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Application of Water Surface Discharge for the Treatment of Chlamydomonas Algae

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ABSTRACT: This research deals with the treatment of Chlamydomonas by generating of high voltage discharge plasma at water surface. Chlamydomonas is unicellular green algae that grow on water surface of ponds or lakes across Bangladesh and in other countries. These are causing several adverse effects on human and other animal beings. We have applied discharge plasma generated by high voltage for the treatment of Chlamydomonas using a point-to-plane electrode configuration. A high voltage test transformer (Model: HV 9000, TERCO, 50Hz, rated secondary voltage: 140kV) is used as a high voltage supply source for generation of discharge at water surface. High voltage electrical discharge in or above water has been considered as an effective method for the water treatment to kill microorganisms, negating the use of chemicals such as chlorine that leads to harmful by-products which may additionally affect human health. It is found that water surface discharge plasma plays a key role in the removal of harmful algae from water.

KEY WORDS: Algae; Chlamydomonas; Discharge plasma; High Voltage; water surface.

I. INTRODUCTION

The high voltage discharge plasma has been used for many applications, including for treatment of polluted water, exhaust gas, surface modifications, material processing and killing microorganisms (such as viruses, bacteria, algae and yeast) [1-3]. Different constructions of plasma reactors including point-point, point-plane, plane-plane electrodes configurations are used for generation of discharge under atmospheric pressure [2-3]. Discharge plasma systems have a higher removal efficiency for organic pollutants and removal efficiency increased by enhancing the pulsed high voltage supplied into the reactor [3-5]. When a high voltage is applied across electrodes, a strong electric field is concentrated at the sharp tip of positive electrode. With increased field density, filamentary conducting channel is initiated as a form of discharge and propagates between electrode gaps. Many researchers studied corona or streamer, spark or arc for various applications [6-8]. In water surface discharge, a high voltage electrode is placed above the water with the ground electrode placed in water, while both are submerged in water in case of underwater discharge [4]. Previous studies have shown that high voltage discharges generated in or above water initiate a variety of physical and chemical processes such as high electric field, intense ultraviolet radiation, overpressure shock waves, temperature and especially, formation of various reactive chemical species such as radicals ($\text{OH}\cdot$, $\text{H}\cdot$, $\text{O}\cdot$, $\text{HO}_2\cdot$) and molecular species (H_2O_2 , H_2 , O_2 , O_3) [2-4, 6-8]. These physical and chemical processes can be employed to destroy or degrade microorganisms or biological cells and chemical pollutants dissolved in water. If discharges take place in close proximity to the water surface, the strongly oxidizing compounds such as $\text{OH}\cdot$ and $\text{O}\cdot$ radicals and their reaction products O_3 and H_2O_2 are formed in the gas phase or at the gas-liquid interface [3, 7]. These are then dissolved in water and degrade the organic compound or deactivate microorganisms via oxidation processes. Also the attack of strong electric field, ultraviolet rays, heating and shock waves being produced by discharge may cause the destruction of microorganism's body cell [8]. Previous work confirmed that the generation of a UV photon flux of $(2.5 \pm 0.3) \times 10^{15} \text{ cm}^{-2} \text{ s}^{-1}$ with an energy density of $(2 \pm 0.3) \times 10^{-3} \text{ J cm}^{-1} \text{ s}^{-1}$ and the emission spectrum maximum at 220 nm by spark discharge [3]. Numerous organic and inorganic pollutants are present in nearby water bodies from the effluents of textile, fertilizer, drug or chemical industries, agricultural lands and also from city sewage. Many undesired microorganisms especially bacteria, viruses, fungi, protozoa and algae grow up in pond, lake and canal water [9]. Nutrients-rich water leads to a massive boom to the growth of algae that overwhelms huge areas at the water surface and it looks like a blue-green or green powder known as algal bloom. These cause a strong biological toxicity, contamination of water and lead to many hazards to humans, animal beings and the environment.

