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Review

Advantages and disadvantages on photosynthesis measurement techniques: A review

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Through photosynthesis, green plants and cyanobacteria are able to transfer sunlight energy to molecular reaction centers for conversion into chemical energy with nearly 100% efficiency. Speed is the key as the transfer of the solar energy takes place almost instantaneously such that little energy is wasted as heat. How photosynthesis achieves this near instantaneous energy transfer is a long-standing mystery that may have finally been solved. Measurements of this process are useful in order to understand how it might be controlled and how the phytomonitoring of plant development to increase productivity can be carried out. Techniques in this sense have evolved and nowadays several have been used for this purpose. Thus, the aim of this paper is to present a review of the various methods and principles that have been used in measuring photosynthesis presenting the advantages and disadvantages of various existing measurement methodologies in order to recommend the most appropriate method according to the needs of specific investigations.

Key words: Photosynthesis measurement, gas exchange, chlorophyll fluorescence, phytomonitoring.

INTRODUCTION

Higher plants transform sunlight energy to chemical energy by means of photosynthesis. During the process, plants fix carbon dioxide (CO_2) and release oxygen (O_2) while coping with the loss of water (H_2O). Measurements of photosynthesis are needed for comparing and understanding productivity (biomass accumulation) of vegetal systems at the leaf, plant or community level as well as their response to environmental stresses. Gas exchange (CO_2 and H_2O) by leaves constitute the basis for the design of most photosynthesis meters. Since CO_2 intake and H_2O release share the same biochemical pathway, photosynthesis measurements commonly include the estimation of photosynthesis itself (assimilation or CO_2 uptake), stomatal conductance and transpiration (Field et al., 1989). Due to the great importance of photosynthesis, measurement methods are required to gather more knowledge about this process. In this review, information is provided about modern methods for estimating photosynthesis used by commercial and experimental measurement systems which can be taken into account as criteria for designing new systems for photosynthesis monitoring. The discussed methods are organized in a block diagram (Figure 1). Finally, some applications and future work areas of photosynthesis monitoring in biological research and agriculture are discussed.

DRY MATTER ACCUMULATION METHOD

Historically reported techniques of measuring photosynthesis were originally estimated based on the accumulation of dry matter from a plant from the point of germination to the time it is cut in order to make the measurement as was aforementioned by De Saussure (Hodson et al., 2005). This procedure involves cutting the entire plant or only the portion that is going to be measured. Fresh weight measurement is optional. Subse-

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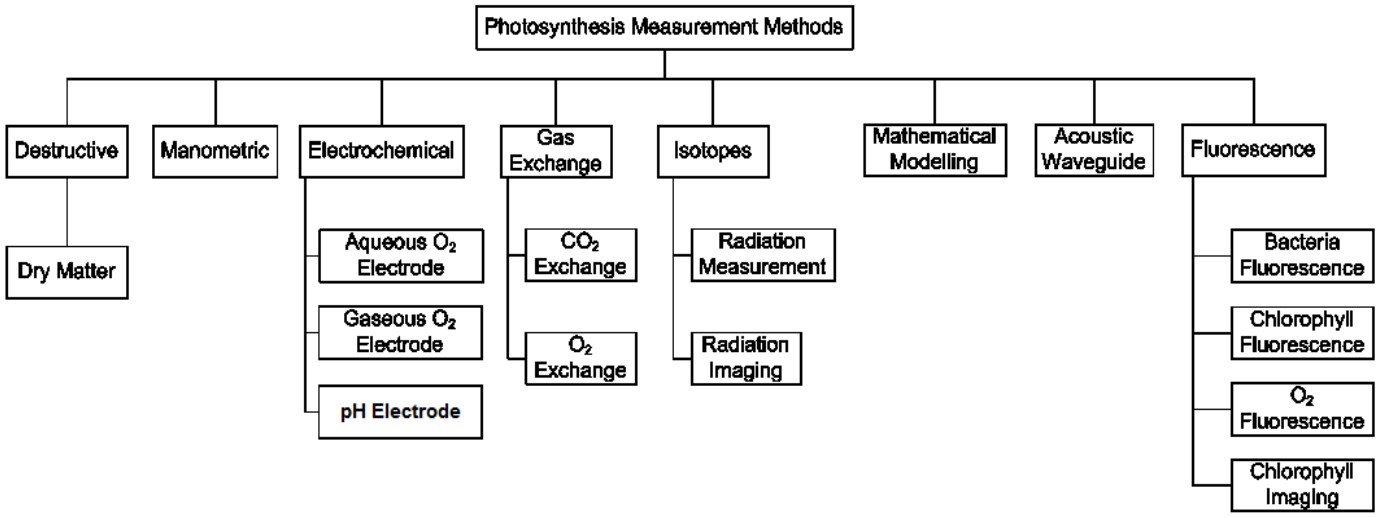

