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Short Communication

Hydrolysis treatments of fruit and vegetable waste for production of biofuel precursors

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HIGHLIGHTS

- Food and vegetable waste hydrolysates support growth of the oleaginous yeast, *Rhodotorula glutinis*.
- FVW hydrolysate-reared *R. glutinis* fatty acids met bio-diesel quality standards.
- Biomethane potential of FVW-residual solids were sufficiently high for use in co-digestion.
- Macerated FVW 24 h 4 °C leachate provides the best opportunity for *R. glutinis* biodiesel production.

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ABSTRACT

This study investigated hydrolysis approaches for cultivation of the oleaginous red yeast *Rhodotorula glutinis* for biodiesel production, whilst utilising the residual solids (RS) for biogas production. Macerated fruit and vegetable waste (FVW) (24 h–4 °C-leachate served as the control, *Pcon*) was hydrolysed chemically (*Chem*), thermally (*Therm*) and using a combined thermo-chemical treatment (*T-Chem*). All cleared hydrolysates supported growth of *R. glutinis*, which was nitrogen-limited. *T-Chem* hydrolysates yielded highest biomass, total fatty acids (TotFA) and RS-derived biogas yields, biomass TotFA failed to meet standards for fuel density and higher heating values, met by the other treatments. Even though *Pcon*-derived yields were slightly lower, it is recommended for FVW treatment for local biogas and biodiesel production due to energy and environmental impact considerations.

1. Introduction

Continual expansion of the global population results in increasing need for energy security due to its pivotal role in current technology-dependant societies. In general, an estimated four-fifth (~87%) of the global population continue to rely on energy derived from fossil fuels such as coal and natural gas (Doll and Pachauri, 2010). Contemporary predictions based on remaining fossil reserves and rates of global consumptions forecast 30–50 residual years, necessitating a fervent push for the development of alternative energy sources (Berg and Boland, 2014). In this sense, biofuels – mainly biodiesel, produced through transesterification of fatty acids from oil plants/microalgae, offers a highly attractive substitute to fossil-derived diesels and petroleum, with associated advantages i.e., similarities of physicochemical properties such as cetane number, density and viscosity, no sulphur and aromatic compounds, etc. The global biodiesel outputs were ~29.1 billion litres, which was 24–26% of the total biofuel production in 2013–2014 (IEA, 2014). Biodiesel production is assumed to be non-toxic, sustainable and energy-efficient, with production costs being estimated to be similar to petroleum-derived diesel (0.914–1.039 USD L^{-1}). Sustainability, however, and economic factors for biodiesel production are still being debated on the grounds of (a) food or fuels; (b) deforestation and land use pattern change; (c) fertiliser demand and fossil fuel consumption; and (d) water use and demand (Timilsina and Shrestha, 2014).

In recent years, much attention has therefore been paid to utilise organic waste resources as feed-stock for biodiesel. Of the organic landfill waste, 40% is fruit and vegetable waste (FVW), which cannot be simply digested for biogas recovery by

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conventional anaerobic digestion processes, due to low cellulose content, which limits methanogenic processes through the rapid accumulation of volatile organic acids (Bouallagui et al., 2009). As an alternative, FVW can be bio-refined by oleaginous microbes such as microalgae, yeast, bacteria and fungi for microbial lipid production, producing precursor molecules for bio-diesel production (Hao et al., 2015). Among them, the oleaginous red yeast *Rhodotorula glutinis* is superior for bio-refinery projects, since the organism is capable of (a) valorising organic carbon as biomass lipids (~70% dry biomass); (b) accumulating other valuable products such as carotenoids (β-carotene; 1 kg ~1600 USD) and biopolymers (PHA–PHB; 1 kg ~4–16 USD); and (c) rapid growth rates, tolerance to fluctuating culture conditions, self-settling properties and offers the possibility for co-cultivation with microalgae (Chi et al., 2011; Hao et al., 2015). However, appropriate pre-treatment of FVW is still required for a bio-refinery-based biodiesel production approach using the yeast *R. glutinis*.

Pre-treatment of FVW alters the physio-chemical-biological characteristics resulting in release of simple sugars and other essential nutrients for the growth of *R. glutinis* (Hao et al., 2015). Thereby carbon assimilation into biolipids is facilitated and therefore potentially improved. However, a ratio of C_{16}/C_{18} poly unsaturated fatty acids $\leqslant 0.5$ in the accumulated biolipids must be achieved to obtain good quality biodiesel. The yeast's fatty acid profile could be influenced by the growth conditions provided, i.e. the growth medium. As growth medium characteristics are interlinked with the pre-treatment efficiency of FVW, selection of appropriate pre-treatment is essential to produce a growth medium that is suitable for high biomass growth rates and yields, optimal carbon assimilation for improved C_{16} and C_{18} contents. Different pre-treatment technologies for organic wastes have been reported in the literature, but these are not universally applicable and efficiencies depend on organic waste characteristics (Karthikeyan et al., 2016). Therefore pre-treatment processes require optimisation based on the end-use process applications. Very few studies compared and reported on the efficiencies of different pre-treatment technologies for organic waste valorisation into biolipids using *R. glutinis* or other yeasts (Hao et al., 2015). Therefore, the present study tested three different pre-treatment technologies for FVW to develop a suitable bio-refinery workflow for biolipid production using *R. glutinis*. The results are compared and discussed on the basis of carbohydrate yields for the pre-treatments, biolipid accumulation by *R. glutinis* and the residual solids for assessment of biomethane potential.