Water bloomed algae may cause noxious bed smell of water, produces toxin, causing several human and animal diseases, even the death of livestock if they drink this contaminated water. In addition, algal blooms cause oxygen depletion in the water of pond or lake, thus causing death of aquatic animals present in water. Harmful algal blooms are reported as a major environmental problem in 50 states in the USA [10]. The adverse effects of algal blooms were reported (Rockville, Maryland in 2012) producing extremely dangerous toxins that created dead zones in the Need Wood lake water for peoples and pets [11]. Polluted water may cause typhoid, cholera, jaundice, dysentery and amoebiasis. Human illness related to kidney, liver [12], ciliary [13], axonemal [14] are associated with algae [15]. To investigate an effective way for removing these algae is a very important issue now a day.

The main objective of this study is to generate water surface discharge plasma at water surface, using a point-to-plane electrode configuration and to understand its physical and chemical activity in the removal of microorganisms from water. We have collected polluted water sample containing Chlamydomonas algae from nearby pond. Because Chlamydomonas is a biological model organism and it is very common and widely distributed in soil and fresh water of Bangladesh. In addition, the conductivity and pH of water are measured before and after discharge generation to confirm the generation of chemical species during discharge in water.

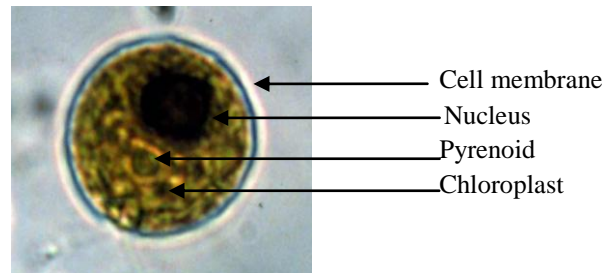


Fig. 1: A typical microscopic image of Chlamydomonas.

Chlamydomonas is a single-cell green algae about 10 micrometers in diameter that floats with two flagella. It contains a cell wall, a nucleus, a large cup-shaped chloroplast, a large pyrenoid and an "eyespot" that senses light. A schematic cell structure and a typical microscopic image of a Chlamydomonas is shown in Fig.1.

II. EXPERIMENTAL SET UP

A block diagram of experimental setup is shown in Fig. 2. A high voltage test transformer (Model No HV 9000, TERCO, 50Hz, rated secondary voltage: 140kV) consists of three windings with insulating shell and corona free aluminium shielded electrodes are used as a high voltage supply source. A control desk is used to control and operate supply voltage across positive and negative electrodes of discharge reactor. The control desk contains all control elements for operation of high voltage test equipments and measuring instruments. The control desk contains a motor operated regulating unit, consisting of ring-core regulating transformer and an isolating transformer.

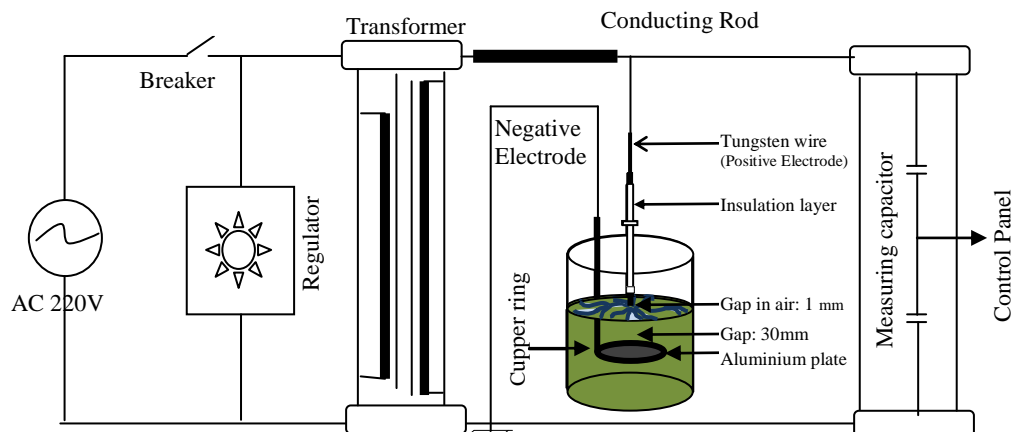


Fig. 2: A block diagram of experimental setup for generation of high voltage discharge at water surface.

