Carbon dioxide sequestration and bio-oil production by 
*Stichococcus* strains

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Abstract

The present study reports results of an experimental campaign of autotrophic cultures of *Stichococcus* strains in lab-scale bubble columns operated as photobioreactors. Air was sparged at the bottom through a porous ceramic distributor. Microalgae growth and lipid production were investigated at 23°C and 140 μE/(m²s) light irradiance. Lipids were extracted from dried microalgae and transesterified with alkaline methanol. The lipid content/distribution of the bio-oil was assessed. The effects of the air volumetric flow rate and operation modality with respect to the liquid phase – batch, fed-batch and semicontinuous - were investigated.

1.Introduction

Biofuels production coupled with carbon dioxide sequestration by means of photosynthetic microorganisms appeared a promising process since the end of the last century [1]. In particular, microalgae biomass may be burned/gasified as crude dry matter in combustors/gasifiers or may be processed to produce liquid fuels [2,3]. In the latter case, bio-oil is extracted from microalgae and it may be either adopted as crude fuels or transesterified to biodiesel. Chisti [2] has recently reported that biodiesel production rate from microalgae cultures may be 1-3 order of magnitude larger than that worldwide available from current oil crops. Just as a data example, oil yield per acre per year from microalgae is ten times higher than that from palm oil, microalgae grow extremely rapidly and many are exceedingly rich in oil (50-80%) [2]. Moreover, the microalgal biomass fixes a large amount of carbon dioxide – 1.83 kg of CO₂ per kilogram of dry microalgae – and strongly contributes to the reduction of greenhouse gas emission [4,5].

In spite of the reported environmental advantages in biodiesel production by microalgae cultures, the process is still unfavourable with respect to biodiesel from oil-crops from an economic point of view. Infact, nowadays microalgae have failed to be economically competitive with other sources of energy. More than 100 such algae-to fuel companies have popped up worldwide, but not a single commercial facility has been built [6]. Strong discussion associated with economical and technical evaluations are continuously proposed [7-9].

Key aspects for industrialization of microalgae-to-biofuel processes are: selection and improvements of algal strains through genetic and metabolic engineering; development of high-performance photobioreactor; improvement of oil extraction and transesterification processes; development of microalgae biorefineries [2]. Biomass yield and productivity and total lipid content are strongly affected by nutrient and light supply strategy.
The present study reports results of an experimental campaign with autotrophic culture in lab-scale bubble columns. Selection of *Stichococcus* strains and photobioreactor operation - batch, fed batch and semi-continuous - are reported and compared in terms of productivity and total lipid content.

2. Materials and Methods

2.1. Microorganism and medium

*Stichococcus* strains (*bacillaris*, *sequoieti*, *cylindricus*, *fragilis*, *minor*) were from the ACUF collection of the Biological Science Department of the University of Studies of Napoli “Federico II”. Bold Basal Medium (BBM) supplemented with NaNO$_3$ as nitrogen source was adopted. Nitrogen content in BBM was fixed at 40 mg/L. Carbon dioxide was supplemented by sparging filtered air into the medium. No organic carbon source was supplemented to the medium.

2.2. Experimental apparatus

Photobioreactors were of 1 L bubble column made of glass (0.04 m ID, 0.8 m high). The working volume was set at 0.9 L. Air was sparged at the photobioreactor bottom by means of a porous ceramic diffuser at volumetric flow rate ranging between 20 and 200 mL/h. 0.2 μm filters were adopted to sterilize air flow inlet and outlet. Photobioreactors were housed in a thermostated chamber (23°C) equipped with lamps (M2M engineering). The light irradiance was set at 140 μE/(m$^2$ s).

2.2. Oil extraction and transesterification

Microalgae biomass was harvested by centrifugation and freeze dried. Total lipid fraction was extracted in CH$_3$Cl:CH$_3$OH (2:1) for 7 h from 400 mg of dried microalgae by adopting a soxhlet. The solvent fraction was separated by evaporation and lipid fraction weighted. Recovered total lipids were resuspended in 12 mL CH$_3$OH and transesterified with 1.5% NaOH at 65°C for 3 min.

2.2. Analytical methods

Biomass concentration was measured on line as optical density at 600 nm. Dry weight was measured on pre-weighted whatman filters. Total organic carbon (TOC), total nitrogen (TN) and inorganic carbon (IC = carbon dioxide + bicarbonate + carbonate) were measured using a Shimadzu TOC-TN-V-CSH analyzer. Photon flux density at the photobioreactor wall was measured by a Li-Cor probe. pH was measured on 1 mL sample by means of a Sentex glass electrode. Oxygen transfer rate was measured by dynamic method as gas-liquid mass transfer coefficient. Oxygen photosynthesis and respiration rates were monitored on 1 mL culture sample with Oxygraph (Hansatech). HPLC was adopted to characterize the methyl ester mixtures obtained from transesterification. The instrument was equipped with a reverse-phase column in which a mobile phase 70/30 CH$_3$CN/H$_2$O was pumped. External standards were adopted to identify the components of the transesterification process.

3. Results and discussion

3.1. Batch tests

A test campaign was carried out to select the strain characterized by the highest growth rate among the investigated ones. Microalgae cultures were carried out in BBM for 32 days and
sparging 50 nL/h of air. Initial TN was made up three times during each test by supplementing the photobioreactors with concentrated BBM. *S. sequoieti* was characterized by the largest final biomass concentration (9.5 OD, 3 g/L) after 900 h of incubation. The total lipid content was 12% under growth conditions characterized by no-nitrogen starvation, and it increased up to 20% at the end of 200 h culture carried out under nitrogen starvation. The HPLC chromatogram of the methyl ester mixture from a *S. bacillaris* culture is reported in figure 1 and the mass fraction - with respect to the lipid total mass - of the main components are reported in table 1. Phytol, a precursor of vitamin K1 and E, was also detected in the analyzed mixture.

