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Influence of Herbicide 2,4-D Dimethylamine 865SL on Photosynthetic Mechanism of Algae Species *Chlorella Kessleri* immobilized in a Biochip

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Abstract. Algae are very important to the life of human as a bio-indicator of water pollution. The observation of algae vitality can give information of the environment changes of the algae, which are shown by the changes of parameter in its environment. This study is aimed to observe the influence of toxicity of herbicide 2,4-D dimethylamine 865 SL on the vitality of algae species *Chlorella Kessleri* in producing dissolved oxygen as a result of photosynthetic mechanism. A 50μL herbicide 2,4-D dimethylamine 865 SL with a concentration of 10 % was added into immobilized algae in the Biochip-C from cellasys GmbH and stimulated with artificial light of 400-700 nm for photosynthetic process. The effect of herbicide 2,4-D dimethylamine 865 SL was observed and shows the increase of the basal potential after 1500 s, which indicates the dissolved oxygen reduction in the environment of the algae. This effect is reversible and a restoration of the photosynthetic activity take place after the substance removal. The use of this toxin is systemic, which slowly kill the living cells.

INTRODUCTION

Pollution caused by agricultural activities is one of the environmental problems due to the excessive use of chemicals without seeing long-term effects. Numerous plantation managers prefer to use chemical techniques, for the sake of time and energy efficiency. Continuous herbicide pollution not only causes harm to the agricultural environment but also can endanger the life in the aquatic environment. In the soil environment, herbicides can contaminate the soil which then enters the ground water and eventually pollutes rivers around the land. In agriculture, the application of herbicide is frequently used to meet the growing farming yields; it compromises the value of aquatic and soil performance. The pesticide 2,4-D dimethylamine is of made synthetic herbicides and categorized as most widely used herbicide. Non-target organisms are exposed to the pesticide via several ways, which could produce toxic effects depending on the dose, frequency of exposure, and the host factors that influence susceptibility and sensitivity [1].

The use of chemicals in modern civilization has contaminated the water sources with numerous substances which have the potential to affect microalgae at the base of the food chain. The observation of algae vitality could give information for environment changes of the algae, which are shown by the changes of parameter in its environment [2]. Due to their specific effect on photosynthesis, herbicides pose a potential threat to estuarine microalgae. However, comprehensive understanding of the risk of these contaminants is currently inadequate.

Algae are very important as a bio-indicator of water pollution. Pollutants cause alteration of the photosynthetic capacity due to then effect of herbicides on inhibition of photosynthesis and algal growth rate which is detected using

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water samples and chemicals mixed in the definite concentration [3, 4]. Boij et al. [5] have investigated the toxic effects of four ubiquitous herbicides (atrazine, diuron, Irgarol®1051 and isoproturon) and herbicide mixtures on marine microalgae.

The risk of groundwater contamination by herbicide 24-D is presented in [6] using adsorption, desorption, degradation and displacement mechanism. Some techniques have been developed to identify the compound's effect on the algae using 96-well plates while long-term effects of herbicide diuron on autotrophic activity of biofilms reflected by a marked decrease in the photosynthetic efficiency was shown in [7]. In vitro algal growth inhibitory effects were also quantified in specific mixtures using the green alga species Desmodesmus, Subspicatus and *Chlorella* Pyrenoidosa as target organism which show an equally sensitive effect to herbicides including atrazine, simetryn, bromacil and hexazinone and algae metabolism [8,9]. An algae species *Chlorella kessleri* purchased from SAG Gottingen Germany was used in this work as a bioreceptor for dissolved oxygen (DO) level detection, which has high sensitivity to pollutants [10, 11]. As the algae is exposed to an artificial daylight source with photosynthesis active radiation wavelength at 450 nm up to 750 nm, the algae will produce DO from the photosynthesis process and it can be used as a biosensor for environment pollution [2, 12].