The point-to-plane electrode configuration consists of a tungsten wire of approximately 0.4 mm diameter with a sharp tip used as positive electrode, and an aluminium plate of 20 mm diameter and 0.2 mm thickness as a ground placed in a cylindrical glass reactor. Tungsten wire is placed in ambient air at 1 mm distance from the water surface, while the ground plate is immersed in water at 30 mm distance below the water surface. A capacitor of 1000 pF is charged with the conducting rod and energy charged by the capacitor is released to the electrodes. Voltage and current changes during discharge were recorded by voltmeter and ammeter in control panel.

The water sample containing *Chlamydomonas* algae was collected from the nearby pond (Vurolia, Gazipur, Bangladesh). The conductivity of water was adjusted to $150\mu\text{S}/\text{cm}$ by adding pure distilled water to reduce the conduction current. The conductivity and pH of solution sample were measured by a conductivity meter (Lutron, PCD-431) and a digital pH meter (Lutron, PH-208). The *Chlamydomonas* cells were observed using a microscope (model no U-TVO, 5×C-3, Olympus, Japan) before and after application of discharge.

III. RESULTS AND DISCUSSIONS

For generation of high voltage discharge, we placed positive electrode in ambient air and a plane ground electrode being submerged in water. When we increase positive high voltage, the air medium around the positive electrode gets ionized and causes breakdown of the medium between the electrodes. The discharge is initiated from sharp needle tip in air, then starts to propagate directly through the air gap to water surface and finally radially spreads over the water surface. At first discharge appears as streamer-like with small branching and then converted to spark discharge reaching expanded streamer channel to the ground electrode. The value of secondary voltage and conduction current was recorded as 2.32kV and 9.7A during propagation of discharge. When streamer branch reached to the ground, it is converted to spark-like discharge. At that time conduction current is increased to 14.1 A and voltage is reduced to 0.89 kV; also the light intensity became brighter than that at streamer. It is expected that physical processes such as high electric field, UV light emission, heating and chemical processes such as formation of various ions and radicals take place during the propagation of discharges. A schematic of fundamental discharge is shown in Fig. 3 (a and b) to understand the physical and chemical processes happened by the discharge propagation from air to water surface.

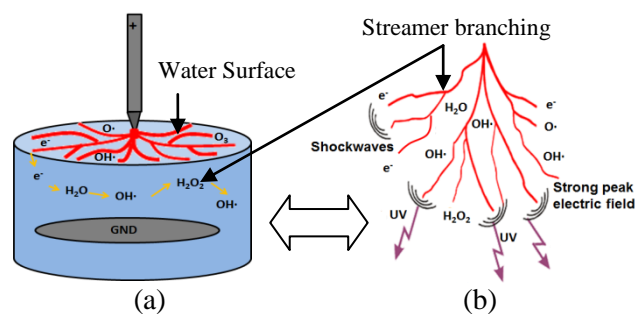


Fig. 3: (a) A Schematic of fundamental discharge at water surface and (b) Physical and chemical processes of discharge.

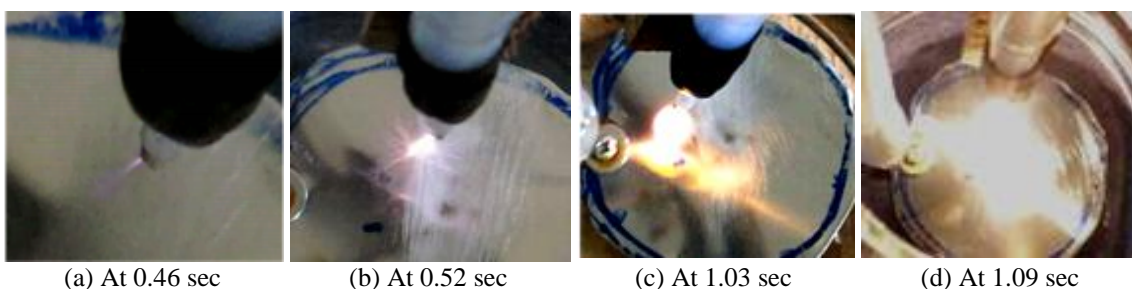
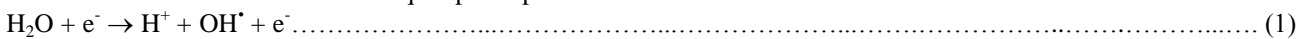


