred to as WWTP with moderate sanitation risk. The third level is WWTP with high sanitation risk because they meet three criteria. The fourth level is WWTP with very high sanitation risks because it meets the four criteria. According to the classification carried out by WWTPs with level two, 55 WWTPs were found from 163 Communal WWTPs in Sleman Regency. This study will investigate the second level WWTP-moderate sanitation risk WWTP namely namely Candi Indah Communal WWTP, Tirta Asri Communal WWTP, Gading Indah Communal WWTP, Guyup Rukun WWTP, Wahana Bina Lingkungan WWTP, and Banyu Bening Comunal WWTP.

2.2 Study sites and sampling locations

The Communal WWTPs used as the sampling location is in Sleman Regency, Yogyakarta which is spread over several sub-districts. In 2021 the area of Sleman Regency is 574.82 km²sq.km with 17 sub-districts and a population of 1,087,339 people. Sleman Regency is directly adjacent to Boyolali Regency in the North, Klaten Regency in the East, Bantul Regency in the South and Kulonprogo Regency in the West. After the distribution of the



Communal WWTPs classification was carried out, there were six Communal WWTPs selected, namely WWTP Candi Indah, WWTP Tirto Asri, WWTP Gading Indah, WWTP Guyup Rukun, Wahana Bina Lingkungan WWTP, and Banyu Bening WWTP.

Table 1 Sampling Location

NO	WASTEWATER TREATMENT PLANT	COORDINATE
1	Candi Indah	7°42'17.1"S 110°24'41.8"E
2	Tirto Asri	7°42'13.4"S 110°25'09.6"E
3	Gading Indah	7°43'29.3"S 110°24'45.6"E
4	Guyup Rukun	7°43'29.3"S 110°24'45.6"E
5	Wahana Bina Lingkungan	7°77'05.381122711518"S 110°39'37.3171381045"E
6	Banyu Bening	7°71'52.63550491678"S 110°34'66.6959919454"E

2.3 Sample Collection

Samples were taken from Communal WWTPs with moderate sanitation risk in Sleman Regency, Yogyakarta using Candi Indah WWTP, Gading Indah, Tirto Asri, Banyu Bening, Wahana Bina Lingkungan and Guyup Rukun using aseptic grab sampling method. Samples were taken from 3 points in each WWTP, there was Inlet, Process and Outlet which taken from March 2021 to March 2022.

2.4 Media Preparation

In this study two kinds of media were used namely *Dilute Nutrient Broth* (DNB) agar, and *Nutrient agar* (NA) (MERCK, GERMANY). DNB agar was used for bacterial dominant isolation. NA media were used for isolate cultivation. NA media were made according to manufacturer's instructions. While DNB agar were made by 100-fold diluted *Nutrient Broth* (NB) media (MERCK, GERMANY adding with 2% *Bacto agar* (OXOID, UK).

2.5 Bacterial Dominant Analysis

2.5.1 Bacterial isolation and plate count

The sample was diluted by the serial dilution method, which was diluted using 9 ml sterile distilled water to which 1 ml of sample was added. The dilution was carried out seven times and coded $10^{-1} - 10^{-7}$ in each test tube. The serially diluted samples were then plated on a Petri dish, and the DNB media were added. Dilution is carried out to reduce the concentration of the sample owned so that the number of bacteria in the sample can also be reduced, which can further assist in observing the type of bacteria and calculating the exact bacterial colony [6]. After 14 days of incubation, bacterial were counted using a colony counter and recultured onto NA media to obtain pure cultures [7].

2.5.2 Bacterial Identification

The bacterial identification were carried out based on the morphological colony and cell of the bacteria. The observation of the morphology of the bacterial colonies was carried out using a colony counter to clarify the identification of the shape, color, elevation, and margins of the colonies as well as to calculate the total bacterial colonies based on colony morphology. Furthermore, the identification of colony morphology was compared with the reference [8] Further to identify the bacterial cell for clarifying the cell shape and gram characteristic, the gram staining test were conducted according to [9].



3. RESULT AND DISCUSSION

3.1 Total Plate Count Results

The total plate count is calculated to determine the density of the number of bacteria contained in each sample. The direct Plating method observes the dominant bacteria in a sample. The Dilute Nutrient Broth (DNB) was used as media because DNB media is a medium with minimal nutrient content. It can reduce the speed of typical bacterial growth and the possibility of further unwanted microbial growth. The results of this study showed the differences in the total number of colonies in each WWTP (Figure 1). It can be caused by environmental conditions where bacteria grow, such as temperature, pH, and availability of nutrients in the growth media. Bacteria have an optimum temperature and pH so that bacteria can grow well. Generally, bacteria will grow when the pH is close to neutral, not too acidic, and not too alkaline, some bacteria cannot grow if they are in an environment with pH conditions below four or above 9.5, but some bacteria can live in acidic or basic environmental conditions [10].

