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Potential of Autotrophic Microalgae (*Spirulina plantensis*) in Decreased Coliform Bacteria

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***Abstract*— Objectives: Dense settlements spend the distance between the well and the toilet. So, many sources of clean water are contaminated by coliform bacteria. Microalgae is an important micro-plant which is a bioindicator of clean water. Besides that microalgae can also decrease the number of coliform bacteria. One potential microalga that is not yet known for its potential in decreased the number of coliform bacteria is Spirulina platensis. The purpose of this study was to determine the potential of Spirulina platensis in decreased the number of bacteria in polluted well water so that it is expected to decrease the number of pathogenic bacteria that can cause infectious diseases. This research used Spirulina platensis, which was cultured, and then it was remediated to water wells. The value of the Most Probable Number (MPN) is calculated in water before and after remediation. The results obtained showed that the number of microalgae decreased as much as coliforms, with a mean decrease is 94.6%. The MPN values of sample 1 decrease from 75 cells / 100 mL to 1.8 cells / 100 mL and in sample 3 from 93 cells / 100 mL to 7.8 cells / 100 mL.**

Keywords: microalgae, MPN, coliform bacteria

I. INTRODUCTION

Coliform bacteria often contaminate water, food, or other materials for human needs. The presence of this bacterium is not expected and even highly avoided because it is suspected to originate from human or animal feces [1]. These pathogenic bacteria if consumed, can cause problems for human health such as diarrhea, vomiting, and other digestive problems [2].

To reduce the number of coliform bacteria in water, can using microalgae on remediation. Remediation can used potential indogen bacteria [3] and microalgae can also be used for remediation of photobioreactors (FBR) [4].

Microalgae is also a bioindicator of water cleanliness. If there is a change in water quality, then it will affect the existence and behavior of the organism so that it can be used as a marker of environmental quality [5].

Microalgae or phytoplankton are microorganisms, which has a distinctive characteristic that distinguishes it, each of these microorganisms can be distinguished by cell size, color, their habitat, unicellular cells and can be photoautotrophic or heterotrophic [6].

Microalgae has ability as a phototropic microorganism, which can supply oxygen to degrade organic aerobic waste and enhance the ability to eliminate pathogenic bacteria. In addition, it is a conventional method for removing heavy metals, chemical compounds that are precipitated and coagulated, as ion exchangers, and adsorption on activated carbon [7].

The use of microalgae in the process of treating wastewater takes a relatively long time but has several advantages, including the principle of the processing process running naturally such as the principle of natural ecosystems, so it is very environmentally friendly and does not produce secondary waste. Another advantage is that by using microalgae, nutrient recycling works very efficiently and produces biomass that can be used for various purposes [4].

II. MATERIAL AND METHOD

A. Procedure

a) Spirulina platensis SubCulture on Media

Spirulina platensis which will be inoculated in well water. The culture container used was a 1000 ml Erlenmeyer placed in a room with a temperature of 25-270 C, and placed on a rack that had been equipped with aeration and TL lamps with a wattage of 18 watts, arranged so that each treatment received light intensity according to the level of treatment. The culture was placed on the culture rack and incubated for approximately 14 days with photoperiodicity of 14 hours of light and 10 hours of darkness. Cultures that grow well and isolats are reproduced gradually.

b) Remediation of Spirulina platensis in Samples

Spirulina platensis taken from subculture added to well water samples, where each ml sample contained 2,041,666 cells. Cell calculation using Haemocytometer. Then remediation was carried out for 14 days with aeration and light settings.

c) Most Probable Number (MPN)

Presumptive Test.

3 test tubes containing double Lactose Broth media and 6 test tubes containing single Lactose Broth media where shaking

until homogeneous. Insert 10 mL of sample into test tubes containing double Lactose Broth media, and 1 mL each into 3 test tubes containing single Lactose Broth media. Insert the entire tube into the incubator at 35°C for 2 x 24 hours. Then the formation of gas that occurs in the Durham tube is observed, positive results continued to Confirmatory test.

Confirmatory test

The tubes which were tested positive from the estimated test were inoculated into tubes containing BGLB (Brilliant Green Lactose Broth) then incubated at 35 °C for 2x24 hours. The reading of the results is done by counting the number of positive tubes. The numbers obtained are matched with the MPN Table [8].