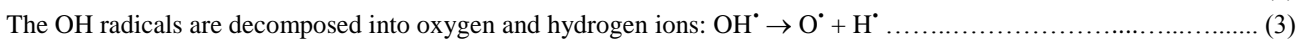
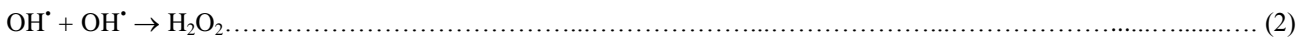
Fig. 4: A typical discharge initiation, propagation and development from positive electrode needle tip to water surface generated by high voltage ac supply: at (a) 0.46, (b) 0.52, (c) 1.03 and (d) 1.09 second.

A typical discharge, initiation, propagation and development from atmospheric air to water surface is shown in Fig. 4 (a, b, c and d). The reactive radicals or charges can be generated in air, and then deposited to water surface which can react with water molecules to initiate chemical reactions in air-water interface [16]. At that time the chemical species are generated in water due to the direct interaction of discharge plasma and water molecules at water surface [17]. Some possible reactions that occur are given below [18]:

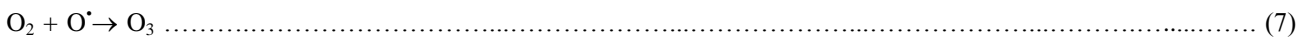
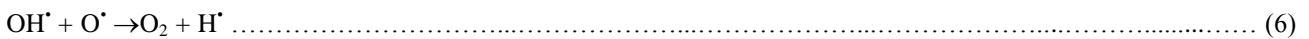
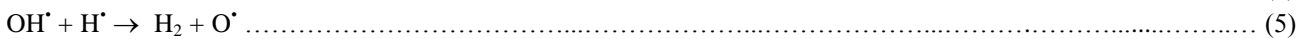
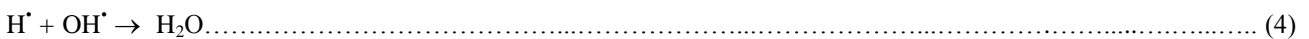
Electron collisions also occur from liquid phase plasma with water molecules:



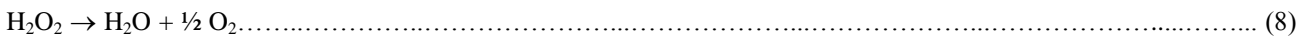
Hydrogen peroxide can be formed from the recombination of hydroxyl radicals:



Other recombination reactions are:



Again, hydrogen peroxide decomposition leads to the formation of molecular oxygen by:



The radicals and molecular species can be generated in air, air-water interface and water by contact of discharge plasma. It is suggested that for plasma propagating over a water surface, OH is formed in the air or gas phase, after which it diffuses into liquid to form hydrogen peroxide [16-19].

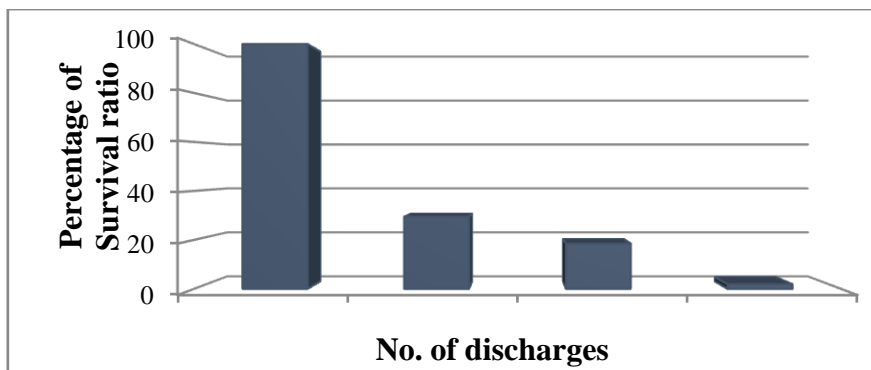


Fig. 5: Percentage of survival ratio of Chlamydomonas with no of discharges generated at water surface.