Figure 1. Photosynthesis measurement methods included in this review.

quently, the sample is placed in a drying oven at a controlled temperature to avoid damage to the carbon content in the sample. This process effectively removes any water from the tissue of the specimen to be analyzed. Once the sample has been dried, it is weighed to determine the amount of dry material accumulated. Considering that photosynthesis produces the bulk of dry matter, this method of dry weight measurement is used to estimate the cumulative photosynthetic activity throughout the life of the plant.

By improving the measurement technique of dry matter, it is possible to be more accurate when measuring the accumulation of biomass by expanding the procedure. Firstly, it is necessary to remove the sample or plant from the soil or cut it. Then store it in a sealed plastic bag that is kept at 2 to 5°C to avoid loss of matter through respiration. Once the sample has being gathered, it is necessary to classify the living and dead parts. The tissues with necrosis should be removed from the sample (Hussey and Long, 1982). The classified living tissue sample should be weighed and then, placed in a dryer at a constant temperature of 80°C with forced air ventilation (Leith, 1968). Once sample has been dried, it needs to be weighed again with an accuracy of three significant digits. Next, it is necessary to incinerate the sample in an oven at a temperature of 500°C for 6 h, with the objective of removing carbon from the dried sample leaving only the inorganic matter. It is important that the temperature do not rise above 500°C to avoid destruction of inorganic compounds. This makes it possible to record the weight proportions of the organic and inorganic matter (Rodin and Basilovic, 1965). The root analysis can be conducted using incineration to avoid contamination of the inorganic salts from the soil. This method is relatively simple. Destructive methods are still used in various procedures (Coombs et al., 1988), for example, to correlate findings recorded with new methodologies for measuring photosynthesis with applications in growth modeling, mainly using variables such as fresh and dry weight tickets to the models.

MANOMETRIC METHOD

The manometric technique for measuring photosynthesis is based on direct measurement of the pressure of CO_2 or O_2 in an isolated chamber with photosynthetic organisms. Based on the pressure change in a gas pressure monitor which occurred at O_2 or CO_2 exchanged with the environment, photosynthetic activity can be studied (Warburg, 1919; Hunt, 2003).The procedure of the technique consists in maintaining a constant pressure from one of the two gases involved in the exchange with the atmosphere, either pressure of O_2 (pO_2) or pressure of CO_2 (pCO_2), through the use of some chemical buffer. It must be made by placing the specimen under test in a glass with two outings coupled with a pressure gauge in the form of U. It has been suggested that the cultivation of algae Chlorella as a case of study for this measuring technique should be done by keeping the tested specimen in an aqueous solution to allow for the observation of many biochemical reactions (Warburg, 1919; Geider and Osborne, 1989).

In the case of measuring photosynthesis when the pO_2 remains constant, $CrCl_2$ is placed in a sub-container vessel to absorb the dissolved O_2 in the air. In this way, it will be possible to let only CO_2 into the pressure gauge to measure changes in the pCO_2 produced by the photosynthetic activity of the algae solution or any biological unit (Hunt, 2003). In the case of maintaining constant pCO_2, a carbonate-bicarbonate buffer was used (Warburg, 1919), to measure the variation of pO_2. The

use of this technique allowed the discovering of the key phenomena for the understanding of photosynthesis, such as quantum efficiency, that requires 4 photons to release a molecule of O_2 (Warburg, 1948), though some requires 3 to 5 photons (Burk et al., 1949) while some other studies of other phenomena related to quantum efficiency in photosynthesis and catalysis made use of the manometric technique as analysis tool (Warburg, 1958, 1969). But the use of this technique for photosynthesis research has some disadvantages in terms of accuracy. This is due to its sensitivity to environmental disturbances such as temperature, composition of the air and abiotic changes in the pressure of the vessel, which requires maintaining very stable temperature and environmental conditions of the vessel, in addition to the very slow changes in pressure and liquid to gas phase change. Therefore, it is not a useful technique for monitoring rapid changing photosynthetic phenomena (Hunt, 2003).

