Bioresources and Bioprocessing

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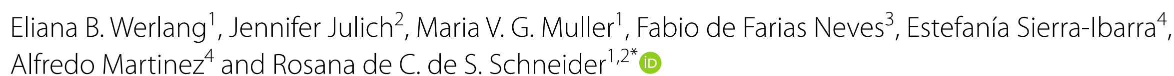
Bioethanol from hydrolyzed Spirulina (*Arthrospira platensis*) biomass using ethanologenic bacteria



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Abstract

Photosynthetic microorganisms are considered excellent feedstock for biofuel production in developing biomass production technologies. A study was conducted to evaluate ethanol production with the sequential enzymatic saccharification and fermentation of *Arthrospira platensis* (*Spirulina*) biomass with the metabolically engineered *Escherichia coli* strain MS04. *A. platensis* was cultivated semicontinuously in an open raceway pond, and the carbohydrate content was determined to be as high as 40%. The enzymatic saccharification was designed to release the maximum amount of glucose. After 40 h of enzymatic saccharification, 27 g L^{-1} of monosaccharides was obtained. These slurries were fermented with ethanologenic bacteria, achieving 12.7 g L^{-1} ethanol after 9 h of fermentation, which corresponds to 92% conversion yield of the glucose content in the hydrolysate, 0.13 g of ethanol per 1 g of *Spirulina* biomass and a volumetric productivity of 1.4 g of ethanol L^{-1} h^{-1}. Therefore, we conclude that it is possible, in a short time, to obtain a high ethanol yield corresponding to 160 L per ton of dry biomass with a high productivity.

Keywords: *A. platensis*, Enzymatic saccharification, *Escherichia coli*, Bioethanol, Biomass

Introduction

The depletion of petroleum oil and the rapid growth of the global population have increased the demand for renewable energies. Ethanol is probably the most popular non-fossil biofuel, which production has been broadly studied. Currently, ethanol can be produced from agricultural crops such as corn and sugarcane (first generation), agroindustrial residues, i.e., lignocellulosic materials (second generation), and photosynthetic microorganisms (third generation) (Lee and Lee 2016; Tan et al. 2019). Bioethanol derived from microalgae and cyanobacteria is an excellent alternative compared to first- and second-generation bioethanol since these photosynthetic microorganisms do not compete with food crops, fix carbon dioxide, can grow on non-arable land, have fast growth compared to agricultural crops, lack lignin in their cell structures, and contain high amounts of carbohydrates, lipids, vitamins and proteins (Hamouda et al. 2018). In the context of biofuel production, carbohydrates are one of the major compounds produced by *Spirulina* from photosynthesis, largely in the form of starch and cellulose, which can be hydrolyzed to obtain fermentable monosaccharides (Sivaramakrishnan and Incharoensakdi 2018; Bastos 2018; Shokrkar et al. 2018; Velazquez-Lucio et al. 2018).

A promising source of photosynthetic microbial biomass for biofuel production is that obtained from *A. platensis*, which shows high carbohydrate, protein and lipid contents (Braga et al. 2018; Vargas-Tah et al. 2015). Depending on the growth conditions, the carbohydrate content can reach up to 60% of the dry cell mass (Silva et al. 2018; Rempel et al. 2018; Li et al. 2018). In the *A.*

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platensis biomass, the composition of polysaccharides is mainly starch, which is an arrangement of glucose monomers linked by α-1,4 glycosidic linkages.

In this sense, the *A. platensis* intracellular carbohydrates can be hydrolyzed and used for bioethanol production by fermentation with an ethanologenic microorganism. To obtain fermentable sugars from this biomass, a pretreatment for cell breakdown must be applied; this process could be chemical, physical or mechanical (Günerken et al. 2015); then, the polysaccharides can be hydrolyzed into fermentable sugars by enzymatic saccharification (Dixon and Wilken 2018). In starch-type polysaccharides (starch, Floridean starch, and glycogen) linked together by α-(1,4) and α-(1,6) glycosidic bonds, enzymes such as α-amylase and glucoamylase are required to cleave the polysaccharide into glucose units (Al Abdallah et al. 2016). The endo-acting α-amylases hydrolyze starch or glycogen into oligosaccharides, glucose, maltose and maltodextrins. The glucoamylase enzyme removes, in a stepwise manner, the glucose units from the nonreducing ends of poly- and oligosaccharides. On the other hand, in the case of cellulose, the action of endoglucanases, exoglucanases, and β-glucosidases is required as cellulolytic enzymes to cleave β-1,4 glycosidic linkages into glucose molecules (Al Abdallah et al. 2016).