Figure 5 shows the experimental results of chlamydomonas survival ratio with the number of discharges at water surface. It is observed that the percentage of survival ratio decreased significantly with increasing number of discharges. Initially alive chlamydomonas decreased at a higher rate from 100% to 29.45% with 3 times discharge and then it gradually decreased to 18.40% and 1.84% with 6 and 9 times discharge. In all cases, discharge was initiated as a very thin streamer at water surface and then converted to spark like discharge with increasing streamer length that touches the ground electrode. Survival ratio is calculated using the following equation:

$$\text{Percentage of survival ratio} = \frac{\text{No. of Algae after discharge}}{\text{No. of Algae before discharge}} \times 100$$

The reason behind the reduction of percentage of survival ratio is that Chlamydomonas cell was destroyed by the attack of discharge at water surface, because streamer head contains highly energetic electron.

The destruction of Chlamydomonas can also be occurred by UV light from discharge, damage of chloroplast or chemical reaction with cell membrane by OH[•] radicals or other active species generated by discharge. The chemical species formation was confirmed by measurement of pH and conductivity change of treated solution after discharge. The analysis of microscopic image of Chlamydomonas cell confirmed its destruction by discharge at water surface.

Specially, it is expected that O_3 or O^- radicals are to be generated in air and OH^- or H_2O_2 are to be generated in water [19]. Therefore, after high voltage discharge conductivity of water increases and pH decreases, which indicates the chemical change of water due to interaction of discharge with water molecules; i.e. OH^- radical's increased or H^+ ion decreased [20]. The change of pH and conductivity of water sample before and after discharge are given in table 1.

TABLE 1. A Typical Change of pH and Conductivity of sample solution before and after generation of water surface discharge.

Condition	Time (s)	pH	Conductivity ($\mu S/cm$)
Before Discharge	0	7.16	150
After 1st time Discharge	20	6.85	176
After 2nd time Discharge	40	6.25	390

It confirms that chemical species were produced in water due to propagation of discharge at water surface. The destruction of Chlamydomonas was confirmed from the analysis of microscopic images as shown in Figure 6. Typical microscopic images of Chlamydomonas contained solution before discharge and after discharge is shown in Figure 7. It is seen that with increasing the no. of discharge, the number of Chlamydomonas decreased significantly.

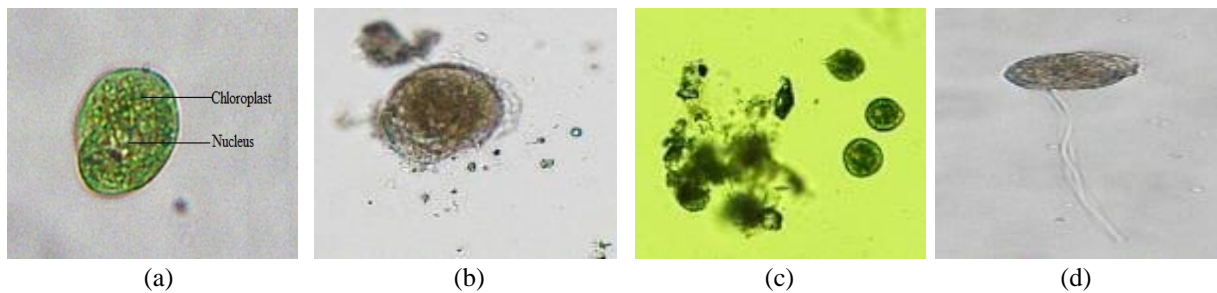


Fig. 6: The destruction of Chlamydomonas cell by attack of water surface discharge; (a) Live cell before applying discharge; after applying discharge (b) death cell with damaged chloroplast, (c) cell destruction and (d) leak out of body protein through damaged cell membrane.