ELECTROCHEMICAL SENSOR METHODS

This technique is based on the use of O_2 and pH electrochemical electrodes to measure the O_2, CO_2 or pH aqueous concentrations of the analyzed sample in detecting variations on those variables as a function of the photosynthetic activity. Initially, these sensors were used for monitoring O_2 in blood testing (Hunt, 2003). Later they were used to monitor cell organelles such isolated chloroplasts and mitochondria (Deleiu and Walker, 1972). There were also CO_2 sensors based on pH and amperometric electrochemical changes of the solution in the presence of CO_2 that have been used for measuring photosynthesis and respiration in aqueous environments (Talling, 1973; Axelsson, 1988).

Aqueous oxygen electrochemical sensors

The electrochemical electrodes for oxygen measurement are commonly manufactured using a platinum cathode and a silver anode separated by an electrolyte (Hunt, 2003). The sensor elements are separated from the test sample by a membrane permeable to oxygen, typically teflon or polyethylene (Takahashi et al., 2001). The terminals of the sensor are polarized with 0.7 Volts and in the cause a chemical reaction, O_2 is converted into hydrogen peroxide (H_2O_2) and then subsequently reduced to OH ions. The four electrons required to carry out this reaction are donated by the silver oxidation in the anode (Hunt, 2003). The electric current flowing from anode to cathode is directly proportional to the oxygen concentration in the environment outside the O_2 permeable membrane. This signal flow can be converted to a voltage signal by a conditioning electronic circuit. Data acquisition systems are used to convert the data into digital form and the measurements obtained by the O_2 electrode are stored on some specialized commercial equipment (DW1, Hansatech Instruments Ltd, King's Lynn, UK, Rank Brothers Ltd. Cambridge UK; RC650, Strathkelvin Instruments, Glasgow, Scotland) and low-cost systems (Tank and Musson, 1993).

The main advantage of this method is its low cost and simplicity. It also has features that make it ideal for academic teaching of photosynthesis processes (Deleiu and Walker, 1972; Takahashi et al., 2001). However, it has some limitations. These types of electrochemical sensors consume oxygen from the sample, so after a prolonged period of measurement, this problem can produce unreliable results. Furthermore, the sample must also be well agitated, as the electrochemical reaction can lead to a concentration of oxygen around the cathode and this can interfere with the measurement of the non-homogeneous O_2 concentration. Another disadvantage in terms of materials is that it requires a periodic change of the teflon membrane in order to maintain maximum accuracy. Yet, another restriction is that by using electrodes for measuring oxygen in aqueous solutions of photosynthesis of algae or isolated organelles, it is necessary that the concentration of chlorophyll is less than 100 µg ml^{-1} because at higher concentrations, the photosynthetic activity produces bubbles that interfere with the current signal outputs (Hunt, 2003).

Gaseous oxygen measurements and leaf disc electrodes

As an alternative, O_2 electrodes have been used to measure photosynthesis in gaseous conditions. This technique has some advantages over the measurement of aqueous O_2 because in gaseous condition, it is possible to measure photosynthesis not only in solutions with isola-ted organelles and algae but in plant tissues (Hunt, 2003). The electrodes are composed of a disc-shaped chamber containing a leaf fragment inside and an electrode for measuring O_2 concentration in order to measure gas changes due to photosynthetic activity of the tissue under test (Deleiu and Walker, 1972).

GAS EXCHANGE METHODS

The gas exchange method is currently the most commonly utilized technique for photosynthesis measurement at present for commercial equipment and experimental setups in order to measure individual leaves, whole plants, plant canopy and even forests (Schulze, 1972; Bassow and Bazzaz, 1998). This procedure consists of isolating the specimen or sample under test in a closed chamber to measure the gas concentration at the point when the chamber is closed. After a few minutes the chamber has been closed, recording changes in the

proportions of gases from the air inside the chamber produced by the plant is also carried out. Consequently the O_2 or CO_2 exchange can be measured (Schulze, 1972; Takahashi et al., 2001). There are two types of gas exchange: closed chambers: where the sample is completely enclosed to measure the difference in gas without contact with outside air and the open chambers where air can freely enter and leave the chamber flowing through the sample (Hunt, 2003).