This paper presents a method that can be used to provide some information about the interactions between immobilized algae cells and active substances (herbicide) in the biochip chamber. The main advantage of this system is the possibility to utilize a marker-free object for long-term measurement, without any external influences on the cells, and providing the observation of long-term effects after chronic exposure to toxins [13]. Effects of the herbicide 2,4-D dimethylamine toxicity on the vitality of green algae species *Chlorella Kessleri* were measured which indicates the metabolism process of the algae to produce DO as a result of the photosynthetic mechanism. Chlorella Algae has a very high level of sensitivity to the presence of pollutants or contaminants around the environment so that it can become a bio indicator of water pollution in the ecosystem. The visible impact can be a decrease in the level of DO produced by the algae. These changes occur due to the transition of photosynthetic algae metabolism into an aerobic respiration, so that the DO is not produced but consumed to break down carbons [14]. A volume of 50 µL Herbicide 2,4-D dimethylamine with the concentration of 0%, 10%, 50%, 75% and 100% were added into immobilized *Chlorella Kessleri* in the Biochip-C (cellasys GmbH, Kronburg, Germany) and stimulated with fluorescent lamp with a wavelength range of 400-700 nm for its photosynthetic process. The change of the oxygen level produced from photosynthetic mechanism is then observed.

MATERIAL AND METHODS

Strains and Culture Conditions

The immobilized alga in biosensor devices is used as bioreceptor and is observed using an integrated Biochip-C with a micro-sized measurement electrode [15]. Biochip electrodes are capable of having multi-parameter measurements which one of its functions is detecting DO changes in the form of electric potential, supported by analog signal processing circuits to change the size of measurement parameters into readings in an electricity parameters [16].

An algae species *Chlorella Kessleri* purchased from SAG Goetingen with the algae strain number of 211-11h was used as test organisms and biological components in this work with a diameter ranging from 2 µm to 9 µm [17]. *Chlorella Kessleri* grows in 16-hour intervals up to 20 hours in optimal environmental conditions and can be cultured at the laboratory scale. The use of the *Chlorella* algae must go through a cultivation process where algae seedlings are bred using the algal culture broth in accordance with such a cultivation process [14]. The Alga Culture Broth (ACB) from Sigma Aldrich SKU-17124 was used as nutrient for alga growth, and the cultivation was prepared by mixing a 1.86gr ACB powder for a liter of distilled water before autoclave process for sterilizing the medium. The final pH of the ACB used is 7.

The *Chlorella Kessleri* was cultivated phototrophically under continuous white light of T521W Fluorescent Lamp (72.63 µmol photons m^{-2} s^{-1}) with bubbling air using a mini generator. The cultivation process of *Chlorella Kessleri* was carried out for 15 days. Furthermore, the increase in cell density was counted using a *haemocytometer* (Neubauer Improved) and microscope. Specific growth rates μ were calculated for the exponential phase using the following equation, where N_2 represents the cell number per ml at t_2 and N_1 the respective number at t_1:

$$\mu = \frac{\ln(N_2/N_1)}{t_2 - t_1} \tag{1}$$

Dissolved Oxygen Sensor Using Biochip-C

The detection device used is the micro-sized Bio Chip-C from cellasys GmbH which is capable to measure four different parameters; dissolved oxygen, temperature, pH and impedance simultaneously. In this work, only the oxygen sensor is used to detect the presence of herbicide as a pollutant in the biochip using alga photosynthesis mechanism.

A light-dark experiments are made to check the mechanism of photosynthetic algae cells using the Biochip-C and the biosensor module while the DO changes in the chip chamber environment is observed. A 150µL ACB medium is added into the Bio Chip-C chamber until the biosensor basal voltage reached a stationary condition [15]. A colony of 10^7 cell/mL green algae were immobilized into the biochip and illuminated using artificial light from a T521W fluorescent lamp at a wavelength of 400-700nm, which results in photosynthetically active radiation intensity of 72.63 $\mu mols^{-1}m^{-2}$. The algae photosynthesis mechanism is detected through the light-dark cycle in the biochip reaction room controlled using a computer by a period of 15 minutes "ON" and 15 minutes "OFF". This experiment will cause the algae to trigger the photosynthesis process which will produce dissolved oxygen. Figure 1 shows the experiment set up using the Bio Chip-C and immobilized algae.

Herbicides Preparation

The pollutant sample used is one of the herbicides for green vegetation with the toxic substance 2,4-D dimethyl lamina which works with systemic growth and is easily absorbed by plants and also can be used as a plant growth regulator. The herbicide is a soluble solution with a value of 865SL, which means that the solution is easily soluble in water and has a clear color solution with 2,4-D dimethylalamine active ingredient of 720g/L solution so that the mixing process can be done using distilled water and an incubator shaker.

