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Laser reflectance measurement for the online monitoring of *Chlorella sorokiniana* biomass concentration

Patricio López Expósito*, Angeles Blanco Suárez, Carlos Negro Álvarez

Chemical Engineering Department, Complutentse University of Madrid, Faculty of Chemical Sciences, Avda. Complutense s/n, Madrid 28040, Spain

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ABSTRACT

Fast and reliable methods to determine biomass concentration are necessary to facilitate the large scale production of microalgae. A method for the rapid estimation of *Chlorella sorokiniana* biomass concentration was developed. The method translates the suspension particle size spectrum gathered though laser reflectance into biomass concentration by means of two machine learning modelling techniques. In each case, the model hyper-parameters were selected applying a simulated annealing algorithm. The results show that dry biomass concentration can be estimated with a very good accuracy ($R^2 = 0.87$). The presented method seems to be suited to perform fast estimations of biomass concentration in suspensions of microalgae cultivated in moderately turbid media with tendency to aggregate.

1. Introduction

The production of microalgal biomass at industrial scale requires fast, reliable methods to monitor the biomass concentration (BC) of the cultures. The gravimetric determination of BC is very time consuming since it requires sampling, centrifugation, drying and weighting. Indirect determinations of BC are preferred at industrial scale since they are faster. Most indirect determination methods rely on establishing a calibration that associates BC with certain optical properties of the medium, such as light attenuation or light scattering (Havlik et al., 2013). The correlation of the medium optical density (OD) with the dry weight concentration is the most popular method to quantify microalgal biomass because it can easily be implemented online. The method has, however, several drawbacks: OD measurements are affected by changes in shape, size of particles due to aggregation of cells, and biomass (Chioccioli et al., 2014); in highly concentrated algal suspensions, OD yields inaccurate measurements due to the shadowing effect of cells; changes in the quantity and type of pigments produced inside microalgal cells, which depend on the cultivation conditions and growth phase may induce inaccuracies in the measurements (Griffiths et al., 2011); changes in culture medium turbidity may also lead to false estimations of biomass concentration (Marose et al., 1999). This latter aspect is of special relevance given the current interest in producing microalgal biomass employing wastewater or process water as cultivation medium (Caporgno et al., 2015; Chiu et al., 2015). In such productions, medium turbidity is usually not-negligible and it may vary in the course of the cultivation influencing the measurements. Although measuring OD of the medium at certain wavelengths may eliminate the effects of changing microalgal pigments, the source of inaccuracies entailed by the other factors cannot be avoided. On the other hand, the literature identifies the processing of chord length distribution (CLD) data to estimate biomass concentration as a potential method to develop new on-line monitoring techniques for bioprocesses (Höpfner et al., 2010). The determination of chord length distribution of particulate suspensions are commonly based on optical sizing techniques such as laser diffraction, dynamic laser scattering and laser reflectance. Although laser diffraction performs accurately over a wide range of particle sizes, it tends to produce inaccurate measurements at small size flocs, it is affected by particle irregular shapes (Govoreanu et al., 2009) and requires low density suspensions <1% vol. of particles (Heinrich and Ulrich, 2012). The sizing through dynamic light scattering also requires low concentration of particles in the suspension and the measurement is affected by submicron sized particles. Laser reflectance tech-

Abbreviations: BC, biomass concentration; COD, chemical oxygen demand; CLD, chord length distribution; MSE, mean squared error; NTU, nephelometric turbidity unit; OD, optical density; ppm, parts per million; PBR, photobiorreactor; RF, random forest; rpm, revolutions per minute; RCF, relative centrifugal force; SVR, support vector regression; TAP, tris acetate-phosphate medium.

* Corresponding author.
E-mail address: plopezex@ucm.es (P. López Expósito).

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niques are especially suited to monitor microalgal cultures given that these techniques have a very ample measuring range, from particulate densities of 0.1–40% vol. (Preikschat and Preikschat, 1991; Mersmann, 2001) and can detect particles over 1 μm. Moreover, although laser reflectance measurements tend to downsize the particles measured, they are not so affected by the shape factor of particles as laser diffraction and yield accurate measurements in high density suspensions. These techniques are therefore promising to estimate dry biomass concentration, as we have shown in our previous work (Lopez-Exposito et al., 2015). In that investigation the biomass concentration of TAP cultivated *Chlamydomonas reinhardtii* was estimated at different states of aggregation up to floc sizes smaller than 1 mm. The mentioned method, based on artificial neural networks, required a relatively long period of calibration and a large number of data points to train the system adequately. To facilitate the implementation of these on-line methods at industrial scale, in this work we study the estimation of dry BC of *Chlorella sorokiniana* suspensions based on laser reflectance by means of machine learning techniques that require very low number of data points to construct the models. This latter aspect implies shorter data collection and calibration times. Furthermore, the present work widens the scope of the estimations of our previous study to microalgal aggregate sizes over 2 mm.

