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Biodiesel production using *Chlorella sorokiniana* a green microalga

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Abstract - *Several commercial applications of microalgae are identified. They can be used to enhance the nutritional value of food owing their chemical composition, they play a crucial role in aquaculture and they can be incorporated into cosmetics. Currently, they are cultivated as a source of renewable energy. Indeed, biodiesel, one form of biofuels can be produced by microalgae. This biofuel has attracting increasing attention worldwide as a clean energy for the future to substitute conventional fuel. However, their production derived from conventional ways that are so expensive and polluant because they used fossil fuels and oilseeds (vegetable oils) or animal fats. In this paper, wild/type strains of chlorella "green microalgae" isolated from Algerian Sahara soil are tested for their ability to produce biodiesel. The results show that Chlorella sorokiniana strain Ce, under different culture conditions, accumulates some fatty acids which make the most suitable for the production of good quality biodiesel.*

Résumé - *Plusieurs applications commerciales de micro-algues sont identifiées. Elles peuvent être utilisées pour améliorer la valeur nutritionnelle des aliments en raison de leur composition chimique, et elles jouent un rôle crucial dans l'aquaculture et peuvent être ainsi incorporées dans des produits cosmétiques. Actuellement, ces micro-algues sont cultivées comme source d'énergie renouvelable. En effet, le biodiesel, une forme de biocarburants, peut être produit par des micro-algues. Une attention croissante existe à travers le monde sur ce biocarburant, lequel est considéré comme une énergie propre pour l'avenir, pour remplacer les combustibles classiques. Toutefois, leur production provenant de façons conventionnelles, qui sont coûteuses et polluantes, parce qu'ils utilisaient des combustibles fossiles et d'oléagineux (huiles végétales) ou de graisses animales. Dans cet article, des souches sauvages de type 'Chlorella' 'microalgues vert' isolées dans le sol du Sahara algérien, sont testées pour leur capacité à produire du biodiesel. Les résultats montrent que la souche de Chlorella sorokiniana Ce, sous différentes conditions de culture, accumule certains acides gras qui sont plus appropriés pour la production de biodiesel de bonne qualité.*

Keywords: Biodiesel – Production - *Chlorella* - Renewable energy.

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1. INTRODUCTION

The growing preoccupation for environment and global warming, in conjunction with the adherence of signatory countries to Kyoto Protocol including Algeria, leads to use clean and renewable energy. Currently, several technologies developed energy systems based on the use of biohydrogen (Chader *et al.*, 2009) and biodiesel (Meng *et al.*, 2009, Basha *et al.*, 2009) as a clean energy for the future.

Biodiesel is one of the renewable energy resources, which is produced from renewable biomass by transesterification of triacylglycerols. In several countries such as South East Asia, Europe, United States and China, palm oil, rapeseed oil, transgenetic soybeans and wasting oil are used to produce biodiesel (Meng *et al.*, 2009).

However, all these plant oil materials require energy and large agricultural acreage. In spite of the favourable impacts that its commercialization provide, the economic aspect of biodiesel production has restricted by the cost of oil raw materials (Antolin, 2002). If plant oil is used for biodiesel production, the cost of source has account to 70 – 85 % of the whole production cost (Miao *et al.*, 2006).

Therefore, taking into account of these inhibition factors, exploring ways to reduce the high cost of biodiesel is of much interest in recent research. Microalgae have often considered for the production of oils and fats as an alternative to agricultural and animal sources. Indeed, Biodiesel produced from microalgae will not compromise the production of food and other products derived from crops.

In addition, oil productivity of microalgae cultures exceeds the yield of the best oil seed crops, e.g. biodiesel yield of 12 000 liter/ha for microalgae (open pond production) compared with 1190 liter/ha for rapeseed (Schenk *et al.*, 2008).

Also, microalgae are grown in aqueous media, but need less water than terrestrial crops therefore reducing the load on freshwater sources and are capable production of all year round (Dismukes *et al.*, 2008).

Finally, microalgae can be cultivated in brackish water on non-arable land, and therefore may not incur land-use change, minimising associated environmental impacts (Huang *et al.*, 2010; Searchinger *et al.*, 2008).

In the same context, the present study attempts to study the ability of biodiesel production from green microalgae. The main purpose of this paper is to estimate and measure the amount of biodiesel produced by isolated strains of Chlorella.

2. MATERIALS AND METHODS

2.1 Strains of microalgae

Local wild type strains of the green microalgae species Chlorella, isolated from soil and foggaras's water of Touat, located in Adrar in the Algerian Sahara on March 2004 were used; they were identified as *Chlorella sorokiniana* strain Ce (Fig. 1).

2.2 Cultivation medium

Four medium are used for biodiesel production from algae. The first one is BG11 media (photoautotrophic conditions) where salts are in mg/l: $NaNO_3$ 1.5, $K_2HPO_4.3H_2O$ 0.04, $MgSO_4.7H_2O$ 0.075, $CaCl_2.2H_2O$ 0.036, citrique acid 0.006, citrate ammoniac ferric 0.006, EDTA 0.001, Na_2CO_3 0.02, Trace metal mix A5+Co 1 ml with pH adjusted at 7.4. The second media is under heterotrophic condition using acetate as organic substrate in Tri Acetate Phosphate medium (Harris, 1989).

