Multidisciplinary Applied Research and Innovation Vol. 4 No. 3 (2023) 151–157

MARI

Homepage: http://penerbit.uthm.edu.my/periodicals/index.php/mari
e-ISSN: 2773-4773

The Study of Plasma Activated Water Treatment for Microorganism in Soil

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DOI: https://doi.org/10.30880/mari.2023.04.03.021
Received 01 March 2023; Accepted 01 May 2023; Available online 30 June 2023

Abstract: To grow and develop, plants need food, just like all other living things. Plants require sixteen essential elements. Carbon, hydrogen, and oxygen are all found in the atmosphere and in soil water. The remaining 13 elements which are nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine are either supplied by soil minerals and soil organic matter or by organic or inorganic fertilizers. Thus, to increase the quality and growth rate of crops, farmers will use fertilizers. However, the usage of excess fertilizers leads to soil pollution and water pollution due to leaching of chemicals. With the usage of Plasma Activated Water (PAW), nutrients can be supplied to crops more organically and produce much less harm. PAW is also a good medium to stimulate the growth of nitrogen fixation bacteria such as *nitrobacter,* which is important in supplying continuous dissolved nitrogen to plants. PAW contain various gaseous reactive species like OH, H_2O_2, NO and HNO as the result of plasma contact with the water surface. This study is being carried out by treating different distilled water samples with different times of plasma treatment to find the optimal time of treatment. The treated water is then poured to microorganisms in soil to check whether it can stimulate the growth of microorganisms that boost plant growth. The significance of this study is to increase the nutrient quality of the soils without using any chemicals such as fertilizer as well to improve the nitrogen fixation process for plants. To summarize, the plasma treated water contains a high amount of N=O bond which also shows the presence of nitrate ion and the plasma treated water also enhances the growth of microorganism in soil.

Keywords: Plasma Activated Water (PAW), Plants, Growth, Nitrogen fixation

1. Introduction

Plant needs sixteen essential elements to continue its growth. Only three elements can be found in the atmosphere and in soil water which are carbon, hydrogen, and oxygen. While the other 13 elements are obtained from mineral soil, organic matter, or fertilizer [1][2]. The usage of fertilizer is important in agriculture for to improve the quality of our crops. Fertilizer will act as a nutrition supplement for

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the crops as there are some elements that cannot be supplied naturally by the soil, water, or mineral soils [3]. However, the excessive and improper usage of fertilizer causes a variety of environmental issues due to the presence of radionuclides and heavy metals in some fertilizers [3].

Towards water, the rate of pollution is very devastative if fertilizer is being used excessively. Excess nitrogen can pollute the water in three ways which are leeching, draining, and directly flowing to the water surface. Therefore, it leads to a phenomenon called eutrophication [4]. When eutrophication occurs, it brings more harmful effects towards our environment like the massive growth of harmful algae that can cause oxygen depletion in the aquatic ecosystem.

Thus, this study proposed Plasma Activated Water (PAW) as a substitute for fertilizer. To obtain PAW, the water will be treated by supplying plasma contact with the surface of distilled water. The treated water then will contain free radicals like nitrite ion [5]. Nitrogen is a crucial element in the help of plants growth [1]. Even for some species of legumes, nitrogen supplementation is crucial to increase seedling biomass during the early development stages [6]. The plasma treatment also supplies nutrients for the growth of important microorganisms in soils that help in nitrogen fixation such as *nitrobacter sp* [2]. With the help of better nitrogen fixation, there will be also better, and more dissolved nitrogen supplied to the plants.

2. Materials and Methods

The materials and methods section, otherwise known as methodology, describes all the necessary information that is required to obtain the results of the study.

2.1 Preparing PAW

In preparing PAW, the distilled water sample was treated using cold atmospheric plasma treatment.

