



## Evaluation of microalgae cell disruption by ultrasonic treatment

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### HIGHLIGHTS

- ▶ Ultrasonic algae cell disruption was monitored by intracellular material release.
- ▶ Lipid–Nile red fluorescence or cell pigments were used to quantify cell disruption.
- ▶ The energy input to maximize cell disruption was approximately 800 J/10 mL.
- ▶ Sonication at increasing energy inputs induced the formation of free radicals.
- ▶ The formation of lipid hydroperoxides is shown but more in-depth study is needed.

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### ABSTRACT

Microalgae are a promising feedstock for biofuels because of their capability to produce lipids. Cell disruption is necessary to maximize lipid extraction. Sonication conditions were evaluated for breaking heterotrophic (*Schizochytrium limacinum*) and autotrophic (*Chlamydomonas reinhardtii*) microalgae cells. Cell disruption was estimated by Nile red–lipids fluorescence quantification in *S. limacinum* and by the release of intracellular chlorophyll and carotenoids in green microalga *C. reinhardtii*. In both species, approximately 800 J/10 mL was the energy input necessary to maximize cell disruption, regardless of the cell concentrations studied. Increasing sonication time produced increasing amount of free radicals, quantified by the formation of hydroxyterephthalate. Sonication energy beyond the level needed for cell disruption induced oxidation of arachidonic acid, a polyunsaturated fatty acid typically found in marine lipids. Careful control of sonication conditions is necessary to maximize oil extraction at the lowest operational cost and to prevent oil from free radical-induced degradation.

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## 1. Introduction

Because of their potential to produce oil and consume CO<sub>2</sub>, microalgae are being actively studied as a possible feedstock for biofuel production. These photosynthetic species have the advantage of capturing CO<sub>2</sub> from the environment and combustion processes (Chisti, 2007), thereby reducing greenhouse gases. Heterogeneous microalgae use reasonably inexpensive carbon sources for growth and can be used in both food and non-food applications (Chi et al., 2007; Johnson and Wen, 2009). There are a number of microalgal strains that have been found to produce 50% of their biomass as oil, mostly in the form of triglycerides (Chisti, 2007; Hu

et al., 2008; Pienkos and Darzins, 2009). These triglycerides are the starting material to produce high-density energy fuel such as biodiesel.

In order to increase the efficiency of the extraction of oil and other valuable components from algae, the cells need to be disrupted, just as the cell distortion by flaking for soybeans before solvent extraction (Johnson, 2008). Cell disruption can be assessed by several methods. Microscopic cell counting and flow cytometry are ways to measure cell disruption. The first one, however, is tedious and time-consuming while the second requires expensive equipment. Another way to measure cell disruption is by measuring the release of a major intracellular component such as lipid, especially if such a component is of interest. Nile red is a dye that can fluoresce emitting at 580 nm when excited at 529 nm in the presence of non-polar moieties such as triacylglycerol-rich droplets (Greenspan and Fowler, 1985). This property may be useful in the detection and quantification of intracellular non-polar materials released during the disruption process. In the case of photosynthetic algae species, chlorophyll and carotenoids, which are

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