Bioresource Technology 271 (2019) 316–324

Contents lists available at ScienceDirect

Bioresource Technology

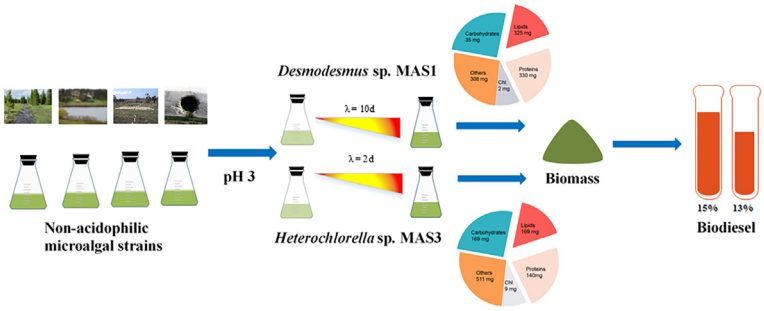
journal homepage: www.elsevier.com/locate/biortech

Sustainable production of biomass and biodiesel by acclimation of non-acidophilic microalgae to acidic conditions

Sudharsanam Abinandan[a], Suresh R. Subashchandrabose[a,c], Nicole Cole[b], Rajarathnam Dharmarajan[a,c], Kadiyala Venkateswarlu[d], Mallavarapu Megharaj[a,c,*]

[a] Global Centre for Environmental Remediation (GCER), Faculty of Science, ATC Building, University of Newcastle, Callaghan, NSW 2308, Australia
[b] Analytical and Biomolecular Research Facility (ABRF), University of Newcastle, Callaghan, NSW 2308, Australia
[c] Cooperative Research Centre for Contamination Assessment and Remediation of Environment (CRC CARE), University of Newcastle, ATC Building, Callaghan, NSW 2308, Australia
[d] Formerly Department of Microbiology, Sri Krishnadevaraya University, Anantapur 515055, India

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Microalgae
Non-acidophiles
Acclimation
Biochemical response
Lipids
Biodiesel

ABSTRACT

The overwhelming response towards algal biodiesel production has been well-recognized recently as a sustainable alternative to conventional fuels. Most microalgae cannot grow well at acidic pH. The present study, therefore, investigated whether non-acidophilic microalgae *Desmodesmus* sp. MAS1 and *Heterochlorella* sp. MAS3 can be acclimated to extreme-acidic pH for sustainable production of biomass and biodiesel. Growth analysis indicated that both the microalgal strains possessed a passive uptake of CO_2 at pH 3.0 with biomass production of 0.25 g dry wt. L^{-1} in *Desmodemus* sp. and 0.45 g dry wt. L^{-1} in *Heterochlorella* sp.. Flow-cytometry analysis for reactive oxygen species, membrane permeability and neutral-lipids revealed the capabilities of both strains to adapt to the stress imposed by acidic pH. Lipid production was doubled in both the strains when grown at pH 3.0. *In-situ* transesterification of biomass resulted in 13–15% FAME yield in the selected microalgae, indicating their great potential in biofuel production.

1. Introduction

Microalgae significantly contribute to the environment through CO_2 fixation, contaminant reduction and production of biomass as a promising feedstock for biofuel. As ubiquitous primary producers, microalgae are crucial to the ecological biota. Recent research widely

* Corresponding author at: Global Centre for Environmental Remediation (GCER), Cooperative Research Centre for Contamination Assessment and Remediation of Environment (CRC CARE), Faculty of Science, The University of Newcastle, ATC Building, University Drive, Callaghan, NSW 2308, Australia.
E-mail address: megh.mallavarapu@newcastle.edu.au (M. Megharaj).

https://doi.org/10.1016/j.biortech.2018.09.140
Received 16 August 2018; Received in revised form 27 September 2018; Accepted 28 September 2018
Available online 29 September 2018
0960-8524/

acknowledged the influence of extreme environments such as ocean acidification and acid mine drainage on microalgal communities (Sassenhagen et al., 2015; Abinandan et al., 2018a). The most common phenomenon is the extent of pH that plays a critical role in algal growth dynamics. Several studies combined the effect of pH, nutrient starvation or cultivation modes for enhancing algal biomass preferably for increased biofuel production (Abinandan et al., 2018b). For instance, the addition of molasses (9.82 g L^{-1}) to serve as an organic carbon source at pH 6.7 resulted in higher yield (2 g L^{-1}) of microalgal biomass (Kose Engin et al., 2018). Cheirsilp and Torpee (2012) observed increased lipid content at a circumneutral pH upon exposure of microalgae to higher concentrations of glucose. Huang et al. (2017) demonstrated that microalgae turned the medium acidic with glucose when ammonium was predominantly present.

Most of the microalgae cannot survive at low pH (< 6.0) as the transporters become inactive (Perez-Garcia et al., 2011). Several studies indicated that exogenous supply of pure CO_2 or flue gas to enhance microalgal biomass productivity is favorable only at controlled pH maintained with bicarbonate availability (Ma et al., 2017). Jiang et al. (2012) reported that microalgae non-adapted to acidic conditions could not survive at pH 3.0, indicating that acid adaptation is imminent for survival. Also, microalgae, when grown at pH 4.0, exhibited a drastic decline in the biomass, suggesting the significant toxicity of pH (Khalil et al., 2010). Interestingly, even pH 4.5 inhibited 50% of growth in acid-tolerant microalgae (Nalewajko et al., 1997). El-Ansari and Colman (2015) also reported that acid-tolerant microalgae could not grow at pH 3.0 due to a decrease in intracellular pH. Thus, acid-tolerant microalgae are also sensitive to low pH, implying that only acidophiles are capable of growth under such extreme conditions due to the gene inheritance through evolutionary response (Hirooka et al., 2017). Sassenhagen et al. (2015) noted that microalgae could grow under a wide range of environmental conditions due to high phenotypic plasticity. An exogenous supply of carbon source (organic or inorganic) may be imminent for biofuel production (Kose Engin et al., 2018). Ma et al. (2017) suggested that pH of the medium (irrespective of carbon source) should be maintained at near neutral for microalgal cultivation. But, addition of hydroxides is required for maintenance of neutral pH and is not cost-effective (Abinandan et al., 2018b). However, available information suggests that non-acidophilic microalgae can withstand naturally-occurring acidic events such as ocean acidification by expressing high phenotypic plasticity or through adaptation process (Jiang et al., 2012).

While perusing the literature on remediation of acid mine drainage (AMD) by microalgae–bacteria biofilms, it was hypothesized that acclimation of non-acidophilic microalgae to acidic conditions might be a better option than applying acidophilic counterparts for reclamation of AMDs (Abinandan et al., 2018a). This is because under different environmental pressures such as acidic conditions, only limited strains of non-acidophilic microalgae could phenotypically adjust to thrive and grow (Abinandan et al., 2018a). To validate this hypothesis, four microalgae isolated from natural habitats of soil and lake waters with near neutrality exposed to pH 3.0 to investigate the microalgal growth response to acclimation at this acidic condition. Subsequently, two microalgal strains capable of growth at pH 3.0 were selected to assess the potential for sustained production of biomass under the environmental pressures imposed by extreme acidic conditions following flow cytometry, and yield of biodiesel following FTIR-based microalgal fatty acid methyl esters (FAME) analysis. The present study reports for the first time on acclimation of non-acidophilic microalgae to extreme acidic pH for the sustainable production of biomass and biodiesel.

2. Materials and methods

2.1. Microalgal strains and determination of growth rate

Microalgae were isolated from local soil and lake water samples by streaking onto agar with modified Bold's basal medium (BBM) with low phosphate. Cell sorting (BD FACSAria IIu) was done to obtain axenic cultures of the isolates. Briefly, log phase cells were sampled to measure chlorophyll dependent autofluorescence (FL3, 670 nm LP). The channel estimates at log scale and the sensitivity was set at 300 mV. Measurements of 10,000 events and 10^5 cells were sorted in sterile BBM and plated subsequently. The cells took nearly two weeks to develop axenic colonies. These isolates were grown at pH 3.0 (experimental) and pH 6.7 (control) in 30 mL BBM contained in 100 mL conical flasks under continuous illumination of 60 µmol m^{-2} s^{-1} at 23 ± 1 °C with 100 rpm shaking. The pH of the culture medium was monitored using LAQUA PC1100 pH meter (Horiba scientific, Japan).

Genomic DNA from algal strains was isolated using microbial DNA isolation kit (Mo Bio Laboratories, Inc.) as per the instructions provided. The DNA was amplified with 18S universal primers, the amplicons were cleaned using PCR and Gel kit (Bioline Laboratories, Inc.), and sequenced at Ramaciotti Centre, UNSW, Australia. The preliminary sequence identification was carried out for three isolates of microalgae using the NCBI Blast nucleotide search tool and a phylogenetic tree was constructed using MEGA 5.0 (Kumar et al., 2016). Phylogenetic analysis obtained from 1000 replicates as per the bootstrap test of clustal muscle alignment indicated that two of the microalgal isolates belong to the genus, *Desmodesmus*, with a slight difference of 3% similarity among nucleotides and hence designated as *Desmodesmus* sp. MAS1 and *Desmodesmus* sp. MAS2 (Fig. 1a). Since the third isolate is closely related to the genus, *Heterochlorella*, it has been designated as *Heterochlorella* sp. MAS3 (Fig. 1b). A well-studied *Chlorella* sp. MM3 (Ramadass et al., 2017; Subashchandrabose et al., 2017a,b; Ganeshkumar et al., 2018), obtained from in-house Phycology laboratory, was used in the present study as a reference microalga.

Microalgal growth, in terms of cell density, was determined in triplicate samples every alternate day using Neubauer hemocytometer (Bright line, Hausser Scientific, USA) under a light microscope (Olympus CX31, Japan). The growth rate was calculated using data at the exponential phase following the equation:

$$\mu = \frac{\ln N_1 - \ln N_0}{T_1 - T_0}$$

where N_1, N_0 are the final and initial cell densities, and T_1, T_0 are the times taken, in days.

2.2. Determination of growth response

Triplicate samples from microalgal cultures were withdrawn every week for determining the activity of carbonic anhydrase (CA), chlorophyll, biomass and metabolic biomarkers such as carbohydrates, proteins and total lipids. After sonicating the microalgal cell suspension, the activity of CA was measured in terms of esterase activity (Ores et al., 2016), and expressed as U L^{-1}. One unit (U) of enzyme activity is defined as the quantity of enzyme needed to release 1 µmol of *p*-nitrophenol min^{-1} in the assay conditions. Total chlorophyll and carbohydrates were estimated after methanol extraction (Chen and Vaidyanathan, 2013). Bradford bioassay was carried out to determine proteins using Bio-Rad kit (Bio-Rad Protein Assay Dye Reagent Concentration; Protein Standard II), and the color intensity was read in a spectrophotometer (Orion AquaMate 7000, Thermofisher Scientific, USA). Chloroform from the extracts was dried before gravimetric analysis of total lipids. Chlorophyll, carbohydrates and total lipids are expressed as mg g^{-1} dry wt. respectively. Microalgal biomass, in triplicate samples, was determined by the gravimetric method and expressed as g dry wt. L^{-1}.

2.3. Assay of reactive oxygen species (ROS), membrane permeability and neutral lipids

Aqueous stock solution (0.5 mg mL^{-1}) of DCFH-DA (Sigma, USA)