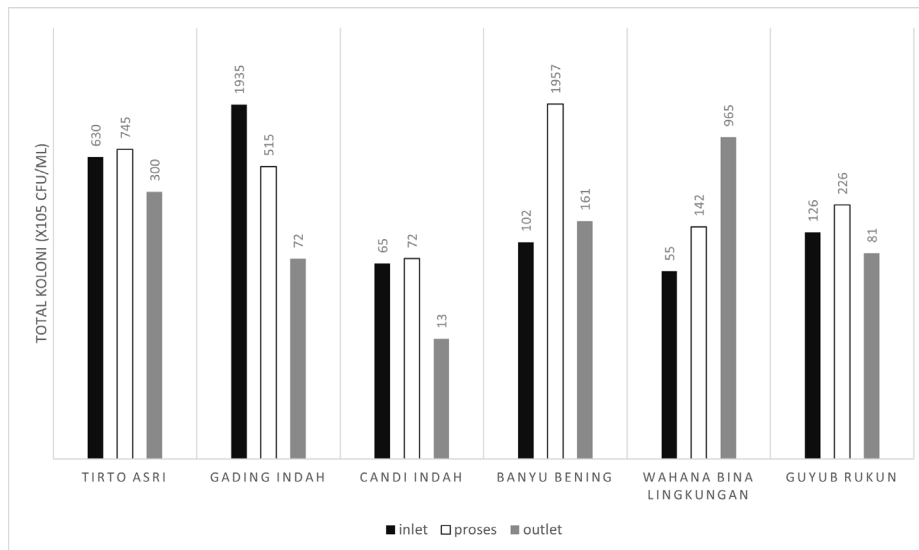


Figure 1 The Total Plate Count results of 6 WWTPs samples

3.2 Bacterial Characterisation Results

Morphological identification is the next step after the sample is incubated for 14-28 days on DNB media. This identification aims to determine the dominant bacteria that grow in one WWTP, which is then grouped according to the similarity of characteristics of each bacterial colony to be grown in sloping Nutrient Agar (NA). The same bacteria were grouped based on the shape of the colony, the shape of the periphery of the colony, and the color of the colonies found (Table 2).



Table 2 The morphological colony of the dominant bacteria

WWTP	SECTION	SHAPE	EDGE	ELEVATION	COLOR
CANDI INDAH	Inlet	spindle	entire	flat	milky white
	Process	circular	undulate	flat	milky white
	Outlet	circular	undulate	flat	milky white
TIRTO ASRI	Inlet	circular	undulate	flat	white
	Process	circular	entire	flat	white
	Outlet	circular	entire	flat	white
GADING INDAH	Inlet	spindle	entire	flat	milky white
	Process	circular	entire	flat	white
	Outlet	circular	entire	flat	white
GUYUP RUKUN	Inlet	filamentous	undulate	flat	white
	Process	circular	entire	flat	white
	Outlet	circular	entire	flat	white
WAHANA BINA LINGKUNGAN	Inlet	circular	undulate	convex	white
	Process	filamentous	undulate	convex	white
	Outlet	filamentous	undulate	convex	white
BANYU BENING	Inlet	filamentous	undulate	convex	white
	Process	filamentous	undulate	flat	white
	Outlet	filamentous	undulate	flat	white

The bacteria that had been grouped based on their morphological similarities were then transferred to slanted NA media and incubated for 24-48 hours in an incubator at a temperature of 35° C. The bacteria that grow in the slanted NA will then be subjected to gram staining and identification of the shape of the cells seen with a microscope with a magnification of 40-1000 times from each grouped colony [11].



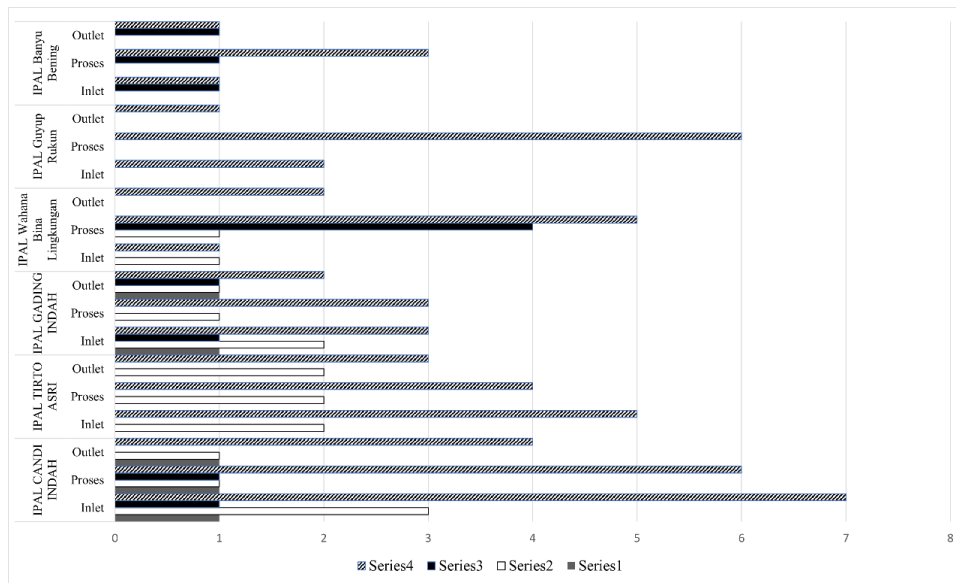


Figure 2 Distribution of Bacteria based on Bacterial Gram Stain, black bars (■) represent negative coccus bacteria, white bars (□) represent positive bacilli, grey bars (▒) represent negative bacilli, pattern bars (▨) represent positive coccus

The results showed that a wide variety of bacteria were found in this study (Figure 2). Positive coccus bacteria were found at all points in Communal WWTPs, while bacilli-negative bacteria were found at least among all points in Communal WWTPs. The variety of bacteria found in each WWTP can be caused by the existing conditions of each WWTP, organic loads, and community activities around the WWTP environment.

