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Heterotrophic growth and lipid accumulation of *Chlorella protothecoides* in whey permeate, a dairy by-product stream, for biofuel production

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HIGHLIGHTS

- Whey permeate, a waste stream, was used to produce microalgae for biofuels.
- High lipid-algal biomass was obtained with whey permeate and yeast extract at low C/N.
- Batch, fed-batch and SSF bioprocesses modes were investigated for algal culture.
- Algal fatty acid profile when grown in whey permeate was similar to that of glucose.

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ABSTRACT

This study proposes a novel alternative for the utilization of whey permeate, a by-product stream from the dairy industry, as the feedstock for the biomass and lipid production of the microalgae *Chlorella protothecoides*. Glucose and galactose from the pre-hydrolyzed whey permeate were used as main carbon sources in a base mineral media for establishing batch and fed batch cultures. Batch cultures reached a biomass production of 9.1 ± 0.2 g/L with a total lipid accumulation of 42.0 ± 6.6% (dry weight basis), while in the fed batch cultures 17.2 ± 1.3 g/L of biomass with 20.5 ± 0.3% lipid accumulation (dry weight basis) were obtained. A third strategy for the direct utilization of whey permeate was investigated by simultaneous saccharification and fermentation (SSF), wherein, 7.3 ± 1.3 g/L of biomass with 49.9 ± 3.3% lipid accumulation (dry weight basis) was obtained in batch mode using immobilized enzyme.

1. Introduction

The use of microalgae for producing biofuels has been highlighted for its potential to be easily integrated into a biorefinery for obtaining diverse value added products with priority towards lipids. Broadly, the advantages that some microalgae offer include high lipid productivity, negligible competition for arable land, and adaptability to grow in inexpensive media including waste water streams (Chisti, 2007; Rodolfi et al., 2009; Stephens et al., 2010; Xavier Malcata, 2011; Menetrez, 2012). Heterotrophic systems offer technological advantages for the cultivation of microalgae including homogeneous, consistent and reproducible processes with overall higher rates of biomass and lipid production (Bumbak et al., 2011; Perez-Garcia et al., 2011). One of the best lipid producing microalgae to date is *Chlorella protothecoides* (Bumbak et al., 2011), which is able to use several carbon sources with preference towards glucose since it promotes the highest respiration and growth rates (Perez-Garcia et al., 2011). However, the use of pure glucose as carbon source can represent around 60–75% of the total cost of the culture media (Li et al., 2007; Menetrez, 2012). An effective strategy to lower the cost of culturing *C. protothecoides* is the utilization of inexpensive glucose-containing substrates (Cheng et al., 2009; Gao et al., 2010; Lu et al., 2010, 2011; Yan et al., 2011; Sun et al., 2013). An ideal scenario for the cost-effective heterotrophic cultivation of microalgae would be the utilization of abundant industrial by-product streams that have limited competing applications.

One such by-product stream is the whey permeate from the dairy industry. Whey permeate is obtained by ultrafiltration and removal of protein from whey that is generated during cheese manufacturing and represents about 85% of the total milk used in the process (Panesar and Kennedy, 2012). Whey permeate displays an overall composition of mostly lactose along with salts and non-protein nitrogen (Jelen, 2009). Considering the large amounts of whey permeate generated, its utilization and disposal becomes crucial. The use of whey permeate as a direct lactose

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source has been neglected due to the extensive processing required for its recovery such as demineralization and dewatering (Jelen, 2009).

Whey derivatives have been used to grow some microalgae for evaluating their potential to decrease organic matter and deplete nutrients from dairy and other manufacturing industrial effluents (Durmus et al., 1999; Woertz et al., 2009). More recently, a dual approach for treating a dairy waste stream from a specific medium-scale industry and producing algal biomass for biofuel was proposed (Kothari et al., 2012). However, the direct use of whey permeate, a consistent and readily available feedstock from the dairy industry, for the controlled heterotrophic microalgal cultivation has not been reported.

The aim of this study was to evaluate the use of whey permeate as the main carbon source for the heterotrophic growth and lipid accumulation of the microalgae *C. protothecoides*. The potential application of pre-hydrolyzed whey permeate as feedstock for batch and high cell density fed-batch microalgal cultures was investigated along with the direct use of non-hydrolyzed whey permeate by simultaneous saccharification and fermentation with immobilized hydrolysing enzyme.