Infra red gas analysis sensors

Infrared sensors for gas analysis (IRGA) are the most common for CO_2 measurement and are based on an infrared emitter-photodetector par whose light beam is used to measure the concentration of gas molecules in the air. This is based on the absorption phenomenon of the light beam by molecules in a gaseous state (Hunt, 2003). This phenomenon of absorption occurs because the heteroatomic gas molecules with odd number of atoms such as CO_2, CH_4, NH_3, to name a few, absorb a portion of the infrared light while the homoatomic gas molecules such as N_2 and O_2 do not. The CO_2 has a maximum detection at a wavelength of 4.25 µm, with peaks side of 2.66, 2.77 and 14.99 µm (Hill and Powell, 1968). The calibration of these sensors to zero adjustment requires air free CO_2 and other heteroatomic gases, therefore N_2 is most often used. Also, the adjustment requires a span of known concentration of CO_2 to be carried out with precision pumps (Hunt, 2003).

CO_2 exchange method

In terms of photosynthesis measurement, the CO_2 exchange is the most commonly used for building commercial and experimental photosynthesis monitoring systems. In this technique, closed systems are used with IRGA CO_2 sensors to measure the initial concentration in an isolation chamber where the sample is placed under test to measure the final concentration after a period of time to allow for the photosynthesis of the plant (PTM-48M, Phytech Inc., Yad Mordechai, Israel). The open-flow systems have great advantages in comparison with closed systems because they do not require waiting for photosynthesis to occur in order to record the final concentration of CO_2. Instead, they permit photosynthesis sampling on higher frequencies than closed systems. Therefore, they are more useful for fast monitoring of photosynthetic phenomena. This also enables the chamber to be interchange by other shape of different leaf sizes or shapes, depending on the specie of the plant that needs to be monitored. There are also systems that allow the measurement of soil respiration. The kind of instrumentation used in these systems varies with the manufacturer. There are systems that use a single sensor to perform the measurement. In that case, it is necessary to change the air flow entering the IRGA without passing through the leaf chamber in order to measure the CO_2 input (CO_2 in) and then change the flow passing through the chamber of the leaves, waiting a while to stabilize the gas transient (Iqbal, 2003), to get steady state conditions for the measurement (Phytech PTM-48M, Phytech Inc., Yad Mordechai, Israel). Other systems use a change in differential settlement of instrumentation that uses two sensors for CO_2, one for the air intake and another for air exhaust, allowing for higher sampling rate of photosynthesis (LI-COR 6400XT, Lincoln, NE, USA).

In previous investigations, this technique has been used for measuring photosynthesis in biological units in isolated chloroplasts using solid supports with the aim of observing CO_2 fixation and O_2 evolution (Cerovic et al., 1987).On the other hand, light was variable to induce changes in photosynthesis, which is a phenomenon that requires speed to be measured. Consequently, the differential CO_2 analyzer is the most suitable equipment (Peterson et al., 1988). In some cases, it has mixed combination of monitoring CO_2 exchange and O_2 that has been used to correlate their variations (Chen, 2006).

Canopy CO_2 exchange measurement

This method is a variant of CO_2 exchange technique, which measures complete sets of plants. There are several researches where this technique has been used to study crops in population form and not individually. Some researchers have designed experimental arrangements that are acrylic boxes that house plant canopies, with an open flow system to measure the exchange of CO_2 within the population under investigation. Another application has been photosynthesis measuring through IRGAs placed on towers in a forest containing different species of trees to determine the canopy photosynthesis (Bassow and Bazzaz, 1998). In other investigations, a complete greenhouse has been designed to permit canopy photosynthesis measurement with air conditioning units, air flow, among other applications to test new growth models (Korner et al., 2007; Takahashi et al., 2008).

