Bioresource Technology 218 (2016) 667–673

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Detergent assisted lipid extraction from wet yeast biomass for biodiesel: A response surface methodology approach

Sravan Kumar Yellapu, Jyothi Bezawada, Rajwinder Kaur, Mathiazhakan Kuttiraja, Rajeshwar D. Tyagi *

INRS Eau, Terre et Environnement, 490, rue de la Couronne, Québec G1K 9A9, Canada

HIGHLIGHTS

- Wet oleaginous yeast biomass was used for lipid extraction.
- Biodegradable anionic detergent (N-lauroyl sarcosine) was used for cell disruption.
- N-lauroyl sarcosine minimized lipid extraction time.
- N-lauroyl sarcosine had no effect on fatty acid profiles.

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 2 July 2016
Accepted 4 July 2016
Available online 5 July 2016

Keywords:
Lipid extraction
N-lauroyl sarcosine
Box-Behnken design
Biodiesel

ABSTRACT

The lipid extraction from the microbial biomass is a tedious and high cost dependent process. In the present study, detergent assisted lipids extraction from the culture of the yeast *Yarrowia lipolytica* SKY-7 was carried out. Response surface methodology (RSM) was used to investigate the effect of three principle parameters (N-LS concentration, time and temperature) on microbial lipid extraction efficiency % (w/w). The results obtained by statistical analysis showed that the quadratic model fits in all cases. Maximum lipid recovery of 95.3 ± 0.3% w/w was obtained at the optimum level of process variables [N-LS concentration 24.42 mg (equal to 48 mg N-LS/g dry biomass), treatment time 8.8 min and reaction temperature 30.2 °C]. Whereas the conventional chloroform and methanol extraction to achieve total lipid recovery required 12 h at 60 °C. The study confirmed that oleaginous yeast biomass treatment with N-lauroyl sarcosine would be a promising approach for industrial scale microbial lipid recovery.

1. Introduction

Biomass based biodiesel is a recent promoting approach to study alternative fossil based fuel due to concerns of decreasing oil reservoirs and less emission of greenhouse gasses (GHG) (Martínez et al., 2015; Moser, 2011). The current research is focused on biotransforming industrial waste like crude glycerol, lignocellulosic waste, and municipal secondary sludge to renewable fuel (biodiesel) using heterotrophic oleaginous microorganisms (Capus et al., 2016; Johnson and Taconi, 2007; Kumar et al., 2009; Seo et al., 2013). The microbial production of lipid will occupy less arable land and will not affect the food supply chain (Martínez et al., 2015).

Biodiesel production utilizing oleaginous yeast consists of three major steps, microorganism cultivation (lipid accumulation), cell wall disruption and lipid extraction from the biomass, and transesterification. Lipid is energy storage (lipid droplets) and structural components of the cell membrane. The lipid droplets are enveloped by phospholipid membrane and outer cell membrane has to be disrupted to free the microbial lipid. There are two widely known methods, i.e. organic solvent extraction and mechanical pressing that have been used to extract lipid from lipid bearing substances. The main disadvantages of these methods are the low lipid yield and long process time required for extraction (Cheng et al., 2011). Therefore, method with high lipid yield and less process time is required.

Traditionally chloroform and methanol based lipid extraction is effective, but it is time consuming (8–12 h), needs temperature up to 60 °C and solvents are toxic having safety concern. Therefore, decreasing solvent volume and time of extraction are main factors for cost effective lipid extraction and safety reasons. Consequently, lipid extraction from dried biomass employing mechanical cell disruption processes such as bead milling, homogenization, microwave, ultra-sonication is an energy intensive process (Garoma and Janda, 2016; Jin et al., 2012; Lee et al., 2010; Zhang

* Corresponding author.
E-mail address: rd.tyagi@ete.inrs.ca (R.D. Tyagi).

http://dx.doi.org/10.1016/j.biortech.2016.07.011
0960-8524/

et al., 2014b). The non mechanical methods such as lytic enzyme treatment, alkali and acid (Jin et al., 2012; Miranda et al., 2012) are cost prohibitive for large-scale microbial lipid extraction.

The moisture content of the cell biomass (which is more than 80% on weight basis) needs to be removed by oven drying or lyophilization (dewatering) process before lipid extraction. Various researchers have investigated different methods of lipid extraction and in-situ transesterification from wet biomass (Table 1) including supercritical methanol (Patil et al., 2011), Enzyme assisted extraction (Jin et al., 2012). Ethanol (Yang et al., 2014), Simultaneous distillation and extraction process (SDEP) (Dejoye Tanzi et al., 2013), osmotic shock (Yoo et al., 2012), acid and base hydrolysis (Sathish and Sims, 2012) and 3_DAPS (Lai et al., 2016). Unfortunately, the most cases still require high energy input, time. Therefore, lipid extraction technologies are up to now within the laboratory scale. Therefore, there is lack of suitable industrial scale lipid extraction has not been developed.

