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# Microbial Fuel Cell: Sustainable Approach for Reservoir Eutrophication

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**Abstract:** Cirata is one of the three reservoirs of Citarum where its function has developed from hydroelectric power generator to aquaculture and tourism in recent times. A number of floating net cage keeps increasing and they have reached 53,031 cages, which have actually exceeded the maximum amount under local government regulation. This condition has triggered eutrophication in the form of ammonium deposited in aquatic sediments. A study with a 30-day treatment of oxidation catalysis of inorganic and organic matter by bacteria called Microbial Fuel Cell (MFC) technology had been conducted to reduce contamination of ammonium in the water. Various treatments were applied in the form of single and dual chambers, with and without aeration. Parameters measurements were decreased in ammonium levels in the sediment, and the same applied to the electricity generated as the by-product of microbial activity. The results showed that MFC technology was proven to reduce the levels of ammonium in the sediments up to 96.12%. The electricity output reached 333 $mA.m^{-2}$ for a single chamber treatment with aeration. Overall, it can be indicated that better results appeared in all measured parameters with single-chamber treatments compared to dual-chamber ones.

**Key words:** Ammonium, Cirata, eutrophication, microbial fuel cell.

## Introduction

Cirata is one of the cascades in three watershed reservoirs of River Citarum, the biggest river in West Java. Cirata (107°14′15″–107°22′03″ LS and 06°41′30″–06°48′07″ BT) is located between two reservoirs, namely Saguling at its upstream and Jatiluhur at the downstream. Cirata is categorised as multipurpose reservoir. The main objective of this development is to serve as a hydropower to cater for the electrical energy throughout Java and Bali, with its installed power generator capacity of 1008 MW. However, the current utilisation of the reservoirs continues to evolve more from its initial purpose as hydropower to aquaculture and tourism activities.

The development in aquaculture using floating net cage system in Cirata has significantly increased in numbers in recent years. Existing number of floating net cages reached 53,031 plots, while the allowed maximum limit is set to 12,000 plots as per government regulations No. 41/2002. In recent years, the growth of floating net cage has exceeded rapidly and as a result, reduced the carrying capacity of the reservoir (BPWC, 2011). The unutilised feed and excretion of the fish remain at the bottom of the water column and thus accumulate in the sediments. This organic waste

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resulting from aquaculture activities has been reaching 6.365 tons/year (Indriani, 2005). The waste has been accumulating to 279.91 tons ever since and reaching more than two metres in thickness. Basically speaking, this accumulated waste contains nitrogen in the form of ammonium, which was highly toxic to aquatic biota. Nitrogen waste has also caused the massive deaths of the local fish in Cirata some years ago due to its upwelling process, which brought toxic compounds from the sediment to the water column, along with nitrogen (Prihadi, 2005).

The increasing number of floating net cage is not always directly proportional to the production of farmed fish. During 1988 and 1996, data showed increasing production volume due to the increasing number of floating net cage. On the other hand, another data captured between 1997 and 2000 showed that the production volume has decreased with the increasing number of floating net cage (BPWC, 2011).

The growth of fish being cultured in Cirata was categorised as having negatively allometric growth, meaning that the fish were gaining more on their length than the weight. This condition surely became a lot less beneficial for cultivation purpose. Fish growth was hampered due to physiological disturbance, decreased appetite, and pain. Those conditions were triggered by the polluted environment of organic materials. Increasing numbers of floating net cage will exceed environmental pollution in the water column. Pollution from fish farming can also increase the amount and concentration of phosphorus and, therefore, cause eutrophication (Kibria et al., 1996).

Bioremediation technique can work well to reduce eutrophication including the toxicans, although they may involve such high energy input and lead to the production of sludge (Morris et al., 2009). One of bioremediation method is Microbial Fuel Cell (MFC), which showed up as a promising technology for wastewater treatment (Boghani et al., 2017). MFC is a bio-electrochemical system that drives a current using bacteria and mimicking bacterial interaction found in nature. The electricigens and its activity have important influence on the power generation capacity and organic matter degradation ability (Fan and Xue, 2016). This technology is renewable and nature friendly; it could be chosen as one of alternative energy source (Gezginci and Uysal, 2016).