According to Lopes et al. (2019), the use of genetically engineering ethanologenic microorganisms assists in the ethanol yield, and in the use of the residual biomass after fermentation due to the improvement of sugar conversion, leaving less residual biomass. A wide range of microorganisms are commonly used to produce bioethanol (Singh et al. 2018) from different sugars obtained from agroindustrial residues and photosynthetic microbial biomass (Shankar et al. 2019). Previous reports showed that it is possible to produce ethanol from carbohydrates contained in different *A. platensis* biomasses. Ethanol yields ranging from 34 to 93%, from released sugars, were reached after carbohydrate hydrolysis of *A. platensis* biomasses and their fermentation with different bacteria and yeast, and the volumetric productivities ranged from 0.14 and 1.0 g_{EtOH} $L^{-1}h^{-1}$ (Efremenko et al. 2012; Aikawa et al. 2018; Markou et al. 2013).

On the other hand, bacteria could be an effective alternative to efficiently produce bioethanol. *Escherichia coli* is a bacterium able to grow on mineral media as well as in starch, glycogen and lignocellulosic hydrolysates, with the capability to efficiently ferment different sugars among hexoses and pentoses. Furthermore, *E. coli* MS04 (*ΔpflB, ΔadhE, ΔfrdA, ΔxylFGH, ΔldhA*, P*pflB::pdc*$_{Zm}$-*adhB*$_{Zm}$, *ΔxylFGH*, km^r, evolved) is a strain that was genetically engineered and evolved to generate ethanol as the main fermentation product (Fernandez-Sandoval et al. 2012). This strain has not been previously studied for the conversion of *A platensis* biomass to ethanol. Further, the technology to produce *A platensis* biomass in open ponds is well-developed and is applied at industrial scale (Ye et al. 2018). Even more, the use of metabolically engineered ethanologenic *E. coli* strains for ethanol production from lignocellulosic hydrolysates has shown high ethanol yields (75–80%) and volumetric productivities (1 to 2 g_{EtOH} L^{-1} h^{-1}) (Pedraza 2016; Vargas-Tah et al. 2015). Since the feasibility of using *E. coli* to ferment *A. platensis* biomass for high ethanol productivity and yield is not reported elsewhere, a confirmation study is required.

Therefore, the aim of this work was to study the effectiveness of pretreated and saccharified biomass from *A. platensis* as a culture medium for ethanol production. The dried *A. platensis* biomass was characterized, and the conditions for maximizing glucose release in the hydrolysate produced from enzymatic hydrolysis with amylases and cellulases were determined. The resulting slurry was used, as culture medium for ethanol production, with the metabolically engineered ethanologenic *E. coli* MS04, aiming to reach a high ethanol yield and productivity.

Materials and methods

Biomass source and preparation

Arthrospira platensis was cultivated in a semicontinuous system in an open raceway pond inside a greenhouse (open system) at the University of Santa Catarina State (UDESC), Brazil. The raceway pond was 6 m long and 1.2 m wide. The water column was maintained at 0.15 m deep, and the culture volume was 1200 L. The raceway culture was homogenized by a paddle wheel working at 16 rpm with a water velocity of approximately 0.3 m s^{-1}. The culture was carried out in a greenhouse under measured, but not controlled, temperature and natural illumination. The cultivation temperature during the year varied between 15.8 °C and 42.7 °C. The pH of the culture media was 10.24 ± 0.90. The culture medium contained freshwater and was composed of 10 g L^{-1} $NaHCO_3$, 1 g L^{-1} commercial soluble fertilizer with a nitrogen:phosphate:potassium ratio of 18:6:18, and a high amount of NaCl (30 g L^{-1}).

The biomass was collected three times a week by filtration with a 20 µm net and then dried in a laboratory oven at 50 °C for 24 h. The dried biomass was stored in a refrigerator at 4 °C. This material was milled with a cryogenic spray mill (MA775, Marconi) prior to the experiments described below.

Characterization of *A. platensis* biomass

The water content of the milled biomass was determined in an electronic heater balance (Brainweigh, Ohaus MB 301) at 60 °C. Determination of ashes and structural

carbohydrates was performed according to the National Renewable Energy Laboratory's (NREL) methods (Sluiter et al. 2005). The lipid content was determined by an adapted method (Bligh and Dyer 1959), and the fatty acid profile was determined as described by Souza et al. (2017) with a gas chromatograph equipped with a mass spectrometer (GC/MS) (Shimadzu, Model QP2010 plus, Japan).

The concentration of the monosaccharides was determined by concentrated acid hydrolysis (CAH) by reaction with sulfuric acid (Sluiter et al. 2012). The acid hydrolysis product was quantified using high-performance liquid chromatography (HPLC, Shimadzu, Model 20A, Japan) with an RHM monosaccharide H^+ ion exclusion column (300 × 7,8 mm, Phenomenex, 00H-0132-K0, EUA), ultrapure water (Milli-Q, USA) as the mobile phase (0.6 mL min^{-1}) at 85 °C and a refractive index detector (Shimadzu, Model 20A, Japan).

For immediate quantification of the glucose contained in the enzymatic saccharified hydrolysates and fermentations, glucose was measured with a biochemical analyzer (YSI model 2700, YSI Inc., Yellow Springs, OH, USA) (Vargas-Tah et al. 2015).

Enzymatic saccharification of *A. platensis* carbohydrates

Enzyme concentration experiments were carried out to verify the most favorable concentration needed to perform the enzymatic saccharification. The tests were carried out in 100-mL Schott flasks with a 0.5% (w/v) load of biomass (0.1 g) and 20 mL of total volume. The pH of the distilled water was adjusted to 6.0 with 0.1% H_3PO_4. The first step was performed at 90 °C by adding 1.9 Kilo Novo Units (KNU) of the α-amylase enzyme (Novozymes–Liquozyme Supra 2.2X) for 2 h, under static condition. In the second step, 1.8 Amiloglucosydase Units (AGU) of glucoamylase enzyme (Novozymes—AMG 300L) were added at 60 °C for 2 h, under periodic manual agitation. Both enzymes were used to cleave the molecules of polysaccharides into glucose via polysaccharide hydrolysis. After that, different concentrations (2, 12, 15 and 30 FPU) of cellulase cocktail NS22086 (Novozymes) were added to perform the enzyme concentration test. The tests were performed over 24, 48 and 72 h at 200 rpm. The HPLC analysis of monosaccharides was performed as described above after 2, 4, 24, 48 and 72 h of enzymatic saccharification. The saccharification yield percent was calculated according to Eq. (1).

$$\text{Saccharification yield (\%)} = \frac{(\text{Enzyme saccharification value})}{(\text{Total carbohydrate value})} 100 \quad (1)$$

To use the hydrolysate in the fermentation study, 20 g of milled biomass was saccharified at a 10% load (w/w). After optimization, the best condition was used with more biomass in relation to liquid phase due to the minireactor capacity. The reactions were performed using three 300-mL mini-reactors fitted with a magnetic stirrer and a working volume of 200 mL (Fernandez-Sandoval et al. 2012). The pH of the distilled water was adjusted to 6.0 with 0.1% H_3PO_4. In this experiment, the load of biomass was increased 20 times and the enzyme concentrations were proportionally adjusted. The first step of enzymatic saccharification was performed at 90 °C by adding 37.8 KNU of α-amylase (Novozymes) enzyme for 2 h. In the second step of saccharification, a load of 30 AGU of glucoamylase enzyme (NS22035–Novozymes) was added at 60 °C for 2 h. The third step was performed by adding 12 FPU g_{glucan}^{-1} of enzymatic cocktail NS22086 (Novozymes) at 50 °C for 40 h. Saccharification was performed at 200 rpm. To avoid microbial contamination, 30 mg L^{-1} of kanamycin was added to the mixture. The HPLC analysis of monosaccharides was performed, as described above, at 0, 2, 4, 24 and 40 h of cellulase saccharification. The yield of enzymatic saccharification was calculated in relation to the total carbohydrate content obtained by CAH.

Fermentation

The fermentation for ethanol production from the hydrolysate of *A. platensis* biomass was performed with the metabolically engineered *E. coli* MS04 (*ΔpflB, ΔadhE, ΔfrdA, ΔxylFGH, ΔldhA,* P*pflB::pdc*$_{Zm}$-*adhB*$_{Zm}$, *ΔxylFGH,* km^r, evolved). In brief, strain MS04 was engineered to produce ethanol as the main fermentation product. Since *pflB*, *frdA* and *ldhA* encode enzymes involved in the synthesis of other fermentation metabolites, such genes were deleted. Furthermore, the native ethanol dehydrogenase gene (*adhE*) was deleted, and the heterologous ethanol pathway (genes *pdc* and *adh*) from *Zymomonas mobilis* was chromosomally introduced with the aim of having redox balance under non-aerated conditions and improving ethanol production. Finally, the *xylFGH* gene was deleted, and the strain was evolved to improve the xylose consumption, acetate tolerance and retain its ability to consume a wide array of sugars (Fernandez-Sandoval et al. 2012). The preinoculum was prepared by transferring the preserved strain from a cryovial (40% glycerol, w/v) to a test tube with 4 mL of rich medium (Luria–Bertani medium) supplemented with kanamycin (30 mg L^{-1}). The cells were grown for 4 h at 37 °C and 300 rpm. Mini-fermenters containing 200 mL of AM1 mineral medium as described by Martinez et al. (2007) and supplemented with 20 g L^{-1} of glucose, 2 g L^{-1} of sodium acetate and 0.1 g L^{-1} of sodium citrate were used to grow the inoculum (Fernandez-Sandoval et al. 2012). The cells were grown in the mini-fermenters at 37 °C,

pH 6.8 and 150 rpm, until an OD_{600} of 1.5 was reached (approximately 20 h). The pH was controlled by automatic addition of 2 mol L^{-1} KOH, and the cells were harvested by centrifugation (4 °C, 10 min, 10,000 rpm) to start the cultures in the fermenters with an OD_{600} of 0.1 (0.037 g_{dcw} L^{-1}).

