Plasma Activated Water as a Source of Nitrogen for Algae Growth

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Abstract

Use of algal biomass as a source of renewable energy and food is gaining more interests. Algal biomass finds its application in a wide range of sectors such as renewable biofuel generation, protein substitution in food industry, in cosmetics and in pharma industry. For growing algae, resources such as water, light and other nutrients including nitrogen are necessary. Nitrogen is the major resources needed for algae growth. Conventionally, this source of nitrogen used to support algal growth is from chemical fertilizers which are derived from fossil fuels. This work reports a preliminary study to quantify the algae growth parameters using Plasma activated water, which is an alternative source of nitrogen. In this study mixed algae culture and Bold's Basal Medium was used for algae growth using 10 klx light source over a period of 6 days. Sodium nitrate, sodium nitrite and a mixture of both were used as comparative controls. Biomass yield and chlorophyll content were used as comparing parameters in this work. The chlorophyll yield of the microalgae grown in PAW was comparable to that of sodium nitrite solution indicating that PAW can be used to grow algae without using fossil fuel derived fertilizers. Biomass yield of PAW, Nitrate, Nitrite and combination were 311±58 mg/l, 227±25 mg/l, 434±94 mg/l and 362±138 mg/l respectively. These experiments provide a proof of concept evidence to support the claim that PAW can be used as a good source of nitrogen for algae growth. Copyright © VBRI Press.

Keywords: Plasma activation water, microalgae, nitrite, nitrate, chlorophyll, biomass yield.

Introduction

According to a recent BP energy outlook 2019 report, the global carbon dioxide emission is projected to increase from the current levels of 37 Gtons to around 40 Gtons by 2040 (10%increase) [**1**]. A recent study estimates that almost 1.8% of the global fossil fuel consumption is used for ammonia production [**2**]. Increase in the global food demand is expected to double from its current levels by 2050 [**3**]. To tackle these challenges, shifting towards renewable sources of energy and food is necessary. Production of microalgae using renewable sources is an interesting option to consider.

Microalgae are microscopic in nature, grow in suspended water and are driven by photosynthesis similar to plants. Microalgae are known to fix the carbon dioxide present in the atmosphere in the presence of light and get converted into biomass. Its carbon capture efficiency is almost ten times greater than terrestrial plants [**4**]. Microalgae is rich in useful sugars, proteins and fats, which can all be harvested. These algae can be used as raw materials for biodiesel [**5, 6**] and bioethanol [**6**] production. The protein present in these microalgae can be used as a good substitute for animal feeds [**7**] and can serve as food for humans [**8**]. In addition, algae can also be used in cosmetic and pharmaceuticals [**9**] industry for substituting a varieties of chemicals. These algae can also be used in waste water treatment applications as they can efficiently remove the nutrients present in the waste water [**10**].

These versatile microalgae can be grown in varieties of methods under a wide range of operating conditions. However, efficient growth of microalgae does require proper temperature control, enough nutrients and adequate light for photosynthesis reactions. The major nutrients needed for microalgae growth are nitrogen and phosphorus, which accounts up to 20% of algal biomass [**11**]. Other macronutrients needed are K, Ca, Mg and Na. In addition, some micronutrients such as Co, Fe, Zn Mo, Mn and B are also needed. Kim *et al.* [**12**] have studied the effect of nitrogen sources on the cell growth and biochemical composition of the marine microalgae. In their work, they have studied different source of nitrogen namely potassium nitrate, ammonium nitrate, ammonium bi-carbonate, ammonium chloride, ammonium acetate, urea, glycine and yeast extract. They report that, higher biomass yield was achieved using yeast extract as a source of nitrogen where in the yield was 2.23 g L^{-1}. Using glycine resulted in the biomass yield of 1.62 g L^{-1} whereas, other nitrate sources only resulted in 1.45 g L^{-1} and ammonium yielded 0.98 g L^{-1}. Katsuya *et al.* [**13**] studied the effect of nitrate and nitrite nutrient utilization using microalga *Trentepohliaaurea*. The nitrite consumption rate by the microalgae was 0.28 mg NO_2-N $L^{-1}day^{-1}$ when the starting concentration of nitrate was 51 mg NO_2-N L^{-1} in the culture media. Higher consumption rates of nitrite and nitrate from medium were shown in a 30-day culture, reaching 37% and 32%, respectively, indicating *Trentepohliaaurea* can efficiently utilize the nitrite and nitrate present in the water leading to higher yield. Mahboobeh Taziki *et al.* [**14**] have studied the effect of nitrate, nitrite and its combination on Chlorella vulgaris(algae). In their work [**14**], they have found that nitrate shows a biomass yield of 3.6 g L^{-1} at a concentration of 2400 mg L^{-1}. Using nitrite, the yield was achieved was 3.16 g L^{-1} at a concentration of 400 mg L^{-1}. When both nitrate and nitrite were combined in concentrations of 800 and 150 mg L^{-1}, maximum growth rate and biomass production of 7.8 g L^{-1} was achieved.

In this work, for the first time, an attempt is made to use nitrogen rich plasma activated water (PAW) for algae production. When an electric discharge in oxygen-nitrogen (air plasma) is created above a water column, which inevitably interacts with the nitrogen and oxygen molecule results in the formation of nitrogen oxides, i.e., NO, NO_2, N_2O_3, and N_2O_5. These eventually dissolve into the water column below leading to the formation of PAW [**15, 16**]. The PAW is a mixture of Nitrate& Nitrite, present in higher concentrations of the order of few 10 to 500 mg/L and small fraction of hydrogen peroxide, which is typically present at a few mg/l. The PAW can be generated using different varieties of plasma sources [**17, 18**]. The PAW, which is rich in nitrogen species can be generated in-situ, at the point of consumption and can be generated using renewable sources of power namely solar panels. Dayonna *et al.* [**17**] have generated PAW starting from tap water, using spark discharge plasma and transferred gliding arc plasma. They report that, they have achieved a maximum of 56 mg/l and 12 mg/l of nitrate and nitrite respectively. F. Jud *et al.* [**18**] used DBD plasma for PAW generation using tap water. They report a maximum nitrate and nitrite concentration of 220.1 mg/l and 5.78 mg/l in PAW respectively. L. Sivachandiran *et al.* [**19**] have used DBD plasma source for PAW generation. In their study, the PAW was generated using DM water and they report a maximum of 16.22 mg/l of Nitrate and 0 mg/l of nitrite. Nowadays, Plasma was applied to different applications namely agriculture [**20, 21**], waste water treatment [**22**], gas reforming [**23, 24, 25**], material application [**26, 27, 28**] etc.

In this work, tap water was used to generate the PAW. Photo-bioreactor was used to compare the algae growth using PAW, in comparison with nitrate and nitrite ions supplied using cultural media. The algae growth was quantified by measure of chlorophyll and biomass yield.

Experimental setup and procedure

Plasma activated water generation - direct discharge method

Fig. 1 shows a picture of the PAW generation setup used in this study. The setup consisted of a 5 L glass container with a closing lid, a commercially available off the shelf 10 kV, 20 kHz AC neon power supply and a recirculating pump-spray system. The spray nozzle was fixed onto the lid of the 5 L jar. Metal electrodes were used in this study. One electrode was submerged inside the water column, and the other electrode was placed about 18 mm above the water surface.

To generate PAW, 2 L of tap water to be activated was taken in the glass jar. A plasma arc was created between the free surface of the water and the hanging electrode. To ensure proper mixing of the plasma species generated into the water a recirculation pump-spray mechanism was used, with which the water was drawn from the jar using a pump and sprayed from the lid of the glass jar which ensured that the plasma species formed were well mixed into the water. The tap water was activated for a fixed time interval of 30 minutes. This 30-minute activation was chosen to maintain the pH of the PAW near neutral conditions, as higher activation times above 30 mins resulted in PAW having a pH < 5. The power consumption was measured using a power meter. For all the experiments, the average power consumption was 37 ± 1W. A minimum of three repeats were performed and the average values and standard deviations were incorporated in all the plots.

Plasma activated water characterization

Nitrite concentration in PAW was measured using standard USEPA diazotization method [**29**] using spectrophotometer at 507 nm. Nitrate concentration in PAW was measured using Metrohm-861 advanced compact ion chromatography. pH was measured using Thermo Fisher-8172BNWP Ross Sure-Flow Glass pH probe.

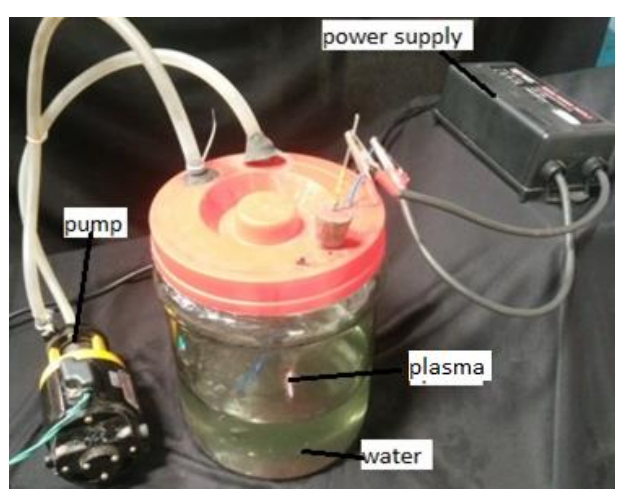

Fig. 1. Direct discharge PAW generator.

Table 1. Nitrogen sources and their composition used for algae growth.

