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Biorefining of by-product streams from sunflower-based biodiesel production plants for integrated synthesis of microbial oil and value-added co-products

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HIGHLIGHTS

- Integrated biorefinery development from first generation biodiesel plants.
- Value-added products and microbial oil were produced from by-products streams.
- High microbial oil production was achieved using entirely renewable resources.
- Biodiesel properties conformed with EN 14214 and ASTM D 6751 standards.

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ABSTRACT

This study focuses on the valorisation of crude glycerol and sunflower meal (SFM) from conventional biodiesel production plants for the separation of value-added co-products (antioxidant-rich extracts and protein isolate) and for enhancing biodiesel production through microbial oil synthesis. Microbial oil production was evaluated using three oleaginous yeast strains (*Rhodosporidium toruloides*, *Lipomyces starkeyi* and *Cryptococcus curvatus*) cultivated on crude glycerol and nutrient-rich hydrolysates derived from either whole SFM or SFM fractions that remained after separation of value-added co-products. Fed-batch bioreactor cultures with *R. toruloides* led to the production of 37.4 g L^{-1} of total dry weight with a microbial oil content of 51.3% (w w^{-1}) when a biorefinery concept based on SFM fractionation was employed. The estimated biodiesel properties conformed with the limits set by the EN 14214 and ASTM D 6751 standards. The estimated cold filter plugging point (7.3–8.6 °C) of the lipids produced by *R. toruloides* is closer to that of biodiesel derived from palm oil.

1. Introduction

The development of sustainable biodiesel production processes could be achieved through valorisation of by-product streams, i.e. crude glycerol and oilseed meals. Crude glycerol, generated as a 10% (w w^{-1}) by-product stream during fatty acid methyl ester (FAME) production, contains mainly glycerol with a purity higher than 77% (w w^{-1}), water, low quantities of residual methanol, salts (e.g. NaCl, K_2SO_4) generated via neutralisation of the catalyst, mono-, di- and tri-glycerides, and various impurities depending on the raw material (Mothes et al., 2007). Oilseed meals are currently used as animal feed, but higher value-added applications are recently envisaged through fractionation of protein, carbohydrates and other components (e.g. antioxidants). Kachrimanidou et al. (2013) have developed a two-stage bioprocess for the production of nutrient-rich fermentation supplements from sunflower meal (SFM). Solid state fermentation (SSF) was employed for the production of crude enzyme consortia that were subsequently employed for hydrolysis of SFM. This medium has been successfully utilised as the sole nutrient supplement for fermentative production of poly(hydroxyalkanoates) (Kachrimanidou et al., 2014a). An advanced process focusing on fractionation of SFM for the extraction of protein isolate and antioxidants as value-added co-products and utilisation of remaining fractions for the

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production of fermentation nutrient supplements has been developed (Kachrimanidou et al., 2014b, 2015). These two processing schemes involving the production of nutrient-rich fermentation supplements from either whole SFM (Process I) or fractionated SFM (Process II) have been evaluated in this study for the production of microbial oil (MO).

MO is a novel source of triglycerides that could be used for biodiesel production due to similar physical and chemical characteristics to vegetable oils. It is produced by oleaginous microorganisms that can accumulate intracellularly lipid bodies in quantities higher than 20% of their dry cell weight. Oleaginous yeasts are heterotrophic microorganisms that consume organic carbon sources (e.g. glucose, xylose, lactose, sucrose, glycerol) for growth and production of MO. An important advantage of oleaginous yeasts is their ability to achieve significantly higher growth rates and lipid productivities than autotrophic algae (Li et al., 2008). MO is a secondary metabolite produced under excess carbon and limiting nutrient conditions (e.g. nitrogen).

Crude glycerol has been employed for the production of MO by some oleaginous yeast strains including *Cryptococcus curvatus* and *Rhodosporidium toruloides* (Thiru et al., 2011; Uckun Kiran et al., 2013). In order to achieve high cell densities that will subsequently lead to high MO concentrations, a high concentration of commercial nutrient sources (e.g. yeast extract) is required. To avoid the use of expensive commercial nutrient supplements, Uckun Kiran et al. (2013) employed hydrolysates of whole rapeseed meal mixed with crude glycerol in fed-batch fermentations by *R. toruloides* Y4 for the production of 43 g L^{-1} of total dry weight (TDW) with a MO content of 45.8% (w w^{-1}). Besides replacement of commercial nutrient supplements, higher MO concentration and glycerol to lipid conversion yield were reported than those achieved when yeast extract was used as nutrient supplement (Uckun Kiran et al., 2013). Thiru et al. (2011) carried out fed-batch fermentations with *C. curvatus* cultivated on crude glycerol, corn steep liquor and recycled de-oiled yeast autolysate resulting in the production of 69.2 g L^{-1} of TDW with an intracellular MO content of 48% (w w^{-1}). Recycling of nutrients derived via autolysis of de-oiled yeast cells could effectively replace baker's yeast autolysate and malt extract.

