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Energy efficient bead milling of microalgae: Effect of bead size on disintegration and release of proteins and carbohydrates

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HIGHLIGHTS

- Bead milling is an energy efficient and mild microalgae disintegration method.
- A smaller bead size results in a lower specific energy consumption.
- The specific energy consumption was decreased from >1.7 to <0.5 kWh kg_{DW}^{-1}.
- Product yields were unaffected using smaller bead sizes.

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ABSTRACT

The disintegration of three industry relevant algae (*Chlorella vulgaris*, *Neochloris oleoabundans* and *Tetraselmis suecica*) was studied in a lab scale bead mill at different bead sizes (0.3–1 mm). Cell disintegration, proteins and carbohydrates released into the water phase followed a first order kinetics. The process is selective towards proteins over carbohydrates during early stages of milling. In general, smaller beads led to higher kinetic rates, with a minimum specific energy consumption of ⩽0.47 kWh kg_{DW}^{-1} for 0.3 mm beads. After analysis of the stress parameters (stress number and stress intensity), it appears that optimal disintegration and energy usage for all strains occurs in the 0.3–0.4 mm range. During the course of bead milling, the native structure of the marker protein Rubisco was retained, confirming the mildness of the disruption process.

1. Introduction

There is a growing demand for sustainable protein sources and bio-based products as an alternative for traditional agricultural crops. Microalgae are a potential source of renewable high value proteins, carbohydrates, lipids and pigments for food, feed and chemical industries (Vanthoor-Koopmans et al., 2013). Such products are typically located intracellular, either in the cytoplasm, in internal organelles or bound to cell membranes, and in most cases, the cells need to be disintegrated before extraction. This step can be done by chemical hydrolysis (Safi et al., 2014), high pressure homogenization (Safi et al., 2014), ultrasonication (Grimi et al., 2014), pulsed electric fields (Goettel et al., 2013; Grimi et al., 2014; Postma et al., 2016) or bead milling (Doucha and Lívanský, 2008; Günerken et al., 2015; Montalescot et al., 2015; Postma et al., 2015).

Bead mills are commonly applied in the chemical industry for the manufacture of paints/lacquers and grinding of minerals (Kula and Schütte, 1987) and have been successfully applied for the disintegration of yeast (Bunge et al., 1992), cyanobacteria (Balasundaram et al., 2012) and microalgae (Günerken et al., 2016; Postma et al., 2015) for the release of intracellular products, under low energy inputs and mild conditions. The efficiency of cell disintegration in bead mills depends on several parameters such as chamber and agitator geometry, biomass concentration, agitator speed (i.e., tip speed of agitator), suspension flow rate, bead filling ratio, bead type and bead diameter. A high bead filling ratio (>55% v/v) was found to be optimal for disruption according to

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Abbreviations

A	peak area [AU]
C	constant [–]
C_i	concentration of component i in supernatant [g L^{-1}]
$C_{i,Biomass}$	total concentration of component i in biomass [g L^{-1}]
C_V	volume cell concentration [–]
d_b	bead diameter [m]
E_M	specific energy consumption [kWh kg_{DW}^{-1}]
$\tilde{E}_M$	theoretical specific energy consumption [kWh kg_{DW}^{-1}]
$E_{M,3\tau}$	specific energy consumption at 3τ [kWh kg_{DW}^{-1}]
$E_{M,min}$	minimal specific energy consumption [kWh kg_{DW}^{-1}]
k_{carb}	carbohydrate release first order kinetic constant [s^{-1}]
k_{dis}	disintegration first order kinetic constant [s^{-1}]
k_{prot}	protein release first order kinetic constant [s^{-1}]
M	mass of biomass on dry weight [kg]
n	agitator speed (revolutions) [s^{-1}]
SN	stress number [–]
SN_D	reduced Stress number for disintegration [–]
SN_G	reduced Stress number for grinding [–]
SI	stress intensity [J/Nm]
SI_{opt}	optimal stress intensity [J/Nm]
t	disruption/milling time [s]
u_s	agitator tip speed [m s^{-1}]
V	volume [mL]
X_i	degree of disintegration (Dis), protein concentration or carbohydrate concentration [–]
$X_{i,max}$	maximal degree of disintegration (Dis), protein concentration or carbohydrate concentration [–]
Y_{carb}	carbohydrate yield [%]
Y_{prot}	protein yield [%]
ε	bead bulk density [kg m^{-3}]
φ_b	bead filling ratio [–]
ρ_b	specific density beads [kg m^{-3}]
τ	characteristic time of process kinetic [s]

Montalescot et al. (2015). In addition, Doucha and Lívanský (2008) found that zirconium oxide (ZrO_2) beads are more efficient than glass beads because of their higher specific density. Postma et al. (2015) investigated the disintegration of *C. vulgaris* by bead milling at lab scale using ZrO_2 beads with a diameter of 1 mm (65% v/v bead filling) and found that an agitator speed u_s of 6 m s^{-1} provides a lower specific energy consumption; though with a biomass concentration of 145 g kg_{DW}^{-1} a lower specific energy input could be obtained, a concentration of 87.5 g kg_{DW}^{-1} showed to have a better biomass suspension handling and higher protein yields.