III. RESULTS

Based on testing the value of MPN after remediation with microalgae showed a decrease in the number of coliform bacteria contained in the sample. In sample 1 decrease from 93 cells / 100 mL to 3.0 cells / 100 mL, in sample 2 decrease from 23 cells / 100 mL to 0 cells / 100 mL and in sample 3 there decrease from 120 cells / 100 mL to 0 cells / 100 mL 9.4 cells / 100 mL. The mean decrease in coliform bacteria in the sample was 96.31%. Based on the results, sample 1 and sample 3 showed positive results, so the test continued to Confirmatory test.

TABLE 1: MPN PRESUMPTIVE TEST RESULTS FROM WELL WATER SAMPLES AFTER REMEDIATION

Sample	Positive Tube			MPN/100 ml
	3 tubes of 10 ml	3 tubes of 1 ml	3 Tabung 0,1 ml	
1	-	1	-	3,0
2	-	-	-	0
3	-	3	-	9,4

Based on the results of tests showing the value of MPN, after remediation with microalgae showed a decrease in the number of coliform bacteria found in the sample. In sample 1 there was a decrease from 75 cells / 100 mL to 1.8 cells / 100 mL and in sample 3 there was a decrease from 93 cells / 100 mL

to 7.8 cells / 100 mL. The mean decrease in coliform bacteria in the sample was 94.6%.

TABLE 2: MPN CONFIRMATORY TEST. RESULTS FROM WELL WATER SAMPLES AFTER REMEDIATION

Sample	Positive Tube			MPN/100 ml
	3 tubes of 10 ml	3 tubes of 1 ml	3 tubes of 0,1 ml	
1	-	-	1	1,8
3	-	-	3	7,8

IV. DISCUSSION

MPN (Most Probable Number) testing is a method used to estimate the concentration of coliform bacteria contained in a sample. MPN is usually applied to water samples. The tests include Presumptive and Confirmatory tests using 3-3-3 tube method.

Coliform bacteria estimation test uses LB (Lactose Broth) fermentation media to determine the presence or absence of coliform bacteria (gram-negative bacteria) in samples marked with gas formation, indicating that the process of coliform bacterial fermentation has occurred. LB media is used to detect the presence of coliform bacteria in water, food, and milk products. Lactose provides a fermentable source of carbohydrates for coliform bacteria [9].

The method used is 3 series tube method. Positive results are characterized by turbidity and gas formed in the Durham tube, which is kept in reverse condition in the test tube. The results can then be viewed based on the MPN table to see the coliform MPN values that are in the sample.

Confirmatory test uses BGLB (Brilliant Green Lactose Broth) media. This media is used as a fertilizing medium for coliform bacteria and at the same time as a selective media so that gram-negative bacteria other than coliform will not give positive results. Positive results are indicated by the formation of gas and turbidity formed, and it is caused by lactose fermentation by coliform bacteria [9].

Microalgae produce secondary metabolites allelopathic, which is able to inhibit the growth of competitors, both microorganisms and other predators [10].

Microalgae can be applied to the processing of human waste, livestock waste, industrial waste, industrial waste, and food factory waste, because it can eliminate toxic minerals such as lead, cadmium, mercury, scandium, lead, arsenic and bromine. In addition, microalgae can also inhibit the growth of coliform bacteria, in the stabilization ponds decrease of 88.8% in 11.4 days and in high-rate ponds decrease 99% of coliform bacteria [11].

Fecal coliform bacteria require nutrients in the form of phosphorus and nitrogen [12]. With the presence of microalgae, the growth of coliform bacteria can be inhibited because microalgae can reduce the amount of phosphorus and nitrogen efficiently in about 1 hour. Autotrophic microalgae have the ability to photosynthesis which converts solar energy into useful biomass and absorbs nutrients in the form of nitrogen and phosphorus, which cause eutrophication [13].

V. CONCLUSION

Autotrophic microalgae *Spirulina platensis* has potential to decrease the number of coliform bacteria in well water samples, with mean coliform bacteria decrease on samples is 94.6%. MPN values after remediation by microalgae showed decrease in the number of coliform bacteria. In sample 1 decrease from 75 cells / 100 mL to 1.8 cells / 100 mL and in sample 3 decrease from 93 cells / 100 mL to 7.8 cells / 100 mL

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