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Laboratory extraction of microalgal lipids using sugarcane bagasse derived green solvents

Sérgio S. de Jesus*, Gabriela F. Ferreira, Leonardo V. Fregolente, Rubens Maciel Filho

Laboratory of Optimization, Design and Advanced Control, School of Chemical Engineering, University of Campinas, P.O. 6066, 13083-852 Campinas, SP, Brazil

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ABSTRACT

Three green solvents were tested for lipid extraction based on the Bligh and Dyer method. Lipids from the microalgae *Chlorella pyrenoidosa* were extracted through the tertiary systems 2-methyltetrahydrofuran:isoamylalcohol:water (1:3, 1:2, 1:1, 2:1, 3:1 v/v) and 2-methyltetrahydrofuran:ethanol:water (3:1 v/v). For comparative effect, extractions with chloroform:methanol:water (3:1, 2:1, 1:1, 1:2, 1:3 v/v), with binary systems 2-methyltetrahydrofuran:water, isoamyl alcohol:water, chloroform:water, n-hexane:water, and monophasic systems 2-methyltetrahydrofuran (2-MeTHF), isoamyl alcohol, chloroform and n-hexane were used. The Bligh and Dyer method using green solvents was highly efficient, although some undesirable components were extracted, especially when larger volumes of isoamyl alcohol were used. System 2-MeTHF:ethanol:water formed only a biphasic system when it was used in a 3:1 (v/v) proportion, obtaining 64% of fatty acids. The chloroform:methanol (1:2 v/v) system extracted 122.2 ± 1.9 mg g^{-1}, in which 92.4% consisted of fatty acids. In systems using green solvents, the extractions with the system 2-MeTHF:isoamyl alcohol (2:1 v/v) achieved a 78% selectivity (99.6 ± 7.6 mg $g^{-1}$of fatty acids) and an 88.2% efficiency when compared with the reference system chloroform:methanol 1:2 (v/v).The fatty acids profile showed that extractions with 2-MeTHF:isoamyl alcohol had higher percentages of fatty acids with low degree of unsaturation, which is ideal for the production of better quality biodiesel. The results obtained suggest that2-MeTHF:isoamyl alcohol (2:1 v/v) can replace the chloroform:methanol system currently used. At an industrial level, this system still requires some technical improvements related to the energy expenditure for solvent evaporation, and at an economic level, the high price of the 2-MeTHF makes the process impracticable compared with hexane.

1. Introduction

More than 60% of the world chemical industry uses solvents in its processes, primarily in the pharmaceutical and fine chemistry fields [1]. Recently, with increasing global demand for fuels and the growth of greenhouse effects, new studies have focused on microalgae as a potential source of biofuel production (biodiesel and bioethanol) [2,3]. However, to produce biodiesel, microalgal oil needs to be extracted after its growth and harvest, which requires large amounts of organic solvents.

The industrial extraction of these oils has been conducted with the solvent n-hexane due to its low cost, ease of recovery and high solubility in oil [4]. N-hexane is a non-renewable solvent made from petroleum and identified by the U.S. Environmental Protection Agency (EPA) as a dangerous air pollutant, responsible for causing neurotoxic effects at high exposure levels [5,6].

The increasing demand for these solvents raised questions not only from environmentalists but also from the scientific community regarding the environmental impact of these compounds, since, besides being inflammable, most are highly toxic and harmful to human beings and to the environment [7]. Recently, new classes of solvents emerged in the market aiming to minimize environmental impacts; the so-called “green solvents” intend to replace the ones currently used, since most of them are toxic. To be considered green, a solvent must originate from renewable raw materials such as biomass and deemed harmless or easily reused by the environment [8].

In the bioethanol production process, several byproducts can be obtained, including a large quantity of solvents that achieve the minimum requirements to be considered green solvents. Among these solvents, 2-methyltetrahydrofuran (2-MeTHF) was obtained by catalytic hydrogenation of the furfural from sugarcane bagasse (Fig. 1) [9,10], and isoamyl alcohol (3-methyl-1-butanol) was obtained from the residue of the bioethanol purification process (Fig. 1).In laboratories, the most common and effective methods for the extraction of oils

* Corresponding author.
E-mail address: ssjesus@gmail.com (S.S. de Jesus).

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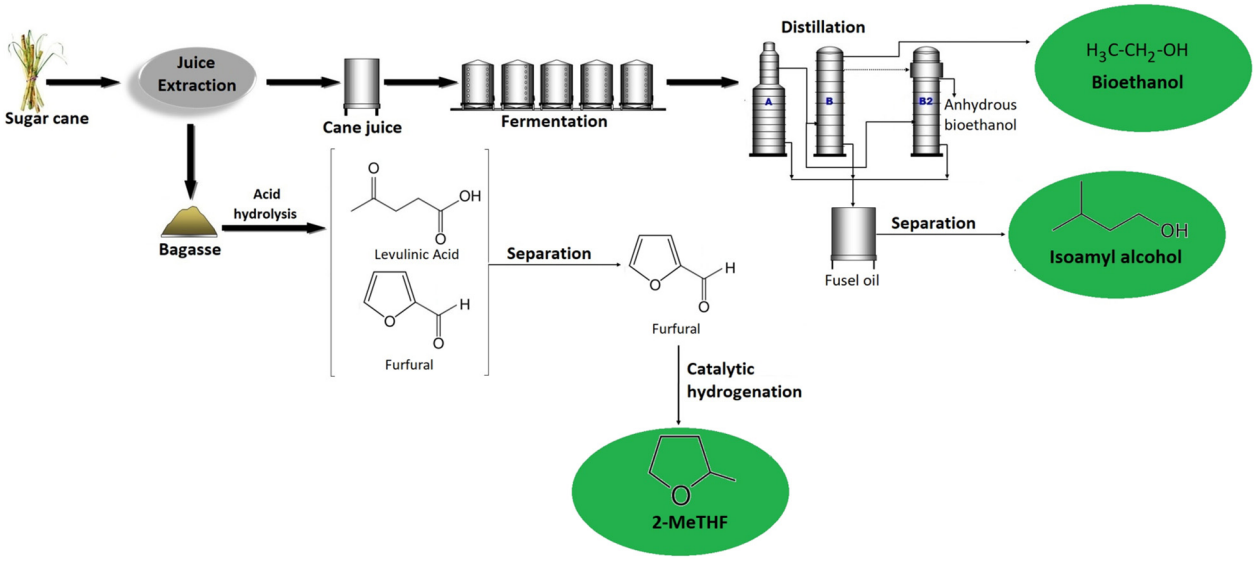

