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Utilization of biodiesel-derived glycerol or xylose for increased growth and lipid production by indigenous microalgae

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HIGHLIGHTS

- Indigenous strains were screened for utilization of glycerol and xylose.
- Yields of lipids and biomass are likely to be improved in future optimization studies.
- Lipid productivity from glycerol of some was higher than previously reported.
- One strain gained increased biomass in the light with both xylose and glycerol.
- For the first time xylose utilization by a microalga is shown.

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ABSTRACT

Microalgae are a promising alternative for sustainable biofuel production, but production yields and costs present a significant bottleneck. Here, the use of glycerol and xylose to boost the lipid yield was evaluated using ten strains from the Université de Montréal collection of microalgae. This report shows that some microalgal strains are capable of mixotrophic and heterotrophic growth on xylose, the major carbon source found in wastewater streams from pulp and paper industries, with an increase in growth rate of 2.8-fold in comparison to photoautotrophic growth, reaching up to $\mu = 1.1$/d. On glycerol, growth rates reached as high as $\mu = 1.52$/d. Lipid productivity increased up to 370% on glycerol and 180% on xylose for the strain LB1H10, showing the suitability of this strain for further development of biofuels production through mixotrophic cultivation.

1. Introduction

After the industrial revolution, the development of the new social system required a mobile, cheap and easy to use energy source. Petroleum was the most obvious choice, fueling the exponential growth of the world population from one billion in the eighteen hundreds to more than seven billion people in 2014. However, the versatility of crude oil has proved to be a double-edged sword, creating a society dependent upon oil as the main source of energy and the most important feedstock for the chemical industry. Today, the full consequences of an abrupt interruption in crude oil supplies are unimaginable, but the era of cheap and abundant oil is already gone. The ever growing demand, with ramped-up prices over the last few decades, and the perspective of a decline in oil production rates (peak oil) in the near future, indicate that we are reaching a critical point (Nashawi et al., 2010). Moreover, the direct and indirect toll on the environment and health systems is beginning to change the cost-benefit calculus of using this black gold. The transportation sector alone consumes more than 70% of the crude oil produced, making the development of biofuels an essential element in any strategy to decrease fossil fuel dependency (Abdelaziz et al., 2013).

Deriving biofuels from microalgae is an interesting proposition since they can be grown using sustainable cultivation systems, which do not require arable land and therefore don't displace food crops. In general, microalgae have faster growth rates and higher lipid yields than traditional oil crops, producing drop-in fuels which do not require modification of the present storage and distribution systems, and little to no modification of current internal combustion engines. Many algal species produce considerable amounts of triacylglycerol (TAG), easily converted into biodiesel, as an energy reserve (Cerón-García et al., 2013). However, like more traditional agriculture, microalgal productivity is of course limited by photosynthetic efficiency, a fact very relevant to geographical locations with low annual solar irradiation.

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Many algal species are capable of assimilating different carbon sources while harvesting light energy (Ukeles and Rose, 1976). Mixotrophic cultivation could represent an important strategy for microalgal production in many situations, including locations in high latitudes. However, the addition of organic carbon to the culture medium could significantly impact the operational cost, constituting between 35% and 80% of the medium price depending on the choice of carbon source (Cheng et al., 2009; Li et al., 2007). In addition, supplementation with organic fixed carbon can negatively impact the sustainability footprint if its production competes with that of food crops. Thus, carbon sources to be used in algal medium should preferably be industrial wastes. Among the possible options, a very obvious one is glycerol, an important biofuel waste since it represents about 10% of the products of TAG transesterification. With the vast amount of biodiesel presently made from oil crops, more than a billion kilograms of crude glycerol are produced annually, with a corresponding drastic decline in its value. The availability of crude (or technical) glycerol as a cheap carbon source has led to many studies evaluating the use of crude glycerol as feedstock for the biological production of valuable chemicals as; dihydroxyacetone, citric acid, cephalosporin and others (Dobson et al., 2012; Liu et al., 2013; Morgunov et al., 2013; Shin et al., 2011). However, if these conversion processes are successful, the value of their products, produced at enormous levels, would also fall precipitously. The only product for which there is a nearly insatiable market is some type of fuel. Indeed, the heterotrophic production of ethanol, butanol and hydrogen using fungi or bacteria has also been reported (Dobson et al., 2012; Ghosh et al., 2012; Sabourin-Provost and Hallenbeck, 2009). Heterotrophic cultivation of the alga *Chlorella protothecoides* showed equivalent growth on pure or crude glycerol (Chen and Walker, 2011). Recently, mixotrophic cultivation of *Chlorella vulgaris* on glycerol and glucose was shown to give higher yields (Kong et al., 2013), while another study with *Chlorella pyrenoidosa* showed a 20-fold increase in lipid productivity under mixotrophic conditions (Rai et al., 2013).

