Algal Research 2 (2013) 113–121

Contents lists available at SciVerse ScienceDirect

Algal Research

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

Strategic enhancement of algal biomass and lipid in *Chlorococcum infusionum* as bioenergy feedstock

Ankush Karemore [a], Ruma Pal [b], Ramkrishna Sen [a,*]

[a] Department of Biotechnology, Indian Institute of Technology, Kharagpur, India
[b] Department of Botany, Calcutta University, Kolkata, India

ARTICLE INFO

Article history:
Received 16 February 2012
Received in revised form 16 November 2012
Accepted 29 January 2013
Available online 5 March 2013

Keywords:
Microalgae
Plackett–Burman design
Lipid
Biomass
Chlorococcum infusionum
Culture medium

ABSTRACT

Algal biomass can serve as rich source of bioproducts including lipids for diverse commercial applications. Both biomass production and lipid accumulation are limited by several factors, of which nutrients play a vital role. In the present investigation, the nutritional requirement for the growth by a (an autotrophic) *Chlorococcum infusionum* was determined using a Plackett–Burman based statistical screening experiment. Five out of the fifteen factors of a reported production medium were found to be significantly affecting the biomass growth. The components $NaNO_3$, K_2HPO_4, $FeSO_4.7H_2O$ and KOH had direct proportional correlation with biomass production, while $MgSO_4$ showed inverse proportional relationship in the selected experimental range. Nitrogen was the most influential factor with an effect contribution of 45.77% and a very low p-value of <0.001. The most favorable nitrogen source was potassium nitrate which could replace both sodium nitrate and potassium hydroxide. More than two fold increase in biomass concentration was achieved by screening and standardizing the media components of Bold Basal Medium. Lipid accumulation under normal condition was 12–15% dry cell weight (dcw). Under nitrogen starvation condition, it was 30–35% dcw. However, a semi-starvation condition at 1.75 mM of sodium nitrate induced lipid production as high as 40 ± 2% dcw. FAME analysis in GC showed the presence of more saturated fatty acids. Results obtained in this work can further be applied to optimize production of algal biomass and lipid for applications like biofuel, fish or animal feed, fertilizer, etc. Also information obtained could be exploited for wastewater treatment processes.

1. Introduction

Microalgal biomass can be used as feedstock for biofuel and other valuable products. Sustainable and eco-friendly feedstock is needed to overcome food security problem and address environmental concerns. Microalgae have been attracting much attention from the scientific community and have emerged as a potential sustainable biomass source due to their neutrality towards nature and versatility with respect to applications. Microalgae have the ability to convert inorganic matter into organically rich biomass by sequestering atmospheric CO_2. Thus, the resulting microalgal biomass, which comprises important components like lipid, carbohydrate; pigments, proteins, etc., can be utilized in the field of therapeutics, cosmetics, nutraceuticals, animal feed, food, biofuel and wastewater treatment [1]. As a result of depletion of the world's fossil fuel reserves and increasing environmental concerns, there is a great demand for alternative renewable fuels and efficient environmentally friendly strategies for the treatment of industrial, municipal and domestic wastewaters. Advantages of microalgae include higher photosynthetic efficiency, enhanced biomass production, and faster growth-rates compared to other terrestrial crops without compromising landmass, making it an attractive option for biofuel and other commercially useful products [2].

Manipulations of the physicochemical parameters can have profound influence on biomass growth, fatty acid content and other bioactive metabolites [3]. The role of medium composition has been well recognized as very significant in influencing growth rate, product yield and biochemical composition of specific microalgae, since standard media generally result in low biomass productivities [4]. Among numerous macro and micro elements, some are rate limiting for algal growth, whereas others are important and involved in various enzymatic reactions for the biosynthesis of many compounds [5]. Conventional method for formulation of culture medium is a time-consuming and labor-intensive process. Statistical methods of media design have been successfully employed to deal with a large number of variables simultaneously in order to reduce the number of experimental runs that result in manifold improvement of the process performance [6,7]. The Plackett–Burman experimental design is a useful tool to screen the most influential factors among numerous parameters with minimal number of experiments. It also determines the relative significance of various parameters and indicates the effects and concentration level

* Corresponding author at: Department of Biotechnology, Indian Institute of Technology Kharagpur, West Bengal 721302, India. Tel.: +91 3222283752; fax: +91 3222278707.
E-mail address: rksen@hijli.iitkgp.ernet.in (R. Sen).

2211-9264/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.algal.2013.01.005

of the various medium components [7,8]. The disadvantages of this method are that it cannot determine the optimal concentration of each component and the interaction between different variables.

In any biomass cultivation system the presence of nutrients in the growth medium have influence on the nature, quantity and the composition of the product. In recent years microalgal cultivation has gained attention due to their ability to store high amount of lipids, which can further be extracted for the production of poly unsaturated fatty acid (PUFA) and biodiesel. It has been reported that the desired fatty-acid composition of lipid intended for a particular application can be achieved by screening critical media components and process parameters such as temperature, light intensity and concentration of various nutrients [9]. Among various approaches, exposing media to nitrogen limiting conditions, has been proved to be effective in accumulation of neutral lipid by algae for biodiesel application [9].