CO_2 exchange systems design

The design of these kinds of systems should include several considerations, including the operating range of CO_2 of the phenomenon being studied to ensure the selection of an appropriate IRGA according to the application. It has been reported that the range of physiological importance is 50 to 800 ppm (Hanstein et al., 2001). Electrochemical sensors for CO_2 are not appropriate because of their poor sensitivity to low CO_2 concentrations. Therefore, non-dispersive infrared sensors

(NDIR) are the most appropriate. Another important aspect to consider is the air flow. It has previously been found that the range more appropriate for minor variations in the accuracy of a photosynthetic rate is 0.3 to 1.0 m s^{-1} (Kitaya et al., 2000). Another aspect to analyze is the design of the chamber seal. Earlier, different systems were designed with a seal of black neoprene and a transparent surface so that light falls on the leaf for photosynthesis process. This is problematic as the black surface of the seal obscures a portion of the road tested and causes dark respiration. This, in turn produces CO_2 that seeps into the leaf chamber of the leaves and produces miscalculation of the photosynthetic rate based on the CO_2 exchange in the chamber without taking into account the parasitic dark respiration (Pons and Welschen, 2002; Long and Bernacchi, 2003). The design of the shape of the leaf chamber should be selected according to the needs of the morphological species to be studied. There are chambers ranging from those used for small leaves all the way up to soil analysis chambers (LI-COR Corporation, Lincoln, NE, USA). Finally, the technological platform that allow electronic control for electrical and mechanical systems that are needed to make an entire photosynthesis monitoring system and the export and storage of data to a computer for future offline analysis on a personal computer (PC) or a microcontroller (µC) be carried out.

O_2 exchange method

This method provides an alternative to the CO_2 exchange, which can be used as an additional tool combined with CO_2 exchange in order to observe these phenolmena. The procedure utilized in this method is basically the same as using CO_2. Nevertheless, this method has serious disadvantages. The first is that the O_2 exchange technique is the difference between the initial and final concentrations, this is, it is smaller compared to CO_2 exchange systems and by this reason, the O_2 exchange systems require high precision sensors and expensive data acquisition devices (Hunt, 2003). Another disadvantage is that the oxygen gas is more unstable than CO_2 and has to be maintained at a high and very stable temperature (around 700°C) to maintain a stable molar concentration. Most recent research recommend a combination of an exchange of CO_2 and O_2 in order to obtain a more precise estimate of photosynthetic rate than using a single gas (Chen, 2006).

CARBON DIOXIDE ISOTOPES METHOD

This methodology is based on the use of carbon isotopes like ^{11}C, ^{12}C and ^{14}C for marked CO_2 production that is applied to algae samples or plants in isolated chambers and illuminated to produce CO_2 fixation marked by the sample during photosynthetic activity. Initially, this was used to follow the path of carbon within the plants (Calvin and Bassham, 1962). This technique is useful for academic teaching of the process of photosynthesis and sugar production in plants and algae (Taiz and Zeiger, 2002; Kawachi et al., 2006).

Photosynthesis measurement by $^{14}CO_2$

This technique was previously used to study carbon distribution in the form of sugars in plants and it has been extended to the use of measurement of photosynthesis (Irvine, 1967). This procedure typically involves applying $^{14}CO_2$ to the sample being studied for a period of 15 to 60 s. Then the sample is subjected to a system that performs the counting of beta particles in the sample using the fixed isotope radiation (Hunt, 2003). This makes it possible to perform photosynthesis estimation on the basis of the amount of beta particles emitted. Several alternatives have been proposed for estimating photosynthesis on the basis of isotopes such as the use of a scintillation counter (Lupton, 1967) or using an ionization chamber to measure the setting of ^{14}C in the plant (Ludwig and Canvin, 1971). This technique has been used for specific research such as in the study of starch storage in isolated chloroplasts (Williams and Cobb, 1985) and for measuring fixation on algae (Jespersen, 1994). The main drawback of this method is that it is destructive to the sample under test and it is not accurate for photosynthesis measurements in low light, conditions as the loss of $^{14}CO_2$ by photorespiration is relatively large and affects the estimation.