2. Methods

2.1. Materials

C. protothecoides (UTEX 256) was directly obtained from The Culture Collection of Algae (UTEX; University of Texas, Austin, TX). Axenic stocks were stored at room temperature (23 ± 2 °C) with 25 μmol/m^{2}s at 12/12 h light/dark cycles as the long-term storage conditions (4 weeks; new agar slants were made every 4 weeks). All chemicals used for media preparation were purchased from Sigma–Aldrich (St. Louis, MO). Sulphuric acid and hexane (HPLC grade) were obtained from Fisher Scientific (Fairlawn, NJ). Whey permeate was procured from a large dairy producer (liquid form; pH 5.5) with lactose concentration of 182 g/L and nutrient composition: phosphorous (0.8% dry weight basis), magnesium (0.2% dry weight basis) and calcium (0.8% dry weight basis). Whey permeate was used 'as is' or hydrolyzed as described below.

2.2. Methods

2.2.1. Whey permeate hydrolysis

Whey permeate was neutralized using 2 M KOH and hydrolyzed at 30 °C for 24 h in shake flask at 200 rpm using 65 U of Lactozyme 3000 (Sigma–Aldrich, St. Louis, MO) per g lactose quantified in whey permeate. Hydrolyzed whey permeate (HWP) with a final composition of 95 g/L glucose and 85 g/L galactose was filtered (0.22 μm) and stored at 2 °C.

2.2.2. Analytical methods

Lactose, glucose and galactose content was determined using an Agilent 1200 series High Performance Liquid Chromatography (HPLC) instrument (Agilent, Santa Clara, CA), with a refractive index detector, Bio-Rad HP87H column (Bio-Rad Laboratories, Hercules, CA) at 60 °C (300 × 7.8 mm), using 0.005 M sulfuric acid as mobile phase with a flow rate of 0.5 mL/min. Algal growth was monitored by measuring $OD_{600\ nm}$ with a standard curve for dry biomass, in which $y = 2.2899\ x + 0.0523$ ($R^2 = 0.9944$); where $y = OD_{600\ nm}$ and x = dry weight (g/L). Lipid accumulation kinetics was determined by measuring fluorescence using Nile Red with a reference standard curve for lipid content hexane extraction; lipid content is reported as percent dry weight basis (DelaHoz Siegler et al., 2012). Briefly, freeze dried algal biomass was crushed with mortar and pestle followed by hexane washes of the resulting paste. Lipid percentage was determined gravimetrically from hexane supernatants. Fluorescence with Nile red was measured in reference algal biomasses of known lipid content (6.3–48.9% dry weight basis) at specific concentration, which were used to build up a standard curve with fluorescence signal vs. lipid percentage for each experiment. Total fatty acid composition in freeze-dried algal biomass was determined by esterification using methanolic hydrochloric acid 3 N (Sigma–Aldrich, St. Louis, MO) according to manufacturer instructions, with nonadecanoic acid methyl ester as internal standard. GC–FID for fatty acid quantification analysis was done in a 30 m × 0.25 mm × 0.25 μm, a column from SGE Analytical Science (Melbourne, Australia) was used. The initial injection temperature was 50 °C held for 0.2 min before ramping to 230 °C in the following program: 50–170 °C/20 min; 170 °C/5 min; 170–230 °C at rate 10 °C/min; 230 °C for 13 min. The injection volume was 1 μL in splitless mode. Total nitrogen and total carbon were measured by the Dumas Combustion Method at the Natural Resources Analytical Laboratory, University of Alberta.

2.2.3. Batch fermentations

C. protothecoides was cultured in 250 mL Erlenmeyer flasks with 100 mL base mineral media (Siegler et al., 2011). The amount of glucose, galactose, lactose, whey permeate or HWP used as main carbon sources was standardized based on total C mole amount of sugar from 0.33 C mol/L to 1 C mol/L which corresponds to 10 g/L and 30 g/L monomeric sugar, respectively. To trigger lipid accumulation, low nitrogen concentration was used in the growth media: $NaNO_3$ (2.5 g/L) for preliminary experiments and yeast extract for all other experiments (Xiong et al., 2008). Yeast extract amounts were varied from 1 to 1.2 g/L ($7.8E^{-3}$ mol/L–$1E^{-2}$ mol/L; based on results from preliminary experiments) for establishing a carbon to nitrogen ratio from 50/1 to 100/1 (DelaHoz, 2012). Flasks were inoculated with 5% (v/v) starter inoculum obtained from a shake flask culture at the late exponential phase (DelaHoz, 2012). Cultures were grown in the dark at 25 °C, 150 rpm with 24 h sampling intervals for a total of 168 or 216 h. pH of the cultures was monitored and adjusted to 6.4 with 2 M KOH. Each experimental unit was monitored for contamination by microscopic examination and streaking on Luria Bertani (LB) agar plates with subsequent incubation. All experiments were done in triplicate.

