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Silver nanofiber assisted lipid extraction from biomass of a Louisiana *Chlorella vulgaris*/*Leptolyngbya* sp. co-culture

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HIGHLIGHTS

- Silver nanofibers (AgNF) enhance the lipid extraction from microalgal biomass.
- 1000 μg g^{-1} of silver nanofibers increased up to 136% the microwave lipid extraction.
- The fatty acid profile of the extracted lipids meets ASTM D 6751 biodiesel standard.
- Microwave heating at 70 °C for 5 min and 1000 μg g^{-1} AgNF extracted the most lipids.

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ABSTRACT

The presence of bulk water and the resistant cell walls significantly limit the efficiency of lipid extraction from microalgal biomass paste. Current methods to rupture the cell walls (i.e. grinding after freeze-drying, osmotic shock, sonication) are energy intensive and time consuming. Due to their high surface energy concentration and high surface to volume ratio, silver nanoparticles can enhanced the cell wall rupture to increase the extraction efficiency of cell components. In this study, silver nanofibers were added as enhancers for the Folch's extraction method and microwave assisted extraction of lipids from wet biomass paste (water content of 80.9%). Nanofibers concentrations of 0–1000 μg g^{-1} were tested. Two solvent:biomass ratios were tested in the Folch's extraction method. Two temperatures (70 and 90 °C) and three treatment times (2, 5 and 10 min) were compared in the microwave assisted extraction. The results showed that the extraction efficiency increased with increased concentration of the nanofibers in the range tested. At concentrations of 1000 μg g^{-1} silver nanofibers (w/w based on the solvent and biomass solution) the efficiency of lipid extraction increased by ~30% and 50% for the Folch's and microwave assisted lipid extraction respectively. Treatment with $AgNO_3$ in the same concentration as the nanofibers did not improve the extraction compared with no silver or nanofibers addition. The extraction method affected the lipid fatty acids profile. The Folch's extraction with no silver nanofibers resulted in proportionally higher short chain saturated fatty acids, but lower lipid extraction. The microwave assisted lipid extraction provides the best results considering fatty acid profile, treatment time, solvent use and lipid extraction efficiency.

1. Introduction

Over the last several decades, microalgae have garnered significant attention as a feedstock of transportation fuels for the future primarily due to their higher growth rate than land crops, photosynthetic efficiency, lipid productivity and the ability to use non-arable lands, eliminating competition with food crops [1,2].

Microalgal biodiesel production is mainly comprised of five steps including strain selection, cultivation, biomass harvesting (dewatering), lipid extraction and transesterification [3]. Although technical issues remain in almost every of these steps for microalgal biodiesel production [4,5], life cycle analyses indicate that lipid extraction is one of the most energy intensive and thus costly processes in the chain [6]. Organic solvent based lipid extraction

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is the most commonly used method for microalgal lipid extraction [7].

Halim et al. [7] have described organic solvent-based lipid extraction from algal cells as a five-step process: (1) the solvent enters the cytoplasm by diffusion through the cell walls and membrane structure; (2) solvent and the lipid interact via van der Waals forces and (3) form a solvent–lipids complex; (4) the solvent–lipids complex diffuses through the cell wall via a concentration gradient; (5) the solvent–lipid complex passes through a static solvent film surrounding the cells and mixes with the bulk solvent. The process requires solvent to diffuse through the cell wall twice. The multilayer microalgal cell walls [8] can significantly limit the diffusion rate of both solvent and lipid. Therefore, disruption of these cell walls would drastically increase the efficiency of solvent-based lipid extraction from microalgae.

Microalgal cell walls are mainly comprised of linear and branched polysaccharides that form networks of microfibrils with strong semi-crystalline patterns [9]. Thus, the tensile strength of cell walls can be as high as 95–100 atmospheres [10]. In order to increase the lipid extraction efficiency, mechanical grinding is usually applied to break the cell wall of oil producing crops like soybean, rapeseed and canola prior to the lipid extraction process. However, the typical microalgal paste after centrifugation contains ~80% water (w/w) compared to ~10% for soybean seeds [11]. The large amount of bulk water not only generates a barrier between the solvent and the lipid/oil, it also limits the effectiveness of mechanical grinding to break the cell walls. The cells flow through the microchannels in the bulk water instead of being disrupted [12]. Many energy intensive and time-consuming methods have been utilized in an attempt to disrupt microalgal cell walls for increased lipid extraction efficiency, including sonication, manual grinding, and microwaves [7,12].