Photosynthesis imaging by $^{14}CO_2$

This methodology for measuring photosynthesis is based on the use of this isotope to observe its fixation on the plant (Kume et al., 1997), that is placed under a Possitron Emitting Tracer Imaging System (PETIS) using low lighting conditions ranging from 0 to 250 mol m^{-2} s^{-1}, where it is possible to see the picture of the distribution of radiation emitted by the isotope in the plant, (Kawachi et al., 2006). Using this technique it is possible to see the setting of various nutrients, by using different isotopes such as ^{11}C, ^{13}N, ^{15}O, ^{52}Fe, ^{52}Mn, ^{62}Zn and ^{107}Cd. This technique allows investigators to observe an image of the absorption of sugars depending on the photosynthetic activity.

PHOTOSYNTHESIS ESTIMATION BY MODELLING

As has been aforementioned, photosynthesis is a variable that cannot be measured directly, so it is necessary to measure using other variables and some specific

equations or methodologies are used to estimate the photosynthetic rate. The specific case of this methodology is based on developing mathematical models that permit the estimation of photosynthetic rate based on behavioral patterns obtained by monitoring other variables that are usually environmental. It has been proposed that a model of canopy photosynthesis can be divided into two sub-models. One is used as an empirical basis for the autocalibrating of the second. The second model is a black box responsible for calculating photosynthesis, making daily regressions (Ehler, 1991). Later a methodology was proposed in which it invert AliBio model for the estimation of canopy evapotranspiration in two soybean crops under water stress, using variables such as spectral reflectance, thermal infrared and microwave as entrances to a model of ground vegetation atomsphere transfer (SVAT) (Olioso et al., 1999). Others have proposed the use of information from a Landsat system as input to a Daisy model (Daisy Quote) in order to estimate the leaf area index and the concentration of nitrogen in plant leaves (Abrahamsen and Hansen, 2002), showing the importance of estimating these parameters in the process of estimating photosynthesis (Boegh et al., 2003). Another variant of this technique has been the simulation of photosynthesis using a model of integrated ecosystem (CoupModel) in two different seasons of the year and with a varied range of saline stress on tomato plants (Karlberg et al., 2006). Other researchers however, (Rehak et al., 2008), proposed the use of a mathematical model using least squares to predict the growth of algae, comparing experimental data with simulations, with the objective of optimizing the photosynthetic activity in the production of algae. This technique, although difficult, is use because of the complexity of some models. It is a powerful tool in the generation of new models to accurately predict photosynthesis, based on parameters monitored in various aspects.

ACOUSTIC WAVEGUIDE METHOD

This method is an unconventional way to measure the photosynthesis of algae in the sea. It is based on the principle of distortion of sound waves by the medium in which they spread. The technique involves placing an acoustic transmitter on the seabed in the area where photosynthetic activity monitoring is desired. A hydrophone is placed near the transmitter of sound to make the reference sound signal. At the other end of the area being monitored, a second hydrophone is placed which serves as an acoustic sensor. Observing the phenolmenon of O_2 small bubble production by the algae photosynthetic activity in the sea basis, there can be noticed a difference between the signals of the reference and measurement hydrophones. So it can be observed that it produces a higher sound difference in the hydrophones in day than night, because the sunlight on the water permits algae photosynthetic activity and consequently the release of O_2 micro bubbles that interfere with sound propagation (Hermand et al., 1998). Improvements to this technique have been made to change the conditions of the seabed and distance to source hydrophone of measuring, checking again the usefulness of acoustic techniques in the measurement of sea grass photosynthesis (Hermand, 2004).

FLUORESCENCE BASED TECHNIQUES

Fluorescence is the phenomenon whereby a certain amount of light energy is retained from illumination and subsequently released light after the light exposure time ceases to illuminate the sample (Taiz and Zeiger, 2002). Within the investigations of photosynthesis, the fluorescence is used in various forms and has varied applications.

Bacteria fluorescence

Luminescent bacteria in the presence of photosynthetic O_2 are used as a biosensor technique (Tchan et al., 1977). The procedure is initiated by placing the bacteria in a chamber and combining it with a solution of microalgae. Once this solution is combined, light conditions are applied to start photosynthesis in the algae of the solution. Later, the developed instrument turns mechanically the bacteria container in which the sample is placed in a measurement mode in order to quantify the bacteria fluorescence. In this way, the amount of fluorescence of the bacteria is proportional to the amount of O_2 dissolved in the solution under test. Consequently, the concentration of O_2 in water depends on the photosynthetic activity carried out by microalgae. This technique has been applied for rapid detection of herbicides that inhibit the activity of photosynthetic cultivation in microalgae.