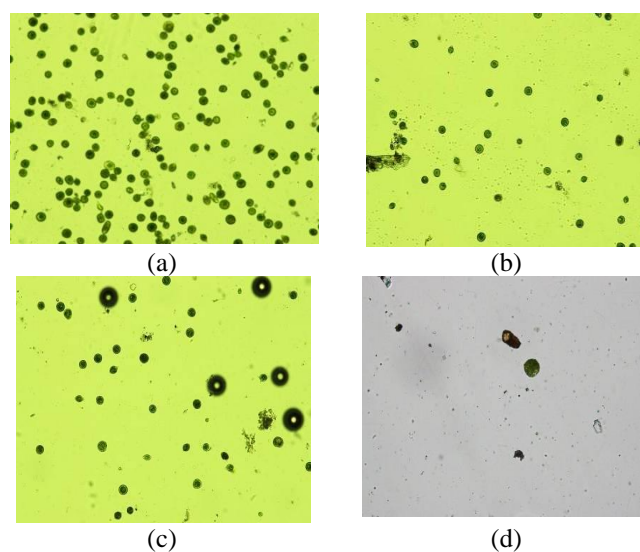


Fig. 7: Chlamydomonas contained solution (a) Before discharge, (b) after 3 times discharge, (c) after 6 times discharge and (d) after 9 times discharge.

Chlamydomonas cell contains chloroplast and nucleus inside its cell membrane. Fig. 6 (b and c) confirm the damage of chloroplast and destruction of body cell by the attack of discharge. Figure 6 (d) body protein comes out through damaged cell membrane. All of the above factors are the reasons for the death of Chlamydomonas. Previous study reported that various active and reactive chemical species such as O, H, OH radicals and O₃, H₂O₂ molecular species are very reactive towards microorganism body cell [21-24].

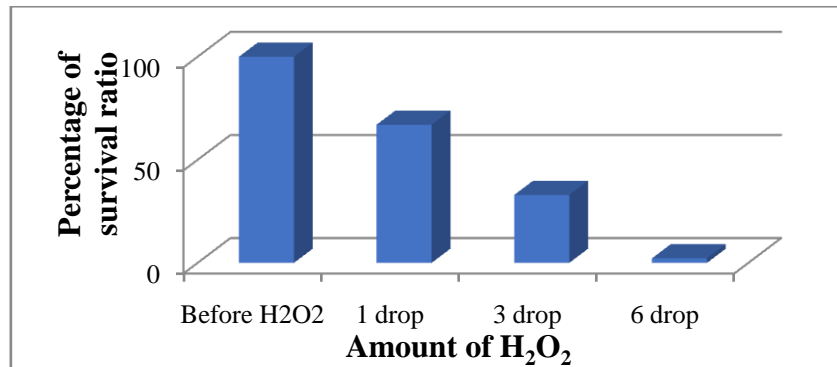


Figure 8: Graphical representation of Survival ratio of Chlamydomonas before and after addition of H₂O₂.

Survival ratio of chlamydomonas before and after addition of H₂O₂ is shown in Figure 8. To confirm the chemical attack of discharge on chlamydomonas cell, we have added a little amount of H₂O₂ solution in 200ml of chlamydomonas containing water sample. After that collected sample was tested by microscope after one minute of H₂O₂ addition. It is observed that the percentages of survival ratio were reduced sharply to 67.03%, 32.97% and 2.20% with the addition of 1 drop, 3 drop and 6 drops of H₂O₂ respectively. The probable reason is that H₂O₂ or its relative species chemically react with Chlamydomonas causing damage to chloroplast or destruction of DNA. It can also directly attack to cell membrane and leak out body protein leading to the reduction in survival ratio. Microscopic images of Chlamydomonas contained solution before and after addition of H₂O₂ are shown in Figure 9.

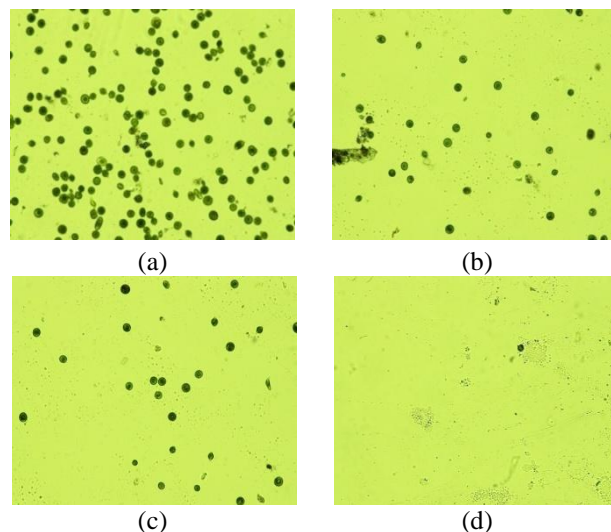


Fig. 9: Typical microscopic images of Chlamydomonas contained solution (a) before, after addition of (b) 1 drop, (c) 3 drop and (d) 6 drop of H₂O₂.