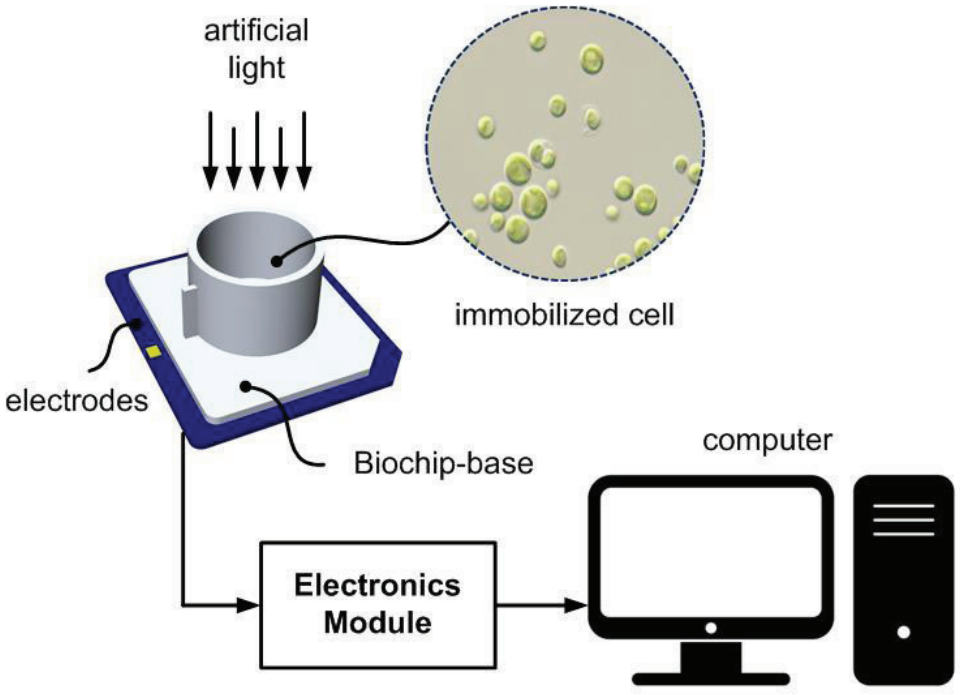

FIGURE 1. Experiment set up with immobilized green algae influenced by herbicide

The pesticide sample was prepared using a mixture of the herbicide dilution with 100mL of distilled water to make herbicides with concentrations of 10%, 50%, 75% and 100% while the sample with no active toxic substance or 0% was fixed as control. A 50µL 2,4-D dimethylamine with different concentration was then added inside the biochip chamber filled with immobilized alga and illuminated using artificial light to stimulate the *Chlorella Kessleri.*

RESULTS

The dynamic model of the algae curve is divided into light-dependent conditions (LD) which state the stages of photosynthetic production (photosynthetic phase) and dark conditions. As a photon unit arrives at PSF, a resting state is activated which triggers the production of dissolved oxygen to be detected by the biochip. Figure 2 shows the results of measurements for 15 minutes of artificial lighting.

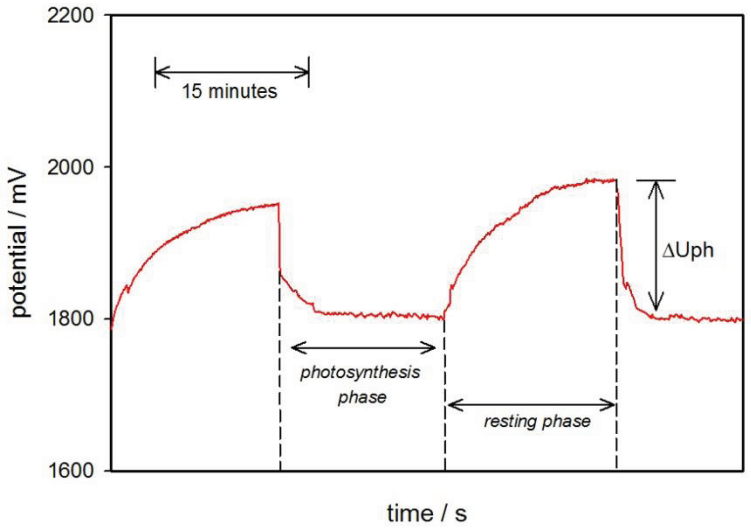

FIGURE 2. The algae photosynthesis mechanism shows the transition status to activated state

The basal potential detected by the pO2 sensor has a value of 1984 mV as the artificial lighting in OFF status (dark stage) and there is no photosynthesis activity. As the lamp is ON, the algae obtains photon energy to produce more oxygen due to photosynthetic mechanisms resulted in a change of sensor's basal potential to a ΔUph of 184 mV. This results show that immobilized algae in biosensors reacts with stimulated artificial light to produce oxygen.

