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Validation of rapid algal bioassay using delayed fluorescence in an interlaboratory ring study

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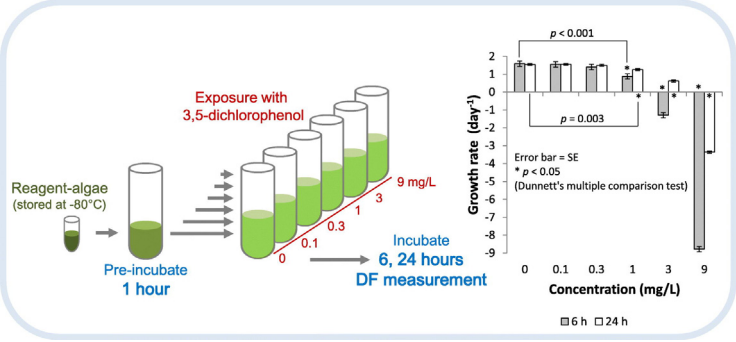
HIGHLIGHTS

- Rapid algal bioassay using delayed fluorescence (DF) from green alga
- The DF has potential to be used as a surrogate for the measurement of algal biomass.
- The method is statistically characterized in an interlaboratory ring study using reference toxicant, 3,5-dichlorophenol.
- EC_{50}–values of the test after 24 h are close enough to the conventional test to be useful for screening tests.
- The intralab and interlab variabilities of EC_{50} at 24 h are 12% and 28%.

GRAPHICAL ABSTRACT



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ABSTRACT

Algal growth inhibition tests are generally used to determine the toxic effects of chemical substances on algae growth. In this report, we describe a rapid and simple test procedure using delayed fluorescence (DF) to determine chemical toxicities more rapidly than the conventional 72 h or 96 h growth inhibition tests. We assess the suitability of DF to serve as an alternative endpoint for biomass production and determine the variability by an interlaboratory ring study using a typical reference toxicant 3,5-dichlorophenol (DCP). The results suggest that DF has the potential to be used as a surrogate measure of photosynthetically-active biomass in the algal growth inhibition tests. The half maximal effective concentration (EC_{50}) values of DCP determined from the DF inhibition test in 6 h and 24 h (1.2 ± 0.3 mg/L and 2.7 ± 0.5 mg/L respectively) are in reasonable agreement with the EC_{50} value of DCP determined by the 72 h conventional method (1.8 mg/L). In the interlaboratory ring study, the intralaboratory and interlaboratory variabilities of the EC_{50} of the DF inhibition test for a 24 h exposure period are 12% and 28% respectively. DF intensity can be considered as a surrogate of living biomass with active photosynthesis, and we conclude that a 24 h exposure duration better estimates the toxic effects measured using conventional surrogate measures for dry weight such as cell counts, volume, optical density or fluorescence.

Abbreviations: ANOVA, analysis of variance; CV, coefficient of variation; DCP, 3,5-dichlorophenol; DF, delayed fluorescence; DFI, delayed fluorescence intensity; DW, dry weight; EC_X, X% effective concentration; GR, growth rate; ISO, International Organization for Standardization; NOEC, non-observed effect concentration; OECD, Organization for Economic Co-operation and Development; SETAC, Society of Environmental Toxicology and Chemistry; USEPA, United States Environmental Protection Agency.

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1. Introduction

Algal growth inhibition tests are commonly applied for assessing toxic chemical effects on algae, a primary producer in aquatic ecosystems. Usually, growth is measured for 72 h (OECD, 2011; ISO, 2004) or 96 h (USEPA, 2012) using standard test guidelines, such as the OECD test guideline 201 (TG201) which describes the principle and criteria of the test (OECD, 2011). Growth and growth inhibition are quantified by the measurement of dry weight algal biomass as a function of time (*i.e.* the growth rate). Because of the difficulties measuring "dry weight" accurately, surrogate measures for the biomass such as cell counts and/or volume, fluorescence, or optical density are described in various guidelines. TG201 requires a known conversion factor between the measured surrogate parameter and biomass. Flow cytometry was reported to be useful in the microalgae test to accurately estimate biomass dry weight (Chioccioli et al., 2014) and to analyze mixed algal populations in multispecies assays (Franklin et al., 2004). Nagai et al. (2011) proposed the utility of flow cytometry for distinguishing living from dead cells in algal growth inhibition studies.

The TG201 and other standard guidelines, however, are labor-intensive, time-consuming, and require tight control of algal quality. Therefore, there is a need to improve the throughput of the test, specifically by developing a rapid screening test to support the conventional 72 h growth inhibition test. The ISO test guideline (ISO, 2004) describes a shorter test protocol in its annex A; rapid screening of wastewater algal growth inhibition with a minimum test duration of 48 h. There are also several suggestions for shorter exposure tests. Effective fluorescence quantum yield ($[F'_m - F] / F'_m$), defined below, is most commonly used for toxicological investigation to evaluate photosynthesis inhibition that may result in growth inhibition (reviewed by Ralph et al. (2007)). F'_m is the maximum fluorescence (with strong pulse light excitation in addition to the standard illumination for photosynthesis) and F is fluorescence from standard photosynthesis illumination. The effective quantum yield provides an indication of the photosystem activity. However, the effective quantum yield ($[F'_m - F] / F'_m$) evaluates the ratio of two chlorophyll fluorescence signals from the same sample under different light conditions. Therefore, algal cell suspensions that have a specific photosystem activity (and correspondingly, a specific ratio between F'_m and F) exhibit similar effective quantum yield at different cell densities. As a result, interpretation of effective quantum yield may be complicated for the evaluation of algal suspensions with varying cell densities (*i.e.* growth tests) (Katsumata et al., 2012a).

