Algal Research 13 (2016) 16–29

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Effect of CO_2 in the aeration gas on cultivation of the microalga *Nannochloropsis oculata*: Experimental study and mathematical modeling of CO_2 assimilation

Carolina Shene [a,b,*], Yusuf Chisti [c], Mariela Bustamante [a], Mónica Rubilar [a]

[a] *Department of Chemical Engineering and Center of Food Biotechnology and Bioseparations, BIOREN, Universidad de La Frontera, Casilla 54-D, Temuco, Chile*
[b] *Centre for Biotechnology and Bioengineering (CeBiB), Universidad de La Frontera, Temuco, Chile*
[c] *School of Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand*

A R T I C L E   I N F O

Article history:
Received 25 June 2015
Received in revised form 7 October 2015
Accepted 14 November 2015
Available online xxxx

Keywords:
Microalgae
pH control
Nannochloropsis oculata
Carbon assimilation

A B S T R A C T

The effects of CO_2-supplementation on growth and biomass productivity of the microalga *Nannochloropsis oculata* are discussed for cultures with and without pH control. In otherwise non-limiting photoautotrophic cultures, the supply of inorganic carbon controls the algal biomass concentration and productivity. Inorganic carbon is nearly always supplied as CO_2, but is taken up by the cells mainly as bicarbonate. The culture pH determines the speciation of the dissolved inorganic carbon and its availability for uptake. In air-sparged batch cultures, the pH control at 6.5, 7.0 and 8.0 by injection of CO_2 as needed, did not affect the biomass concentration and productivity relative to the control culture (no pH control) sparged with air. In the absence of pH control, the supplementation of air with CO_2 at 0.34 to 1.34% v/v levels had no effect on the biomass concentration, but the pH oscillated with the day–night cycle. Compared to the control culture, the range of pH oscillations was reduced if CO_2 was added to air at the specified levels. A mathematical model was developed to explain the effect of the culture pH and the CO_2 level in the aeration gas, on the rates of photosynthetic CO_2 assimilation and CO_2 absorption.

1. Introduction

Microalgae are being extensively examined as potential sources of lipids for making biodiesel. In addition to oils, the microalgal biomass contains proteins, carbohydrates and other materials. Consequently, microalgae are potential sources of foods, feeds and feedstock for producing diverse chemicals and fuels. Microalgae transform sunlight into chemical energy (ATP and NADPH) that is used in the photosynthetic carbon reduction cycle, or Calvin–Benson cycle, to produce organic carbon. The first step of the Calvin–Benson cycle is catalyzed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). In both carboxylation and oxygenation reactions one of the substrates is ribulose-1,5-bisphosphate (RuP_2). The other substrate is either oxygen (in oxygenase reaction) or carbon dioxide (for carboxylation reaction). Because of the low level of carbon dioxide (10–12 μM) in seawater in equilibrium with the atmosphere and the relatively low affinity of RuBisCO for carbon dioxide, algal biomass production in industrial processes requires a CO_2-enriched medium to enhance the rate of photosynthesis and reduce photorespiration. Notwithstanding this, some marine phytoplanktonic species possess mechanisms that increase the concentration of CO_2 in the proximity of the active site of RuBisCO [22].

A CO_2-enrichment mechanism involves external carbonic anhydrases for dehydration of bicarbonate (HCO_3^-) to maintain a constant equilibrium concentration of CO_2 at the site of the CO_2 transporter [51]. In some species, bicarbonate, the main form of the dissolved inorganic carbon (DIC), is taken up via an active transport system [12,13,15,33,46]. Inside the cells, HCO_3^- is dehydrated by internal carbonic anhydrases located near the active site of RuBisCO to generate CO_2, the required substrate.

The inorganic carbon required by a culture system is typically supplied by bubbling, or sparging, CO_2-enriched air through the culture medium. The effect of a CO_2-enriched air supply on the biomass production parameters of a microalgal culture depends not only on the algal strain, but also on the culture conditions (e.g. temperature, salinity, ionic strength of the medium, concentrations of the other nutrients, and the pH) being used. The culture conditions define the main form of the dissolved inorganic carbon available, its concentration, and the expression of any carbon concentrating mechanisms [22]. CO_2-enriched air is commonly used to reduce the culture pH that would otherwise increase as a consequence of the uptake nutrients such as HCO_3^- and NO_3^- [49]. In addition to the effect of pH on carbon speciation, pH can also affect the growth rate of microalgae by influencing the rates of biochemical reactions and the properties of the cell membrane [47].

