New Methods of Quantifying Microalgae in Biofuel Production and Wastewater Treatment

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Background

There is growing interest in the use of microalgae for wastewater treatment and biofuel production. However, there is not yet a reliable, user-friendly method of quantifying the biomass and lipid production of a large sample of algae. This study aims to establish and validate new methods for algae quantification using the Microplate reader and the Coulter Counter.

1. Microalgae and wastewater

Commercial algae production for biofuels can be too cumbersome and expensive to present a viable fuel source. This problem could be partially mitigated by extracting nutrients from domestic wastewater for algal growth. Combining algae production for biofuels and wastewater treatment has the following benefits:

- Reduce water requirement
- Eliminate need for fertilizer input
- Reduce land use
- Defray cost of water treatment

2. *Chlorella vulgaris*

Chlorella vulgaris was chosen in this research for the following reasons:

- Small, spherical, relatively uniform cells
- High lipid production
- Ability to remove nutrients and metals from wastewater

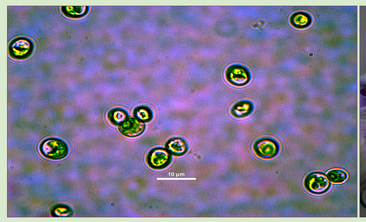


Figure 1. Microscopy picture of *C. vulgaris*, the green microalgae used in this study.

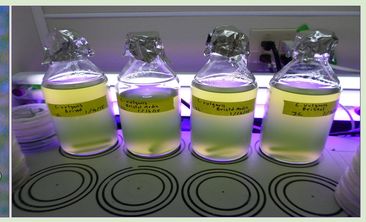


Figure 2. Algae cultivation used in this study. Each inoculated bottle was capped with an autoclaved cotton ball and aluminum foil. The culture was maintained on a stir plate in front of 3 high-output lights in a 25-26°C room.

Methods and Materials

The Microplate reader and Coulter Counter were chosen in this study as the new algae quantification methods. These methods were compared with traditional methods based on data accuracy and reliability (via standard deviation and r^2 values), required sample volume, required volume of hazardous chemicals, and processing time.

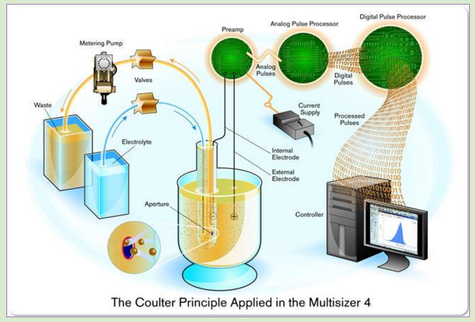


Figure 3. The Coulter principle applied in the Beckman Multisizer 4 Coulter Counter (Image from Beckman Coulter). It was used for measuring the number and concentration of *C. vulgaris* cells.

Traditional Methods	New Methods

Biomass/Cell Count

Microscope Method

- Vacuum filter 1 mL sample
- Multiple pictures taken at 100x magnification
- Results requires further calculation

Coulter Counter Method

- 100 µL or less sample required
- Analyzed with 100 µm aperture
- Particles between 2.2 and 7 µm considered *C. vulgaris* cells

Chlorophyll Measurement

Spectrometer Method

- Vacuum filter 50 mL sample
- Filters placed in 13 mL 90% acetone
- Vortexed, sonicated, and centrifuged
- Analyzed by spectrometer

Microplate Method

- 100 µL or less sample required
- Analyzed for fluorescence at 440 nm/685 nm by microplate reader.

Lipid Production

Modified Bligh/Mujtaba Method

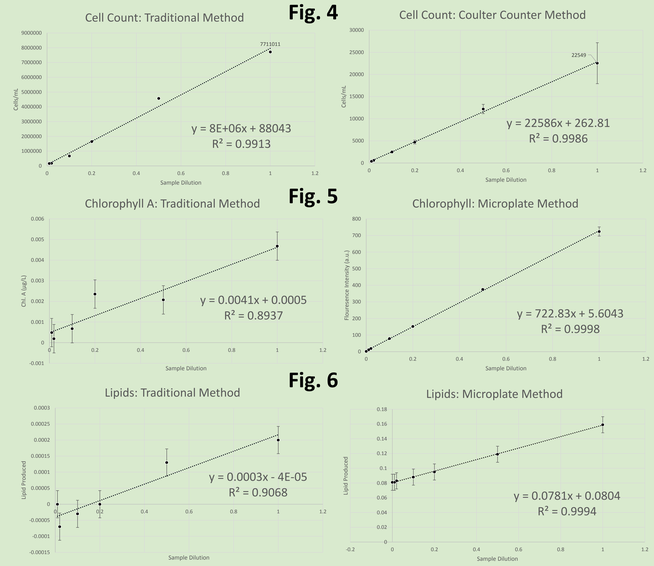
- 50 mL sample
- Disrupt cells and extract lipids
- Chloroform-based extraction
- Dry and weigh lipids

Microplate Analysis

- 100 µL of sample required
- 100 µL Red Nile Dye
- Analyzed for fluorescence at 530 nm/570 nm by microplate reader.

Results

Results showed these new methods can provide accurate and reliable data for algae quantification (Figures 4-6), and a summary for method comparison is shown below (Table 1).



Figures 4-6. Cell count (Fig. 4), chlorophyll (Fig. 5), and lipid (Fig. 6) measurements are shown at various algae concentrations. With traditional methods shown in Figures a (left) Microplate and Coulter Counter methods shown in Figures b (right). Statistical R-values and relative standard errors are also shown here.

Table 1. A comparison between the traditional and novel methods for microalgae quantification.

Parameters	Method/Instrument	Volume Req. (mL)	Time Req.	Chemicals Req.
Chlorophyll A	SM 10200-H, ESS 150.1 / Spectrophotometer	50	2 days	13 mL 90% Acetone
Chlorophyll	Held / Microplate	0.1*	1 minute	None
Cell Count	Coulter Counter	0.1	5 min/sample	9.9 mL Isotone
Cell Count	Microscope Method	1 to 2	3 hours	None
Lipids	Modified Bligh-Mutjaba	50	2 days	2.5 mL Chloroform, 3 mL Methanol
Lipids	Held/Microplate	0.1*	12 minutes	100 µL Nile Red Dye
* These variables are measured using the same 0.1 mL of sample.				

Acknowledgements

The authors would like to thank Arkansas Biosciences Institute, the SURF Grant Program, and the University of Arkansas Doctoral Academy Fellowship for their financial support. We would also like to thank Connie Moloney for her assistance in the lab.