2.2.4. Fed batch fermentations

2.2.4.1. Small scale. The carbon sources used for fed batch fermentations, done in 250 mL Erlenmeyer flasks, were either HWP or a control mixture of glucose and galactose prepared at the same concentration as HWP. Initial C mole from sugar was standardized to 0.33 C mol/L (10 g/L of each monomeric sugar) and N was supplied at 2 g/L yeast extract added at the beginning. Inoculation, incubation, pH control and contamination checks were done as described for batch cultures. Feeding with HWP or control mixture along with yeast extract (0.5 g/L) was done as required. Triplicate cultures were monitored for a total of 240 h.

2.2.4.2. Scale up. 5 L bioreactors (Infors, Einsbach, Germany) were used for the scale-up of fed batch fermentations done with either HWP or glucose control, which was prepared at the same concentration as HWP. Initial fermentation volume was 3 L with a C mole amount from sugar doubled from the small scale experiments (HWP: 10 g/L glucose, 10 g/L galactose; glucose control: 20 g/L glucose) and initial yeast extract at 4 g/L. Inoculation, pH control and contamination checks were done as indicted for batch cultures. HWP or glucose along with yeast extract (2 g/L) was fed as required. During fermentation, aeration rate and stirrer speed were varied between 1–2 vvm and 100–200 rpm, respectively, to keep

air saturation over 20% (Xiong et al., 2008). When necessary, pure O_2 was mixed with air to maintain saturation. Duplicate cultures were monitored for a total of 240 h.

2.2.5. β-Galactosidase immobilization

The enzyme β-galactosidase from *Aspergillus oryzae* (Sigma–Aldrich, St. Louis, MO) was immobilized by encapsulation using polyvinyl alcohol (PVA) and polyethylene glycol (PEG) according to the manufacturer's instructions (Lentikat's, Prague, Czech Republic). Briefly, PVA 10% (w/w) and PEG 6% (w/w) were solubilized in water by boiling, and the solution was cooled to 35 °C before adding 0.1 mg/L β-galactosidase enzyme solution (5% w/w). Immobilized enzyme beads were produced using a LentiPrinter device (Lentikat's, Prague, Czech Republic), dried and subsequently rehydrated with 0.1 M Na_2SO_4 for 2 h before transferring into 0.1 M potassium phosphate buffer with 2 mM $MgCl_2$, pH 6.5 for storage at 4 °C. Enzymatic activity of the immobilized enzyme was measured according to manufacturer's instructions.

2.2.6. Simultaneous saccharification and fermentation (SSF)

C. protothecoides was cultured in 250 mL Erlenmeyer flasks with100 mL media containing 4× concentrated mineral base (25 mL; Siegler et al., 2011), 0.25 g/L yeast extract, non-hydrolyzed whey permeate and autoclaved double distilled water (MilliQ, Millipore, Billerica, MA). Whey permeate was filtered (0.22 μm) and added based on C mole from sugar considering the lactose content. At inoculation, 3 U of encapsulated β-galactosidase activity were added to the flasks and yeast extract (2 g/L) was supplemented as required. Inoculation, incubation, pH control and contamination checks were done as indicted for batch cultures. Triplicate cultures were monitored for a total of 216 h.

2.2.7. Calculations

Statistical analysis of variance for biomass and lipid production as well as fatty acid composition was done using one or two way-ANOVA (GradPad software, La Jolla, CA). Yields (dry weight basis) were calculated at harvesting time as follows:

$$\text{Biomass yield } (Y_{b/s}) = \text{Biomass (g)}/\text{Utilized substrate(initial} - \text{residual) (g)}$$

$$\text{Lipid yield } (Y_{l/s}) = \text{Lipid (g)}/\text{Utilized substrate(initial} - \text{residual) (g)}$$

3. Results and discussion

3.1. Growth of C. protothecoides in whey permeate

The ability of *C. protothecoides* to utilize lactose in whey permeate or glucose and galactose in HWP was evaluated in batch cultures. Pure lactose, glucose and galactose were included as controls, and $NaNO_3$ was used as the inorganic nitrogen source. *C. protothecoides* was unable to utilize lactose as a carbon source in whey permeate or control, but was able to grow well in HWP assimilating glucose and galactose. Hydrolyzed whey permeate acted as a favourable substrate for the algal growth and had no inhibitory effects; biomass production of 2.8 ± 0.4 g/L was observed. With glucose and galactose, a lower biomass production of 1.5 ± 0.1 and 1.7 ± 0.4 g/L, respectively was observed. The assimilation of disaccharides, specifically lactose, by *Chlorella* has previously been reported to be negligible (Rodriguez, 1966; Perez-Garcia et al., 2011).

