Monitoring cell-specific neutral lipid accumulation in Phaeodactylum *tricornutum* (Bacillariophyceae) with Nile Red staining - a new method for FlowCam

Journal of Phycology
2016 | Impact Factor 2.239 | Peer-Reviewed

Katariina Natunen, Jukka Seppälä

The objective of this work was to investigate whether cell-specific phytoplankton lipid accumulation could be monitored with the image-based particle analyser FlowCam and NR [nile red] staining. Applying Phaeodactylum tricornutum as a model species, we compared the FlowCam method to two established lipid quantification methods: spectrofluorometric NR fluorescence measurement and total lipid analysis by gas chromatography.... We showed significant correlation between the three different lipid quantification methods confirming the applicability of the novel FlowCam method in cell-specific and near real-time lipid quantification.

With the lipid-soluble fluorescent dye NR, phytoplankton lipids can be measured rapidly and in situ, based on the linear relationship between the amount of lipids and the NR fluorescence intensity. ... The fluorescence from polar membrane lipids and neutral storage lipds can be distinguished from each other.

The primary results describe cell-specific variations in lipid content, and when supplemented with data of cell counts, also measured with FlowCam, an estimate of lipid concentration in a sample is obtained.

Early detection and quantification of zooplankton grazers in algal cultures by FlowCam

Algal Research
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Yifei Wang, Maria Castillo-Keller, Everett Eustance, Milton Sommerfeld

There is an urgent need for the development of methods and procedures for early detection and quantification of zooplankton in algal cultures. In this study, the FlowCam was able to detect and quantify Brachionus calyciflorus, a model organism in algal cultures with a detection limit of <1 individual/mL for B. calyciflorus in an algal (Chlorella sp.) cell density of 10^7 cells/mL. The methodology also allowed successful monitoring of rotifer population growth at low density (<1 individual/mL of B. calyciflorus) in dense algal cultures. Furthermore, the methodology was also effective in detecting and quantifying other zooplankton grazers including ciliates, Poterioodhromonoas sp., and Euplotes sp. In outdoor open raceway cultures.

DISCUSSION Ciliates, Poterioochromonas sp., and Euplotes sp. were used to assess the accuracy of enumeration by the FlowCam, compared to microscopy.... When the density was lower than 10 individuals/mL, the FlowCam could still detect and quantify those protozoa species in the algae culture, while the hemocytometer method typically could not, let alone quantify the contaminant. This indicates that the FlowCam could provide a 3- to 5-day earlier warning system for the producers of

algae biomass to take effective actions to control the contaminating grazers and prevent culture crash.

Effect of *Brachionus rubens* on the growth characteristics of various species of microalgae

Electronic Journal of Biotechnology
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Reda A.I. About-Shanab, Manjinder Singh, Anangelica Rivera-Cruz, Grace Power, Thomas Bagby-Moon, Keshav Das

"A major disadvantage of outdoor cultivation is susceptibility of algal crops to attack by predatory rotifers. In order to quantify the impact of rotifer attack on different species of algae, we evaluated the growth of eleven microalgal species over a 21-d period after being infected by the predatory rotifer *Brachionus rubens*."

METHOD "We use[d] a digital flow cytometer (FlowCam) to measure the changes in cell density (number of cell/mL), cell size and shape of microalgal species in the culture medium, and quantify the effects of the presence of rotifers on growth characteristics (such as cell densities and biomass productivity) of eleven different microalgal species (green and cyanobacteria)." Measured values of algal biomass dry cell density (g/L) were related to the measured algal cell counts (cell/mL) using a linear regression.

DISCUSSION "The system clearly differentiated between particles of different diameter as equivalent spherical diameter (ESD), length, and width."

Sorting cells of the microalga *Chlorococcum littorale* with increased triacylglycerol productivity

Biotechnology for Biofuels
2016 | Impact Factor 6.44 | Peer-Reviewed

Iago Teles Domingues Cabanelas, Mathijs van der Zwart, Dorinde M. M. Kleinegris, Rene H. Wijffels and Maria Barbosa

"Strain improvement has multiple approaches that could be used: selection of cells, adaptive laboratory evolution, random mutagenesis and genetic manipulation.... We present a strategy to find and sort microalgae cells with increased TAG productivity."

METHOD "FlowCam...was used to complement the assessment of the population characteristics, providing the actual cell diameter (µm) and photomicrographs of the cells.... [The] FlowCam was used daily [to measure] autofluorescence (chlorophyll fluorescence) and lipid-dependent fluorescence (BODIPY 505/515 fluorescence).... Bodipy (505/515) is a lipophilic fluorophore that binds to neutral lipids, being a relative measurement of total TAGs.

"Cell analysis from the FlowCam were exported to Microsoft Excel to plot graphs and estimate descriptive statistics.... We can conclude, combining all results above, that the sorting carried out in the present research was successful in producing new cell lines with increased lipid productivity."

Low-Energy Input Continuous Flow Rapid Pre-Concentration of Microalgae through Electro-Coagulation Flocculation

Chemical Engineering Journal
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Teodora Rutar Shuman, Gregory Mason, Daniel Reeve, Alexander Schacht, Ann Goodrich, Katerine Napan, Jason Quinn

Microalgae cells are small (typically 1-10 µm) and grow in

low concentration. These biological parameters raise operational costs for algae producers by increasing the input of energy and water, as well as harvest time. Teodora et al. studied alternatives to current systems by "rapidly [processing] algae in a continuous flow process and [pre-concentrating] them in a separate collection vessel…. The effect of the ECF process on cell viability was examined using optical data collected using a Fluid Imaging Technologies FlowCam® to quantify fluorescence from Sytox Green Nucleic Acid Stain (Invitrogen, Molecular Probes). Sytox Green penetrates cells with compromised plasma membranes and does not cross the membranes of live cells."

DISCUSSION "The ECF Efficiency is seen to rapidly increase in the first 30 min after treated saltwater is mixed with algae suspension…. Most of the settling occurs in the first 5 min. This is a desirable result for commercial applications of a continuous flow apparatus…. Once the treated saltwater is mixed into the algae solution, algae flocs begin to form, seen with FlowCam and the hydroxides collect flocs and sweep them to the bottom in fairly short time."

"The data show that "increased input voltage and slower flow rate reduce algal cell viability and that samples do not tend to recover over time. At higher flow rates and lower voltages, the number of viable cells increases over time along with an observed increase in OD. The increase in OD is attributed to regrowth and not biological contamination as no other cells were observed on FlowCam® photos."