It is still suggested that different microbial sources should be experimented for getting better-performing community (Tran et al., 2016). Reported source of electro-active microorganisms have been domestic wastewater, activated and anaerobic sludge and marine sediments, garden compost, and manure (Erable et al., 2009; Liu et al., 2004; Parot et al., 2008; Rezaei et al., 2007; Scott et al., 2007). From various source of material for MFC, use of sediment is still scanty while it holds great promises as alternative energy source. Some researchers were uncovering that the greatest value of MFC technology may not be the production of electricity but the ability of electrode associated microbes to degrade wastes and toxic chemicals (Franks and Nevin, 2010). Thus, this study aimed at the potentiality of MFC using eutrophic sediment as source, in terms of electricity production and ammonium reduction.

## Material and Methods

### Water and Sediment

Media used in this study were water and sediments from Cirata reservoir. The location was adjacent to the floating net cage with its history of frequent mass fish deaths, which can be concluded as an indicator of high ammonium contain in the sediments. Sediments were taken using Eickman Grab as much as 40 litres of water from the location approximately 10–13 metres below the surface, and then stored in sterile plastic jar with airtight lid. Water was taken from the water column at the same location with sediments (Holmes et al., 2004). Water samples were taken in the amount of 30 litres, which was then collected in sterile jerry cans. Water and sediment samples were stored in a cool box during the trip, and kept at refrigerator under the temperature of 10°C to slow down the metabolism of microorganisms and also to avoid any unexpected chemical reactions until the experiment was conducted.

### Microbial Fuel Cell Circuit

The MFC series was made in three consecutive stages: preparation of the electrode, the setting up for single and dual chambers, and the setting up for aeration. Rod-shaped carbon electrode was used with its dimension of $6\times1.5\times1$ $cm^3$ (Logan, 2008). Prior to the application, carbon electrodes were neutralised using soaking treatment in succession with 1N NaOH after initial soaking in 1N HCl solution for 24 hours each respectively. The electrodes were then rinsed at each soaking treatment with distilled water and dried via air. Electrodes were soaked in distilled water until the experiments. Shortly before the experiment began, neutralised electrodes were wounded with copper wire, which had first been opened and closed with a rubber

isolator. The wires and electrodes were then isolated using silicone rubber so that it became watertight.

The single-chamber MFC is referred to Holmes et al. (2004), where the sediment samples are inserted into the vessel at 3-cm thick. An electrode made of carbon (anode) was placed on the sediments and covered by 2-cm thick sediments. The vessel was then filled with 400 ml of water sample and stored in room temperature for 24 hours to precipitate the particles of sediments. On the next day, a carbon electrode (cathode) was placed 1 cm above the sediment surface and kept surfacing on the water column. Cables from the anode and the cathode were connected to the resistor (820 Ω ± 5%) to form a closed circuit. MFC was then operated in dark conditions (without lighting) and at room temperature (27°C ± 1), with and without aeration. Water loss due to evaporation during the measurement of electric current was replaced with sterilised distilled water demineralisation.

The used dual chamber circuits were identical plastic vessels. The vessel pair were connected with a pipeline with 2.5 cm diameter and 10 cm length, contained salt bridge as the cation exchange medium. The composition of the salt bridge material contained 15-ml $KNO_3$ 0.1 M dissolved in 250 ml of distilled water. Cathode chamber was filled with water sample at full volume, as well as the anode chamber, filled with sediments in the other vessel. Carbon electrode was stored on each of the cathode and anode. Cathode chamber was then coupled with aeration to provide oxygen for the treatment using aeration.