3.2. Biomass production and lipid accumulation

To evaluate biomass production and lipid accumulation of *C. protothecoides* grown in HWP, batch cultures were done using 10 g/L total monomeric sugar (0.33 C mol/L), yeast extract as the main nitrogen source (1 g/L = $7.8E^{-3}$ N mol/L) with a C to N ratio of 50/1. No contamination was observed in cultures during the entire growth period till harvest. Glucose and galactose were used individually and in a mixture as controls. *C. protothecoides* was able to assimilate glucose and galactose simultaneously with a slight preference for glucose; the substrate utilization rate was higher for the control mixture compared to HWP (Fig. 1A and B). A biomass of 4.3 ± 0.6 g/L with lipid content of 24.6 ± 1.5% was obtained when grown in HWP. Lipid content in all control cultures was not significantly different ($\alpha = 0.05$) from the HWP culture. To promote higher lipid accumulation, cultures were then grown using 30 g/L total monomeric sugar and reduced yeast extract with a C to N ratio of 100/1 (Heredia-Arroyo et al., 2010). Higher concentrations of glucose have previously shown to cause substrate inhibition in batch cultures (Shi et al., 1999; Xiong et al., 2008). At 30 g/L substrate concentration, slower growth rates and residual sugars were observed for all controls in contrast to that of HWP (Fig. 2). Higher biomass was obtained using HWP (9.1 ± 0.2 g/L) compared to the controls: glucose (5.9 ± 0.2 g/L), galactose (6.2 ± 0.2 g/L), and glucose and galactose mixture (5.9 ± 0.1 g/L) that were not significantly different ($\alpha = 0.05$) from each other. Increased lipid accumulation of 42.0 ± 4.5% was also observed using HWP which was not significantly different ($\alpha = 0.05$) from the other culture conditions. Biomass and lipid production yields suggested comparable substrate utilization efficiencies for all the groups (Table 1). The shorter lag phase and faster substrate utilization observed with HWP suggests its beneficial role in promoting algal growth, which likely is due to other nutrients present in the whey permeate, including nitrogen and minerals.

3.3. Fatty acid composition

The composition of the fatty acids from the lipids accumulated by *C. protothecoides* was analyzed to assess any potential alteration promoted by the use of different feedstock. The amount of fatty acids in the freeze-dried algal biomass was normalized to determine their composition relative to the total. The fatty acid profile of *C. protothecoides* grown in whey permeate was found to be oleic (54.5%), linoleic (21.8%), stearic (13.5%), followed by palmitic, myristic, linolenic, palmotoleic and arachidic acids (Fig. 3). The fatty acid composition of *C. protothecoides* was both qualitatively and quantitatively invariant compared to glucose or galactose (no significantly different $\alpha = 0.05$). It has been previously reported that the composition of algal lipids can be altered by changing various physical conditions during culturing, including feedstock (Gao et al., 2010; Knothe, 2011). However, depending on the intended final application of the microalgal lipids, consistency in the fatty acid profile may be a desired relevant property. From these results, whey permeate offers alternative feedstock for obtaining a reproducible process that offers the same lipids as those obtained with a pure glucose based system.

3.4. Fed batch culture and scale up

To further increase biomass and lipid production, fed batch cultures were established using HWP and control mixture of glucose and galactose. After depletion of the initial carbon (72 h), HWP (5.2 g/L glucose, 4.6 g/L galactose) and yeast extract (0.5 g/L) were added, and then subsequently HWP (2.3 g/L glucose, 2.0 g/L galactose) with 0.5 g/L yeast extract was added at 96, 120 and 168 h to a total of 32.3 g/L glucose and galactose from HWP and 34.9 g/L in

