The Basics of Chlorophyll Measurement

Tech Note

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What is Chlorophyll?

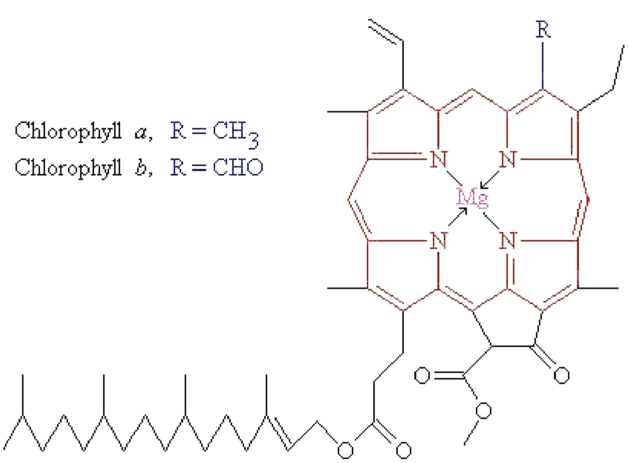
Chlorophyll, in various forms, is bound within the living cells of algae and other phytoplankton found in surface water. Chlorophyll is a key biochemical component in the molecular apparatus that is responsible for photosynthesis, the critical process in which the energy from sunlight is used to produce life-sustaining oxygen. In the photosynthetic reaction below, carbon dioxide is reduced by water, and chlorophyll assists this transfer.

$$6\ CO_2 + 6H_2O \xrightarrow[\text{Chlorophyll}]{\text{Sunlight}} C_6H_{12}O_6 + 6O_2$$

Photosynthetic Reaction

Chlorophyll is present in many organisms including algae and some species of bacteria. Chlorophyll a is the most abundant form of chlorophyll within photosynthetic organisms and, for the most part, gives plants their green color. However, there are other forms of chlorophyll, coded b, c, and d, which augment the overall fluorescent signal. These types of chlorophyll, including chlorophyll a, can be present in all photosynthetic organisms but vary in concentrations.

Chlorophyll enables plants and other chlorophyll-containing organisms to perform photosynthesis. Chlorophyll is a chelate, or a central metal ion, in this case magnesium, which is bonded to a larger organic molecule called a porphyrin. The porphyrin molecule is composed of carbon, hydrogen, and other elements such as nitrogen and oxygen. The magnesium ion bonded within this ring is thought to be responsible for electron transfer during photosynthesis (below).



Chlorophyll Molecule

The Importance of Chlorophyll as a Water Quality Parameter

The measurement and distribution of microscopic living plant matter, commonly referred to as phytoplankton or algae, have been of interest to scientists, researchers, and aquatic resource managers for decades. An understanding of the phytoplankton population and its distribution enables researchers to draw conclusions about a water body's health, composition, and ecological status.

Although researchers often refer to microscopic living plant matter as either algae or phytoplankton, and the two terms are often used interchangeably, each has distinct meaning. Algae refer to simple aquatic organisms, such as seaweed, pond scum, and plankton, that are plantlike and contain chlorophyll. Phytoplankton are a subset of algae and are the suspended aquatic microorganisms that contain chlorophyll. For *in-situ* monitoring, the measured parameter is the chlorophyll contained within the phytoplankton.

Chlorophyll is essential to the existence of phytoplankton. Phytoplankton can be used as an indicator organism for the health of a particular body of water. Monitoring chlorophyll levels is a direct way of tracking algal growth. Surface waters that have high chlorophyll conditions are typically high in nutrients, generally phosphorus and nitrogen. These nutrients cause the algae to grow or bloom. When algae populations bloom, then crash and die in response to changing environmental conditions, they deplete dissolved oxygen levels — a primary cause of most fish kills. High levels of nitrogen and phosphorus can be indicators of pollution from man-made sources, such as septic system leakage, poorly functioning wastewater treatment plants, or fertilizer runoff. Thus, chlorophyll measurement can be utilized as an indirect indicator of nutrient levels.

Reasons to Measure Chlorophyll

Algal content can be tracked in surface water and, with time, databases and quality assurance protocols can be developed to characterize lakes or streams. These characterizations can be used for the indirect monitoring and detection of indicator pollutants, including phosphorus and nitrogen. Monitoring phytoplankton concentration is a less expensive alternative to frequent collection of grab samples for costly and labor-intensive laboratory analysis.

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In general, the amount of chlorophyll in a collected water sample is used as a measure of the concentration of suspended phytoplankton. The use of the measurement of phytoplankton as an indicator of water quality is described in Section 10200 A. of *Standard Methods for the Examination of Water and Wastewater*. Currently, chlorophyll determinations are made on lakes, rivers, reservoirs, and coastal and ocean waters across the globe.