The third nutrition medium enriched or no with nitrogen (fourth medium) where composition is in mg/l: urea 1050, K_2HPO_4 265, $MgSO_4.7H_2O$ 185, Fe_2 $(SO_4)_3$ 10, H_3BO_3 0.5, $ZnSO_4.7H_2O$ 0.5, $MnSO_4.4H_2O$ 0.5, $CuSO_4.5H_2O$ 0.1, $(NH_4)_2$ MoO_4 0.01, $CoSO_4.7H_2O$ 0.001 with pH maintained at 7.3 (Petkov *et al.,* 2007).

The Chlorella biomass was grown at 25 °C (±1°C) in 5 l flasks in an environmental growth chamber with a light intensity of ≈ 100 photons$/\mathrm{m}^2$.s and a 16 h photoperiod per day.

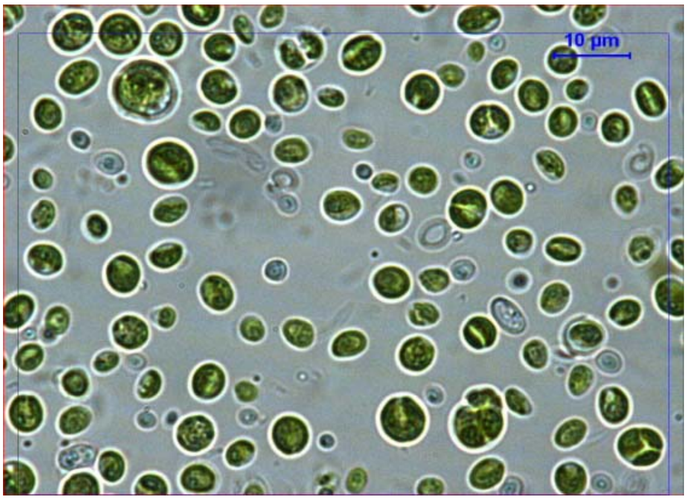

Fig. 1: *Chlorella sorokiniana* strain
Ce observed under optic microscope (G x 40)

2.3 Lyophilization of Chlorella culture

The biomass sample was harvested by centrifugation and dried to a constant weight. The cell mass obtained after filtration was lyophilized. The wet cell mass was frozen overnight at - 70 °C and then freeze-dried at - 52 °C under vacuum. The dry biomass obtained after freeze-drying was stored in air-tight containers at 20 °C.

2.4 Extraction of lipids and transmethylation

The total lipids were estimated gravimetrically using extraction Folch method (Folch *et al.*, 1957) and modified by Christie (1989). Parts of the lipid samples were converted to fatty acid methyl esters by heating in methanol containing 6 % anhydrous HCl at 70 °C for 1 h. The fatty acid methyl esters were extracted with hexane and purified by decantation.

2.5 Fatty acid analysis

Gas chromatography of fatty acid methyl esters was carried out on a Trace GC (Thermo Finnigan) equipped with a flame ionization detector (FID), using 30 m long supelcowax-10 capillary column at 225 °C. Helium was a carrier gas at flow rate of 20 ml/min. Chromatographic data was recorded and integrated using Agilent data analysis software. The components were identified by comparing their retention times and fragmentation patterns with those for standards (C16:0, C16:1, C17:0, C18:0, C18:1, C18:2 and C18:3).

3. RESULTS AND DISCUSSION

The major fatty acid composition of the tested microalgae was determined using a GC analysis (**Table 1**). It is mainly composed of mixture of unsaturated fatty acids, such as oleic (18:1), linoleic (18:2) and linoleic acid (18:3). Saturated fatty acids, palmitic (16:0) and stearic (18:0) are also present to a small extent. In a study of Knothe (2008), palmitic, stearic, oleic, and linoleic acid were recognized as the most common fatty acids contained in biodiesel.

In the three media tested (TAP, BG 11 and media +N), oleic acid (C18:1) and linoleic acid (C18:2) were commonly dominant. The results shown that the most important fatty acids, are C16:0, C18:0, C18:1, C18:2 and C18:3 whatever the medium.

In the fourth media (media +N), we observe that additional quantity of fatty acids C18:2 are produced when the strain is cultivated under nutrition medium enriched with nitrogen as it's reported in then literature (Petkov *et al.*, 2007 and Xu *et al.*, 2001). These acids make the most suitable for the production of good quality biodiesel (Lee *et al.*, 2010).

Table 1: Percentage of main fatty acids composition of *Chlorella sorokiniana* strain Ce cultivated under different medium

Fatty acid	**TAP media**	**BG 11 media**	**Media +N**	**Media -N**
Palmitic acid C16:0	17.14	23.24	16.73	16.85
Stearic acid C18:0	0.34	2.58	2.41	0.21
Oleic acid C18:1	17.40	30.99	28.74	17.30
Linoleic acid C18:2	24.66	23.72	20.82	17.67
Linoleic acid C18:2 in transposition	0	0	16.78	6.99
Linoleic acid C18:3	26.63	1285	10.21	30.34

4. CONCLUSION

The present study introduced an integrated method for the production of biodiesel from locally isolated strain of *Chlorella sorokiniana*.

The study strain culture produced a same quality composition of fatty acids compared with another species of Chlorella. This cultivation is interesting in the case of biodiesel production as diesel engines in alternative use.

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