3.3 Bacteria Mapping

Identification of bacterial genera was carried out based on the similarity of cell morphology and the results of gram staining, obtained four genera of bacteria, namely *Bifidobacterium*, *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Microthrix*, and *Methanobacteria* scattered in the inlet, process, and outlet of Communal WWTP. *Bifidobacterium* is commonly found in the vagina and gastrointestinal tract in both humans and animals [12]. *Bifidobacterium* is a gram-positive bacillus and non-motile, with circular colonies, white to milky white, and flat elevation. *Bifidobacterium* can utilize glucose, galactose, lactose, fructose, and maltose as their carbon source [13]. Bacteria that have morphological similarities with bacteria of the genus *Streptococcus* were found in the process and outlet of the Candi Indah WWTP and Gading Indah WWTP. *Streptococcus* is a gram-positive bacterium with a coccus cell shape, some species are facultatively anaerobic, and some are obligate anaerobic. [14]

Another bacteria identified in the Communal WWTP with moderate sanitation risk is *Staphylococcus*, according to research conducted by [15], *Staphylococcus* is found in domestic wastewater treatment and many influents and effluents of WWTP. *Staphylococcus* is a spherical, gram-positive bacterium that belongs to facultatively anaerobic bacteria and does not form spores [16]. One of its species, *Staphylococcus aureus*, is a bacterial pathogen and can cause infection and inflammation of the skin but can produce the enzyme catalase, where hydrogen peroxide (H₂O₂) is converted into Hydrogen (H²) and Oxygen (O²). [17]. Bacteria with similar morphology in the genus *Lactobacillus* bacteria were also found in the process and outlet of Tirta Asri WWTP. *Lactobacillus* was found to have



white to milky white circular colonies with flat and convex elevations. *Lactobacillus* is a gram-positive bacterium with a bacillus cell shape, non-motile, does not produce spores, and belongs to lactic acid-producing bacteria. These bacteria play a role in helping the process of acidogenesis. [18]

Microthrix bacteria were found to dominate in the WWTP Banyu Bening. In contrast, in the WWTP Guyup Rukun and WWTP Wahana Bina Lingkungan, bacteria were found with morphological similarities to bacteria in the genus *Micrococcus*. *Microthrix* is a gram-positive bacterium with filamentous bacterial colonies. *Microthrix* often causes bulking and foaming in wastewater treatment processes using activated sludge [19]. Furthermore, bacteria in the genus *Micrococcus* have a white circular colony morphology with entire edges. *Micrococcus* is a gram-positive bacterium with a coccus cell shape, non-motile, and can help catalase and oxidation commonly found in soil and water sources. [20].

There are various bacteria in anaerobic treatment at the Communal WWTP, and these bacteria have a role in helping the wastewater treatment process. [21]. In the hydrolysis stage, complex organic matter will be broken down into simple monomers by hydrolytic enzymes and excreted by bacteria [22]. The bacteria that play a role in helping the processing of this process are *Lactobacillus* [23]. In the acidogenesis stage, simple organic matter will be processed into volatile fatty acids. *Bifidobacterium* bacteria have a role in helping this acidogenesis process [24]. Next are acetogenesis and methanogenesis. At the stage of acetogenesis, acid and alcohol will be converted into acetic acid and H₂ gas. In contrast, at the stage of methanogenesis where the compounds produced from the acetogenesis process will be converted into methane gas by methanogenic bacteria. [25] However, in this study, methanogenic bacteria were not found to be dominant. Of the six bacteria that were found to be able to assist the anaerobic process, there were no bacteria that could assist the methanogenesis process. It might be due to the anaerobic process in the Communal WWTP that had not been fully implemented, so the methanogenic bacteria were not found to dominate in the three WWTPs studied.

4. CONCLUSIONS

The dominant bacteria found in the Candi Indah WWTP, Gading Indah WWTP, Tirta Asri WWTP, Banyu Bening WWTP, Guyup Rukun WWTP and Wahana Bina Lingkungan WWTP were quite diverse. The dominant bacteria have morphological similarities with bacteria in the genera *Bifidobacterium*, *Streptococcus*, *Lactobacillus*, *Microthrix*, *Micrococcus* and *Staphylococcus*. Bacteria of the genus *Bifidobacterium* help in the process of acidogenesis, while bacteria of the genus *Lactobacillus*, *Staphylococcus* play a role in assisting the process of hydrolysis. *Microthrix* bacteria cause bulking and foaming in wastewater and can inhibit wastewater treatment processes.

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