**Electricity Measurement**

Measurement of the electrical current was done every day during the 30-day experimental period while digital multimeter (Fisher brand digital auto-ranged multimeter) was also applied at the same time. Electrical components were observed in the form of voltage (mV) and currency (mA). The values obtained were then converted to power (watts), current density ($mA.m^{-2}$) and power density ($mW.m^{-3}$), which were measured from the conversion of voltage, current, and the surface area of the electrodes being used.

**Ammonium Analyses**

Ammonium analyses were measured after 30 days of MFC treatments where the amount of ammonium concentration decreased and efficiency was removed. Measurements were conducted at initial ($H_0$) and final ($H_{30}$) experiments. Extraction of sediment samples for nitrate measurements refers to the US EPA (1981). Samples of 1 g dry sediments were extracted and put into 200 ml Erlenmeyer flask. Thereupon, 50 ml of distilled water and 1 ml of $H_2SO_4$ were added. Then, another 50 ml of distilled water were added, stirred gently, and heated for 15 minutes. The samples were then transferred to a centrifuge tube and centrifuged for 5-10 minutes with a rotation speed of 2,000 rpm using Benchmark 2000, USA. The supernatant fluid was then poured into 200 mL volumetric flask. The pellets were recentrifuged after having another 50 ml of distilled water for 5-10 minutes at 2,000 rpm. The supernatant fluid was then stored in a 200 ml volumetric flask. These leaching and washing processes were then repeated twice. The supernatant fluid was then filtered with a 0.45µ pore-size membrane filter and used for the next measurement.

Measurements were made with ammonium sulfate bruisin colorimetric method. Samples were neutralised with NaOH or acetic acid. Tubes for reagent blanks, standard solution, and samples were placed in the rack evenly. A total of 10 ml of standard and samples were pipetted into each test tube and then added by 10 ml of $H_2SO_4$. The solution was then shaken gently. Samples and standards were placed in a water bath at 10°C. After that, a 0.5 ml sulfanilik brucine acid reagent was added to each tube and mixed carefully. Samples were placed in water bath at 100°C for 25 minutes, then placed in water bath until the temperature reached and remained at the range of 20-25°C. Samples were measured in spectrophotometer using Thermoscientific, Genesys 60, USA for absorbance at a wavelength of 410 nm.

## Results

After 30 days of experiments, the results showed that the single chamber and dual chamber generated different electricity productions both for the group of aerated and non-aerated processes. Overall, the electricity performance of single chamber in both groups aerated and non-aerated were higher compared to the group with dual chamber (Figure 1).

Production of high voltage at the beginning of the experiments occured in the samples with single aerated chamber, and reached its highest value at 148.67 mV while other treatments resulted in a relatively lower value. Voltage production showed fluctuative decrease during the 30-day experimental periods in all treatments.

Current production density also showed fluctuative decrease, especially for the treatment with single aerated chamber. At the beginning of the experiments, the value of current density showed 85.39, 46.78, 23.39 and 22.85

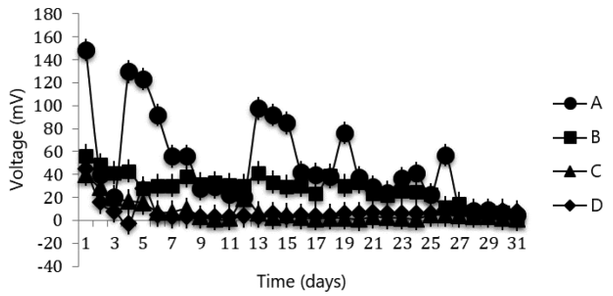


**Figure 1: Graph of production voltage (mV) during the 30-day MFC treatment, for each set of treatment are as follows: A. Aerated single chamber; B. Non-aerated single chamber; C. Aerated dual chamber; D. Non-aerated dual chamber.**

mA.m$^{-2}$ for treatments A to D, each consecutively. During the experimental period, drastic improvement showed only for samples with a single aerated chamber treatment, starting from day 3. It reached 333 mA.m$^{-2}$, and was scored as the highest value during the 30-day experimental period. The other treatments showed fluctuative pattern between 0.1 to 50 mA.m$^{-2}$, and tended to decrease continuously until the end of the experimental period.