Furthermore, for the disintegration of the microalga *Chlorella* sp. it was found that similar specific energy consumptions were achieved for the same flow rate, biomass concentration and agitator speed for beads of 0.3–0.4 and 0.6–0.8 mm (Doucha and Lívanský, 2008). On the other hand, the disintegration of the microalga *Scenedesmus* sp. and *Nannochloropsis oculata* was improved when smaller beads (0.35–0.6 mm) were applied (Hedenskog et al., 1969; Montalescot et al., 2015).

Bunge et al. (1992) studied the release of enzymes from *Arthrobacter* by means of bead milling. It was found that small glass beads (Ø 0.205–0.460 mm) at moderate to high biomass concentrations and low to moderate agitator speeds result in optimal energy utilization. Schütte et al. (1983) found that smaller beads (0.55–0.85 mm) are more beneficial to release intracellular products from the cytoplasm of yeast over larger beads (1 mm). On the other hand, the larger beads are better at releasing products from the periplasm.

To describe the comminution of cells in bead mills as a function of different process parameters, Kwade and Schwedes (2002) and Bunge et al. (1992) presented a very clear description of the so-called Stress Model (SM). The SM assumes that the disruption process in stirred media mills (e.g., bead mill) is governed by the number of stress events (i.e., bead to bead collisions) and by the intensity of such events. Quantitatively, this is expressed by the Stress Number (*SN*) (Eqs. (1) and (2)) and the Stress Intensity (*SI*) (Eq. (4)) (Bunge et al., 1992; Kwade and Schwedes, 2002); two types of behaviors are also recognized: 1) disintegration/deagglomeration of cells, characterized by the fact that a cell is either intact or disrupted; and 2) grinding of crystalline materials, applicable for materials in which the particle size decreases during the milling process.

Accordingly, the *SN* (–) can be calculated for Disintegration (SN_D) and for Grinding (SN_G) as:

$$SN \propto \frac{\varphi_b(1-\varepsilon)}{\{1-\varphi_b(1-\varepsilon)\}c_V}\frac{nt}{d_b} \propto C \cdot SN_D \quad (1)$$

$$SN \propto \frac{\varphi_b(1-\varepsilon)}{\{1-\varphi_b(1-\varepsilon)\}c_V}\frac{nt}{d_b^2} \propto C \cdot SN_G \quad (2)$$

with

$$C = \frac{\varphi_b(1-\varepsilon)}{\{1-\varphi_b(1-\varepsilon)\}c_V} \quad (3)$$

where φ_b is the bead filling ratio (–), ε is the bead bulk porosity (–), c_V the volume cell concentration (–), n the agitator revolutions (s^{-1}), t the milling time (s) and d_b the bead diameter (m).

Furthermore, the *SI* (Nm) can be regarded as the magnitude of the kinetic energy of a single bead and can be calculated as:

$$SI \propto d_b^3 \rho_b u_s^2 \quad (4)$$

in which ρ_b is the specific density of the beads (kg m^{-3}) and u_s is the agitator tip speed (m s^{-1}). A cell can only be intact or disintegrated upon the release of the intracellular products. Therefore, an optimal stress intensity SI_{opt} can be considered. At or above SI_{opt} cells break with a single stress event; below SI_{opt}, multiple stress events are required to break the cell.

Consequently, the theoretical specific energy input is proportional to the product of the number of stress events times the energy of such events:

$$\tilde{E}_M \propto \frac{SN \cdot SI}{M} \quad (5)$$

where M is the mass of biomass (kg_{DW}) in the system and $\tilde{E}_M$ is the theoretical specific energy input (kWh kg_{DW}^{-1}).

The SM was first applied to microalgae by Montalescot et al. (2015). However, to our knowledge, it has not been applied in combination with the release of water soluble microalgae components. In large scale disruption trials for yeasts and bacteria, Schütte et al. (1983) observed that cytoplasmic enzymes were better solubilized by smaller beads, and that periplasmic enzymes were more easily released by larger beads. We therefore hypothesize that smaller beads could interact more effectively with internal organelles over larger beads and thus are better able to release proteins (e.g., Rubisco) from the pyrenoids and carbohydrates from the cell wall or starch granules. If the process is operated above SI_{opt}, smaller