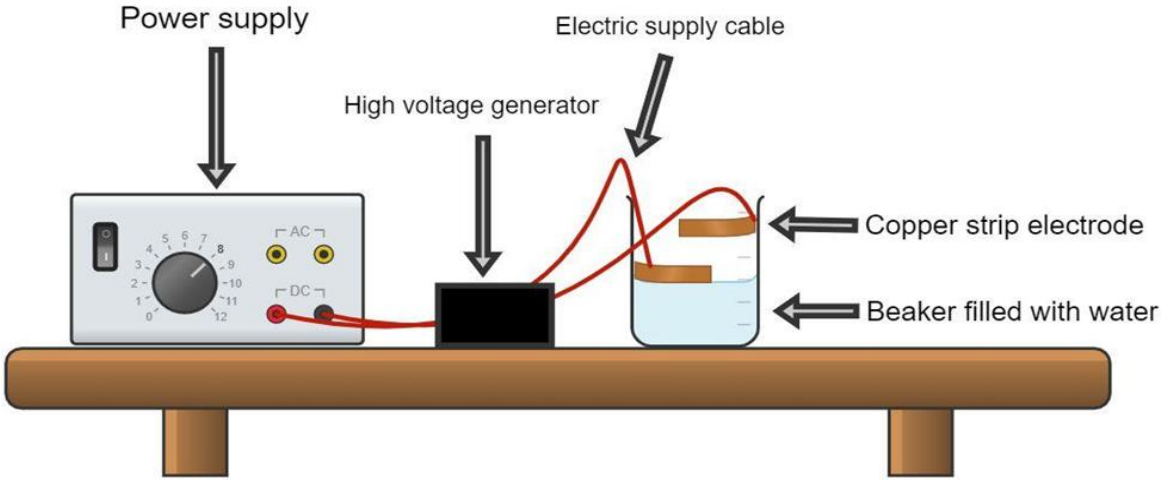

Figure 1: Simplified diagram on the setup of Cold Plasma Atmospheric treatment in producing PAW samples

Figure 1 illustrates the experimental setup for PAW. The treated water samples were prepared into six samples where they had different treatment times or different exposure times with the plasma. The exposure times for the treatment are 10 seconds, 20 seconds, 30 seconds, 40 seconds, 50 seconds, and 60 seconds. Each sample has an interval of 10 seconds compared to the previous sample.

2.2 Analyzing the PAW samples

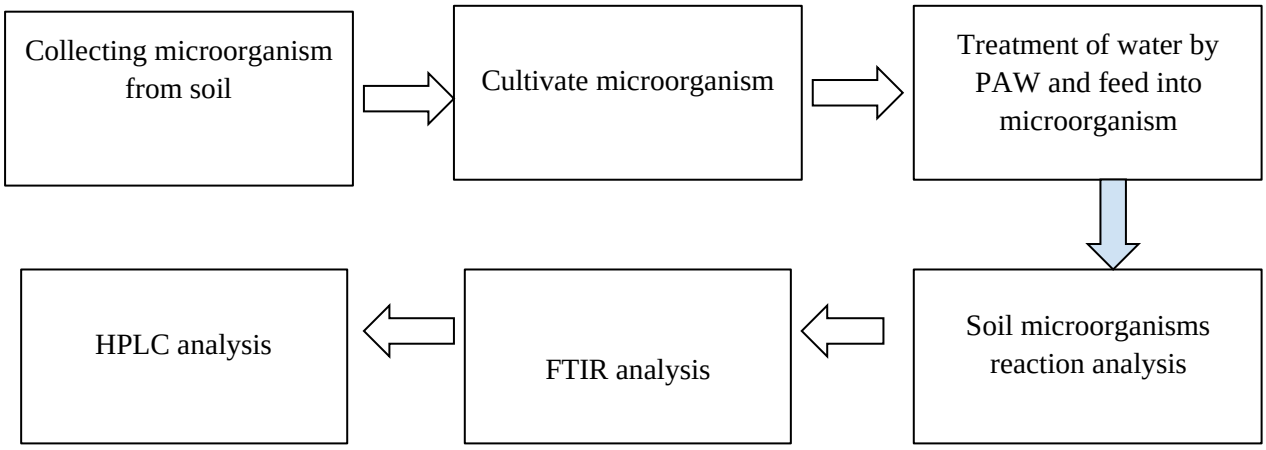

Figure 2: Flow chart of study of PAW treatment on Microorganism

2.2.1 Preparation of Microorganism Sample

To obtain the microorganism sample, a swab of soil samples was taken using swabbing cotton buds in humid soil around UTHM Pagoh. The samples were then stored in a biohazard sample bag.