In this experiment, the amount of ammonium decrease in the sediment was also measured with a given series of MFC, to investigate the best MFC treatment. Generally, the results showed that final ammonium concentration in the sediment samples has decreased at the end of the experiments for all MFC treatments, including control. Samples without MFC treatments showed ammonium concentration decrease ranging from 24.26% to 37.49% from the initial measurement. The ammonium concentration of sediment samples with MFC treatments decreased beyond 50% of the initial concentration for all treatments. The largest decrease was shown by the sediment samples treated with a single aerated chamber, which reached 96,12% of the removal efficiency.

## Discussion

The voltage value at the beginning of the experiments showed a surge which presumably came from the accumulation of electrons in the sediments starting from the beginning. When microorganisms began to perform metabolism on the anode surface, high-energy

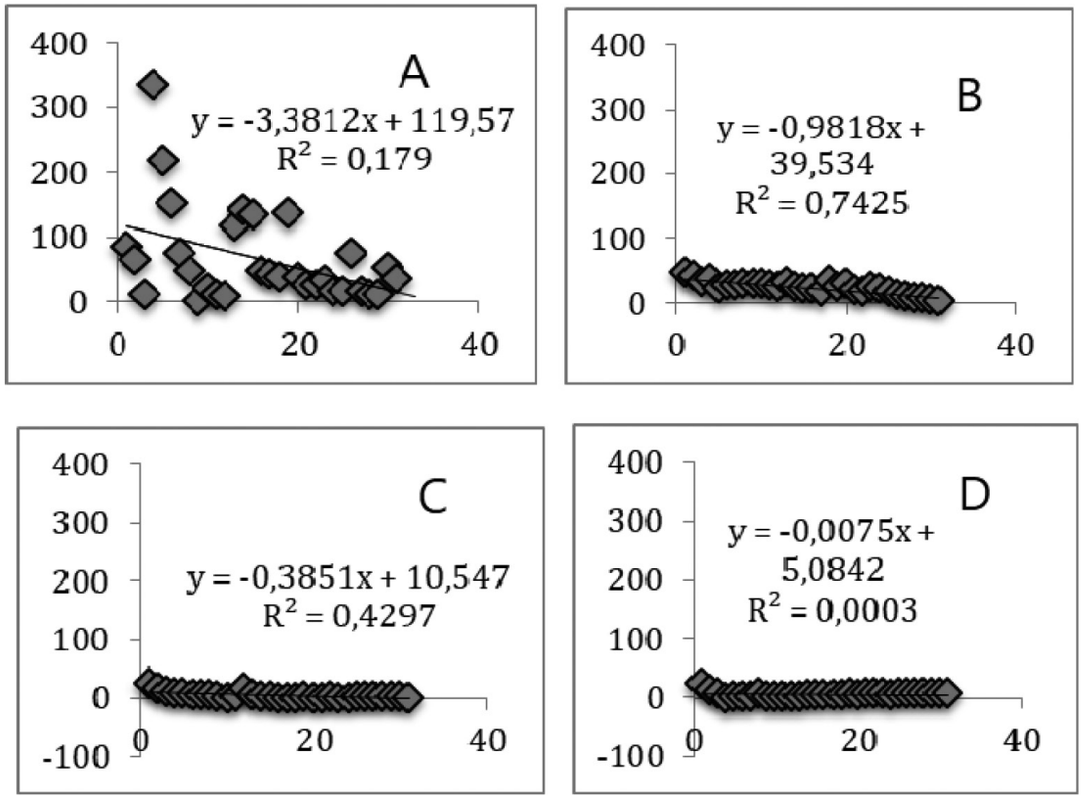


**Figure 2: Production of current density (mA.m$^{-2}$) during the 30-day MFC treatment, for each set of treatment are: A. Aerated single chamber; B. Non-aerated single chamber; C. Aerated dual chamber; D. Non-aerated dual chamber. Vertical axis is for current density (mA.m$^{-2}$) and horizontal axis is for duration of experiment (day-). Line is for trend.**

molecules accumulated around the anode. Electrons then accumulated on the anode while it became more oxic on the cathode. This condition was resulting in a potential difference between the anode and cathode and hence triggered an electric current (Holmes et al., 2004).

Treatment of non-aerated dual chamber reached its lowest value on day 3, –2.33 mV. A negative value for D treatment is thought due to the lack of oxygen in the cathode chamber, which is a non-aerated chamber. Lack of oxygen caused the cathode potential nearly 0, while the potential on the anode was negative, so that the voltage value detected by the multimeter became negative. According to Holmes et al. (2004), MFC with freshwater sediments will experience peak production within five days.

Moreover, the addition of oxygen through aeration also affects the production of electricity. Oxygen plays a role in the completion of the reaction occurring in the chamber, and its cations move through the membrane. Inadequate growth of microorganisms and substrates that do not fit can be one of the cause of low production of electricity in the treatment of dual-chamber MFC (Ozansoy and Heard, 2011).

Sources of sediments that are used as substrate can also be another cause for low electricity production. Sediments used in this study were taken from fresh water. Fresh water has a lower electrical conductivity than sea water, so it does not generate electricity to the fullest. MFC using marine sediments as a substrate will experience peak production within 20 days while the faster result can be reached by the MFC using fresh water sediments, in just five days (Holmes et al., 2004).

Research of Hampannavar et al. (2011) also reported higher current production and power density from single-chamber MFC in the amount of 28.15 $mW.m^{-2}$, while the treatment of dual-chamber MFC produced 17.76 $mW.m^{-2}$ in the alcohol industry wastewater treatments. The higher value of power density was also obtained from single-chamber MFC using acetate as a substrate, scored 880 $mW.m^{-2}$ and 816 $mW.m^{-2}$ for the treatments of dual-chamber MFC.

At the beginning of the treatment, the ammonia concentration of the sediments Cirata amounted to 13,528 $mg.kg^{-1}$, which has actually exceeded the quality standard as per US Environmental Protection Agency (2013) for ammonia content in fresh water sediments.

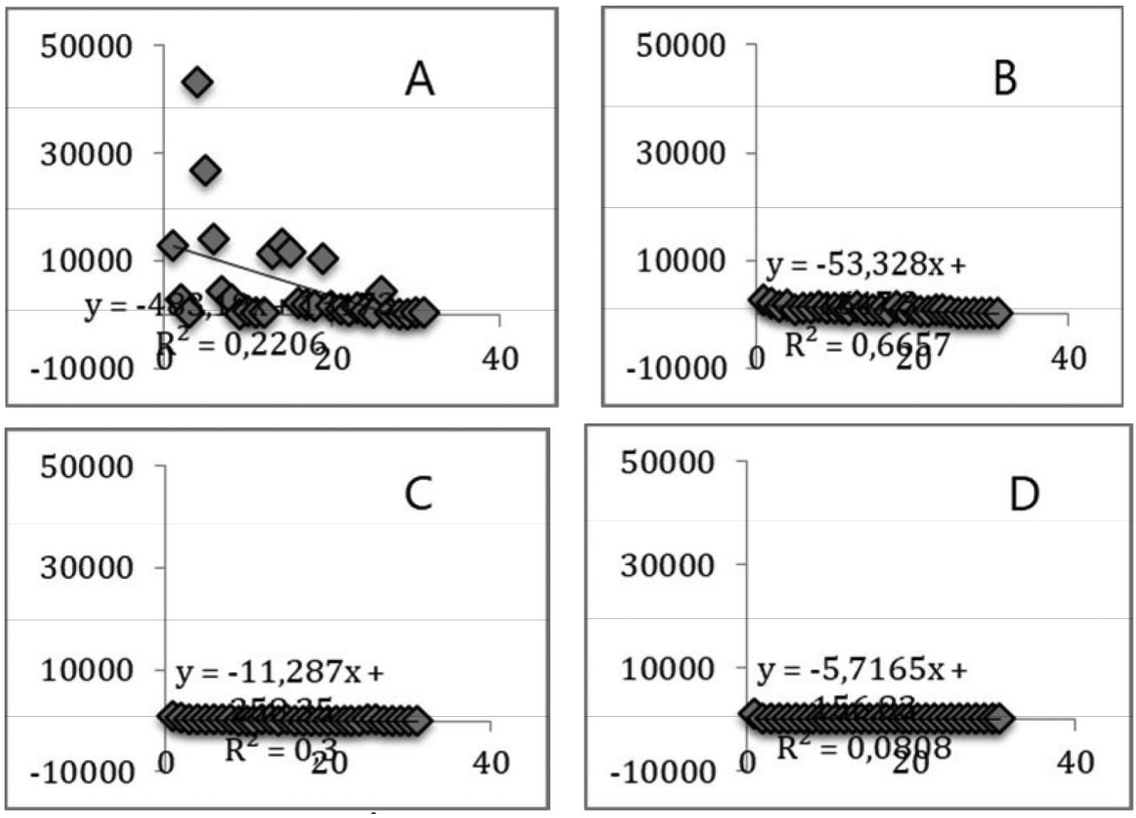


**Figure 3: Production of power density ($mW.m^{-3}$) during the 30-day MFC treatment, for each set of treatment are: A. Aerated single chamber; B. Non-aerated single chamber; C. Aerated dual chamber; D. Non-aerated dual chamber. Vertical axis is for current density ($mA.m^{-2}$) and horizontal axis is for duration (days). Line is for trend.**

The amount should be <75 mg.kg$^{-1}$ for category non-polluted, 75-200 µg/kg for category moderate polluted, and >200 mg.kg$^{-1}$ for category heavily polluted. A very high ammonia concentration showed because the sampling location was surrounded by a number of floating net cage, and there was also waste from floating restaurants. Moreover, the cause for pollution in Cirata was also caused by the entry of pollutants carried by the streams resulting from 15 rivers flooding into Cirata. The pollution in River Citarum has also affected Cirata, which is located in the Watershed of Citarum, and therefore carries domestic sewage, industrial waste and run-off from agricultural waste.

In natural water, organisms can only tolerate maximum 1.5 mg. L$^{-1}$ of ammonia value. The ammonia levels suitable for the life of fish and other aquatic organisms should be less than 1 mg.L$^{-1}$ (Pescod, 1973).

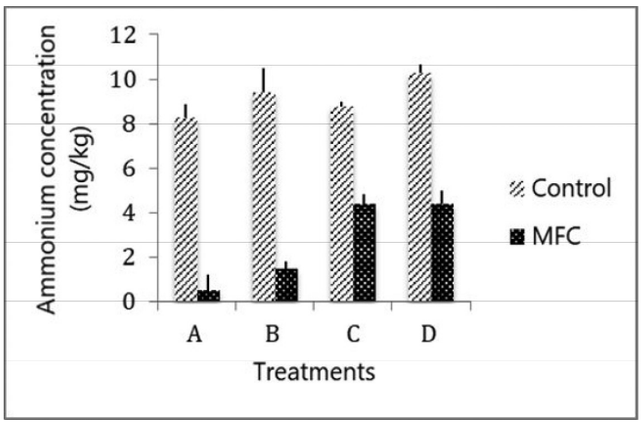


**Figure 4: Final ammonium concentration of the sediments in Cirata reservoir (mg.kg$^{-1}$) during the 30-day MFC treatment, for each set of treatment are: A. Aerated single chamber; B. Non-aerated single chamber; C. Aerated dual chamber; D. Non-aerated dual chamber. Bars are SD.**

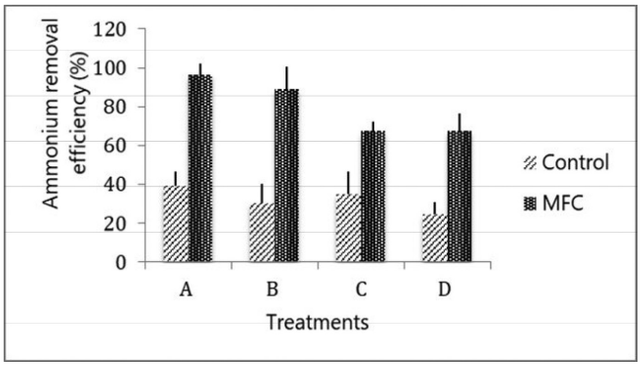


**Figure 5: Ammonium removal efficiency of the sediment from Cirata reservoir (%) after 30 days of the MFC in each experimental treatment: A. Aerated single chamber; B. Non-aerated single chamber; C. Aerated dual chamber; D. Non-aerated dual chamber. Bars are SD.**

As for the purpose of the fishery and animal husbandry, the maximum permissible limit of ammonia is 0.02 mg.L$^{-1}$ (Government Regulation No. 82, 2001).

The concentration of the ammonia decrease in the control treatments A and C was supposedly due to the active oxygen diffusion in both treatments using a single treatment with single and dual aerated chambers, while the treatments B and D are another treatment with single and dual non-aerated chambers each respectively and, therefore, a decline occured, which was supposedly due to the passive diffusion of oxygen that stimulated the aerobic degradation by microorganisms. The same results happened in Jeffrey and Song (2012) as well, where they used a circuit in the control treatment with organic material degradation as much as 2.1%, or about 337 mg.kg$^{-1}$.

All of these results indicate that there is significant aeration used in the study of the degradation of contaminants. Oxygen injection to remediate contaminated land is generally referring to bioventing. However, remediation does not rely on oxygen alone as these bioventing techniques are usually coupled with additional nutrient enrichment. The addition of nutrients and oxygen injection is intended to stimulate the metabolism of aerobic bacteria. Allegedly, tirstimulus controls the treatment with aeration in the water column so that there is diffusion of oxygen in the sediment column.

The end of the ammonia concentration in a single treatment with aeration chamber (A) is equal to 0.523 mg.kg$^{-1}$ or down as much as 96.13%, while the value in dual treatment with aeration chamber (C) is equal to 4403 mg.kg$^{-1}$ or decreasing by 67.45%. A high drop is thought to occur because of the presence of a carbon electrode that serves as an alternative electron acceptor for the microorganisms in the anaerobic process of metabolism in order to stimulate continuous degradation. Zhang et al. (2013) also successfully degraded 90.2% ammonia using MFC with space separation of anode and cathode. In addition to anaerobic respiration, aerobic respiration allegedly occurred in the sediments as well, making it responsible for a large reduction in ammonia. This happens because of the possible diffusion of oxygen from the water column.

Toxic sediment conditions mostly occur in anaerobic metabolism. In cellular respiration, if oxygen is not found, the cells will continue to perform metabolism, namely through anaerobic respiration and fermentation. Anaerobic respiration is generally held on prokaryotic organisms (bacteria) that live in environments without oxygen, or anoxic. These organisms have electron

transport circuits but do not use oxygen as a final electron as captured at the end of a series of electron transport. Oxygen is able to capture electrons very well because it is electronegative, but substances other than oxygen have also the ability to capture electrons although they do not act as good as oxygen. These substances serve as the final electron catcher in anaerobic respiration.

## Conclusion

From this study, it can be concluded that ammonium eutrophication in the sediments of Cirata reservoir can be reduced by applying the process of degradation by indigenous bacteria contained in the sediments and be accelerated by Microbial Fuel Cell method. MFC method with a series of single-chamber type has proved to be accelerating the degradation process, while generating electricity at the same time, that can be used as a source of alternative microenergy. Optimisation of ammonium degrading bacteria by engineering the environment becomes an undeniably important factor for the continuation of this research. Likewise, the stability of the circuit is also maintained in generating electricity.

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