As lipid is considered to be a secondary metabolite, two step strategies are generally adopted to produce algal biomass [10]. Accordingly in the first step optimal conditions best suited for high biomass production are provided, followed by necessitating medium with nutrient limiting conditions to induce lipid biosynthesis. To the best of our knowledge there is scanty information in the literature reporting systematic studies on screening of critical medium components to enhance biomass and lipid yields by *Chlorococcum* sp. So in the current study, with a view to developing a modified medium, the statistical Plackett–Burman design was used to screen the most influential medium components and to determine the optimal ranges of concentration of nutrients. Further, lipid accumulation studies were carried out under nutrient starved conditions to obtain high lipid content for potential biodiesel and healthcare applications.

2. Materials and methods

2.1. Microalga maintenance and culture conditions

The microalgal strain *C. infusionum* was obtained from the Botany Department, Calcutta University, Kolkata. It was isolated from brackish water, grown and maintained in bold basal medium (BBM), whose composition is given in Table 1 [11–13]. Algal strain was grown at 25 ± 2 °C and at pH 6.8–7 under 14:10 light–dark cycle with light intensity of 1500 lx. All the media chemicals used in this study were obtained from Hi-media and Merck, India.

2.2. Inoculum preparation and experiments

Seed culture was prepared by inoculating *C. infusionum* into BBM media and it was grown till it reached early logarithmic phase (6–7 days). The seed culture thus prepared was then inoculated in 150 ml Erlenmeyer flask containing 50 ml culture medium (statistically designed) with inoculum size of 10% (v/v). Algal culture was grown aerobically in the phototrophic culture conditions as mentioned earlier. Algal strain was grown at 25 ± 2 °C and at pH 6.8–7 under 14:10 light–dark cycle with light intensity of 1500 lx. During incubation, the cultures were periodically gently mixed to ensure homogeneous mixing, release of O_2, and to avoid settling and sticking of algae on to the surface of the flask.

Experiments were performed in triplicate by growing the cultures till late-exponential or stationary growth phase and each data point obtained from experiments was expressed as mean with standard deviation (SD).

2.3. Selection of important media components

Defined culture media for freshwater algae is a mixture of macro-elements, micro-elements, vitamins, etc. [12,14]. Several media have been reported to support the growth of algae. Nevertheless, all the nutrients present in the media may not be essential for growth and maintenance as nutrient requirement changes with respect to algal species. Fifteen different media components for PB design were selected based on the Bold Basal Medium (BBM) composition and concentration level (lower and higher range) of each component was fixed based on the different reported media as listed in Table 1 [11–13]. Macro-nutrients include components such as $NaNO_3$, K_2HPO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$, NaCl, $FeSO_4.7H_2O$, KOH, whereas micro-elements have $CuSO_4.5H_2O$, $MnCl_2.4H_2O$, $CoCl_2.6H_2O$, H_3BO_3, $ZnSO_4.7H_2O$, $Na_2MoO_4.2H_2O$, and EDTA.

Table 1
The various reported media composition for microalgal culturing with different nutrients and their concentrations level [11–13].

Components	BG11 (mg/l)	Bold Basal (mg/l)	Chu (mg/l)	RM (mg/l)	Allen's (mg/l)
$NaNO_3$	1500	250	8.5	300	1500
K_2HPO_4	40	7.5	8.7	80	39
KH_2PO_4		175		20	
$MgSO_4$	75	75	36.9	10	75
$CaCl_2$	3.6	25	36.7	58.5	25
NaCl		25		20	
$FeSO_4.7H2O$		4.98			
$FeCl_3.6H2O$					2
$CuSO_4.5H2O$	0.08	1.57	0.02	0.08	0.08
$MnCl_2.4H2O$	1.81	1.44	0.0126		1.81
$FeC_6H_5O_7.5H_2O$	2.62		33.5		
$MnSO_4.4H2O$				1.5	
$Cl_2CO.6H_2O$			0.02		
H_3BO_3	2.86	11.42	0.62	0.3	2.86
$ZnSO_4.7H_2O$	0.22	8.82	0.044	0.1	0.22
MoO_3		0.71			
$Na_2MoO_4.2H_2O$	0.39		0.0126		0.391
$Co(NO_3)_2.6H_2O$	0.05	0.49		0.26	0.05
KOH		31			
Mg EDTA	1	5		7.5	
Citric acid	8				
Ferric ammo. Citrate	6				
Na_2CO_3	20				
Na_2HCO_3			12.6		
$NaSiO_3.9H_2O$			28.4		

2.4. Plackett–Burman experimental design and analysis

The Plackett–Burman experimental design tool was used for the rapid screening of most significant parameters and to determine their relative significance [8]. In the current study, the Plackett–Burman design containing 19 variables was used out of which 15 (A to P) (Table 2) were independent variables and the rest four variables (from Q to T) were designated as dummy variables. Each variable was represented at two levels of concentration: −1 for low level and +1 for high level shown in Table 2. Media for each run was formulated in accordance with the design matrix (Table 3). In Table 3 each row and each column represent 20 different experimental runs and different independent (assigned) or dummy (unassigned) variables respectively. According to the design matrix, the experiments were conducted by incubating the cultures inside a light facility chamber at 25 ± 2 °C for 25 days under 14:10 light–dark cycle with light intensity of 1500 lx. All the experiments were performed in triplicate and the average of biomass production was used as the experimental response. The data obtained were analyzed using the statistical software package Design-Expert® v. 7.1.3. (Stat Ease Inc., Minneapolis, USA).

The analysis of variance (ANOVA) was performed on experimental data to assess the statistical significance of the model. The effect of each variable was determined by the standard equation:

$$Effect = \frac{2[\Sigma R(H) - \Sigma R(L)]}{N} \quad (1)$$