2. Materials and methods

2.1. Microalgal cultures

A strain of *Chlorella sorokiniana* (CCAP No. 211/8 K) was cultivated in tris-acetate-phosphate medium (TAP medium) (Gorman and Levine, 1965) in 250 mL shake flasks (115 rpm, 23 °C and 12 h cool while light). When the cultures reached a dry biomass concentration of around 1 g/L the contents of 4 flasks were inoculated to a 5.5 L photobiorreactor (PBR) operated with 0.22 μm filtered wastewater (COD = 219 mg L^{-1}, Turbidity = 387 NTU) coming from an anaerobic digestion reactor. The culture was maintained at a temperature between 23–25 °C with aeration (2 L min^{-1}) and pH 7.5. pH was controlled through the automatic supply of CO_2 (0.2 L min^{-1}). Light was provided by means of four fluorescent cool while light in a 12 h cycle.

2.2. Flocculant

Crab shell chitosan (Sigma-Aldrich) was employed as promoter of algal cells aggregation. A stock solution of the flocculant was produced dissolving powder chitosan in a 1% solution of glacial acetic acid. The solution was mechanically stirred for 1 h at 400 rpm and left to settle for 24 h prior to its use. The solution was used for a period of two months.

2.3. Culture sampling and measuring of dry biomass concentration

2 L samples were taken from the PBR and maintained in agitation by means of a mechanic stirrer at 200 rpm. From the bulk sample, 20 mL aliquots were taken for the determination of dry BC. Dry biomass was determined in triplicate according to the method described in (Lopez-Exposito et al., 2015). Each sample was centrifuged in a Hettich Universal 320 centrifuge at 6800 RCF for 15 min and washed twice with ultrapure water. The biomass pellets obtained were dried at 101.5 °C for 3 h on previously weighted aluminium dishes. After drying, the dishes were placed in a desiccator for 45 min and then weighted again. The microalga dry biomass concentration was calculated as g per litre of culture medium.

2.4. Characterisation of the cell cultures through chord length distribution

CLD data were gathered by means of a Particle Track 400 (PT400, Mettler-Toledo). The device consists of a probe that projects a rotating laser beam in the medium bulk and detects its reflectance. The occurrence of a reflection is interpreted as a particle by the device software and, given that the beam rotating speed is fixed and known, the apparent length of the particles is calculated based on the time for which the particle is reflecting the incident beam. The device can detect particles of lengths ranging from 1 to 4000 μm. In our study, the PT400 software was set to classify the chord lengths gathered in 200 logarithmic intervals. Nineteen *Chlorella sorokiniana* suspensions of different concentration were considered. For each chord length data collection, a microalgal suspension sample of 200 mL was taken and placed in a 250 mL beaker. The samples were stirred mechanically at 200 rpm.

In order to reproduce situations of non-univocal correspondence between BC and CLD, fresh microalgal samples were partially flocculated with different doses of 1% chitosan, namely 10, 20, 30 ppm. In this way we generated different instances of aggregation data corresponding to the same biomass concentration.

The CLD data of each sample, fresh or flocculated, were gathered every 10 s for a period of 7 min. Each CLD data point obtained consisted of a vector of 200 elements. Each element in the vectors represented to the number of particles detected in the corresponding logarithmically spaced length interval spanning 1–4000 μm. The data for each culture was averaged over 39 consecutive measurements (those after taking out the two first and last points of the 42 measurements obtained in the 7 min sampling time). The dataset consisted of 76 input vectors of 200 parameters corresponding to 19 biomass concentrations, each at 4 aggregation states (one initial culture and 3 partially flocculated cultures), and an output of 76 biomass concentrations having 19 different values.

3. Laser reflectance data processing

Laser reflectance probes operate projecting a rotating laser beam on the sample bulk and measuring the time that the beam is reflected back (time of transition) when intercepting a particle. The number of separate reflecting events per unit of time is associated with the number of particles in the medium. Given the rotation speed is known and much higher than that of the particle (in our case 2 m s^{-1}), with the time of transition it is possible to calculate the length of the particle chord traversed by the beam. The information gathered through the probe enables the construction of a distribution of chord lengths.

This method presents, however, some disadvantages. The most important one is the fact that the probability of a particle being detected by the laser is proportional to its size. Moreover, given that the particles pass by the lens in a random fashion, the segment of the particle surface intersected by the laser will also be random, what implies that the particle lengths gathered by the probe will be downsized. Secondly, chord length distributions of microalgal suspensions are highly heterogeneous in terms of size and shape, i.e. in the same culture it is possible to encounter isolated cells of approximately spherical shape and cell aggregates of irregular shape.

The implementation of a semi-analytical method to estimate dry biomass from CLD would require to know how it translates into the actual particle size distribution (PSD). Agimelen et al. (2015) devised an algorithm capable of getting size distribution and shape from CLD data without additional information. The algorithm, however, applies to cases in which the aspect ratio of particles is homogeneous. In the case concerned, the dispersions of microal-