Ocean and coastal studies investigate the distribution of phytoplankton in marine systems. These studies can help track and predict deadly algae blooms. The nutrient enrichment of water bodies is leading to increased production of organic matter and resulting in low levels of dissolved oxygen that are killing marine life. Also, ocean profiling can track and record chlorophyll readings, which may change vertically along a column of water.

Rivers and streams are monitored for excessive growth of phytoplankton due to high concentrations of plant nutrients. This excessive growth can lead to eutrophication of the river or stream and cause deadly fish kills. For similar reasons, lake, pond, and reservoir monitoring, including lake profiling studies, also observe excessive algae population distribution and growth. Algae control is a major concern in pond management, especially in smaller bodies of water, where excessive algae growth can quickly become a problem.

Measuring chlorophyll concentration is also a step in the process of screening/monitoring for nuisance algal blooms that may influence the taste and odor of drinking water sources. These blooms may actually create conditions that are toxic to fish, wildlife, livestock, and humans. Bodies of water used as drinking water sources are also monitored for phytoplankton concentrations for the early detection of algal blooms to minimize filtration system clogs.

How Chlorophyll is Measured

There are various techniques to measure chlorophyll, including spectrophotometry, high performance liquid chromatography (HPLC), and fluorometry. All of these methods are published in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition.

Spectrophotometry is the classical method of determining the quantity of chlorophyll in surface water. It involves the collection of a fairly large water sample, filtration of the sample to concentrate the chlorophyll-containing organisms, mechanical rupturing of the collected cells, and extraction of the chlorophyll from the disrupted cells into the organic solvent acetone. The extract is then analyzed by either a spectrophotometric method (absorbance or fluorescence), using the known optical properties of chlorophyll, or by HPLC. This general method, detailed in Section 10200 H. of *Standard Methods*, has been shown to be accurate in multiple tests and applications and is the procedure generally accepted for reporting in scientific literature. The fluorometric method also requires the same extraction methods used with spectrophotometry, then uses a fluorometer to measure discrete molecular chlorophyll fluorescence. However, these methods have significant disadvantages. They are time-consuming and usually require an experienced, efficient analyst to generate consistently accurate and reproducible results. In addition, they do not lend themselves readily to continuous monitoring of chlorophyll (and thus phytoplankton) because the collection of samples at reasonable time intervals, e.g., every hour, would be extremely time-consuming.

YSI has developed optical sensors for chlorophyll determinations both in spot sampling and in continuous monitoring applications. The sensors are based on an alternative method for the measurement of chlorophyll that overcomes these disadvantages, albeit with the potential loss of accuracy. In this procedure, chlorophyll is determined *in situ* without disrupting the cells as in the extractive analysis. The YSI 6025 chlorophyll sensor and EXO 599102 total algae sensor are designed for these *in-situ* applications, and their use allows the facile collection of large quantities of chlorophyll data in either spot sampling or continuous monitoring applications.

It is important to remember, however, that the results of *in-situ* analysis will not be as accurate as results from the certified extractive analysis procedure. The limitations of the *in-situ* method should be carefully considered before making chlorophyll determinations with the YSI sonde and sensor. Some sources of inaccuracy can be minimized by combining extractive analysis of a few samples during a sampling or monitoring study with the YSI sensor data. The *in-situ* studies will never replace the standard procedure. The estimates of chlorophyll concentration from the easy-to-use YSI chlorophyll system are designed to complement the more accurate, but more difficult to obtain, results from more traditional methods of chlorophyll determination..

How YSI Measures Chlorophyll

One key characteristic of chlorophyll is that it fluoresces. When irradiated with light of a particular wavelength, it emits light of a higher wavelength (or lower energy). The ability of chlorophyll to fluoresce is the basis for all commercial fluorometers capable of measuring the analyte *in situ*. Fluorometers of this type have been in use for some time. These instruments induce chlorophyll to fluoresce by shining a beam of light of the proper wavelength into the sample, and then measuring the higher wavelength light which is emitted as a result of the fluorescence process. Most chlorophyll systems use a light-emitting diode (LED) as the source of the irradiating light that has a peak wavelength of approximately 470 nm. LEDs with this specification produce radiation in the blue region of the visible spectrum. On irradiation with this blue light, chlorophyll resident in whole cells emits light in the 650-700 nm region of the spectrum. To quantify the fluorescence, the system detector is usually a photodiode of high sensitivity that is screened by an optical filter that restricts the detected light. The filter prevents the 470 nm exciting light from being detected when it is backscattered off particles in the water. Without the filter, turbid (cloudy) water would appear to contain fluorescent phytoplankton, even though none were present.