Source of N	Nitrate-N (mg/l)	Nitrite-N (mg/l)
PAW	1.8	17.5
Nitrate solution	59.2	0.00
Nitrite solution	0.00	59.5
Nitrite + Nitrate solution	42.5	16.8
Blank (DM Water)	0.00	0.00

Fig. 2. Bioreactor for Algae growth.

Algae growth

Fig. 2 shows the photo of the bioreactor used for algae growth. Mixed algae culture available from a local lake was used in this study. Bold's basal media was used for nutrition [**13**] and for carbon source bicarbonate was used [**30**]. The media contained 175 mg KH2PO4, 75 mg K2HPO4, 25 mg MgSO4·7H2O, 25 mg NaCl, 50 mg EDTA, 30 mg KOH, 5 mg FeSO4·7H2O and 11 mg H3BO3 dissolved inn 1 litre of deionised water. The nitrogen substitution used in this study is shown in **Table 1**. As can be seen from **Table 1**, nitrate, nitrite, nitrate+ nitrite and blank solutions were studied as controls, whereas PAW was used as the testing parameter.

Microalgae were grown in the 500 ml capacity conical flasks. To start the growth of algae, a few drops of algae culture were added to 500 ml of mixed solution along with 0.5 g of sodium bicarbonate. The LED light with 10,000 lumens was used and the experiment was conducted for 6 days (150 hrs). The chlorophyll present in the solution was measured daily using a standard trichromatic method [**31**]. The final dry algae weight was measured and tabulated as biomass yield.

Results and discussion

Plasma activated water characterization

The nitrate-N and nitrite-N concentration of tap water, used to generate PAW, was 0.27 mg/l and 0 mg/l respectively. The chemical characterization of PAW obtained after 30 minutes of activation is shown in **Table 2**. It can be seen from table 2 that, with activation time, the nitrate and nitrite concentration in PAW increased to 1.8 ± 0.3 mg/l and 17.5 ± 0.9mg/l respectively. This increase in concentration of nitrate and nitrite in PAW is due to the ionization of air. Generating plasma means ionizing the air around the electrodes which results in the formation of reactive nitrogen and oxygen species in air. These reactive oxygen and nitrogen species formed in air further dissolve into water leading formation of nitrate and nitrites in PAW [**16**]. As can be seen from **Table 2**, 30 minutes of plasma activation resulted in very minor change in the pH of the water i.e., the pH change was only from 7.8 ± 0.1 to 7.0 ± 0.03 after 30 minutes of activation. Similarly, as shown in **Table 2**, the oxidation-reduction potential (ORP) increased from 167.7 ± 13 to 218 ± 18 mV, due to activation. The increases in the ORP (mV) is attributed to the formation of nitrates, nitrites and small amount of hydrogen species in water.

Table 2. PAW Composition.

PAW - 30 min	
pH	7±0.03
Nitrate - N (mg/l)	1.8±0.3
Nitrite - N (mg/l)	17.5±0.9
ORP (mV)	218±18

As can be noticed from **Table 2**, when PAW was generated using arc plasma, higher nitrite ions than that of nitrate ions were formed. Similar observations are also reported by Hoeben W. F. L. M. *et al.* [**32**]. Reasons for such behavior is currently being explored.

Algae growth

Biomass yield – Fig. 3 shows the biomass yield for the different solutions studied. As can be seen from **Fig. 3**, the biomass yield of PAW, nitrate, nitrite and combination was 311 ± 58 mg/l, 227 ± 25 mg/l, 434 ± 94 mg/l and 362 ± 138 mg/l respectively. The biomass yield with just DM water was close to 10 ± 5 mg/l. The results show that the biomass yield with PAW is comparable to that of the nitrate and nitrite mixture. These results show that PAW can be used to grow microalgae and the yield using PAW is comparable to the nitrogen substituted media.

Chlorophyll content in microalgae – The chlorophyll is made up of porphyrin which comprises of 4- pyrrole rings, with single iso-cyclic ring attached to one of the pyrrole rings. Each pyrrole ring has 4-carbon atoms and one nitrogen atom. The nitrogen atoms face inward creating a central hole [**33**]. Since, chlorophyll contains nitrogen as a major component, direct measurement of chlorophyll is an indication of nitrogen uptake by algae. So, chlorophyll is an indirect parameter for nitrogen uptake by the algae. Higher nitrogen uptake would also mean higher growth rate of the algae. In summary, measurement of chlorophyll is an indirect measure of nitrogen uptake and higher growth rate of algae. Higher chlorophyll content means higher nitrogen uptake leading to higher algal growth rate.