* Corresponding author at: Department of Chemical Engineering and Center of Food Biotechnology and Bioseparations, BIOREN, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.
E-mail address: carolina.shene@ufrontera.cl (C. Shene).

http://dx.doi.org/10.1016/j.algal.2015.11.005
2211-9264/

Nomenclature

a_B	total bubble surface area
abs	absorptance
A_c	total cell surface area
ASW	artificial seawater
ATP	adenosine triphosphate
B_e	concentration of HCO_3^- in the medium
B_i	concentration of HCO_3^- in the cell
c_1	parameter defined by Eq. (58)
c_2	parameter defined by Eq. (59)
Ca	carotenoid content of the biomass
$C_{absorption}$	fraction of the supplied CO_2 absorbed in the bioreactor
C_{Chl}	chlorophyll content (mass per unit biomass) in the cell
$C_{Chl,HI}$	chlorophyll content in the cell under high light
$C_{Chl,LI}$	chlorophyll content in the cell under low light
C_e	concentration of CO_2 in the medium
C_H	concentration of protons in the medium
C_{Hi}	concentration of protons in the cell
Chl*a*	chlorophyll *a* content of the biomass
C_i	concentration of CO_2 in the cell
C_{OH}	concentration of hydroxyl anions in the medium
D	dilution rate
DIC	dissolved inorganic carbon
e^-	electron
f	parameter correcting for spectral quality of the light
F	molar flow rate of the aeration gas
F_C^{in}	inlet molar flow rate of CO_2
F_N^{in}	inlet molar flow rate of N_2
F_O^{in}	inlet molar flow rate of O_2
F_C^{out}	outlet molar flow rate of CO_2
F_O^{out}	outlet molar flow rate of O_2
F_C^x	molar flow rate of CO_2 in the gas bubbles reaching the headspace
F_O^x	molar flow rate of O_2 in the gas bubbles reaching the headspace
H_C	Henry's law constant for CO_2
HI	high irradiance
H_O	Henry's law constant for O_2
I	incident irradiance
I_2	fraction of the incident irradiance (*I*) absorbed by photosystem II
j	potential electron transport rate per unit mass of chlorophyll
J	potential electron transport rate
J_{max}	maximum rate of carboxylation allowed by light reactions at given partial pressures of CO_2 and O_2
k_1	forward rate constant of CO_2 hydration
k_{-1}	reverse rate constant of CO_2 hydration
k_3	forward rate constant of CO_2 hydration in acidic conditions
k_{-3}	reverse rate constant of CO_2 hydration in acidic conditions
k_3^*	k_3 value estimated at the intracellular pH
k_4	forward rate constant of CO_2 hydration in alkaline conditions
k_{-4}	reverse rate constant of CO_2 hydration in alkaline conditions
k_4^*	k_{-3} value estimated at the intracellular pH
k_{LB}	mass transfer coefficient between gas bubbles and the liquid
k_{LH}	mass transfer coefficient between gas in the headspace and the medium
K_2	dissociation constant of carbonic acid to bicarbonate
K_3	equilibrium constant of CO_2 hydration in acidic conditions
K_4	equilibrium constant of CO_2 hydration in alkaline conditions
K_5	dissociation constant of the bicarbonate to carbonate
K_6	ion product of water
K_b	Michaelis constant of the intracellular carbonic anhydrase for HCO_3^-
K_c	Michaelis constant of the intracellular carbonic anhydrase for CO_2
K_C	Michaelis constant of RuBisCO for CO_2
K_O	inhibition constant of RuBisCO for O_2
K_r	Michaelis constant of HCO_3^- transport out of the cell
K_t	Michaelis constant of HCO_3^- transport into the cell
L	lipid content of the biomass
LI	low irradiance
N	cell concentration
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
O_e	concentration of O_2 in the medium
O_i	concentration of O_2 in the cell
p_C^{out}	partial pressure of CO_2 in the outlet gas
p_O^{out}	partial pressure of O_2 in the outlet gas
p_C^x	partial pressure of CO_2 in the bubbles reaching the headspace
p_O^x	partial pressure of O_2 in the bubbles reaching the headspace
P_C	cell permeability to CO_2
P_O	cell permeability to O_2
P_T	total pressure
Q	volumetric flow rate of gas
Q_f	volume flow rate of feed
QY	quantum yield
r_1	rate of HCO_3^- dehydration by intracellular carbonic anhydrase
r_2	rate of CO_2 hydration by carbonic anhydrase
r_3	rate of HCO_3^- dehydration in the cell
r_4	rate of CO_2 hydration in the cell
r_6	rate of HCO_3^- transport from the medium to the cell
r_7	rate of HCO_3^- transport from the cell to the medium
r_8	rate of carboxylation
r_9	rate of CO_2 diffusion due to membrane permeability
r_{10}	rate of O_2 diffusion due to membrane permeability
r_{11}	rate of oxygenation by RuBisCO
r_{12}	rate of CO_2 production by dark respiration
r_a	rate of CO_2 assimilation per mole of Chl in the biomass
r_B	mean bubble radius
r_C	mean cell radius
r_{dark}	dark respiration rate per unit leaf surface area
R	gas constant
R_B	rate of HCO_3^- production in the liquid phase
R_C	rate of CO_2 production in the liquid phase
R_{dark}	dark respiration rate
$R_{dark, HI}$	dark respiration rate under high light
$R_{dark, LI}$	dark respiration rate under low light
RuBisCO	ribulose bisphosphate carboxylase-oxygenase
RuP_2	ribulose-1,5-bisphosphate
s_H	area of the interface between the liquid and the headspace in the bioreactor
S	salinity
S_{CO}	specificity of RuBisCO
t	time
T	absolute temperature
TS	total sugar content in the biomass