Chlorophyll fluorescence

This is a variant of the estimation methods of photosynthesis by fluorescence that has been widely used recently. This technique is to harnesses the fluorescence that is produced when the chlorophyll is illuminated and then releases the stored energy as red fluorescence (Taiz and Zeiger, 2002; Long, 1990). The phenomenon is based on the Kautsky principle, which measures the relationship between minimum fluorescence F_o and maximum fluorescence (also known as saturation F_m), to estimate the photochemical efficiency of the sample. The measurement procedure requires placing the sample under test in a dark room, which is then lit up for a while to get pulses through F_o, then light up to get F_m. This is

calculated by F_v variable fluorescence (F_m-F_o) which leads to F_v/ F_m, that permits the estimation of the chlorophyll fluorescence (Fedack et al., 2005). This technique has several advantages compared with the exchange of CO_2, as this technique is virtually immune to environmental perturbations caused by temperature, pressure, or relative humidity (RH). It is therefore considered as a very robust method for measuring photosynthesis based on the amount of chlorophyll, even in difficult environmental conditions (Wang et al., 2004). Another option with this technique is that it is very easy to make the measurement in conjunction with a system of exchange of CO_2, which is interesting because it permits both measurements to be correlated to get a more accurate estimate of photosynthesis of the specimen being studied (Peterson, 1990; Long and Bernacchi, 2003).

Some applications of the technique uses fluorometers and pulse amplitude modulation (PAM). Ralph et al. (1998) used PAM to study the photosynthetic activity of three species of Australian algae. An array of red and white light emitting diodes (LED) was proposed as a tool for measuring chlorophyll in seedling beds (Okamoto et al., 2000). Initially both light colors were used to excite the chlorophyll. Then the red LED was turned off to be used as a photodetector of the light reflected by the green chlorophyll excited by the white LED. To separate the fluorescence from the ambient light, a sine modulated technique within the white light was used, which produced good results. Bulgarea and Boukadoum (2001) and Fedack et al. (2005) reported the design and construction of fluorometers based on the principle of measurement using three types of light which included: radiation, analytical and saturation with the objective of finding F_o, F_v and F_m, respectively. Other researchers have isolated and immobilized biological units like chloroplasts in solid supports to be used as herbicides biosensors in order to analyze samples of contaminated water. This is based on the chlorophyll fluorescence technique to measure the herbicide concentrations in the analyzed sample (Breton et al., 2006).

As an alternative technique, a Laser Induced Fluorescence Transient (LIFT) was used to measure chlorophyll fluorescence at a distance ranging from 5 to 50 m based on a laser pulse width modulation (PWM) to create a transient effect of various intensities on the sample (Kolber et al., 2005). Some researchers combine various techniques for measuring photosynthesis and chlorophyll such as accumulation of dry matter, exchange of CO_2, leaf area and more, with the objective of obtaining a more precise estimate and more information about the tested specimen. The use of a He-Ne laser of 632.8 nm for measuring photosynthesis through chlorophyll fluorescence (Wang et al., 2004) has been proposed and it was compared with a commercial system (LI-6200 LI.COR Biosciences, Lincoln, NE, USA) to validate results. Consequently, technical improvements have changed the laser by a six LED array as a source of excitation of 628 nm; it has permitted a less expensive system to be created as compared with a LI-6400 (LI-COR Biosciences, Lincoln, NE, USA).

Alternatively, some methods of chlorophyll fluorescence in canopy have been developed. IA method capable of measuring photosynthesis by the spectral reflectance measured from the surface of plants and comparing results with an IRGA has also been reported (Tian et al., 2005; Van Gaalen et al., 2007). Another similar method to utilize the solar radiation as excitation source uses the principle of Fraunhofer line to separate the reflectance of the fluorescence and compare results with traditional fluorometers (Liu et al., 2005). This technique is an extremely useful tool, capable of being used in studies of various types of environmental stress in plants because it is a simple technique to understand low cost and possesses great versatility in several applications (Sayed, 2003).