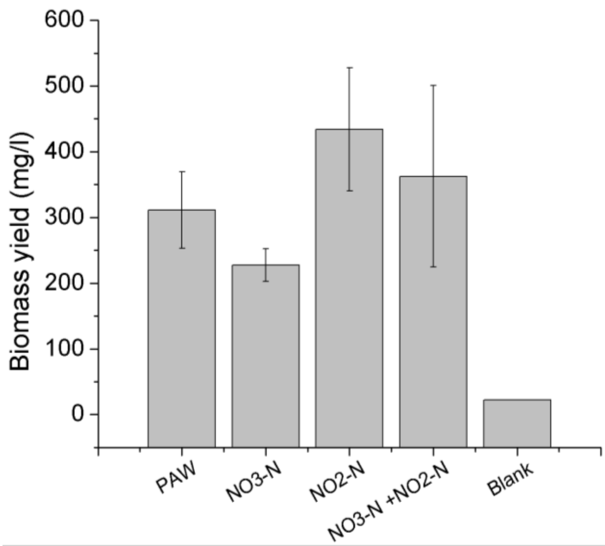

Fig. 3. Biomass yield for different solution.

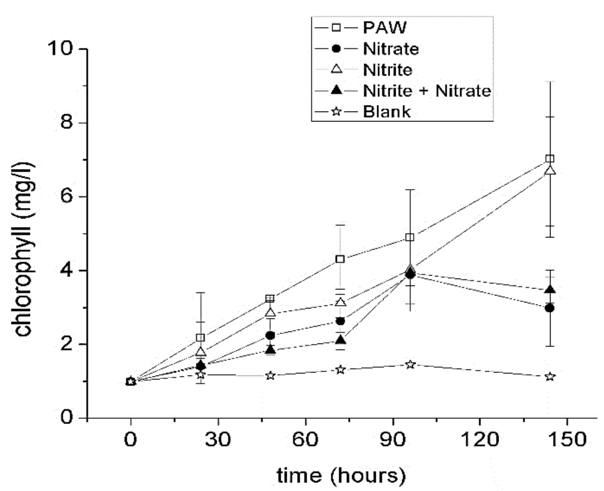

Fig. 4. Chlorophyll variation with time.

Fig. 4 shows the chlorophyll content measured for different solutions studied in this work. As can be seen from **Fig. 4**, the chlorophyll concentration of the blank (DM water) is < 1 mg/l, confirming the fact that there was no source of nitrogen in the DM water. Due to this, the algal growth was very minimal, and the chlorophyll content was also low.

As can be seen from the figure, for both PAW and nitrite substituted solutions, the chlorophyll concentration increased from 0.9 mg/l on day 1 to 7 ± 2 mg/l by day 6.Also, as shown in **Fig. 3**, the biomass yield for PAW was comparable to that of the nitrite + nitrate solutions, despite having much lower nitrate and nitrite concentrations. This result indicates that, PAW which is rich in nitrite, shows similar chlorophyll content as that of the nitrite only solution. Also, the biomass yield of PAW was comparable to that of the nitrogen substituted solutions with overlapping error bars. These results indicate that PAW can be used as an alternative 'green' source of nitrogen for algae production.

Fig. 4 shows the chlorophyll content of nitrate and nitrate+nitrite substituted solutions. As can be seen from **Fig. 4**, the chlorophyll content increased from 0.9 mg/l to 3 ± 1 mg/l in nitrate substituted solutions. A similar trend was observed for combination of nitrate and nitrite solution, wherein the chlorophyll content increased from 0.9 mg/l to 3 ± 0.4 mg/l.

This study shows that, algae can assimilate the nitrogen present in PAW. This preliminary experiment provides a proof of concept that, PAW can be used as a substitute source of nitrogen for algae production and has the potential to replace conventional chemical sources.

There is a tremendous scope to understand the PAW for algae growth. The PAW can be generated by integrating with renewable energy electric grid. The PAW can be a major substitute for nitrogen eliminating the use of fossil fuel-based fertilizers.

Conclusion and future studies

This work reports for the first time, the use of PAW for growing microalgae. This work shows that nitrogen rich PAW can substitute nitrogen fertilizer for growth of microalgae. The PAW is a mixture of nitrate and nitrite with nitrite as a major component. The biomass yield obtained using PAW was comparable to that obtained from the nitrate and nitrite mixed solutions. The chlorophyll content of the micro algae grown using PAW was similar to the nitrite substituted media. Future studies, towards understanding the effect of PAW on algae growth, by quantifying the carbohydrates, lipids and protein content of the algae are being planned. Efforts are also underway to quantify and reduce the specific energy consumption of providing nitrogen fertilizer through PAW route.

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Author's contributions

Punith N. and Lakshminarayana R. wrote the manuscript and designed the experiments. Punith N. and Seema Sukhani performed the experiments and interpreted the results. Lakshminarayana R., and H.N. Chanakya supervised the study, and provided the necessary experimental materials and facilities needed to conduct the experiments.

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