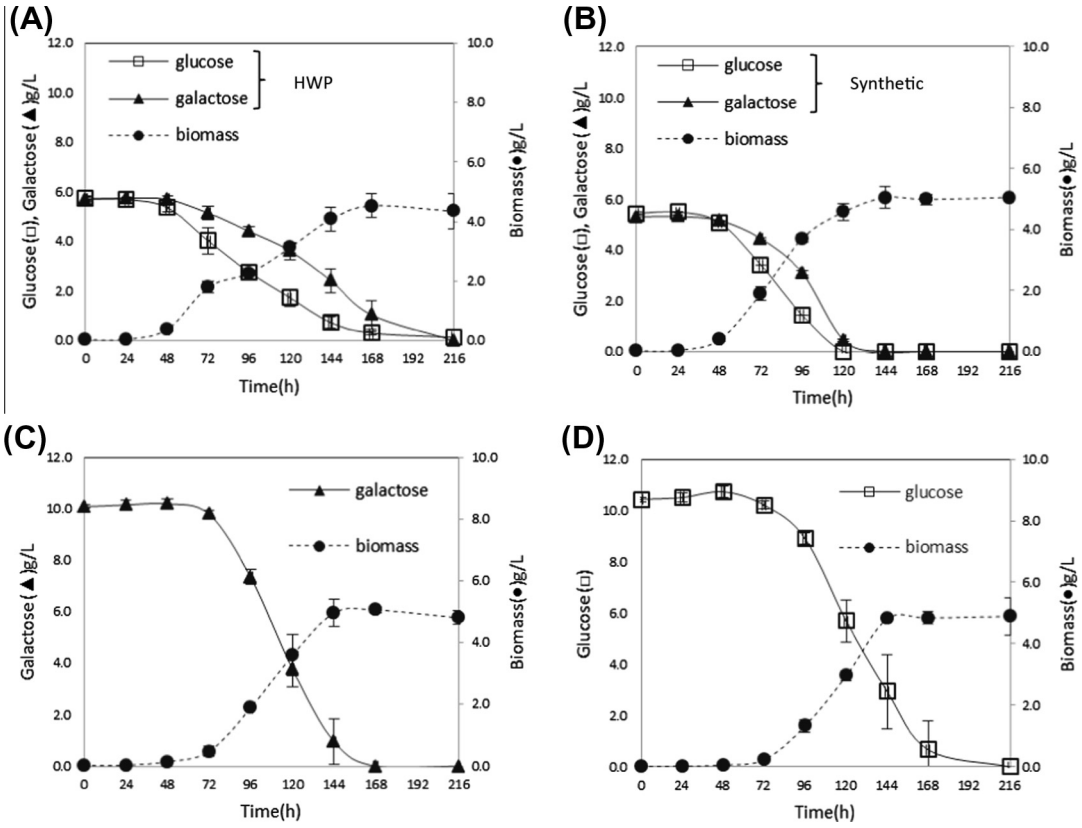

Fig. 1. Kinetics of sugar consumption and growth of *C. protothecoides* in batch cultures for 216 h using different substrates as main carbon sources at a final concentration of 10 g/L monomeric sugar (0.33 C mol/L) and 1 g/L yeast extract ($7.8E^{-3}$ N mol/L) with a C/N ratio of 50/1. (A) HWP; (B) glucose and galactose mixture; (C) glucose; (D) galactose.

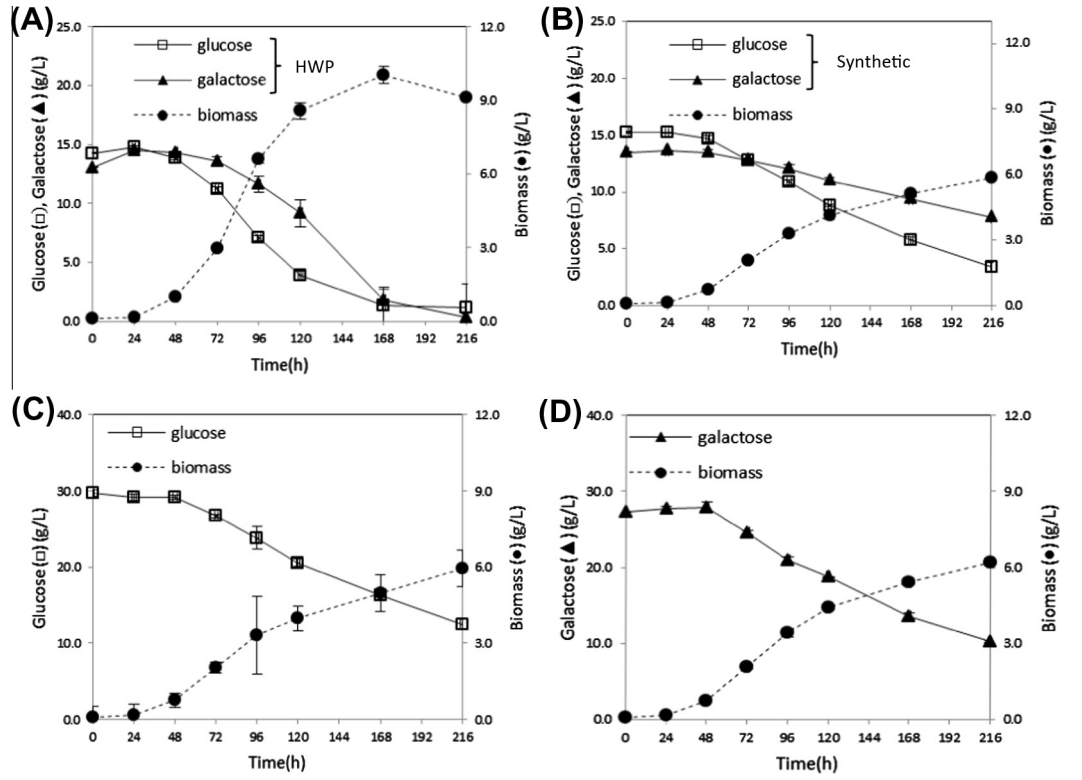

Fig. 2. Kinetics of sugar consumption and growth of *C. protothecoides* in batch cultures for 240 h using different substrates as main carbon sources at a final concentration of 30 g/L monomeric sugar (1 C mol/L) and 1.2 g/L yeast extract ($1E^{-2}$ mol/L) with a C/N ration of 100/1). (A) HWP; (B) glucose and galactose mixture; (C) glucose; (D) galactose.

Table 1
Biomass and lipid yields (Y) of *C. protothecoides* grown at 10 and 30 g/L monomeric sugar, and 1 and 1.2 g/L yeast extract, respectively. Hydrolyzed whey permeate (HWP); Glucose and galactose mixture (glu + gal); glucose (glu); galactose (gal).

	10 g/L C monomer–1 g/L yeast extract		30 g/L C monomer–1.2 g/L yeast extract	
	$Y_{(b/s)}$	$Y_{(l/s)}$	$Y_{(b/s)}$	$Y_{(l/s)}$
HWP	0.41 ± 0.06	0.10 ± 0.01	0.35 ± 0.01	0.15 ± 0.01
Glu + gal	0.47 ± 0.01	0.13 ± 0.02	0.33 ± 0.01	0.15 ± 0.00
Glu	0.49 ± 0.06	0.15 ± 0.01	0.36 ± 0.02	0.15 ± 0.01
Gal	0.46 ± 0.02	0.13 ± 0.01	0.36 ± 0.01	0.13 ± 0.02

b/s = g biomass dry weight basis/g substrate; l/s = g lipid dry weight basis/g substrate.

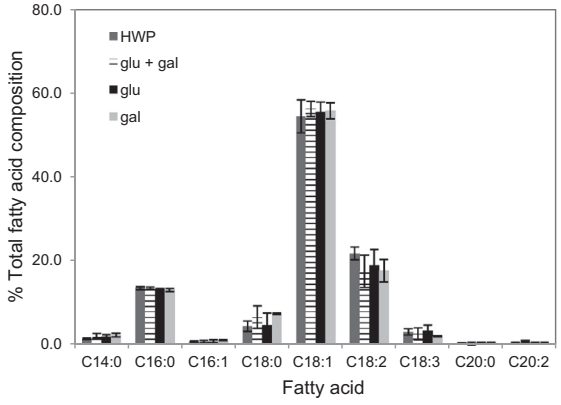

Fig. 3. Fatty acid profile of *C. protothecoides* grown with different substrates at final concentration of 30 g/L monomeric sugar and 1.2 g/L yeast extract.

the control. No contamination was observed in cultures during the entire growth period till harvest. Biomass after 240 h of culture was 8.0 ± 0.3 g/L for HWP and 10.9 ± 0.4 g/L for the control (Fig. 4A and B) and the percentage of glucose and galactose consumed was 91.1 and 69.2% for HWP, and 96.3 and 81.4% for the control. This suggested that the fed batch approach was not superior to the batch cultures at the small scale for HWP compared to the improvement observed for the glucose control.

To overcome limitations associated with fed batch cultures in shake flasks, the process was scaled up to 5 L bioreactors, in which HWP or glucose (control) was used. HWP or glucose was fed into the cultures as early as 48 h to avoid total depletion of the carbon substrate, and yeast extract was added only at 48 h at a concentration of 2 g/L. Feeding of HWP was done at: 48, 72, 84, 108, 120, 144, 156, 168 and 192 h for a total volume of 1.1 L HWP (105.9 g glu, 93.9 g gal). Feeding of glucose solution was done at: 48, 72, 78, 84, 108, 120, 144, 156, 168, 192 and 216 h for a total of 250 g of glucose. No contamination was observed in cultures during the entire growth period till harvest. More than two fold increase in biomass was observed with 17.2 ± 1.3 g/L for HWP and

