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# Effective screening of *Scenedesmus* sp. from environmental microalgae communities using optimal sonication conditions predicted by statistical parameters of fluorescence-activated cell sorting

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ABSTRACT

The effects of the sonication parameters, including the power and time, were investigated for the effective isolation of *Scenedesmus* sp. from environmental microalgae communities when using fluorescence-activated cell sorting (FACS). The selectivity, defined as the percentage of *Scenedesmus* sp. successfully isolated and grown in microplates, appeared as peaks in contour plots spanned by the sonication power and time. For fast screening of the optimal sonication conditions, correlations between the selectivity and the statistical parameters from the FACS analysis were investigated. A graphical comparison analysis of the contour plots showed a pattern similarity of over 82% between the coefficients of variation for the side scatter (SSC-CV) and the selectivity. This predictability of the optimal sonication conditions enabled a *Scenedesmus* sp. selectivity of ca. 2 times using only one-third of the sonication condition sets arbitrarily chosen around the peaks of the SSC-CV, thereby saving resources and time for subsequent processes.


## 1. Introduction

The increasing global demand for alternatives to fossil fuels and recent climate fluctuations have sparked a renaissance in microalgae research based on the expectation that mass cultivation of appropriate microalgae will help reduce climate fluctuations by carbon dioxide fixation, while also producing biofuels without severe competition with food crops (Mata et al., 2010). Yet, despite such optimistic predictions on the potential of microalgae (Brennan and Owende, 2010; Chisti, 2007, 2008; de la Noue and de Pauw, 1988), the technological development and application of microalgae continue to lag due to limited knowledge about their physiology, genetics, and ecology. For example, the diversity of microalgae is huge, with the estimated number of microalgae species exceeding one million (Andersen, 1992), yet the number of microalgal stocks currently available in world-class culture collections is lower than 3000 species, and many of these species are just duplicated among the culture collections. In addition, the current number of full-sequenced microalgae is lower than 100 species (Wijffels and Barbosa, 2010). Thus, the isolation of new microalgae species from various environments is a promising option to increase the genetic pool and identify variants suitable for downstream applications in the production of high-value biomolecules and biofuels. Indeed, indigenous microalgae isolated from local environments are effectively applied for the treatment of a concentrated wastewater and biomass production (Zhou et al., 2011).

Fluorescence-activated cell sorting (FACS) is already used for the analysis and separation of microalgae, where individual particles or cells held in a thin fluid stream are passed through one or more laser beams. These individual particles or cells scatter light, absorb the laser beam, and emit corresponding fluorescence, which is detected using a set of highly sensitive detectors to provide information on the cell size, integrity, and photosynthetic characteristics (Katsuragi and Tani, 2000; Reckermann, 2000). This information is closely related to the morphology and photosynthetic characteristics that are conventionally used for the identification and classification of microalgae. Indeed, the usefulness of FACS has been proven in many microalgae-related studies, including analyzing the developmental and growth-related properties of microalgal cultures (Li et al., 2011) and monitoring phytoplankton communities in the ocean, especially the pico- and nano-plankton fractions (Crosbie et al., 2003; Kuosa, 1991; Moore et al., 1998). Yet, despite of its effectiveness for microalgae-related studies, the full application of FACS remains limited, as many microalgae naturally found as aggregated forms with other microorganisms, called microalgae communities,

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