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Optimisation of microalgal starch formation for the biochemical production of biobutanol

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1. Introduction

Global warming and crude oil depletion have shifted research and industry attention to the area of biofuels and their development. The current sugar-based biofuel market has been dominated by bioethanol, but a higher energy content and compatibility with...
existing infrastructure make biobutanol a more suitable candidate for the energy sector (Kumar and Gayen, 2011). Biobutanol is biochemically produced by the ABE (Acetone-Butanol-Ethanol) fermentation, a microbial process whereby Clostridia species metabolise carbohydrates into fermentation solvents. Although food-based and lignocellulosic feedstocks have been commonly studied for their viability as fermentation substrates, these sugar-rich compounds create challenges due to their competition with food destined for human use and arable land. Recently, microalgae have been considered as a suitable ABE fermentation substrate due to their ability to accumulate starch. Nutrient-limited strategies, such as nitrogen and phosphorus deprivation, have been successfully employed to further increase starch accumulation in microalgal cells, but a simultaneous trade-off in biomass production affects overall productivity (Markou et al., 2012). Optimisation of the microalgal cultivation stage is necessary to establish the optimal initial nutrient concentrations for the formation of starch. Thus, in this work, an integrated experimental and computational study was undertaken to develop a multi-parameter kinetic model for the prediction of starch formation in response to nitrogen, phosphorus, and acetate (carbon) concentrations.

2. Materials and Methods

2.1. Strain and lab-scale cultivation

Lab-scale experiments were carried out with the wild-type strain Chlamydomonas reinhardtii CCAP 11/32c, grown mixotrophically in Tris-Acetate-Phosphate (TAP) medium. In order to evaluate nutrient-limitation and calibrate the proposed model, six different cultures were grown under various nitrogen (0.3543 - 0.3824 gN L^{-1}), phosphorus (0.01 – 0.1 gPO_4 L^{-1}), and acetate (0.42 – 0.84 gC L^{-1}) concentrations. Culture vessels contained 500 mL of medium and 1 mL of algal inoculum, and were kept at 25 °C and 150 rpm while providing constant illumination of 125 μmol m^{-2} s^{-1} in a light/dark cycle of 16/8 hours. Samples of the biomass and residual medium were taken at regular intervals throughout the cultivation period until cells attained the stationary phase.

2.2. Analytical methods

The cell dry weight was determined gravimetrically by drying the wet biomass for 24 h at 70 °C. Starch concentration was measured by means of a colorimetric Total Starch Kit as per the method supplied by Megazyme International, Ireland. Lipid concentration was quantified by solvent extraction in a SOXTEC Unit 1043, where oil extraction was carried out for 2h using hexane at 160 °C. Extraction was then followed by rinsing and evaporating stages of 20 min and 30 min, respectively. Extracted oil was then measured gravimetrically. The residual nutrient concentrations were determined as follows: nitrogen was measured in a TOC-V,C,N,Sh/TNM-1, Shimadzu (Total Organic Carbon/Total Nitrogen); phosphorus was quantified in a Varian Vista MPX ICP-OES at λ=213nm (Inductively Coupled Plasma - Optical Emission Spectroscopy); acetic acid was quantified by HPLC (High Pressure Liquid Chromatography) in a Hi-Plex column (8 m, 300x7.7mm) coupled with a UV detector at λ=210 nm. H_2SO_4 5mM was used as mobile phase at 50 °C and a flow rate of 0.6 mL min^{-1}.

3. Kinetic Modelling

The multi-parameter model presented here is based on our previous modelling work (Figueroa-Torres et al., 2016), which was expanded to account for phosphorus-limited
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growth. The model considers the following 9 state variables: Total biomass (X, gC L⁻¹), starch (S, gC L⁻¹), lipids (L, gC L⁻¹), active biomass (x*, gC L⁻¹), nitrogen (N, gN L⁻¹), nitrogen quota (q_N, gN gC⁻¹), phosphorus (P, gPO₄ L⁻¹), phosphorus quota (q_P, gPO₄ gC⁻¹), and acetate (A, gC L⁻¹). All kinetic parameters are defined and presented in Table 1. Following the approach of Mairet et al. (2011), total biomass is made up of three carbon-based elements: starch, lipids, and active biomass (i.e. X = S + L + x*). Starch and lipid dynamics incorporate formation and degradation rates and depend mainly on the internal nitrogen (N_int = q_N X) and carbon (A_int = A − A_s) concentrations. Accumulation rates for these elements are described by Eq.(1) – Eq.(4).