Oxygen fluorescence

The technique of O_2 fluorescence is a variant which requires the application of cyan dye reactive to oxygen to measure the concentration of intracellular oxygen through a fluorometer applied to plants injected with the dye. Due to the application of an optical multifrequency phase-modulation technique, chlorophyll from O_2 fluorescencencan be discriminated (Schmalzlin et al., 2005).

Chlorophyll imaging

This methodology is based on the same principle used by chlorophyll fluorescence measurement (Fedack et al., 2005). However, it differs from the chlorophyll fluorescence measurements as these are made by measuring fluorescence in a timely manner and it is not possible to fully understand the different photosynthetic areas in the same leaf. Instead, this technique uses a camera as a photodetector, with the significant advantage of providing a complete picture of the fluorescence of the sample making it possible to observe the diversity of photosynthetic activity areas that may exist on the same road, where a spot metering generally provides poor information about this phenomenon (Lichtenthaler et al., 2005). Also it is possible to carry out studies of reactive oxygen species using PAM with image acquisition (Hideg and Schreiber, 2007). As has already been aforementioned, this technique is useful to obtain a complete graphically measurements of the sample and that contains much more information than a spot metering. The disadvantage here is that the cost of traditional fluorometers has increased.

PHYTOMONITORING APPLICATION

Phytomonitoring is a new concept that some authors

attach to the use of plants as low cost biosensors of pollutants of the air or water (Upadhyay and Kobayashi, 2007). This procedure is based on estimating the degree of pollution based on the observed physical damage to the plant. Other authors use this term to refer to a new technology that initially started with photosynthesis monitoring capabilities in order to obtain information about the physiology and morphology of the plant. This idea has been expanded to the use of different sensors in order to gather more information. Ton (1997) has commented that this is the most important role in the physiology of a plant. However, there are also some other morphological and physiological variables that might reveal very important information about the real state of a plant such as stem diameter, fruit size, flow sap to mention but a few. Consequently, it can be said that monitoring of photosynthesis is the activity that led to the beginning of the development of phytomonitoring technology. It has been developed in climatically controlled isolation chambers of plants with the objective of studying the behavior of various specimens under controlled laboratory conditions in order to monitor their physiology and morphology (Mazanti-Hansen and Ehler, 1998). The photosynthesis measurement techniques, made possible the development of plant tissue based biosensors. This kind of sensors provides a cheap and useful platform to gather interesting information about the plant cell biochemistry reactions against different external agents (Campas et al., 2008). Some applications of phytomonitoring in the computerized climate control based on data from a phytomonitoring system have been reported (Schmidt, 2005). On the other hand, it is important to emphasize the importance of phytomonitoring as a tool for detecting stress in plants, based on the phenomena observed during the monitoring of physiology and most notably photosynthesis (Musyimi et al., 2007). Finally, it is important to mention that the development and improvement of techniques for measuring photosynthesis will eventually permit more precise manner of gathering knowledge of the specific requirements of each crop which will consequently maximize production. Therefore, there is great interest in developing new technologies for physiological monitoring of plants, in order to increase the production of food in any particular place and in any season under greenhouse conditions. In the past, it was impossible in unprotected agricultural systems. Furthermore, crop production in outer space could very well be a topic explored in the not too distant future (Kania and Giancomelli, 2000).

CONCLUSION

As mentioned earlier, photosynthesis is a process of extreme importance since it governs all life on earth as it is crucial in terms of the economic interests of growers. Because of this importance, there is a necessity for continuing research to increase the knowledge on photosynthesis. There is also, the necessity of developing new techniques for measuring photosynthesis, so that these become increasingly precise and permit researchers to gather more accurate information. Also, the combination of multiple measurement methods in the photosynthesis research to achieve better results and observe effects that are not visible using only single measurement methodologies is important. One application of the measurement systems of photosynthesis is to use it as a phytomonitoring tool to regulate the photosynthetic activity of the crops to aid farmers in adjusting the conditions that most affect the photosynthetic rate, such as CO_2 and radiation. Also, it gathers the necessary information to perform related research like CO_2 enrichment systems (Matthews et al., 1987; Xiao et al., 2000) and the development of more precise growth models.

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