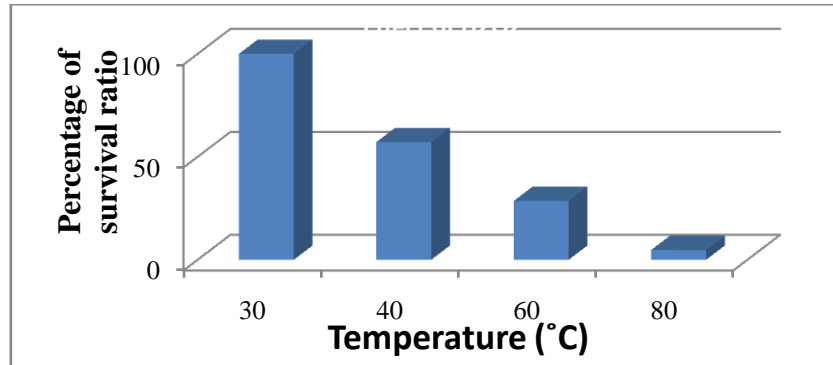


Figure 10: Effects of Temperature on Chlamydomonas.

A high temperature was produced around the discharge channel during its propagation across the water surface. The sudden increase of temperature from discharge can cause the death of chlamydomonas in the reactor. To confirm effect of temperature from discharge, we have treated Chlamydomonas containing solution through rise of temperature on the same sample solution. Ambient temperature was 30°C. Figure 10 shows that the percentage of survival ratio of Chlamydomonas at different temperature. It is found that survival ratio of Chlamydomonas dropped sharply with increasing temperature in reactor. The survival ratio falls down to 57.14%, 28.57%, 4.76% and almost 0.00% with the solution temperature of 40°C, 60°C, 80°C and 100°C respectively. It can be said that temperature rise due to discharge is also an important factor for inactivation of chlamydomonas. Microscopic images of Chlamydomonas containing solution at different temperature are shown in Figure 11.

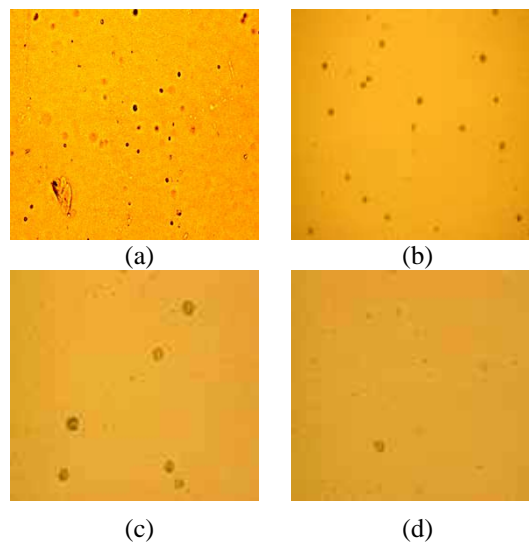


Fig. 11: Typical microscopic images of Chlamydomonas contained solution at: (a) ambient temperature 30°C (b) 40°C, (c) 60°C and (d) 80°C.

VI. CONCLUSION

The discharge plasma was generated successfully at water surface using three phase high voltage test transformer. At first discharge was initiated at the positive electrode tip with a thin light intensity. After that, it developed as a streamer branching spreading all over the water surface and then converted to spark touching the ground electrode.

Chlamydomonas algae were successfully inactivated by applying high voltage discharge at water surface. Experimental results from addition of H₂O₂ and rise of temperature on Chlamydomonas contained solution confirmed that both physical and chemical phenomena of discharge helped to damage Chlamydomonas. Finally, it can be concluded that high voltage discharge plasma at water surface can be an effective method for inactivation of algae contaminated water.



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