![HPLC chromatogram of methyl esters in the mixture obtained from microalgae after extraction and transesterification. Strain: S. bacillaris. Batch test. Total lipid fraction 32%W.](image)

<table>
<thead>
<tr>
<th></th>
<th>Methyl hexadecanoate (methyl palmitate)</th>
<th>Methyl cis,cis,cis-9,12,15-octadecatrienoate (methyl linolenate)</th>
<th>Methyl cis,cis-9,12-octadecadienoate (methyl linoleate)</th>
<th>Methyl cis-9-octadecenoate (methyl oleate)</th>
<th>Phytol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>270.4</td>
<td>292.5</td>
<td>294.5</td>
<td>296.5</td>
<td>296.5</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C₁₇H₃₄O₂</td>
<td>C₁₉H₃₂O₂</td>
<td>C₁₉H₃₄O₂</td>
<td>C₁₉H₃₆O₂</td>
<td></td>
</tr>
<tr>
<td>Mass fraction with respect to the lipid total mass.</td>
<td>6.5%</td>
<td>5.2%</td>
<td>4.6%</td>
<td>13.8</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

**Tab. 1** Methyl esters identified in the mixture obtained from transesterification.

### 3.2. Fed-batch tests
Fed-batch tests were aimed to assess the operating conditions promoting the increase of the biomass concentration in the culture. A fixed volume (100 mL) of concentrated BBM nitrogen supplemented was periodically added to the culture, provided that an equal amount
of medium (without cells) was withdrawn between successive feed. Tests were carried out sparging air at 20, 50 and 200 nL/h.

Figure 2 reports the data regarding a test carried out sparging the *S. bacillaris* culture with air at 20 nL/h. Biomass concentration increased up to 13 OD (4 g/L) in 50 days. TOC and residual TN increased with the time with a maximum after 55 days of incubation. Remarkable, the ratio between the TOC and TN was constant in the time suggesting that protein accumulated in the broth. Th pH increased from 6.8 (typical of buffer in BBM) up to 8. Accordingly, the IC content increased as a result of the CO$_2$/HCO$_3^-$/CO$_3^{2-}$ equilibrium shift. Results of tests carried out sparging air at 50 and 200 nL/h reproduced the behaviour reported in Fig. 2. The maximum biomass concentration was measured sparging air at 20 nL/h.

![Fed-batch test carried out sparging air at 20 nL/h. Strain: S. bacillaris. Arrows mark the concentrated BBM supplements.](image-url)
3.3. Semi-continuous tests

Semi-continuous tests were carried out by weekly replacement of the microalgae suspension fraction with fresh medium. Figure 3 reports data regarding a *S. bacillaris* culture test carried out adopting a progressive change of operating conditions: i) batch for 10 days; ii) fed-batch for 25 days; iii) semicontinuous since the day 37 by replacement of 20% of the suspension. The average dilution rate under semicontinuous conditions was about 0.028 1/day. The biomass concentration increased during the batch phase and approached a steady state value (about 6 OD) during the semicontinuous phase.

![Figure 3](image)

*Fig. 3*  Semicontinuous test. Strain: *S. bacillaris*. Arrows mark BBM addition during fed-batch phase. Dot-lines mark culture replacement during semicontinuous phase. Volume fraction replaced: 20%.

Table 2 reports steady state values of biomass concentration, TN and total lipid fraction measured during the semicontinuous tests carried out at weekly replacement of 20, 35 and 50%
of suspension volume. The biomass concentration decreases with increasing the dilution rate. An unexpected maximum in total lipid fraction was measured at dilution rate of 0.050 1/day.

<table>
<thead>
<tr>
<th>Suspension fraction replaced weekly</th>
<th>D</th>
<th>X</th>
<th>Biomass productivity</th>
<th>Total lipid</th>
<th>Total lipid productivity</th>
<th>TN</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>1/day</td>
<td>g_{\text{DM}}/L</td>
<td>mg_{\text{DM}}/L day</td>
<td>%_{w}</td>
<td>mg/L day</td>
<td>mg/L</td>
</tr>
<tr>
<td>20</td>
<td>0.028</td>
<td>1.8</td>
<td>55</td>
<td>13</td>
<td>7.3</td>
<td>12.4</td>
</tr>
<tr>
<td>35</td>
<td>0.050</td>
<td>1.1</td>
<td>61</td>
<td>25</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>50</td>
<td>0.071</td>
<td>0.78</td>
<td>62</td>
<td>18</td>
<td>11</td>
<td>8.05</td>
</tr>
</tbody>
</table>

Tab. 2 Main data of the semicontinuous tests. Strain: *S. bacillaris*.

4. Conclusions
A preliminary campaign of tests of autotrophic cultures of *Stichococcus* strains in lab-scale bubble photobioreactor have been successfully carried out. The tests allowed to select the optimal operating conditions to maximize the biomass concentration/productivity. Batch test carried out sparging air at flow rate of 20 nL/h (corresponding to 0.37 vvm) was characterized by the highest biomass concentration. Semicontinuous operations with weekly replacement of a suspension fraction with fresh medium resulted a promising strategy to obtain steady state biomass productivity. The maximum total lipid fraction of the microalgae (25\%_{w}) was under semicontinuous operation conditions characterized by 35\% of weekly suspension replacement. Preliminary HPLC analyses on a methyl ester mixture obtained submitting to transesterification the total lipids extracted from the microalgae allowed to identified methyl palmitate, methyl linolenate, methyl linoleate and methyl oleate along with phytol.

6. References