2.2.2 Preparation of Microorganisms in Soil Samples using Nutrient Agar Cultivation

Nutrient agar cultivation method was used to cultivate the microorganism [9]. The microorganism samples were obtained from humid soils in the UTHM region because this type of soil is frequently utilized for crop plantations. 28 g of nutrient agar powder was mixed with 1 L of distilled water to make nutrient agar. Then, heat the solution until it boils. Pour the solution into a glass bottle and autoclave it at 121˚C for 15 minutes. Allow the nutrient agar to cool down and solidify in a petri dish.

After that, swab the sample taken from the soil at the petri dish under a fume hood in aseptic conditions. To provide an aseptic environment, the entire fume hood was cleaned with alcohol solutions, and ignited the three burners during the whole swabbing process. This is to ensure that the sample is not contaminated. Once the sample was swabbed, we taped the petri dishes using parafilm. Lastly, incubate the sample in an incubator at 37˚C for 24 hours.

2.2.3 Analysis of microorganism sample after being sprayed with PAW

After the sample has been incubated and grown, each sample with PAW water samples was sprayed in ascending order from 10 seconds sample to 60 seconds sample respectively. This analysis aims to observe the physical reaction of the microorganism with the PAW. We will observe the microorganism stains increase in colony size, color, smell, and water vapor produced.

2.2.4 Analysis of PAW sample using FTIR machine

The analysis of PAW using FTIR is used to know what kind of functional group that the PAW has [11]. By knowing the functional group, the chemical bond in PAW samples can be identified. We are also able to know what kind of free radicals are being supplied by cold atmospheric plasma treatment toward the water surface. We are using FTIR machine model spotlight 400 from Pelkin Elmer [12]. As we are aiming to find nitrite ions, we need to find any N=O bond in the PAW samples whose peak is at 1650 cm^{-1}.

2.2.5 Analysis of PAW using HPLC

Our aim for the analysis of PAW using HPLC is to know the concentration of nitrite ions in PAW. Basically, HPLC is not suitable for detection of ions [13]. However, we can detect the functional groups of our sample. Hence, we are extracting data on what kind of bond that our sample has and predict the possible ion it must form the specific bond like how we did for the FTIR analysis. Our method on

analyzing this PAW sample is by using the C18 column. This is because the C18 column is one of the most capable columns that can be used to analyze any type of samples as we are worried if our sample could harm other columns but not for C18 as this column is being used for a wide range of samples [13]. For the retention time, we are using 1ml/min. The wavelength for our sample is from 200 nm to 400 nm as the nitrogen bond range is between this range.

3. Results and Discussion

3.1 Results

3.1.1 Microorganism observation

Observation of the cultural microorganisms was done to analyze the growth of microorganism in the petri dish. This observation was done in three days after PAW was sprayed in the petri dish to observe the rate of microorganism growth. There are 7 samples used to observe microorganism growth, one of it was untreated and acts as a control to differentiate between treated microorganism soil and untreated microorganism soil. Then, the petri dish can be observed clearly to see the difference between microorganisms that are treated with PAW and microorganisms that were untreated. **Figure 3** shows images for effect of different PAW treatment time on the grow of microorganism inside Petri dish. It is clearly seen that after 30 s of plasma treatment, in **Figure 3 (d)**, the microorganism growth inside petri dish had become larger than the initial 10 s treatment time. After the microorganism treat with cold atmospheric plasma for 60 s, in **Figure 3 (g)**, the microorganism growth at excessive rate, which fill almost entire petri dish. These results shown that nitrogen oxide released from breakdown of atmospheric under plasma treatment is dissolved in water and give positive impact toward the growth rate of microorganism.