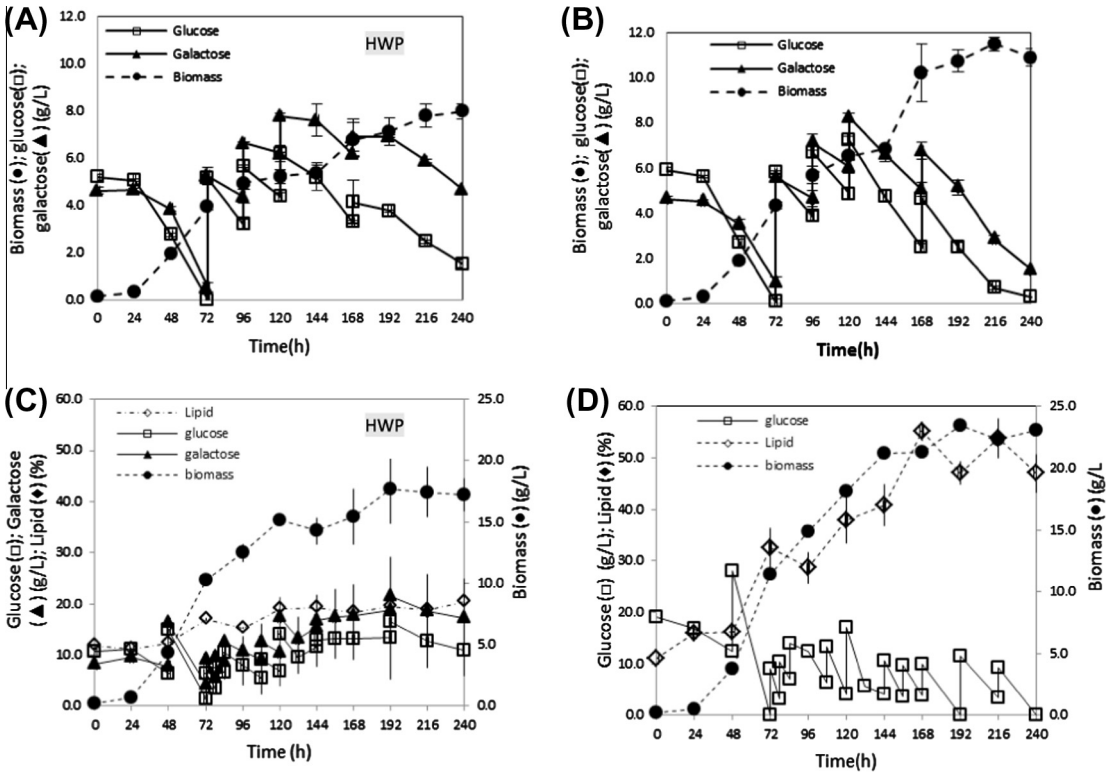

Fig. 4. Kinetics for biomass production and substrate consumption of *C. protothecoides* in fed batch cultures grown for 240 h in 100 mL shake flasks and 5 L reactor with different substrates. (A) Shake flask, HWP (initial 5.19 g/L glucose, 4.61 g/L galactose, 0.5 g/L yeast extract); (B) shake flask, glucose and galactose mixture (equivalent to HWP); (C) 5 L bioreactor, HWP (10 g/L glucose, 10 g/L galactose, 4 g/L yeast extract); (D) 5 L bioreactor, glucose.

23.1 ± 0.7 g/L for the control (Fig. 4C and D), which was proportional to the increased substrate added. Lipid accumulation was less for HWP (20.5 ± 0.4%) compared to the glucose control (47.0 ± 3.7%) likely due to the continued supply of nitrogen from HWP feed during fermentation. Using HWP, fed batch strategy may be more beneficial for increasing biomass rather than lipid content of *C. protothecoides* since nitrogen depletion is essential for promoting lipid accumulation (Jakobsen et al., 2008; Rodolfi et al., 2009; Widjaja et al., 2009).

The growth rate of the microalgae is not as high as some of those reported in the literature since other variables such as temperature, micronutrients, salinity and pH influence growth (Takagi et al., 2006; Heredia-Arroyo et al., 2010; Chen et al., 2011; Bartley et al., 2013; Han et al., 2013) and may have played a role in the observed lower growth rate. It is important to note that some reported high cell density cultures of *C. protothecoides* (Xiong et al., 2008) use undefined "*Chlorella* growth factors" and hormones, which enhance growth rates but negatively impact environment and process economics. Further, our results suggest that C/N ratio is critical for lipid accumulation, which has also been reported to be influenced by the variables described above (Takagi et al., 2006; Heredia-Arroyo et al., 2010; Chen et al., 2011; Bartley et al., 2013; Han et al., 2013).