Fig. 1. Summary of the process flow diagram for obtaining 2-methyltetrahydrofuran and isoamyl alcohol from sugarcane bioethanol production.

or lipids are the ones developed by Folch [11], who used chloroform-methanol solvents, and especially by Bligh and Dyer [12], who enabled the formation of a two-phase system based on the proportions of the solvents added during the extraction process. However, the toxicity of the solvents employed and the extraction of non-lipid contaminants in the organic phase are disadvantages of these two methods. Due to the lack of alternative methodologies that use green solvents, these procedures are still the most used in laboratory extractions. Currently, no effective method that uses green solvents to extract oils from raw materials of animal, plant or microbial origins has managed to obtain a biphasic system that permanently replaces the chloroform used in the Bligh and Dyer method. The use of toxic and hazardous solvents in laboratories and in the chemical industry is considered a very important problem for the health and safety of workers and for the environment.

This study proposes an adaptation of the Bligh and Dyer method [12] for lipid extraction from microalgae aiming at the biodiesel production, using green solvents obtained through byproducts of the bioethanol production process from sugarcane. The choice of isoamyl alcohol and ethanol as substituents of methanol was based on their low toxicity to the environment, in addition to the formation of a two-phase system and absence of emulsion. To assess the efficiency of extraction, the microalgae *Chlorella pyrenoidosa* was used.

2. Experimental

2.1. Materials

2.1.1. Microalgae

Chlorella pyrenoidosa was purchased, in tablet form, from Taiwan Chlorella Manufacturing (Taiwan, China).

Isoamyl alcohol and ethanol were obtained from Dinâmica Ltda. (Brazil). According to the manufacturer these solvents are produced through a process that uses sugar cane as raw material. The solvent 2-methyltetrahydrofuran was purchased from the Sigma-Aldrich Corporation and was obtained from a renewable source not declared by the manufacturer.

2.1.2. Chemicals

All chemicals used in the extraction process are listed in Table 1.

Table 1
Chemical used in the extraction process.

Solvent	Formula	CAS no.	Purity	Manufacturer
Chloroform	$CHCl_3$	67-66-3	≥99.8%	Êxodo Científica (Brazil)
Ethanol	C_2H_6O	64-17-5	≥99.5%	Dinâmica Ltda. (Brazil)
Isoamyl alcohol (3-methyl-1-butanol)	$C_5H_{12}O$	123-51-3	≥98.5%	Dinâmica Ltda. (Brazil)
Methanol	CH_4O	67-56-1	≥99.9%	Êxodo Científica (Brazil)
n-Hexane	C_6H_{14}	110-54-3	≥98.5%	Labsynth Ltda. (Brazil)
2-Methyltetrahydrofuran	$C_5H_{10}O$	96-47-9	≥99.0%	Sigma-Aldrich Co. (Germany)

2.2. Methods

2.2.1. Drying

Tablets of *C. pyrenoidosa* were first grounded in a mill (A11 basic analytical mill, IKA, Germany) and sieved to obtain a homogeneous mixture with a particle size of 120 mesh (0.125 mm). Then the powder was dried at 80 °C for 24 h before the extraction procedure, reaching a moisture content lower than 3.5 ± 0.2 wt%.

2.2.2. Lipid extraction

The lipid extraction was performed based on the Bligh and Dyer method [12] adapted to the 2-MeTHF:isoamylalcohol:water and 2-MeTHF:ethanol:water green solvent systems. The proportions used were: 3:1, 2:1, 1:1, 1:2 (v/v) 2-MeTHF:isoamyl alcohol and 3:1 (v/v) 2-MeTHF:ethanol; the water volume was calculated through the dielectric constant of the solvent mixture, having the Bligh and Dyer method as a reference and using the 1:2:0.8 (v/v/v) chloroform:methanol:water system. For comparison purposes, extractions with the chloroform:methanol:water in the proportions 3:1, 2:1, 1:1, 1:2 and 1:3 (v/v) chloroform:methanol were performed, as well as extractions with the 2-MeTHF:water, isoamyl alcohol:water, chloroform:water and n-hexane:water binary systems and 2-MeTHF, isoamyl alcohol, chloroform and n-hexane solvents. To calculate the water volume required in each extraction system, Eq. (1) and data in Table 2 were used.

The dielectric constant of the solutions was calculated according to the equation [13]: