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Residual corn crop hydrolysate and silage juice as alternative carbon sources in microalgae production

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ABSTRACT

Microalgal biomass represents a sustainable alternative to fossil consumption. Microalgae grow in either photoautotrophic, heterotrophic or mixotrophic modes where the latter two trophic modes require organic carbon to grow efficiently. However, supplementation of organic carbon contributes significantly to a higher cost of microalgae production and this can compete with human and animal alimentation. Therefore, the use of second generation carbon sources from agriculture and industry residues offers an interesting solution. This study sought to determine the efficiency of two available and alternative organic carbon sources, residual corn hydrolysate and corn silage juice on the growth and lipid production of a bacteria–*Chlorella* consortium. Residual corn crop hydrolysate was predominantly composed of glucose, xylose, and arabinose, whereas silage juice contained volatile organic acids such as acetic acid. Algal biomass, neutral lipid content, esterase activity and reactive oxygen species were measured by using a flow cytometry. Photosynthetic activity was measured with a Plant Efficiency Analyzer (PEA). Under the mixotrophic condition, the photosynthetic activity remained constant throughout the experiment whereas a decrease was observed in the heterophophic condition. Maximum microalgal biomass of 0.8 g/L was obtained with 1 g/L of residual corn hydrolysate whatever the trophic strategy. Under mixotrophic conditions, the use of residual corn hydrolysate led to an increase of 21% and 22% in comparison with the biomass produced with glucose or silage juice, respectively. This increase varied between 11% and 28% under heterotrophic condition. At the end of the experiment, algae exposed to silage juice decreased significantly. Our study showed that the use of residual corn hydrolysate represents an interesting and efficient alternative as an organic carbon source. However, silage juice needs additional treatments to be implemented as a culture medium. This paper highlights the potential of two agro-industrial co-products as microalgal growth media with consequent production of high-value microalgal oil and biomass.

1. Introduction

As a biodegradable and renewable feedstock for biofuel production, microalgae cultivation has gained tremendous attention, principally as an alternative source due to its efficiency to produce biomass and its ability to produce lipids [8,40]. These large amounts of cellular lipids through transesterification of the alkyl esters of fatty acids provided and produced biofuels [45]. Microalgae can grow under photoautotrophic, heterotrophic or mixotrophic culture conditions, where higher mass production can be achieved in an open-pond system or a fermenter. Photoautotrophic growth requires the conversion of light and CO_2 into energy. On one hand, the addition of organic compounds as the carbon source is required for heterotrophic and mixotrophic cultivation. Several species of microalgae have the ability to assimilate organic carbon compounds, which exponentially stimulate their growth rate and dramatically enhance their lipid content, particularly in strains of *Chlorella protothecoides* and *Chlorella vulgaris* [28,29,46,48]. On the other hand, the uptake of organic carbon enhances algal cell division rate which significantly decreases the cellular energy storage within the cell [47].

As previously mentioned, the requirement to add a carbon source into the medium to accelerate the productivity of microalgae under mixotrophic and heterotrophic conditions increases the cost of production. The output/input ratio in algal cultivation remains a key factor, even when recycling elements are used such as wastewaters. The economic and social needs to replace existing consumable sugars with sustainable alternatives to reduce the cost of production become mandatory. Alternative sources of organic carbon have been successfully tested as supplement sources to heterotrophic and mixotrophic growth such as corn powder hydrolysate [47,48]. Alternative organic substrates have also been investigated to replace classical carbohydrates (glucose and glycerol). Accordingly, new sources of organic carbon have been tested such as Jerusalem artichoke, sugar cane, sweet sorghum, corn

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powder, cassava, dry-grind ethanol thin stillage, soy whey and crude glycerol [6,7,30,32,47,48], with positive biomass productivity in comparison with the use of pure glucose.

Agro-industrial waste could contain a variety of organic carbon, however, most microalgae cannot directly ingest and assimilate this waste due to the complexity of their composition such as polysaccharides, proteins and long fatty acids. Transformation and modification of these agro-industrial wastes may be required and could be accomplished through enzymatic or anaerobic processes which lead to easy-to-assimilate hydrolysates. Hydrolyzed corn powder in heterotrophic cultivation of *C. protothecoides* [48] and also hydrolysates of cassava starch [47] were used as alternative carbon sources. Therefore, residues of corn crop left on field could be an ideal and relatively inexpensive carbon source, once transformed into cellulose-hydrolyzed or sugars solution. Cellulose and hemicellulose contained in corn leaves and straws would be reduced into C5 and C6 sugars such as glucose, xylose, arabinose and mannose, which are simple and facilitate ingestion and assimilation. Silage juice is another agricultural waste material that could be used as an alternative source of organic compounds. Large amounts of silage juice are generated by natural fermentation under anaerobic conditions to preserve crops, such as corn or wheat in silos. Silage juice must be managed properly because it can be an important source of environmental pollution. Silage juice contains high concentrations of organic compounds such as lactic acid, acetic acid and sugars [25,37]. Then, the silage juice could be recovered and used as an alternative organic carbon substrate for the production of microalgae.