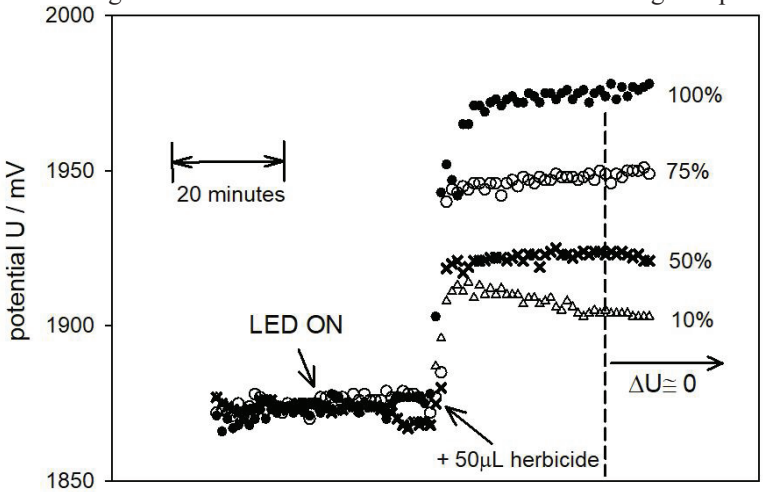

FIGURE 3. Influence of 2,4-D dimethylamine on the photosynthetic phase

Figure 3 depicts the influence of herbicide 2,4-D dimethylamine 865 SL on the photosynthetic activity of the algae which shows significantly the decreasing of the oxygen level represented by the detected basal potential for each concentration of 10%, 50%, 75% and 100%. However, the data was analyzed to prove the difference, as shown in Table 1.

TABLE 1. Group of measured biosensor potential at different concentration of herbicide

Group Concentration	N	Mean	Std Dev	SEM
U0%	23	1873.923	1.093	0.214
U10%	22	1905.750	1.997	0.446
U50%	23	1922.625	1.129	0.200
U75%	22	1948.579	1.305	0.299
U100%	22	1974.500	1.766	0.377

From Table 1, the effect of herbicide concentration on the potential was significant ($p<0.05$). The differences in the mean values among the treatment groups are greater than would be expected by chance. One way analysis of variance (ANOVA) showed significant difference between concentration and output potential. All analyses were performed at 5% statistical significance level which is used all pairwise Multiple Comparisons (Tukey Test). The increasing of the herbicide concentration will produce higher output voltage, which is proportional inversely with the dissolved oxygen level. This may be due to the influence of herbicide concentration affect the algae metabolism to produce dissolved oxygen systemically.

Furthermore, the response time needed for the algae to reach the stationary conditions was slightly slowly for about 30 minutes until the potential relative constant ($\Delta U \sim 0$), since the active substances 2,4D – dimethylamine disturbs the alga metabolism mechanism. Based on [18], the influence of herbicides with active ingredient 2,4D - dimethylamine will decrease the living algae function in the form of photosynthetic pigment reduction, algal population density and oxygen production rate. The 2,4D pollutants active ingredients have also greatly affected algal functions even in small amounts of exposure which can cause instability in the algae habitat environment. This effect is reversible and a restoration of the photosynthetic activity take place after the substance removing. The use of this toxin is systemic, which slowly kills the living cells and after a short period of time, the immune system of the cells becoming active and the cells return to the initial condition.

CONCLUSION

In the environmental research, the system is capable to evaluate the effects of the pollutants herbicide on the relevant metabolic process using photo-synthetically active living cell as signal transducers, for example by measuring the dissolved oxygen produced from the green algae. Dissolved oxygen level shifts significantly due to the addition of herbicides in the algae environment. The Bio Chip-C is also suitable for mobile applications such as the pollution analysis in the areas of environmental research and public services locally.

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