Algal Research 22 (2017) 122–126

Contents lists available at ScienceDirect

Algal Research

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

Short communication

Phycoremediation of cheese whey permeate using directed commensalism between *Scenedesmus obliquus* and *Chlorella protothecoides*

Jean-Michel Girard [a,*], Réjean Tremblay [b], Nathalie Faucheux [c], Michèle Heitz [c], Jean-Sébastien Deschênes [d]

[a] Centre de Recherche sur les Biotechnologies Marines, 265, 2ième rue est, Rimouski, Québec G5L 9H3, Canada
[b] Institut des Sciences de la Mer, 310, allée des Ursulines, Rimouski, Québec G5L 3A1, Canada
[c] Département de Génie Chimique et Génie Biotechnologique, Université de Sherbrooke, 2500, boul. de l'Université, Sherbrooke, Québec J1K 2R1, Canada
[d] Département de mathématiques, informatique et génie, Université du Québec à Rimouski, 300, allée des Ursulines, Rimouski, Québec G5L 3A1, Canada

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form 7 November 2016
Accepted 15 December 2016
Available online xxxx

Keywords:
Lactose
Co-cultivation
Microalgae
Mixotrophic
Heterotrophic

ABSTRACT

The objective of this work is to explore a new phycoremediation strategy to convert a dairy by-product into microalgal biomass. A culture strategy involving two different green microalgae species was used to remove lactose from cheese whey permeate. First a co-cultivation experiment involving *Scenedesmus obliquus* and *Chlorella protothecoides* showed better utilization of the different sugars (lactose, glucose and galactose) than homogeneous *S. obliquus* cultures. In a second experiment, sequential cultivation of the two microalgal species allowed to convert 62% of the total lactose into 6.8 ± 0.2 g L^{-1} and 6.2 ± 0.4 g L^{-1} *S. obliquus* and *C. protothecoides* cultures respectively, for a "lactose-to-biomass" yield ($Y_{x/s}$) of 0.45. This type of beneficial interaction between two different microbial species represents a simple and sustainable way to improve and diversify the possibilities in the field of industrial microbiology.

1. Introduction

Phycoremediation is the process that converts nutrients and contaminants from industrial and municipal wastewaters into microalgal biomass [9,12]. It allows water decontamination while producing biomass that has potential for many useful applications including energy and food production [8,11,21]. One of the major factors acting on the microalgal biomass productivity is the dissolved organic carbon (DOC) contents in the effluents. Indeed, some heterotrophic microalgae species have the ability to use certain DOC sources such as acetate, glycerol or simple sugars [10]. These cultures typically reach much higher biomass densities (>10 g L^{-1} on a dry basis) than strict photoautotrophic cultures, whereas light does not become a limiting factor because of mutual shading above 1–2 g L^{-1} [20].

Whey is an important co-product of the cheese industry that possesses a substantial polluting power, with a biochemical oxygen demand (BOD) some 175-fold higher than typical sewage effluent [13]. This is due to its high DOC content which is mainly lactose (>75% m/m of total solids) and no whey utilization strategy will succeed without suitable attention being paid to lactose [5]. Whey proteins, which represent approximately 10% of total solids, are of high commercial value because of their nutritive qualities and health benefits [14]. During the process of whey protein isolation, a lactose concentrate is produced (also called "whey permeate (WP)") as a result of an ultrafiltration step. We therefore suggested that phycoremediation would be a good strategy to reduce the DOC content of WP (>100 g lactose L^{-1}) and BOD and to valorize a new biomass. The green microalgae *Scenedesmus obliquus* was identified as a good candidate specie for this purpose because of its abilities to hydrolyse extracellular lactose [6] and to produce high quality proteins [1]. However, incomplete utilization of the lactose and accumulation of glucose and galactose in the culture medium of *S. obliquus* were observed previously [7]. On a large scale, this would translate into high residual sugar content in the sewage effluent. The goal of the present study was therefore to address this concern by co-cultivating *S. obliquus* and *Chlorella protothecoides*, a well-known heterotrophic microalgae specie with high potential for lipid production but unable to use lactose for growth [4,7]. The use of these two species in the same culture medium could convert a maximum of sugars into microalgal biomass.

2. Materials and methods

2.1. Culture maintenance and medium

Scenedesmus obliquus was obtained from the Canadian Phycological Culture Center in Waterloo, Canada (CPCC 5) and *C. protothecoides* from the collection of algae at the University of Texas (UTEX 255).

* Corresponding author.
E-mail address: jean-michel_girard@crbm-mbrc.com (J.-M. Girard).

http://dx.doi.org/10.1016/j.algal.2016.12.013
2211-9264/

Cultures were kept in heat sterilized (121 °C, 15 min) Bold's Basal Medium (BBM), adjusted to pH 6.8 [15]. For *C. protothecoides*, BBM was supplemented with 1 g L^{-1} soy protein peptone (Sigma-Aldrich) and hereby called BBMP medium. Cheese whey permeate (WP) was shipped on ice from a dairy transformation plant, filtered (0.2 μm) upon reception and kept at 4 °C until use.

2.2. Experimental procedures

2.2.1. Co-cultivation experiment

At day 0 of the experiment, two sets of triplicate for two treatments were inoculated with *S. obliquus* (5 mL of a culture in exponential stage of growth) for an initial volume of 20 mL and initial medium composition of 60% (v/v) BBM and 40% (v/v) WP. This lactose concentrate widely produced in the industry (WP) was diluted to reduce the lactose concentration to about 40 g L^{-1} due to a "substrate inhibition" phenomenon observed on *S. obliquus* cultures at higher lactose concentrations [7]. All the cultures were first incubated at 22.5 °C with continuous orbital agitation (120 rpm) and continuous illumination (100 μE m^{-2} s^{-1}).

At day 4 of the experiment, one treatment was inoculated with *C. protothecoides* (5 mL of a culture in exponential stage of growth) and supplemented with 2 g L^{-1} soy protein peptone. The *C. protothecoides* inoculum (20% v/v) was from a photoautotrophic culture estimated at 0.5 g L^{-1} for an initial biomass contribution of approximatively 0.1 g L^{-1}. In the other treatment, the *C. protothecoides* inoculum was replaced by 5 mL of BBMP. The cultures were sampled (0.5 mL) each week during six weeks. Samples were centrifuged (5 min, 10,000*g*) and supernatants were kept at −20 °C until sugar determination by infrared absorbance spectra acquisition as previously described [6]. At the end of the experiments, fresh culture samples (0.5 mL) were diluted to 1×10^6 cells mL^{-1} in Tris-buffered Saline (TBS). These solutions were passed into an Epics Altra flow cytometer (Beckman Coulter Inc., Fullerton, BC, CA) fitted with a 488 nm laser operated at 18 mW according to a method previously described [16]. The absence of bacterial contamination was confirmed as previously described [7]. The rest of the biomass was harvested by centrifugation (5 min, 3500*g*). Pellets were washed once with deionized water and then freeze-dried. Final production were determined gravimetrically and expressed in g L^{-1}.

2.2.2. Sequential cultivation experiment

At day 0 of the experiment, five sets of triplicate (treatment 1 to 5) were inoculated with *S. obliquus* (1.4 mL of a culture in exponential stage of growth) for an initial volume of 10 mL and initial medium composition of 60% (v/v) BBM and 40% (v/v) WP. The cultures were incubated at 22.5 °C with continuous orbital agitation (120 rpm) and continuous illumination (100 μE m^{-2} s^{-1}). At day 0 and each week during four weeks a different treatment was harvested by centrifugation (5 min, 3500*g*). The supernatants were kept at −20 °C until further use. The pellets were used to determine the final *S. obliquus* dry biomass. When the last treatment was harvested, the supernatants from all treatments were thawed and volumes were readjusted to 10 mL (initial volume) using sterile deionized water to compensate for evaporation. These media were inoculated with *C. protothecoides* (0.5 mL of a culture in exponential stage of growth) and supplemented with 4 g L^{-1} soy protein peptone. The cultures were incubated at 22.5 °C with continuous orbital agitation (120 rpm) in continuous darkness (heterotrophy) during 6 days. At the end of this incubation, the biomass was harvested by centrifugation (5 min, 3500*g*). The supernatants were kept frozen at −20 °C until acquisition of their infrared absorbance spectra. The pellets were used to determine the final *C. protothecoides* dry biomass. The absence of bacterial contamination was confirmed as previously described [7]

2.3. Data analysis

Biomass production and residual sugars (g L^{-1}) data were analyzed by an analysis of variance (ANOVA). Where differences were detected, posteriori Student-Newman-Keuls (SNK) multiple comparison tests were used to determine which means were significantly different. The normality was verified by a Shapiro-Wilk test and the variances homoscedasticity through direct observations of residuals. If necessary, data were log + 1 or arcsine square-root (for % data) transformed to achieve homogeneity of variances. Analyses were carried out using SAS v.9.2 software (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

Our previous work demonstrated the capacity of *S. obliquus* to hydrolyze extracellular lactose in a WP supplemented culture medium and thus to induce the accumulation of glucose and galactose in the medium [7]. To improve the phycoremediation process of WP, *S. obliquus* and *C. protothecoides* were first co-cultivated and then sequentially cultivated in a culture medium composed of 60% (v/v) BBM and 40% (v/v) WP.

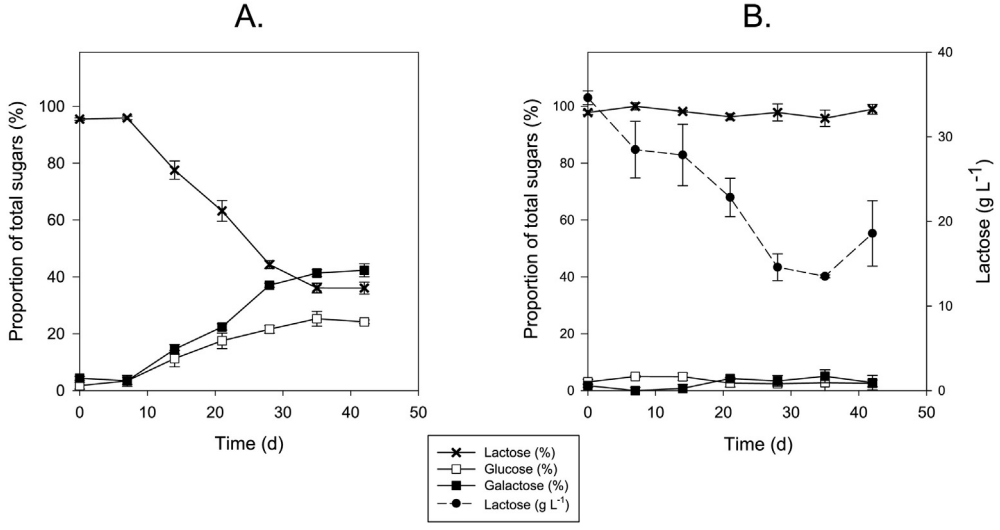

Fig. 1. Concentration of lactose and proportions (%) of lactose, glucose and galactose present in the culture medium of *S. obliquus* cultivated alone (A) or in co-culture with *C. protothecoides* (B). Results are expressed as the mean ± SD ($n = 3$).