Following Soxhlet lipid extraction using sonication and microwave pretreatment of the microalgal biomass, Cravotto et al. [13] reported 4.8–25.9% improvement over Soxhlet lipid extraction without sonication. For Blight Dyer extraction, Lee et al. [14] reported less than 5% increase in lipid extraction by using sonication for 5 min as pretreatment on microalgal cells. Cooney et al. [12] obtained an increase of 45% using grinding-assisted lipid extraction in *Nannochloropsis* sp. compared to the unground freeze-dried samples. However, for the purpose of fuel production from microalgal biomass, grinding combined with freeze-drying is highly energy intensive and has little scale-up potential [7]. Microwave assisted solvent extraction changes the electric field along with the wavelength at high frequency, leading to instantaneous water heating inside the cells, causing rupture of the cell walls, facilitating a more rapid diffusion of microalgal lipids into the extracting organic solvent [7]. Lee et al. [14] reported a ~200% increase in lipid extracted from *Botryococcus* sp. for microwave extraction at 100 °C for 10 min compared to Bligh and Dyer extraction, however, the same procedure resulted in ~5% increase for *Chlorella vulgaris* and *Scenedesmus* sp.

For economic viability, an extraction method requiring less energy input to rupture cell walls in the presence of bulk water is needed. Metals such as copper, cobalt, cadmium, silver have been reported to interact with cell walls and cause structural and morphological changes within cell walls [15]. Silver and copper have been extensively studied for their antimicrobial activity, which is directly related to the interactions between the metals and cell walls [16]. This antimicrobial and cell disruption activity could be significantly enhanced by using nanostructured metals due to increased surface to volume ratio [17]. Ruparelia et al. [16] found that the antimicrobial efficiency for silver nanoparticles, which was directly related to cell wall disruption, was almost 40–50% higher than copper nanoparticles. Although these results are based on prokaryotic organisms, nanostructured silver could be a good choice for microalgal cell disruption based on the effect on cell walls of bacteria.

The mechanisms through which the silver nanomaterials affect the microbial cells is not clear. Some authors have suggested that initially the silver nanoparticles attach and anchor on the surface of the cell wall [18,19]. The electrostatic forces and molecular interactions involved are believed to cause structural and morphology changes, damaging the cell wall [18,20]. The high energy concentration on the surface of silver nanoparticles caused by differential energy absorption could be another factor that attribute the ability of silver nanoparticles to rupture the cell walls [21]. Once attached to the cell wall, the nanoparticles can penetrate the cell wall through damaged areas, perforating the cell membranes and releasing intracellular materials [22]. Other authors have pointed that toxicity may be caused by reactive oxidative compounds formed by the interaction of the nanomaterials with the environment chemistry, damaging the cell wall [20]. Based on the studies above, it is likely the cell disruption activity of silver nanoparticles would significantly increase the lipid extraction efficiency from microalgal cells.

In this study, the ability of silver nanofibers to improve the lipid extraction efficiency of the Folch's [23] and microwave-assisted lipid extraction was assessed for a *C. vulgaris*/*Leptolyngbya* sp. co-culture. The fatty acid profiles of the lipid extracted by each tested method (i.e. Folch's method and microwave extraction with and without nanofibers) were analyzed to investigate the effects of the extraction methods on the fatty acid profile from this co-culture.

2. Materials and methods

The impact of silver nanofiber concentrations (0, 50, 200, 500, 1000 $\mu g\ g^{-1}$, based on the solvent–biomass mixture) on the lipid extraction efficiency of the Folch's method and microwave-assisted lipid extraction from wet paste of the Louisiana *C. vulgaris*/*Leptolyngbya* sp. co-culture was investigated. The fatty acid (FA) profiles of the lipids extracted using each method were determined. The FA profiles provided information on the impact of these extraction methods on the compositions of the biodiesel produced from this Louisiana *C. vulgaris*/*Leptolyngbya* sp. co-culture.

2.1. Strain selection and biomass production

A *C. vulgaris*/*Leptolyngbya* sp. (97:3 by cell count based on flow cytometer) co-culture was used in this research. This co-culture was isolated from the College Lake, Baton Rouge, LA. In previous work (unpublished data) it was found that the presence of the *Leptolyngbya* sp. improved the growth rate of the *C. vulgaris*. In previous studies, the lipid productivity was reported as high as 116 $g\ m^{-3}\ d^{-1}$ with 89% neutral lipids [24]. The fatty acid profile of this co-culture meet the ATSM D 6751 standards for biodiesels and does not vary with irradiance and nitrogen levels [24].

In this study, the microalgal biomass for this co-culture was produced in a hydraulically integrated serial turbid stat algal reactor (HISTAR) developed by Rusch and Malone [25]. HISTAR is comprised of two enclosed turbidostats and a series of eight open-top, continuous flow stirred-tank reactors (CFSTRs). The inoculum culture was prepared in four 10 L carboys with F/2 media and an irradiance level of 200 $\mu mol\ m^{-2}\ s^{-1}$ with 1 $L\ min^{-1}$ aeration. When the optical density (OD) measured at 664 nm of the culture reached 0.5, the co-culture in the carboys was inoculated in the turbidostats with 100 L of F/2 media. The culture grew in the turbidostats in batch mode for 3–4 days until the OD at 664 nm reached 0.6 before it was diluted to 454 L with F/2 media. The HISTAR process controller maintains the pH and inoculum