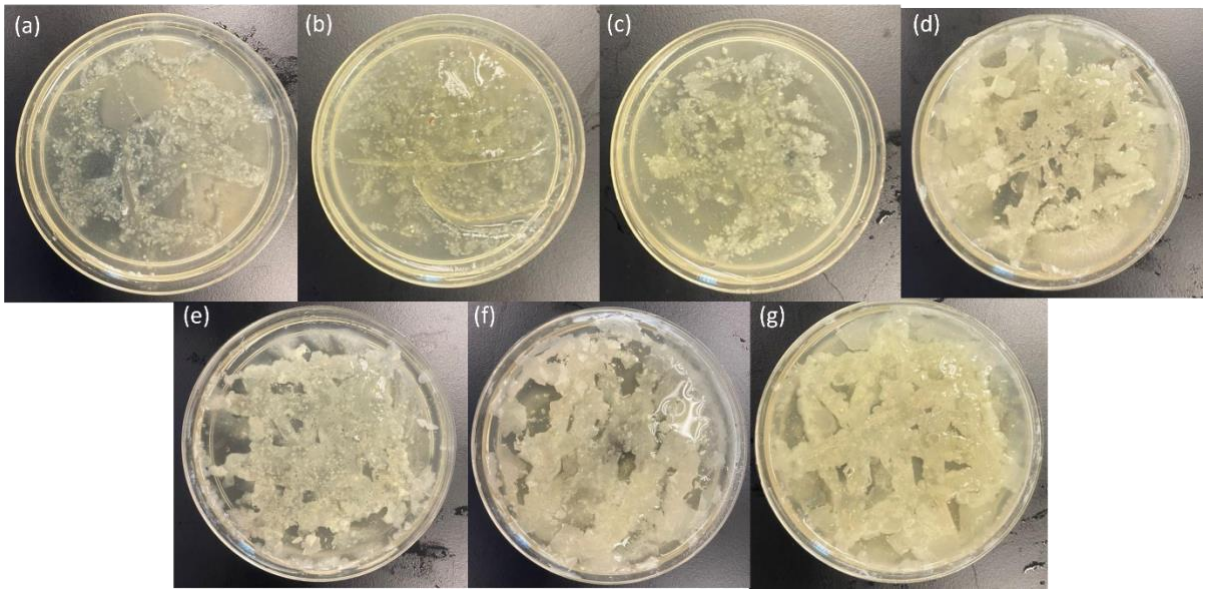

Figure 3: Microorganism soil studies within different PAW treatment (a) controlled sample (b) 10 s (c) 20 s (d) 30 s (e) 40 s (f) 50 s and (g) 60 seconds treated sample

3.2 FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis has been implemented to study existence of functional groups that exist in PAW. Observation of PAW and untreated water were taken to differentiate any functional groups that exist with one and another. There are seven samples taken for FTIR analysis to identify functional group that exists in these samples from the untreated one to 60 s PAW treatment. FTIR result shows the peak at 3500 cm^{-1} refer to absorbance of water while peak at 1636 cm^{-1} correspond to peak of protein, which is from microorganism collected from soil.

3.3 HPLC Analysis

HPLC machine is used to find spectrum in the graph from the sample. It has a broad range of ways to use to find the desired ways to get results.