Delayed fluorescence (DF) has been proposed as an endpoint for evaluation of the influence of chemical substances on the growth of alga and other photosynthetic organisms (*e.g.* duckweed; Drinovec et al., 2004). DF can be detected for up to several minutes after light excitation in the dark (Strehler and Arnold, 1951). The source of the light in DF is charge recombination at a photosystem reaction center *via* trapping of electrons in electron acceptors during the photosynthetic chain reactions (Lavorel, 1975; Jursinic, 1986; Schmidt and Senger, 1987; Goltsev, 2009). DF is sensitive to exposure to chemical substances that disturb photosynthetic reactions (*e.g.* photosynthesis inhibitors, respiration inhibitors). DF represents the total amount of active photosystem (capable of trapping electrons and undergoing recombination) within the sample (*e.g.* algal cell suspension). Briantais et al. (1980) and Joliot and Joliot (1980) reported that the light induced proton gradient across thylakoid membranes is related to DF, and furthermore the typical inhibition of DF is caused by dissipating the proton gradient by an uncoupler. Several studies have demonstrated applicability of DF for the evaluation of toxic effects of chemicals (*e.g.* metals, herbicides, and photosynthetic inhibitors) on alga (Bürger and Schmidt, 1988; Scordino et al., 1996, 2008; Katsumata et al., 2008). Additional studies compare DF with measures for biomass such as cell count/volume, absorbance, and fluorescence. Drinovec et al. (2004) demonstrated DF at 24 h is more sensitive for detection of toxicity than the 72 h growth inhibition on duckweeds (*Lemna minor*). We reported the intensity of DF at relatively long delay times (*e.g.* later than 0.6 s) following short term exposure (15 min or 24 h) is a promising metric to estimate the toxicity of chemicals (herbicides and uncoupler) measured by the 72 h conventional growth test of green alga *Pseudokirchneriella subcapitata* (Katsumata et al., 2006, 2009). We concluded DF at 24 h is a possible endpoint to estimate the 50% effective concentration (EC_{50}). We also demonstrated the correlation between DF and living cell density in mixtures of living and dead cells of *P. subcapitata* (Katsumata et al., 2010). Consequently, DF can probe photosynthetic activity for both quantity (living cells in a mixture) and quality (active photosystems per detectable cell particle), suggesting that DF may be a useful measure of algal productivity. There are numerous studies comparing DF with other surrogate measures for biomass on several algae species. These results demonstrated DF to be a suitable and sensitive method for algal toxicity testing independent of cell size, taxonomic group, and pigment composition (Zrimec et al., 2007; Leunert et al., 2013; Yamagishi et al., 2016; Breuer et al., 2016).

Therefore, DF is a promising endpoint to estimate algal growth with shorter exposure durations than conventional growth tests (*i.e.* provides rapid evaluation). Conventionally, growth tests require preparation of test alga with a pre-incubation period of 2–3 days, which is inconvenient at best, and subject to variability leading to reduced test quality. Consequently, we developed a frozen algal suspension (cryopreservation) for preparation of test alga on the day of the test, and reported preliminary results from a ring study utilizing the frozen alga in a rapid and simple test procedure (Katsumata et al., 2012b). The analysis mainly focused on the variation of the DF and its dose-response. We have also demonstrated the use of DF to evaluate the toxicity of

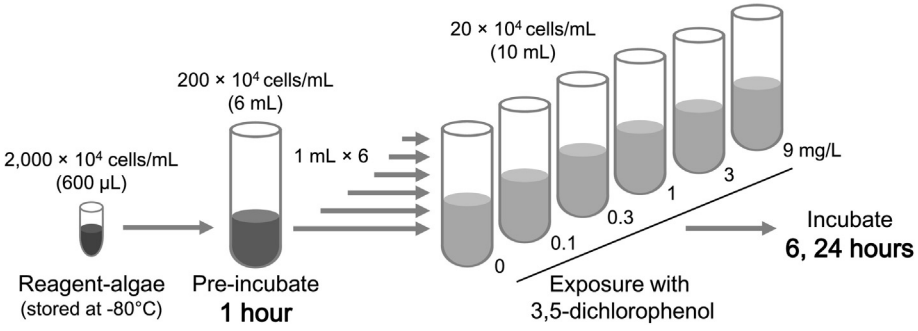

Fig. 1. Preparation and exposure using reagent-algae. Reagent-algae are thawed, diluted 10-fold and pre-incubated for at 1 to 2 h. The pre-incubated algae are used to prepare control and exposure samples. The samples were incubated for up to 24 h.