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Azolla microphylla and *Pseudomonas aeruginosa* for bioremediation of bioethanol wastewater

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Abstract. *Annisa K, Sutarno, Santosa S. 2021. Azolla microphylla and Pseudomonas aeruginosa for bioremediation of bioethanol wastewater. Biodiversitas 22: 1799-1805.* Bioremediation is the right choice in wastewater treatment since it requires has low cost but works optimally. *Azolla microphylla* and *Pseudomonas aeruginosa* are considered to have the ability to optimize waste degradation. The aim of this study was to determine the best treatment for reducing pollutants in bioethanol waste, namely by using *A. microphylla* and *P. aeruginosa*. The twelve treatments in this experimental research included variations in the *A. microphylla* biomass and variations of biomass combinations with the density of the *P. aeruginosa*. Research method used was completely randomized design with three replications, and all 36 samples were taken on the seventh day. Research data collected were the quality parameters of bioethanol wastewater before and after the treatment. The results showed that TSS, BOD, COD were decreased during seven days of treatment, whereas pH and H_2S levels were increased. The combination of 0.2 kg *A. microphylla* and 10^{10} cfu/mL *P. aeruginosa* showed the best result in reducing pollutants. This study showed that *A. microphylla* and *P. aeruginosa* could be used to reduce pollutants in bioethanol wastewater. Results of the research are expected to become an alternative solution to the bioethanol wastewater problem.

Keywords: *Azolla microphylla, Pseudomonas aeruginosa,* bioremediation, wastewater

INTRODUCTION

Waste discharged directly into the environment without treatment will contaminate the environment. In addition, the disposal of waste directly into the environment may get protests from the surrounding community as happened with the waste of the bioethanol industry. Therefore, waste should be treated first before being discharged into the environment. However, the cost for waste treatment is very high, so it is necessary to develop wastewater treatment methods that can process waste optimally at a low budget. A cheap alternative for degrading wastewater pollutants is bioremediation, namely the use of biological interventions to mitigate the effects of toxins caused by pollutants (Soman et al. 2018). Biological interventions can be carried out using microorganisms, plants, or other living things. One of the bioremediation methods utilizes, *Azolla microphylla*, an aquatic fern in the family Salviaceae which has roots without fibers (Sood et al. 2011), a soft leaf surface, which can fix N_2 in the air and live in symbiosis with *Anabaena azollae* or bacteria (Carrapiço 2017). *A. microphylla* rapidly multiplies to form biomass and can be found in the rice fields, so it is easy to obtain this plant.

Previous research conducted by Unisah and Akbari (2020) showed that the use of *A. microphylla* in tofu wastewater treatment reduced 96% of BOD, 96% COD, 97% TSS, and neutralize pH. *A. microphylla* is also able to prevent eutrophication, reducing organic pollutants by 33.3% and organic carbon by 50% in wastewater (Soman et al. 2018). Nurcahyani (2015) states that the bioethanol wastewater industry contains high organic glucose, which is 1.3285%. Therefore, *A. microphylla* is suitable for use in the treatment of bioethanol industrial wastewater. *A. microphylla* growth can be induced by the rhizobacteria such as *Pseudomonas* sp. which acts as a biofertilizer and biostimulant. *Pseudomonas aeruginosa* produces auxin which plays a role in cell division, cell elongation, lateral root formation and provides phosphate for *A. microphylla* nutrition (Mardhiana et al. 2017; Santosa et al. 2018; Astriani et al. 2020). Phosphate is a macro element needed for the growth of *A. microphylla* (Utama et al. 2015). *Pseudomonas sp.* also considered potential as a biocontrol agent in plant diseases, further referred to as endophytic bacteria or endophytic bacteria (Mardhiana et al. 2017; Warzatullisna et al. 2019; Wiratno et al. 2019). The combination of *A. microphylla* and bacteria is considered to optimize the process of waste degradation. Interaction between plants and bacteria in areas contaminated with waste can increase the growth of these plants and the effectiveness of the phytoremediation process (Khan et al. 2015; Furini et al. 2015). Ekyastuti's research (2018) showed that *Pseudomonas sp.* increased the growth of *Jatropha curcas* thereby optimizing waste remediation. *A. microphylla* can live in symbiosis with bacteria to support its growth (Carrapiço 2017).