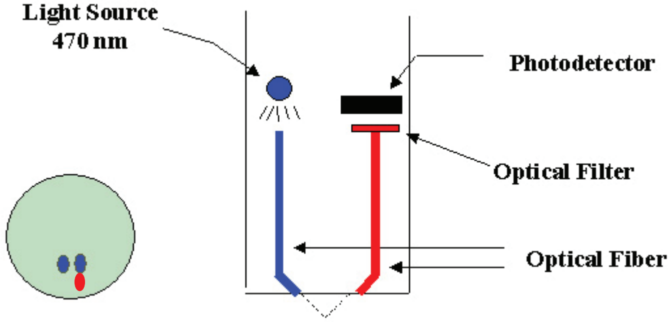

Diagram of YSI 6025 Chlorophyll Probe

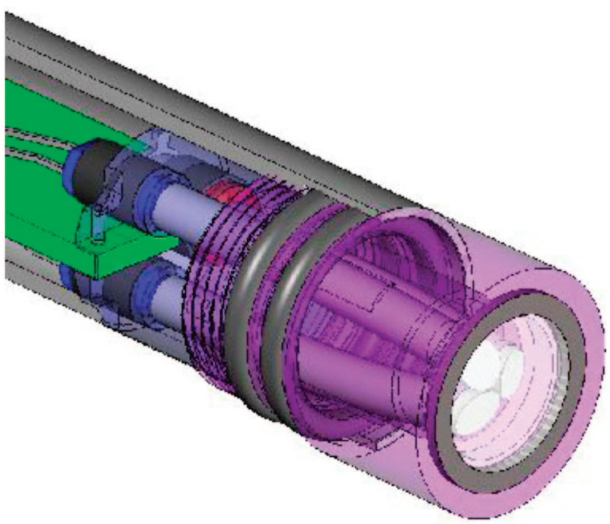

Diagram of EXO Total Algae (Chlorophyll + Blue-green Algae) Probe

Most commercial fluorometers fit into two categories; benchtop instruments that generally have superior optical flexibility and capability but are relatively expensive and can be difficult to use in the field, and the less expensive field-type fluorometers which have a fixed optical configuration, can be more easily used in the field, and are usually compatible with data collection platforms. However, the use of a pump is generally recommended for both benchtop and field-type fluorometers and this can result in the need for large-capacity batteries for field use. These types of fluorometers have other disadvantages. For example, they can only give chlorophyll (fluorescence) readings and cannot measure any other water quality parameter. Also, the output is in mV or fluorescence units, not µg/L.

YSI's unique chlorophyll system consists of a probe similar in concept to the field-type fluorometers but much smaller, making it compatible with the probe ports of the YSI 6-Series and EXO sondes. The output of the chlorophyll sensor is automatically processed via the sonde software to provide readings in either generic fluorescence units (percent full scale; % FS) or µg/L of chlorophyll. No pump is required for the YSI system, allowing the sensor to operate from either the sonde internal batteries or the batteries in a display/logger. In addition, a YSI chlorophyll probe will be a component in YSI and EXO sondes that also have the ability to acquire multiple parameters simultaneously, rather than just providing the single parameter chlorophyll.

Fouling Effects on Optical Measurements

The YSI 6025 chlorophyll probe is equipped with a mechanical wiper to periodically clean the optical face either by manual or automatic activation. The EXO 599102 total algae sensor (combined chlorophyll and blue-green algae sensor, also from YSI) is wiped clean by a central wiper in the EXO2 sonde. With these features, YSI chlorophyll sensors provide the same level of short-term performance as other commercial field-type fluorometers, but are much easier to use and can be deployed in surface water for several weeks without the need for service. Many of the field fluorometers will foul and provide inaccurate data unless maintained at short and constant intervals.

Field optical measurements are particularly susceptible to fouling, not only from long-term buildup of biological and chemical debris but also from short-term formation of bubbles from outgassing of the environmental water. These bubbles can generally be removed in short-term sampling applications by simply agitating the sonde manually. The mechanical wiper makes it ideal for unattended applications. The wiper can be activated in real-time during discrete sampling operations, or will function automatically during long-term unattended monitoring studies. The number of wiper movements and the frequency of the cleaning cycle for the unattended mode can be set in the sonde software. Generally, one wiper movement is sufficient for most environmental applications but, in media with particularly heavy fouling, more frequent cleaning cycles may be necessary.

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