N-lauroyl sarcosine (N-LS), an amino acid derived detergent (an anionic detergent, made up of amino acid sarcosine and fatty acid), is non-toxic, and biodegradable (Kippert, 1995). It can disrupt the cell wall by the formation of micelle at certain specific N-LS concentration, incubation time and temperature (Abraham and Bhat, 2008; Yadav et al., 2014). It can be safely used for permeabilization of yeast cells to release intracellular enzyme activities (Yadav et al., 2014). There are several chemical surfactants like Triton-100, sodium dodecyl sulfate (SDS),toluene and cetyltrimethylammonium bromide (CTAB) that have been used for yeast cells permeabilization (Abraham and Bhat, 2008; Kippert, 1995). But most of them are toxic and environmentally unsafe.

The objective of the present study is to investigate the wet biomass cell disruption for lipid extraction using N-lauroyl sarcosine. Response surface methodology was used to optimize the process parameters to obtain maximum lipid extraction efficiency. Three important parameters (N-LS concentration, incubation time and incubation temperature) were considered to study the impact on lipid extraction.

2. Methodology

2.1. Strain, production and harvesting conditions

Yarrowia lipolytica SKY-7, oleaginous yeast (isolated in our lab INRS-ETE Quebec, Canada) was used in this study (Kuttiraja et al., 2015). The yeast strain was grown in a medium containing 500 mL of crude glycerol solution with 11% (w/v) glycerol (by-product of biodiesel production, obtained from a biodiesel producing industry in Quebec, Canada) and 8.5 L starch industry wastewater (SIW) in a 15 L fermenter with working volume 10 L, (Biogene, Quebec). SIW was obtained from a starch producing industry in Québec. The fermenter was operated at constant pH 6.8–7.0 and temperature 28 °C and dissolved oxygen was maintained above 30% of saturation. After 72 h of fermentation, the broth was heat treated in the fermenter (to kill cells and preserve the accumulated lipid inside the cells) at 80 ± 2 °C for 10 min (Zhang et al., 2015). Thereafter, biomass was harvested by centrifugation at 8000 rpm for 10 min. The biomass was washed with warm water to remove residual glycerol and soap. To perform lipid extraction and to estimate biomass dry weight, 3.1 ± 0.2 g wet biomass (83.8% moisture content) harvested from 25 mL fermented broth was used.

2.2. Conventional (chloroform-methanol assisted) lipid extraction

The standard chloroform and methanol extraction was used to determine the lipid content in the biomass (Bligh and Dyer, 1959; Folch et al., 1957; Vicente et al., 2009). The washed wet biomass pellet (3.1 ± 0.2 g) was mixed with 15 mL solvent mixture of chloroform and methanol (2:1 v/v), and then incubated for 4 h in an agitator water bath at 60 °C and 100 rpm. The mixture was then centrifuged at 4000 rpm for 10 min. The mixture was separated in three different layers. The residual biomass was in the bottom layer, middle phase was lipid in chloroform and top layer methanol and water. The middle layer of chloroform containing lipid was pipetted out and transferred into a pre-weighed glass tube (L_1). The rest of the solution (containing cell debris, methanol) was again fortified with 15 mL solvent mixture of chloroform and methanol (2:1 v/v) and again incubated for 4 h at 60 °C in the agitated water bath. After 4 h incubation, the solution was filtered using vacuum filtration. The filtrate was mixed with previously extracted solution (chloroform solution containing lipid) and the mixed solution was allowed to stand for phase separation. The bottom phase containing lipid in chloroform (the other phase was water and methanol) was collected and subjected to nitrogen sparging until total chloroform evaporated. The samples were further dried in an oven at 60 °C until constant weight (L_2). The lipid recovery from the biomass calculated as:

$$CL\% = \frac{L_2 - L_1}{DBW} \times 100\% \quad (1)$$

The obtained lipid was stored for further transesterification study. Eq. (1) CL represents weight obtained from conventional lipid extraction, L_1 expresses the pre-weighed glass tube and L_2 denotes the oven dried microbial lipid in a pre-weighed glass tube and DBW denotes dry biomass weight.

2.3. N-lauroyl sarcosine assisted lipid extraction

The lipid bearing wet biomass (3.1 ± 0.2 g wet biomass) harvested by centrifugation after fermentation was used in each

Table 1
Comparison of different methods of lipid extraction from wet biomass.

Oleaginous substance	Wet biomass %	Extraction condition	Extraction efficiency (%)	References
Nannochloropsis sp.	Nr[a]	Supercritical methanol	84.15	Patil et al. (2011)
R. toruloides	94	Combination of pretreatment with microwave and recombinant enzyme	95.4	Jin et al. (2012)
Yarrowia lipolytica	89	N-lauryl sarcosine	95.4	This study
Nannochloropsis oculata	80	Simultaneous distillation and extraction process (SDEP) using soxhlet	90.2	Dejoye Tanzi et al. (2013)
Chlamydomonas reinhardtii	99.4	Osmotic shock	9.06	Yoo et al. (2012)
Chlorella and *Scenedesmus* sp.	84	Acid and base hydrolysis	79	Sathish and Sims (2012)
Scenedesmus	Nr[a]	3-DAPS[b]	100	Lai et al. (2016)
Picochlorum	66.96	Ethanol	32.81	Yang et al. (2014)

[A] Nr – not reported.
[b] 3-DAPS – 3-(decyldimethylammonio)-propanesulfonate inner salt.