Previous study conducted by Romayanto et al. (2006) showed that *Pseudomonas* bacteria in domestic waste treatment decreased oil, fat, BOD and TSS levels and increased pH. *P. aeruginosa* belongs to a strain of bacteria that can degrade organic waste (Austin 1988). *P. aeruginosa* is a Gram-negative, rod-shaped bacterium found in agricultural water, sewage and hospital wastes (Chellaiah 2018). *Pseudomonas sp.* is an important factor in the carbon cycle, so these bacteria are often used as waste bioremediation agents. Microorganisms use wastewater as their growth nutrition (Shah et al. 2013). *Pseudomonas sp.* decomposes protein (Wahyudi et al. 2019), cellulose, hemicellulose, starch, chitin, pectin, inulin, lignin (Rao 1994) into simpler compounds and produces enzymes protease, lipase, catalase, chitinase and amylase (Mardhiana et al. 2017). Lipase enzymes decompose oils and fats contained in waste.

This study aimed to determine the best treatment for reducing pollutants in bioethanol waste, using *A. microphylla* and *P. aeruginosa*.

MATERIALS AND METHODS

Study area

The location of this research was the bioethanol home industry center of Sukoharjo District, Central Java, Indonesia, namely Karangwuni Village, Ngombakan Village, and Bekonang Village. This industry has been run since the 1940s and is widely known in Indonesia. Meanwhile, for the process of bacterial dilution, the manufacture of bacteria growing media and the making of bacterial stocks was carried out at the Biology Education Microbiology Laboratory of Sebelas Maret University (UNS), Surakarta, Indonesia. The research was carried out from January to April 2020.

Procedures

Propagation of the bacterium Pseudomonas aeruginosa *inoculum*

The bacterium used in the treatment was *P. aeruginosa*. According to Shah et al. (2013) *Pseudomonas sp*. is a bacterium often used as a bioremediation agent. *P. aeruginosa* bacterial inoculum was obtained from the Laboratory of the Department of Agricultural Microbiology, Gadjah Mada University (UGM), Yogyakarta, Indonesia. Bacteria from Nutrient Agar (NA) slope were grown on 900 mL of Nutrient Broth (NB) for 24 hours and re-incubated for 24 hours at 37^0 C. Transfer of 10% of the inoculum into a new medium was done every day, aimed to maintain the supply of inoculum during the study. The concentrations of inoculums used were 10^8 cfu/mL, 10^9 cfu/mL, 10^{10} cfu/mL. The inoculum was then mixed with the bioethanol wastewater to be used in the experiment.

Azolla microphylla acclimatization

Azolla microphylla was taken from a farmhouse in the Polokarto Subdistrict, Sukoharjo District, Central Java, Indonesia and then put into an acclimation pond. *A. microphylla* was subsequently acclimatized for one week before being used in the experiment. During the acclimatization process, the growth of the plants was observed to determine their resistance to the new environment. Observation and testing of the sample water quality parameters were also carried out before treatment. After the propagation of the *P. aeruginosa* and the acclimatization of *A. microphylla* was completed, the next step was testing the pre-treatment parameters of the bioethanol wastewater samples, i.e., pH, TSS, BOD, COD and sulfide material. These assays were conducted at the Center for Environmental Health and Disease Control Engineering, Yogyakarta.

Bioethanol wastewater treatment process

Each treatment tank was filled with 50 mL of wastewater and added with water until it reached a volume of 10 liters. The original waste was diluted to obtain a waste pH of 6.5 and the concentration of waste to 0.5% because *Azolla sp.* lives at pH 5.5 - 6.5 and *P. aeruginosa* lives at pH 6.5-7.5 (Maslahat et al. 2010). Ewies et al. (1998) say that almost all bacteria like neutral conditions, since strong acidic or alkaline conditions can inhibit the activity of microorganisms. There were twelve treatments in this stage. The treatment consisted of a variety of *A. microphylla* biomass, which weighed: a) 0.1 kg, b) 0.15 kg, c) 0.2 kg and variations in the combination of *A. microphylla* biomass and the density of the *P. aeruginosa* bacteria, i.e., d) 0.1 kg and 10^8 cfu/mL, e) 0.15 kg and 10^8 cfu/mL, f) 0.2 kg and 10^8 cfu/mL, g) 0.1 kg and 10^9 cfu/mL, h) 0.15 kg and 10^9 cfu/mL, i) 0.2 kg and 10^9 cfu/mL, j) 0.1 kg and 10^{10} cfu/mL, k) 0.15 kg and 10^{10} cfu/mL, l) 0.2 kg and 10^{10} cfu/mL. Each treatment was done in three replicates and incubated for seven days at 37^O C. Research by Mentari et. al (2016) showed that the most effective of *Azolla sp.* contact duration was 7 days. On the seventh day, a total of 36 samples of bioethanol wastewater were taken and assayed.

Observation and sample water quality parameters testing after treatment

After the treatment in the experiment, the morphology of *A. microphylla* was observed and documented. Bioethanol wastewater samples were taken to further test water quality parameters for pH, TSS, BOD, COD, and sulfide material (H_2S). The pH test was carried out by researcher using a pH meter while testings the quality parameters of bioethanol wastewater, i.e., TSS, BOD, COD, and sulfide material (H_2S) were conducted at the Center for Environmental Health and Disease Control Engineering, Yogyakarta. Parameter testing methods used were the In House Method for TSS test, SNI 6989.2-2009 for COD test, SNI 6989.72-2009 for BOD test, and SNI 6989.70-2009 for sulfide test (H_2S).

Data analysis

According to the Minister of Environment Regulation number 5 of 2014 concerning Wastewater Quality Standards, the data of wastewater quality were compared to bioethanol wastewater quality standards consisting of pH, TSS, BOD, COD, and sulfide content (H_2S). Data analysis was carried out before (pre-test) and after (post-test) the treatment process. The effectiveness of the reduction in levels was calculated for each parameter of the quality of wastewater in each treatment sample. The formula for calculating the effectiveness of reducing the level of wastewater quality parameters (Rohmah et al. 2019) was as follows,

$$\text{The effectiveness of wastewater quality treatment} = \frac{\text{Pretest level} - \text{posttest level}}{\text{pretest level}} \times 100\%$$

The data were tested with Anova and further analyzed with Borda method to find the best treatment.

RESULTS AND DISCUSSION

The characteristics of bioethanol wastewater taken from bioethanol home industry center of Sukoharjo District, Central Java, Indonesia and the characteristics of diluted wastes are described in Table 1. The levels of TSS, COD, BOD and pH exceeded the quality standard values, so treatment was required to reduce these pollutant levels.

Total Suspended Solids (TSS) Reduction

The highest decrease in TSS levels occurred in the I (0.2 kg and 10^9 cfu/mL), i.e., 31.9 %, while the lowest in the treatment D (0.1 kg and 10^8 cfu/mL), i.e., -90.48 %. ANOVA test showed there was a significant difference between the treatment groups. *Azolla* biomass and a combination of *Azolla* biomass and *Pseudomonas* bacterial density had a significant effect on the TSS. TSS is the amount of suspended substances in wastewater. The decrease of TSS level was caused by the ability of *A. microphylla* as a biofilter (Xin et al. 2008), which can absorb and accumulate nutrients in the roots (Ekyastuti et al. 2018; Parbo et al. 2019). In this case, *A. microphylla* could accumulate large amounts of contaminants into the biomass (Arora et al. 2006). The study by Sheena and Harsha (2018) also revealed that the high reduction in solids was caused by the ability of plant roots to accumulate organic matter in wastewater that supports its growth. Aquatic plants absorb pollutants through direct root contact with contaminated water (Sood et al. 2011). *P. aeruginosa* expresses biosorption characteristics and promotes plant growth and pollutant accumulation by plants (Chellaiah 2018). *P. aeruginosa* can be used as suitable biosorbent for the removal of pollutants from solution, contaminated waste, water and soil. The decrease of TSS level was also caused by bacteria such as *P. aeruginosa* which can digest the organic substances contained in the waste. Previous study conducted by Romayanto et al. (2006) showed that the use of Pseudomonas bacteria in domestic waste treatment decreased TSS levels by 22.09%. Waste has been decomposed by bacteria and produces compounds that can be used for bacterial growth (Romayanto et al. 2006). *Pseudomonas sp.* decomposes protein (Wahyudi et al. 2019), cellulose, hemicellulose, starch, chitin, pectin, inulin, lignin (Rao 1994) into simpler compounds with the help of the enzymes protease, lipase, catalase, chitinase and amylase (Mardhiana et al. 2017). Therefore, solids that are suspended in the waste are reduced.