\[
\frac{dX}{dt} = \mu \cdot X
\]  
(1)

\[
\frac{dS}{dt} = r_1 \cdot \frac{N_{int}^{n_s} \cdot k_1}{N_{int}^{n_s} + K_{s,n_s} + (N_{int}^{n_s}/k_{i,s})} \cdot \left(1 + \frac{1}{\mu} \cdot e^{q_s \cdot A_m} \right) \cdot \mu \cdot X^* - \frac{r_2}{q_N} \cdot X
\]  
(2)

\[
\frac{dL}{dt} = r_3 \cdot \frac{N_{int}^{n_L} \cdot k_2}{N_{int}^{n_L} + K_{L,n_L} + (N_{int}^{n_L}/k_{i,L})} \cdot \left(1 + \frac{1}{\mu} \cdot e^{q_L \cdot A_m} \right) \cdot \mu \cdot X^* - \frac{r_4}{q_N} \cdot X
\]  
(3)

\[
\frac{dx^*}{dt} = \frac{dX}{dt} - \frac{dS}{dt} - \frac{dL}{dt}
\]  
(4)

Here, a new mathematical expression for the algal specific growth rate, \( \mu \), was constructed so as to portray mixotrophic microalgal growth under either nitrogen or phosphorus limitation. Mixotrophic conditions are expressed by the sum of the heterotrophic and phototrophic growth rates, \( \mu_H \) and \( \mu_s \), both modelled as Haldane functions to account for inhibition. The growth rates limited by nitrogen and phosphorus, \( \mu_N \) and \( \mu_P \), are modelled as Droop functions subject to a minimum law as shown in Eq.(5).

\[
\mu = \mu_{\text{max}} \cdot \left[w_H \cdot \mu_H(A) + w_I \cdot \mu_I(I)\right] \cdot \min\left[\mu_N(q_N), \mu_P(q_P)\right]
\]  
(5)

\[
\mu = \mu_{\text{max}} \cdot \left[w_H \cdot \frac{A}{A + K_{s,a} + A^2/k_{s,a}} + w_I \cdot \frac{I}{I + K_{I,I} + I^2/k_{I,I}}\right] \cdot \min\left(1 - \frac{q_{N,0}}{q_N}, 1 - \frac{q_{P,0}}{q_P}\right)
\]  
(6)

In Eq. (5), \( w_H \) and \( w_P \) are weighing functions accounting for the contribution of the heterotrophic or phototrophic rates to overall growth. Light distribution, \( I \), along the culture vessel (with a depth equivalent to \( z \), m) is described by the Beer-Lambert law:

\[
I = I_o \exp(-\alpha z)
\]  
(7)

Consumption of acetate, nitrogen, and phosphorus over the cultivation period is described by Eq.(8) - Eq.(10), respectively.
\[
\frac{dA}{dt} = - \frac{1}{Y_{X/A}} \cdot \frac{\mu_H(A)}{\mu_H(A) + \mu_I(I)} \cdot dX
\]  

(8)

\[
\frac{dN}{dt} = -\rho_N \cdot X = -\frac{\rho_{N,\text{max}}(X)}{N + K_{s,N} + N^2/k_{i,N}} \cdot \frac{A}{A + K_{s,E,N} + A^2/k_{i,E,N}} \cdot X
\]  

(9)

\[
\frac{dP}{dt} = -\rho_P \cdot X = -\frac{\rho_{P,\text{max}}(X)}{P + K_{s,P} + P^2/k_{i,P}} \cdot \frac{A}{A + K_{s,E,P} + A^2/k_{i,E,P}} \cdot X
\]  

(10)