The current study investigates two agricultural waste co-products, corn silage juice and residual corn crop hydrolysate, in their ability to support and to promote mixed microalgal growth using wastewaters from a smelter plant. The goal of this study was to provide insights on how a native microalgae–bacteria consortium would adapt when cultured in two alternative organic carbon sources (residual corn crop hydrolysate and silage juice) compared to glucose. To elucidate the viability of the algal population, esterase activity, chlorophyll *a*, reactive oxygen species (ROS) and neutral lipids (NL) were measured by using flow cytometry. Furthermore, microalgae biomass, lipid peroxidase (LPO), ascorbic peroxidase (AXP) and mitochondrial electron transport (MET) were measured.

2. Methods

2.1. Microalgae–bacteria consortium

The microalgae–bacteria consortium was mainly composed of *Chlorella* spp. and was isolated from the water retention pond of a smelter plant in Quebec province, Canada and was adapted to the smelter plant wastewaters [2].

2.2. Culture medium

Smelter plant wastewaters were used as a culture medium for the microalgae culture with addition of nutrients to mimic Bold's basal medium (BBM) (g/L): KNO_3, 0.75; KH_2PO_4, 0.7; $MgSO_4$, 0.3; and chelated iron, 0.028. The chemical composition of the smelter wastewater was described in Table 1. The carbon sources were either glucose, residual corn crop hydrolysate or corn crop silage juice. Residual corn crop materials were obtained from Agrosphère, Lanoraie, Canada. Stover, cob and leaf were ground to separate cellulose from lignin and hemicellulose by using a twin-screw extruder. The cellulose fraction was then converted into sugars with the enzymatic cocktail, Accelerase® Duet™ [9]. Tetracycline and cycloheximide were added in the hydrolysates to prevent the consumption of sugars by bacteria [42]. Corn silage juice was acquired from Nethasol (Quebec, Canada) and stored at 4 °C. Silage juice was 3 years old. The composition of corn silage juice and residual corn crop hydrolysate is listed in Table 2. Both corn silage juice and residual corn crop hydrolysate were filtered on a sterile 0.22 μm Millipore system prior to usage.

Table 1
Ion concentrations in waster waters from the smelter plant. LOD = limit of detection.

Ion	mg/L
Cl^-	166.28
Br^-	0.58
F^-	5.1
NO_3^-	54.48
PO_4^-	2.01
SO_4^{2-}	<LOD
Al	0.65
Mn	0.01
Mg	6.65
Mo	0.09
Zn	0.389
Co	<LOD
Cr	<LOD
Cu	<LOD
Fe	<LOD
Oil and grease	0.4
Aliphatic hydrocarbons	–
HAP	0.0008

2.3. Cultivation of microalgae consortium

Experiments were performed in 1 L shake flasks. Each flask containing 500 mL of the medium was inoculated (10% v/v) with an active culture of the microalgae consortium and then incubated at room temperature and stirred at 150 rpm. A 12:12 h light/dark cycle with 20 μmol photon/m^2/s was provided by two cool white fluorescent lamps for the photoautotrophic and mixotrophic cultures while heterotrophic cultures were kept in the dark. Two separate doses of 0.5 g/L of organic carbon were added into the media culture at time 1 h and 25 h. All experiments lasted 48 h and were performed in triplicate.

Table 2
Composition of the corn silage juice and the residual corn crop hydrolysate.

Organic components (g/L)	Corn silage juice	Residual corn hydrolysate
Crude protein	2.05	80.6
Elemental analysis		
Total carbon	38.0	42.04
Hydrogen	5.45	4.78
Total nitrogen	2.85	1.29
Sulfur	0.41[a]	0
Lactic acid		
Volatile fatty acids		
Acetic acid	9.1	
Proprionic acid	7.4	
Butyric acid	7.8	
Succinic acid	0.15	
Citric acid	0.76	
Reducing sugars		55.63
Carbohydrates		
Glucose		30
Fructose		
Sucrose		
Arabinose		3.6
Xylose		22.7
Galactose		
Mannose		
Alcohols		
Ethanol	5900	
Methanol	350	
n-Propanol	340	
Methyl iso-butyl ketone	79–89	

[a] Value below the limit of detection.