2. Methods

2.1. Pre-treatment of FVW

The FVW was collected from food outlets, super markets and canteens in and around James Cook University, Townsville, Australia. They are mixture of corn-3.3%; foliage-24.4%; broccoli-7.7%; banana and peals-13.4%; orange-6.4%; pumpkin-7.9%; apple-10.3%; eggplants and carrots-15%; and tomatoes-12.2%. The FVWs were ground to optimise surface area prior to pre-treatment. Size-reduced and homogenised FVW was mixed with deionised water to achieve 250 g solids L^{-1}, then refrigerated at 4 °C for 24 h. This process allows for passive leaching of soluble sugars and essential nutrients, whilst minimising microbial-mediated leaching processes. After 24 h incubation, the FVW slurry was pre-treated by chemical (*Chem*), thermal (*Them*) and combined thermo-chemical (*T-Chem*) hydrolyses. 24 h–4 °C-non-hydrolysed FVW leachate was used as physical controls (*Pcon*) representing only the physical maceration and 4 °C-leaching processes.

For *Chem* hydrolysis, the slurry pH was adjusted to acidic conditions (pH-3) by addition of 2 M hydrochloric acid (HCl) and incubated at 28 °C for 24 h. HCl was used instead of sulphuric acid, to avoid any furfural or hydroxyl-furfural formation during the *T-Chem* hydrolysis, as these have been reported to be toxic to *R. glutinis* (Zhang et al., 2011). Moreover, they also adversely affect anaerobic digestion of residual solids (RS) obtained from the hydrolysed FVW. Hydrolysis *via Them* simply employed autoclaving of the FVW slurry under standardised heat and pressure conditions of 121 °C at 1013.25 hPa for 30 min (Tomy, VWR International, Murarrie, QLD 4172, Australia). *Them* hydrolysis has been widely accepted for hydrolysing organic wastes to solubilise simple sugars and improve the digestibility of organic waste (Karthikeyan et al., 2016). *Them* hydrolysis was expected to produce biologically stable gelatinised starch and hemi-cellulose and/or melanoidins (Liu et al., 2012). Providing sterile incubation conditions is important for *R. glutinis* cultivation in order to maximise biolipid yields. Therefore, *Them* hydrolysis served a dual purpose: (a) sterilisation of FVW and (b) hydrolysis of complex organic polymers for a biorefinery approach to FVW re-utilisation. The *T-Chem* hydrolysis combined the *Chem* and *Them* pre-treatments, where the *Chem* pre-treatment of the FVW slurry preceded the *Them* pre-treatment. Cleared FVW hydrolysates from the individual hydrolytic processes were obtained through centrifugation (15,900*g* for 20 min with 10 min deceleration at 4 °C (Avanti®J-26 XPI, Beckman Coulter, USA) and were used as the growth medium for *R. glutinis* cultivation, while the RS were subjected to anaerobic digestion.

2.2. *R. glutinis* culture preparation, bio-lipid accumulation and bio-diesel property calculations

Cultivation of *R. glutinis* FRR-4522, preparation of hydrolysates, inoculation, replication, freeze-drying, fatty acid extraction and transesterification (FAME) were performed as detailed in our earlier publication (Hao et al., 2015). The resultant specific growth coefficients were determined as per Fogg and Thake (1987)'s equation (Eq. (1)):

$$\mu_x = \frac{\ln(N_2/N_1)}{t_2 - t_1} \tag{1}$$

where, $t_2 - t_1$ are the days of the time period associated with cell concentrations (N_2 & N_1).

The carbohydrate content of the growth medium was monitored using the UV-sulphuric acid method and the potential physico-chemical properties of biodiesel were calculated based on the Eqs. (1)–(4) in Hao et al. (2015). Characterisation of RS, elemental analysis, theoretical biogas (Bth) and yields of RS were calculated using Eqs. (5) and (6) in Hao et al. (2015) and compared with actual CH_4 potential as reported previously (Hao et al., 2015). Statistical analyses were carried out using SPSS 22.0 (IBM Inc.). Statistical analysis of initial total carbohydrate data were carried out using single-factor ANOVA. Homogeneity of variances was confirmed using Levene's test. Where significant differences were obtained, Tukey's Post Hoc tests were used to determine the driver for the significant outcome. Results were deemed significant and highly significant results at $p < 0.05$ and $p < 0.001$, respectively.

3. Results and discussion

The FVW had high TS and VS contents compared to those published for food waste or other organic waste types (Bouallagui et al., 2009; Hao et al., 2015). The average C/N ratio was 22.6 ± 0.13 due to low N content (<2%), which consequentially resulted the high percentages of elements O-51.87 ± 0.25% and H-6.4 ± 0.01%. Similar CHNO contents of FVW have been reported