Chemical Oxygen Demand (COD) reduction

The highest percentage of COD decrease occurred in the treatment F (0.2 kg and 10^8 cfu/mL) which was 62.66 % and the lowest percentage was in the treatment D (0.1 kg and 10^8 cfu/mL), i.e., 54.45 %. Based on ANOVA test, there was no significant difference among the treatment groups. *Azolla* biomass and a combination of *Azolla* biomass and *Pseudomonas* bacterial density had no significant effect on the COD. The COD is the amount of oxygen needed to oxidize organic compounds in chemical waste, indicating the organic content in waste (Jenie and Rahayu 1996). The decrease in level of COD is caused by plants oxidizing the pollutant inside their tissues and bioconcentrate these substances (Varghese and Jacob 2016).

Table 1. Test results of bioethanol wastewater parameter on various treatments

Treatment (kg, cfu/mL)	TSS		COD		BOD		H_2S (mg/L)	pH
	C^* (mg/L)	R^* (%)	C^* (mg/L)	R^* (%)	C^* (mg/L)	R^* (%)		
A0.1	70	7.14	596	56.16	134	79.24	0.17	8.1
A0.15	72	7.62	565	58.49	115	82.23	0.14	8
A0.2	81	26.67	564	58.55	138	78.70	0.15	7.7
A0.1 P10^8	133	-90.48	620	54.45	172	73.36	0.27	7.7
A0.15 P10^8	56	19.52	532	60.88	147	77.23	0.09	7.8
A0.2 P10^8	64	9.05	508	62.66	129	80.01	0.14	7.9
A0.1 P10^9	70	1.90	553	59.35	134	79.29	0.14	7.9
A0.15 P10^9	59	16.19	570	58.13	138	78.62	0.28	7.9
A0.2 P10^9	48	31.90	536	60.59	181	82.38	0.12	8.1
A0.1 P10^{10}	59	17.14	542	60.15	182	71.87	0.11	7.9
A0.15 P10^{10}	59	16.67	557	59.04	181	71.92	0.45	7.8
A0.2 P10^{10}	68	6.19	572	57.94	191	70.48	0.31	7.9
Original waste	11.400		263.500		59.100		4.13	4.4
Diluted waste	70		1360		647		0.0694	6.5

Note: A: Biomass of *Azolla microphylla*, P: Density of *Pseudomonas aeruginosa*, C: The average of pollutant levels, R: Reduction of pollutant

Research by Arora et al. (2006) revealed that *A. microphylla* had the highest bioconcentration potential among the genus *Azolla species.* The presence of these plants in wastewater can reduce CO_2 used for photosynthesis and increase DO water leading to aerobic conditions in wastewater, which results in the reduction of BOD and COD (Sheena and Harsha 2018). Research conducted by Unisah and Akbari (2020) showed that the use of *A. microphylla* in tofu wastewater treatment reduced 96% of COD.

Pseudomonas aeruginosa oxidizes glucose (Jawetz 2010), mostly in bioethanol waste (Nurcahyani 2015), so bioethanol pollutants become decomposed. Organic matter in bioethanol wastewater can be a source of carbon nutrition for microbes (Tangahu et al. 2019). Other researchers also confirm that the reduction in COD can be caused by oxidation of organic matter in wetland systems that provide energy for microbial metabolism. In other words, the reduction of organic components is the result of biological decomposition by microorganisms. Reaction in oxidation of organic waste according to Romayanto (2006) are as follows,

$$(CH_2O)n + nO_2 \longrightarrow nCO_2 + nH_2O + \text{energy}$$

CO_2 and H_2O from that reaction are potentially absorbed by the plants. *P. aeruginosa* plays a key role in phytoextraction process of plant (Chellaiah 2018), biofilm production (Wahyudi et al. 2019) and plant growth-promoting rhizobacteria activity (Santosa et al. 2018). Phytoremediation is influenced by the growth of plants and microorganisms associated with the rhizosphere (Sheena and Harsha 2018). Rhizosphere in plants stimulates microbial activity and community density by providing root surface area for their growth (Tanner 2001; Vymazal and Kropfelov 2009). Many fluorescent *Pseudomonas* strains, for example, *P. aeruginosa* which colonize the rhizosphere exert a protective effect on the roots through the production of *in situ* antibiotic compounds that promote growth and inhibit microbial infections (Jenni et al. 1989; Wackett 2000).

Biochemical Oxygen Demand (BOD) Reduction

The highest percentage of BOD decrease occurred in the treatment I (0.2 kg and 10^9 cfu/mL), i.e., 82.38 % and the lowest percentage occurred in the treatment L (0.2 kg and 10^{10} cfu/mL), i.e., 70.48 %. The Anova analysis showed significant differences among the treatment groups. *Azolla* biomass and a combination of *Azolla* biomass and *Pseudomonas* bacterial density significantly affected the BOD. The decrease in BOD is due to the decrease of oxygen demand by microorganisms for decomposing organic matter and the increase of dissolved oxygen (DO) in the water due to photosynthetic activity (Morrice et al. 2008) which produces oxygen. *P. aeruginosa* plays an important role in the breakdown of organic matter in bioethanol wastewater. Microorganisms decompose organic matter to obtain nutrients (Suhendrayatna et al. 2012). Reaction in cell oxidation according to Romayanto (2006) are as follows,

$$\text{Cell} + O_2 \longrightarrow CO_2 + H_2O + NH_3 + \text{energy}$$

Pseudomonas sp. can decompose organic matter like proteins and carbohydrates into simpler compounds by secreted protease, lipase and amylase enzyme. Organic matter contained in wastewater provides substrate for microbial metabolism and the time of culture incubation can reduce the concentration of BOD in wastewater (Prabu 2007). This indicates that the reduction in BOD concentration in this study was influenced by *P. aeruginosa* which can break down the organic components. Romayanto et al. (2006) showed that the use of *Pseudomonas* bacteria in domestic waste treatment decreased BOD levels to 71,48 %.

The BOD is the amount of O_2 needed to decompose organic compounds in biological waste and indicates the decrease in BOD level in this study also occurred due to decomposition of organic matter by *A. microphylla*. Organic matter evaporates from the stems and leaves through a direct phytovolatilization mechanism or from the soil through root activity called indirect phytovolatilization mechanism (Limmer and Burken 2016; Ekta and Modi 2018). Phytoremediation agents such as *A. microphylla* absorb components from the environment which are then processed in the tissue metabolism to remove pollutants (Alkorta et al. 2004; Tulod et al. 2012; Varghese and Jacob 2016; Napaldet et al. 2019). Unisah and Akbari (2020) showed that the use of *A. microphylla* in tofu wastewater treatment reduced 96% of BOD.

pH

The highest percentage of pH increase occurred in the treatments A (0.1 kg), i.e., 24.1 % and I (0.2 kg and 10^9 cfu/mL), i.e., 24.1 % while the lowest percentage is in the treatments C (0.2 kg) and F (0.1 kg and 10^8 cfu/mL) which were 18.97 % and 18.97 % respectively. ANOVA test showed that there were significant differences among the treatment groups. *Azolla* biomass and a combination of *Azolla* biomass and *Pseudomonas* bacterial density significantly affected the pH. The acidic condition of bioethanol wastewater was due to the addition of sodium metabisulfite ($Na_2S_2O_5$) and $NaHSO_4$ added in the production process of microbial nutrition. The $NaHSO_4$ is a weak electrolyte salt that decreases pH (Sanna et al. 2017). It can be inferred that the incomplete decomposition of molecules causes the acidic condition in weak electrolyte salts. The HSO_4^- ion resulted from dissociation of $NaHSO_4$ will reduce pH when it reacts to the water. This is what causes the bioethanol wastewater tends to be acidic (Kurniawati et al. 2015).

$$NaHSO_4 \longrightarrow Na^+ + HSO_4^-$$

$$HSO_4^- + H2O \rightleftharpoons H_3O^+ + SO_4^{\ 2-}$$

The increase of pH after treatment enabled *A. microphylla* to bind acids in wastewater. Increase in pH occurred due to photosynthesis by plants. Intake of H^+ ions in water for photosynthesis decreases the H^+ ion content, so the pH of the water increases (Ugya et al. 2019). The increase in pH is also caused by the breakdown of organic matter in water by microorganisms. The decomposition of organic matter by microorganisms will produce OH^- in the form of H_2O, thereby supporting an increase in pH. The activity of microorganisms decomposing organic material in waste is also related to photosynthesis activity, which takes dissolved CO_2, resulting in an increase in pH (Morrice et al. 2008). Arnol et al. (2018) showed that the increase in pH is also related to reduce $SO_4{}^{2-}$ to H_2S. The activity of bacteria in reducing sulfate to sulfide causes an increase in pH and sulfide level.

Sulfides (H_2S) reduction

The highest percentage of the decrease of H_2S level occurred in the treatment E (0.15 kg and 10^8 cfu/mL) which is -29 % and the lowest percentage in the treatment K (0.15 kg and 10^{10} cfu/mL) which is -544 %. ANOVA showed no significant difference among the treatment groups. *Azolla* biomass and a combination of *Azolla* biomass and *Pseudomonas* bacterial density had no significant effect on the H_2S. The increase of sulfide (H_2S) in wastewater is caused by the use of wastewater by microorganisms as growth nutrients (Shah et al. 2013) which produces compounds such as H_2S. Hydrogen sulfide (H_2S) is corrosive to metals, forming greenhouse gases (SO_2 or SO_3) if it is burned and toxic at certain concentrations is toxic. H_2S can be found in wastes, quarries and crude oil (Qaisar et al. 2007). H_2S comes from the breakdown of compounds containing sulfur by bacteria. Sulfate-reducing bacteria utilize sulfur ions in the form of sulfate ($SO_4{}^{2-}$), thiosulfate ($S_2O_3{}^{2-}$) and sulfite ($SO_3{}^{2-}$) as terminal electron acceptors in their metabolic respiration, which is then reduced to sulfide. Reaction in releasing sulfides according to Arnol et al. (2018) are as follows,

$$SO_4{}^{2-} \longrightarrow HS^-$$

The activity of bacteria reduces sulfate to sulfide causes an increase in the level of sulfide. Jorgensen (1982) reported that the number and activity of sulfate-reducing bacteria increased with the thickness of the sedimentary layer. Roots of aquatic plants such as *A. microphylla* can bind the nutrients and form the pollutant sedimentation (Arora et al. 2006), which increased the activity of sulfate-reducing bacteria and the level of sulfide. pH and oxygen also affect the formation of sulfides, i.e., at pH <7 forming H_2S, at pH 7-9 HS^- and at pH> 8 S_2^- (Markl 1999; Sanchez et al. 2005).

The results of Borda method showed that the best treatment in reducing pollutants in bioethanol wastewater was 0.2 kg of biomass with a bacterial density of 10^{10} cfu/mL. The treatment with the highest biomass and bacterial density will give the best results in reducing bioethanol wastewater levels. The greater the biomass, the more the organic matter is decomposed by plants. The treatment in the form of a combination of plants and bacteria in waste bioremediation showed better results. Plants and microorganisms are work synergistically for the treatment of waste in various media (Etim 2012). Research by Germaine et al. (2015) showed that plants and microorganisms form an association during reclamation of land polluted by organic contaminants. Interaction between plants and bacteria in contaminated areas can increase plant growth and the effectiveness of the phytoremediation process (Khan et al. 2015; Furini et al. 2015).

Phytoremediation agents such as *A. microphylla* are more tolerant towards pollutants in the environment because they can absorb components from the environment that are metabolized inside the tissues (Alkorta et al. 2004; Tulod et al. 2012; Varghese and Jacob 2016; Napaldet et al. 2019). In this case, *A. microphylla* can accumulate large amounts of contaminants into the biomass (Arora et al. 2006). Research by Arora et al. (2006) revealed that *A. microphylla* had the highest bioconcentration potential among the genus *Azolla* species. *A. microphylla* absorption capacities were 11.17 ppm TSS/100 gram plant biomass, 426 ppm COD/100 gram plant biomass, 354.67 ppm BOD/100 gram plant biomass, and 0.05 ppm H_2S/100 gram plant biomass.

The presence of aquatic plants contributes to the growth of microbes that will degrade organic components (Qu et al. 2017, Riaz et al. 2017). Plants provide nutrients for microbes in the rhizosphere that can enhance the process of waste degradation (Vymazal 2007). *A. microphylla* growth can also be induced by the rhizobacteria of *Pseudomonas sp.* which acts as a biofertilizer and biostimulant. *P. aeruginosa* produces auxin which plays a role in cell division, cell elongation, lateral root formation and provides phosphate for *A. microphylla* nutrition (Mardhiana et al. 2017; Santosa et al. 2018; Astriani et al. 2020). Phosphate is a macro element needed for the growth of *A. microphylla* (Utama et al. 2015). Therefore, the pollutant reduction in *A. microphylla* and *P. aeruginosa* biomass treatment was better than *A. microphylla* biomass alone. It is concluded that the combination of *A. microphylla* and *P. aeruginosa* could be used to reduce pollutants in bioethanol wastewater, with the most effective treatment being 0.2 kg of *A. microphylla* biomass with the density of *P. aeruginosa* of 10^{10} cfu/mL.

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