3.5. SSF using non-hydrolyzed whey permeate

SSF strategy was employed to grow *C. protothecoides* in order to investigate the potential direct use of whey permeate, eliminate pre-hydrolysis and use immobilized enzyme. SSF involved simultaneous enzymatic saccharification of lactose in the whey permeate into glucose and galactose monomers, and parallel fermentation of the monomers by the growing algal culture. Based on previous experiments (data not shown), β-galactosidase from *A. oryzae* was chosen for optimal release of sugars during algal growth. Enzymatic activity in the original re-suspended enzyme preparation was 5 U/mg. Considering the importance of reusability for the better economics of the process, enzyme immobilization was done (5% w/w) using PVA and PEG, and the enzymatic activity of the immobilized beads was 3 U/mg solid beads. A series of experiments done with non-hydrolyzed whey permeate (20–40 g/L lactose) and immobilized enzyme showed that an initial lactose concentration of 25 g/L with yeast extract 0.25 g/L added at 0, 48, 72 and 96 h was ideal for optimal biomass and lipid accumulation. Complete saccharification of lactose was achieved and the monomers were consumed as expected, resulting in a biomass of 7.3 ± 1.3 g/L and lipid accumulation of 49.9 ± 3.3% on a dry weight basis (Fig. 5). No contamination was observed in cultures during the entire growth period till harvest.

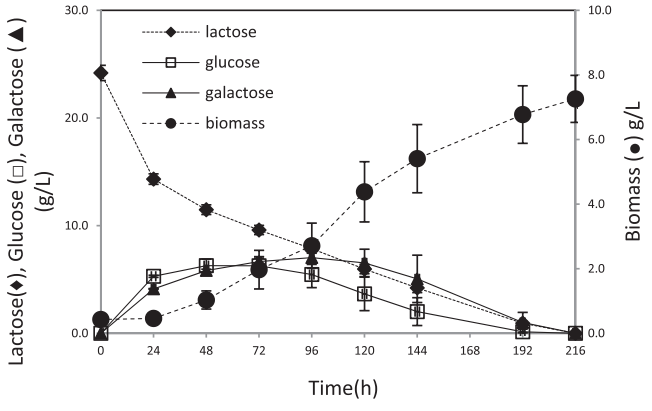

Fig. 5. Kinetics for biomass production and substrate consumption of *C. protothecoides* in SSF mode grown for 216 h in 100 mL shake flasks with non-hydrolyzed whey permeate (final 25 g/L lactose), yeast extract (0.25 g/L initial, 2 g/L feed as required) and 3 U of encapsulated β-galactosidase activity.

Although whey or whey derivatives have been used for the production of industrially important products (Panesar and Kennedy, 2012), its global abundance indicates that there is scope for alternative strategies as the one proposed in this work. The world trade of whey and whey products for 2009 was estimated at 1.2 million tons, of which, the major product was whey powder (International Dairy Federation, 2010). In USA, 190,000 tons of whey protein was produced in 2009 leading to 1.5 million tons of available lactose mostly in the form of whey permeate (lactose estimated from the average ratio of 1:8 protein/lactose; Mawson, 1994). The versatility of *C. protothecoides* to heterotrophically grow and accumulate lipids by using different complex feedstock has been previously demonstrated, however in all cases, the feedstock used was not a waste stream and is more expensive than whey permeate. Biomass concentration and lipid content in batch cultures (Gao et al., 2010; Lu et al., 2010, 2011) using pre-hydrolyzed sweet sorghum (3.3 g/L, 52.3%), cassava starch (4.3 g/L, 50.2%) and corn powder (4.7 g/L, 39.9%) are comparable to those obtained in the present study (9.1 g/L, 42%). In fed-batch cultures (Cheng et al., 2009; Lu et al., 2011; Yan et al., 2011), biomass concentration and lipid content using pre-hydrolyzed sugar cane (121.3 g/L, 45%), cassava starch (53.6 g/L, 53%) and molasses (97.1 g/L, 57%) are higher than those obtained in the present study (17.2 g/L, 20.5%). There are not many reports regarding the use of SSF for microalgal growth; a high biomass concentration (49.3 g/L) and lipid content (54.5%) was reported using cassava starch but required continuous addition of enzyme (Lu et al., 2011). In this study, using SSF strategy, 7.3 g/L biomass with 49.9% lipids was achieved with no residual substrate or hydrolysis products maximizing feedstock utilization. Moreover, the system offers the advantage of potential reusability of the immobilized enzyme.

4. Conclusion

Whey permeate was found to be a promising carbon source for the heterotrophic growth and lipid accumulation of *C. protothecoides* using batch and SSF cultures, however fed batch strategies required additional conditions to limit nitrogen. The lipid accumulation and fatty acid profile of the algal biomass grown using whey permeate feedstock was comparable to the use of pure glucose. These results suggest that this feedstock can be readily integrated into algal cultivation technologies towards biofuel production. Further, this work has demonstrated a value added alternative for the utilization of a dairy by-product stream with current limited applications.

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