This study demonstrates the potential of a novel biorefinery concept for the production of antioxidant-rich extracts, protein isolate and MO that was produced via fermentation using crude glycerol as carbon source and nutrient-rich supplements derived from residual streams produced during fractionation of SFM (Process II). This approach will increase biodiesel production capacity by conventional plants. Furthermore, polyphenol extracts from SFM could be employed as antioxidants in order to increase the oxidative stability of sunflower oil (De Leonardis et al., 2003) or the storage stability of biodiesel (Das Purkayastha et al., 2013). The protein isolate from SFM or its enzymatic hydrolysate could be applied, as other oilseed-derived protein isolates, for food, feed and various industrial applications (Rodriguez et al., 2002). MO production was evaluated in both shake flask and fed-batch bioreactor cultures. The properties of biodiesel produced from MO have been estimated using established equations derived via simulation of the properties estimated based on the fatty acid composition of vegetable oils.

2. Method

2.1. Microorganisms

Fermentations for the production of MO were carried out with *C. curvatus* ATCC 20509, *Lipomyces starkeyi* DSM 70296 and *R. toruloides* DSM 4444. *R. toruloides* was maintained at 4 °C in agar slants containing 10 g L^{-1} glucose, 10 g L^{-1} peptone, 48 g L^{-1} malt extract and 20 g L^{-1} agar. *C. curvatus* and *L. starkeyi* were maintained at 4 °C in agar slants composed of 10 g L^{-1} glucose, 10 g L^{-1} peptone, 10 g L^{-1} yeast extract and 20 g L^{-1} agar. Inocula of *R. toruloides* were prepared in liquid medium (10 g L^{-1} glucose, 48 g L^{-1} malt extract, 10 g L^{-1} peptone) for 24 h at 28 °C in a rotary shaker with an agitation speed of 180 rpm. Inocula of *L. starkeyi* and *C. curvatus* were prepared in liquid medium (10 g L^{-1} glucose, 10 g L^{-1} peptone, 10 g L^{-1} yeast extract) for 48 h and 24 h, respectively, at 30 °C in a rotary shaker with an agitation speed of 180 rpm.

SSF cultures were carried out with a fungal strain of *Aspergillus oryzae* that was kindly provided by Professor Colin Webb (University of Manchester, UK). Isolation, preservation, sporulation and inocula preparation are presented by Kachrimanidou et al. (2013).

2.2. Production of SFM and PSFM hydrolysates

The crude glycerol and SFM used in this study were kindly supplied by the biodiesel industry P. N. Pettas S.A. (Patras, Greece). SFM contains (on a dry basis, db) 26.62% protein, 0.93% oil, 6.83% ash and 19.54% fiber. The original crude glycerol with a purity of 91% (w w^{-1}) was pre-treated via decanting using separation funnels to remove potentially inhibitory components, such as various non-polar compounds. The glycerol content of the pretreated crude glycerol stream was 92.4%. Detailed composition of crude glycerol and SFM was presented by Kachrimanidou et al. (2013, 2014a).

Whole SFM and pretreated SFM (PSFM) hydrolysates were prepared via a two-stage bioprocess involving SSF of *A. oryzae* cultivated for the production of crude enzymes followed by hydrolysis of whole SFM (Process I) and PSFM (Process II), respectively. Kachrimanidou et al. (2013) presented the two-stage bioprocess that has been employed for the production of whole SFM hydrolysates (Hydrolysate I produced by Process I). SSF were carried out in 250 mL Erlenmeyer flasks at 30 °C with SFM as the sole solid substrate. After 48 h, the remaining fermented solids were mixed with unprocessed SFM to achieve hydrolysis of protein and phytic acid and release of various micronutrients. The reaction mixture was placed in 1 L Duran bottles and the hydrolysis was carried out at 45 °C. Agitation was achieved with magnetic stirrers. An initial SFM solid concentration of 50 g L^{-1} and an initial proteolytic activity of 6.4 U mL^{-1} were used. The duration of hydrolysis was 24 h and the free amino nitrogen (FAN) concentration produced was around 850 mg L^{-1}.

SFM was fractionated (Process II) according to the method described by Parrado et al. (1991). This process has been described by Kachrimanidou et al. (2015). SFM was initially suspended in water in order to produce three distinct fractions:

1. A lignocellulose-rich fraction (LF) aggregated at the surface of the aqueous suspension.
2. A protein-rich fraction (PF) precipitated at the bottom of the vessel.
3. An intermediate liquid fraction that contained soluble components of SFM.

A phenolic-rich extract with antioxidant activity was extracted from SFM after treatment for 1 h using 10 mL of ethanol:water (1:1, v/v) mixture in an ultrasonic bath. This procedure was repeated twice for 30 min. The antioxidant activity and quantitative analysis of phenolic compounds has been presented by Kachrimanidou et al. (2015).

The protein isolate was produced via sequential treatment of the PF under alkaline (pH 10 using 5 M NaOH) conditions followed