Another abundant waste that is available worldwide comes from the pulp and paper industry. The waste stream from pulp production is rich in xylose, turning it into a putative cheap source of carbon with high chemical energy content (Pérez et al., 2002). One suggested strategy is the chemical transformation of this waste directly into fuels (Xing et al., 2010). However, these processes are highly dependent on the purity of the waste stream and face several challenges before being scaled up. Biological approaches being developed include production of PHB (polyhydroxybutyrate), a feedstock for the production of bioplastics (Garcez Lopes et al., 2011); and biofuels, such as ethanol, isobutanol and triacylglycerol (TAG) (Brat and Boles, 2013; Kurosawa et al., 2013; Li et al., 2008). Nevertheless, all current proposed biological approaches use either prokaryotes or fungi only and until very recently, the utilization of xylose by algae had not been reported (Yang et al., 2014).

The use of these types of substrates can therefore not only provide cheap fixed carbon for potentially augmenting algal growth and lipid production, but can also serve a valuable function in waste treatment. Here, the photoautotrophic, heterotrophic and mixotrophic growth and lipid productivity performance of ten strains indigenous to Quebec (Abdelaziz et al., 2014) was assessed using glycerol or xylose as alternative carbon sources.

2. Methods

2.1. Strains and cultivation

The strains used in this work are part of the collection of the Département de microbiologie, infectiologie et immunology of the Université de Montréal. These are indigenous strains, mainly *Chlorella* sp., isolated in the region of Québec, Canada, and were previously described (Abdelaziz et al., 2014). The medium used for photoautotrophic cultivation was the Bold's Basal Medium (BBM) described by Andersen (Andersen, 2005). For the mixotrophic cultivation, BBM was supplemented with 20 mM xylose or glycerol from reagent grade stocks.

The pre-inoculum was grown under photoautotrophic conditions (BBM medium only) in 12-well plates until mid-log phase. The cultures were diluted to an optical density at 630 nm (OD_{630}) of 0.1 and used as inoculum (5% v/v). Three biological replicates of each algal strain were grown for 17 days in 12 well microtiter plates containing 3.5 mL of culture medium, and illuminated under a light/dark cycle of 12 h using day-light LED boards at an incident light intensity of 40 W/m^2 (approximately 190 $\mu E/m^2/s$). Each strain and medium condition was also carried out in continuous darkness (biological triplicates) to provide a comparison under heterotrophic conditions. Growth was measured by reading the optical density at 630 nm using a microtiter plate reader (Biotek EL800). Growth rates were calculated according to Eq. (1) using the optical density data (OD_{630}) between days 1 and 4 of cultivation.

$$\mu\ d^{-1} = (\ln OD^{f}_{630} - \ln OD^{i}_{630})/t^{f} - t^{i} \quad (1)$$

Growth rate formula used in Eq. (1), where μ/d = growth rate per day; $\ln OD^{f}_{630}$ = final optical density; $\ln OD^{i}_{630}$ = initial optical density; t^{f} = final time in days, t^{i} = initial time in days.

2.2. Spectrophotometric determination of nitrate, glycerol and xylose

The amounts of residual nitrate, glycerol or xylose were assessed in analytical triplicates. Established colorimetric methods were adapted for 96 well plate format and were performed for each of the biological replicates. The results are shown as the mean of the nine values obtained for each strain. At the end of the growth period the samples were centrifuged at 2000*g* for 8 min and the supernatant used for analysis. The classic colorimetric assay for quantification of reducing sugars using 3,5-dinitrosalicylic acid was adapted for measurement of xylose (Miller, 1959). 90 μL of DNS solution (10 g/L dinitrosalicylic acid; 10 g/L sodium hydroxide; 0.5 g/L sodium sulfite) was mixed with 90 μL of the sample or standard and incubated for 15 min in a water bath at 90 °C; immediately cooled down in an ice bath, and then 30 μL of 40% potassium sodium tartrate solution was added to stop the reaction. The optical density at 630 nm (Biotek EL800 microtiter plate reader) was compared to a standard curve obtained under the same conditions.

Glycerol was quantified using the colorimetric method described by Bondioli and Bella (2005), a two-step process with the periodate oxidation of glycerol followed by the formation of formaldehyde through Hantzsch's reaction. Here, 100 μL of samples or standards were placed in a 96 well plate, mixed with 60 μL of 10 mM sodium periodate solution (20 mM $NaIO_4$ in acetic acid, ammonium acetate, 1:1) by 60 μL of 200 mM acetylacetone solution (2% v/v acetylacetone, 49% v/v 1.6 M acetic acid, 49% v/v 4 M ammonium acetate), mixed and incubated in a water bath at 70 °C for 1 min, cooled down immediately in a water bath to room temperature and the optical density at 405 nm was then read in a Biotek EL800 microplate reader. Both these solutions were prepared daily as previously described (Bondioli and Bella, 2005).

The residual nitrate in the culture broth after 17 days of cultivation was detected using the method described by Bartzatt and Donigan (2004). Here, 20 μL of sample was placed in a 96 well plate and mixed with 90 μL of diphenylamine solution (3.34 g of diphenylamine in 14.4 M H_2SO_4) and 85 μL of pure H_2SO_4 was then