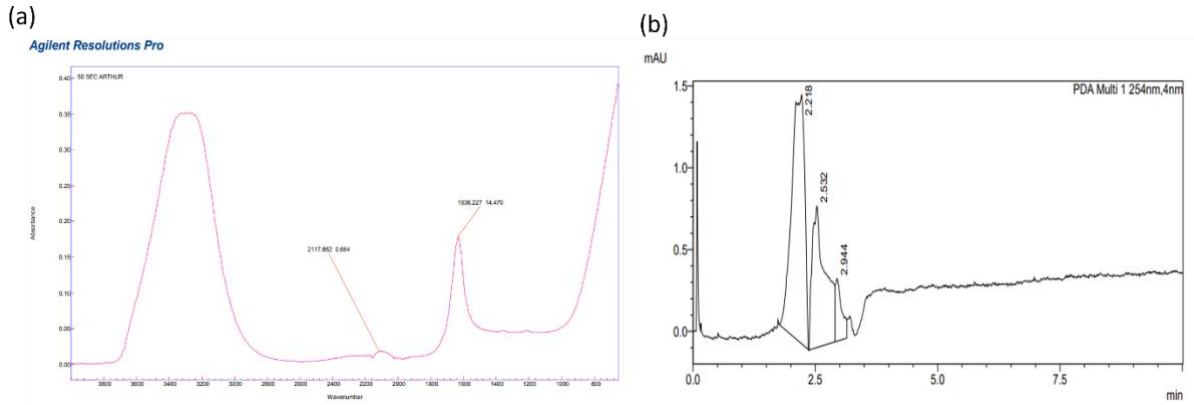

Figure 4: Spectra of (a) FTIR and (b) chromatogram for microorganism soil treatment with 50 s PAW.

3.2 Discussions

3.2.1 Microorganisms observation

After spraying PAW directly into the samples, the samples were put back into the incubator to see it culture and grow. After that, observation was done with all the samples including the untreated sample since that sample will act as control in this study as shown in **Table 1**.

After observation has done, the samples from untreated to treated samples with PAW in 60 seconds were increasing in microorganisms' growth. In the last samples that were treated with PAW in 60 seconds were rapidly growing than other samples with the vapour appearing on top of the petri dish. The vapour itself shows that the microorganism is breathing inside of the petri dish. These samples also release a pungent and unpleasant smell, with the intensity of this smell increasing with duration of treated samples with PAW. While for the untreated sample, it has become inactive after it has sat inside the incubator for 3 days.

Table 1: Observation of microorganism samples after 3 days of incubating

Duration of PAW treatment	Area of microorganism's growth	Smell	Intensity of both variables
Untreated	Inactive	Does not emit smell	None
10	Increase	Unpleasant smell	Low intensity
20	Increase	Unpleasant smell	Higher intensity than 10 seconds
30	Increase	Unpleasant smell	Higher intensity than 20 seconds
40	Increase	Unpleasant smell	Higher intensity than 30 seconds
50	Increase	Unpleasant smell	Higher intensity than 40 seconds
60	Increase	Unpleasant smell	Highest intensity

The longer the duration of PAW treatment on sample, the more microorganism's growth in petri dish. This observation shows that PAW treatment has a significant impact on microorganisms in soil.

3.2.2 Spectrum on FTIR machine

FTIR machine shows a spectrum of functional groups that it can identify. From the spectrum given, the first peak of all samples rises at the same wavenumber. From the graph itself, it shows the presence of nitrogen in the sample on both high peaks. It also shows present of nitrite bond in the graph.

3.2.3 Spectrum on HPLC machine

Chromatogram spectra from HPLC analysis for PAW treatment showing existence of chloride ion, nitrate, and nitrate. After plasma treatment, the result shows that peak for chloride ion Cl^- at retention time 2 min which normally found in tap water start to reduce within plasma treatment and concentration while nitrate NO_3^- at retention time nearly 1.8 min [9] increase by almost 1.5 times higher than the untreated water observed under the PAW treatment for 30 s. This shows that increase of nitrate compound had substantially support growth of living microorganism. In addition, the reduction of chorine also contributes toward healthier growth of microorganism since chorine can be poisonous and kill most of loving microorganism.

4.0 Conclusion

PAW has a lot of potential to give in the agriculture industry since a lot of studies show that it can promote growth in several plants. Since PAW is proven to promote nitrogen fixation to the plant, it can serve as a sub-fertilizer to make the plant grow without any outside nutrients. It helps microorganisms in the soil to grow and culture actively, indirectly helping plants to grow on the soil at the area. This will also lessen the pollution in the drainage system and may not risk humans' life like ever before since the fertilizer will be used in less amount. In future, we must observe the effect of ion formation that contribute toward growth of healthier plant and other living cells.

Acknowledgement

Authors would like to thank Center for Diploma Studies for their utmost support.

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