The specific uptakes rates of nitrogen and phosphorus, \(\rho_N\) and \(\rho_P\), are expressed by inhibited-type kinetics so as to account for the inhibition of growth observed at high nutrient concentrations. Furthermore, since nutrient consumption was found to stop at high cell densities, the maximum uptake rates of nitrogen and phosphorus are both regulated by a decreasing function of total biomass, as shown in Eq.(11) and Eq.(12). In line with experimental observations, the maximum nitrogen uptake is additionally a function of both the initial nitrogen concentration and the phosphorus quota.

\[
\rho_{N,\text{max}}(X) = \rho_{N,\text{max}} \cdot e^{-\phi_X} \cdot \frac{N_0^n}{N_0^n + K_{N_0}^n} \left(1 - \frac{K_{D_P}}{q_P}\right)
\]  

(11)

\[
\rho_{P,\text{max}}(X) = \rho_{P,\text{max}} \cdot e^{-\phi_X}
\]  

(12)

Finally, the dynamics of the intracellular nitrogen and phosphorus quotas are obtained by differentiation with respect to time, which yields:

\[
\frac{dq_N}{dt} = \rho_N - q_N \cdot \mu
\]  

(13)

\[
\frac{dq_P}{dt} = \rho_P - q_P \cdot \mu
\]  

(14)

4. Results and discussion

From the six lab-scale batch cultures grown under various N-P-A regimes, four experimental datasets were used to fit model parameters and two were used for validation. The values of each parameter (Table 1) were estimated by minimizing an objective function defined as the sum of squared relative error (Error) between experimental data and model predictions. Minimization was carried out through a stochastic optimization algorithm coupled with a deterministic method as per the methodology of Vlysidis et al., (2011). The results from the preceding method are presented in Figure 1, showing predicted time-profiles of biomass, starch and lipids against their corresponding experimental values only for two different datasets.

Table 1. Kinetic parameters: definitions and corresponding values.
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As seen in Figure 1, a good level of agreement between predictions and experimental data was achieved (Error = 1.956), indicating the model’s potential as a robust tool in the establishment of optimized cultivation strategies. Given that microalgal starch is a potential substrate for biobutanol production, a cultivation strategy for optimal starch formation was established in terms of the initial set of nitrogen, phosphorus, and acetate (N-P-A) concentrations.

![Figure 1](image)

Figure 1. Predicted time-profile for biomass, starch, and lipids, against experimental data. *Fitting*: culture grown in TAP (0.3824 gN L⁻¹, 0.1 gPO₄ L⁻¹, and 1.05 gC L⁻¹); *Validation*: culture grown in A-enrichment and P-limitation (0.3824 gN L⁻¹, 0.01 gPO₄ L⁻¹, and 1.26 gC L⁻¹). Error bars are the standard deviation from two replicates.

The optimal N-P-A set was identified in a ternary diagram for starch formation, which was constructed from the predictions generated by the validated model at a cultivation time of 190 h (period of stationary phase). The resulting diagram is presented in Figure 2, showing that a starch concentration of up to 0.06 gC L⁻¹ could be obtained by *C. reinhardtii* cultures if grown under the encircled N-P-A concentration sets. When compared with the starch concentration attained by cultures grown in standard TAP medium (0.016 gC L⁻¹), this predicted increase in starch formation translates into a larger availability of the carbohydrate pool that will be ultimately required by Clostridial species to produce biobutanol.
Figure 2. Ternary diagram displaying predicted formation of microalgal starch (t = 190h) with respect to initial nitrogen, phosphorus, and acetate concentrations. Circled area corresponds to optimal N-P-A sets for starch accumulation.

5. Conclusions

A multi-parameter kinetic model responsive to nitrogen, phosphorus, and acetate concentrations was developed to predict starch formation during microalgae cultivation. Parameter fitting was carried out by means of a global optimisation algorithm avoiding getting trapped in local optima. Model predictions, obtained with the set of fitted parameters, were accurately validated with experimental data. Optimal conditions for microalgal starch formation were additionally established to identify improved microalgae-to-biobutanol production routes.

References