Fermentations were performed in the same vessels used for saccharification. Before inoculation, the pH of the saccharified biomass was adjusted to 6.6 with 2 mol L^{-1} KOH, and 1 mmol L^{-1} betaine (an osmoprotectant), 1 g L^{-1} ammonium phosphate salts and 2 g L^{-1} sodium acetate were added. The cultures were incubated at 37 °C and 200 rpm (without aeration) until the glucose was consumed. A positive control was prepared with 25 g L^{-1} of glucose and AM1 medium to verify the strain performance.

The ethanol produced from fermentation was measured by HPLC (Waters U6K, USA) using an Aminex HPX-87H ion exclusion column (300 × 7.8 mm), a 5.0 mmol L^{-1} H_2SO_4 solution as the mobile phase (0.5 mL min^{-1}) at 45 °C, a photodiode array detector at 210 nm (Model 996, Waters, USA) and a refractive index detector (Model 2410, Waters, USA). Calibration curves were prepared from a stock solution of glucose at 200 g L^{-1} (J.T. Baker, batch#V25C64, 1916-01), acetic acid at 100 g L^{-1} (J.T. Baker, batch #V03C71, 9508-02) and ethanol at 200 g L^{-1} (Merck, batch #K49257583, 00,923-2500). The calibration curves were built with 7 points.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 8 software. Significant differences were determined by ANOVA, and $p < 0.05$ was considered significant. The experiments were conducted in triplicate.

Results and discussion

Characterization of *A. platensis* biomass

The biomass composition is shown on Table 1. According to previous reports, the composition of *A. platensis* was high in proteins (64–73%) and low in carbohydrates (12–17%) and lipids (5–7%) (Verdasco-Martín et al. 2019). However, the total carbohydrate content achieved in this study was as high as 40% in terms of total glucan, determined by CAH. These results are similar to those (45% DW) reported by Phélippé et al. (2019). In the context of biofuel production, a high amount of carbohydrates prone to conversion into fermentable sugars is desirable. The lipid content (6.7%) was also similar to the results found by Magro et al. (2018). Table 2 illustrates the fatty acid profile of the biomass determined as methyl esters (FAME) relative to total FAMEs identified using the relative GC/MS peak areas. In the FAME profiles, hexadecanoic acid (C16:0) and linoleic acid (C18:2) were the most abundant fatty acids, as shown also by Lu et al. (2019). In our study, however, oleic acid (C18:1) was the second most abundant fatty acid.

Table 1 Characterization of *A. platensis* biomass produced in raceway ponds

Content (%)	Untreated *A. platensis* biomass
Total carbohydrate	40.02 ± 0.47
Protein	24.95 ± 0.47
Ashes	11.81 ± 0.54
Lipids	6.69 ± 3.56
Other	16.53 ± 5.06

Table 2 Fatty acid profile of *A. platensis* biomass obtained in raceway pond

FAME profile	%
C14:0	0.44 ± 0.10
C16:0	48.72 ± 0.39
C16:1n-7	3.04 ± 0.10
C18:0	4.20 ± 0.26
C18:1n-9	25.57 ± 0.45
C18:2n-6	18.03 ± 0.96

Saccharification of *A. platensis* biomass

For enzymatic saccharification of the pretreated biomass (*A. platensis* milled in a cryogenic spray mill), the performance of sequential treatment with α-amylase (1.9 KNU), glucoamylase (1.8 AGU) and the cellulase cocktail (2, 12, 15 and 30 FPU) was studied with a 0.5% biomass load (Fig. 1). The maximum glucose concentration of 1.89 g L^{-1} was achieved at 72 h using 12 FPU of the cellulase blend, which represents a saccharification yield of 94.5% considering the conversion of starch-type polysaccharides and the cell wall polysaccharides.

Since the best results for polysaccharide hydrolysis were obtained with 12 FPU of the cellulase cocktail (Fig. 1), this concentration was used for the saccharification of a 10% w/w biomass load of *A. platensis* for ethanol production in a bioreactor. The enzymatic hydrolysis produced 27 g L^{-1} glucose after 40 h of saccharification (Fig. 2), which represents a yield of 67.5%, calculated as the ratio between the obtained glucose and the maximum amount of glucose that could be achieved (40 g L^{-1} in this case). The change in glucose concentration from 20 h to 40 h did not show a significant difference ($p > 0.05$), avoiding the need to let the reaction proceed until 72 h, as perceived earlier. Other fermentable sugars, such as xylose,

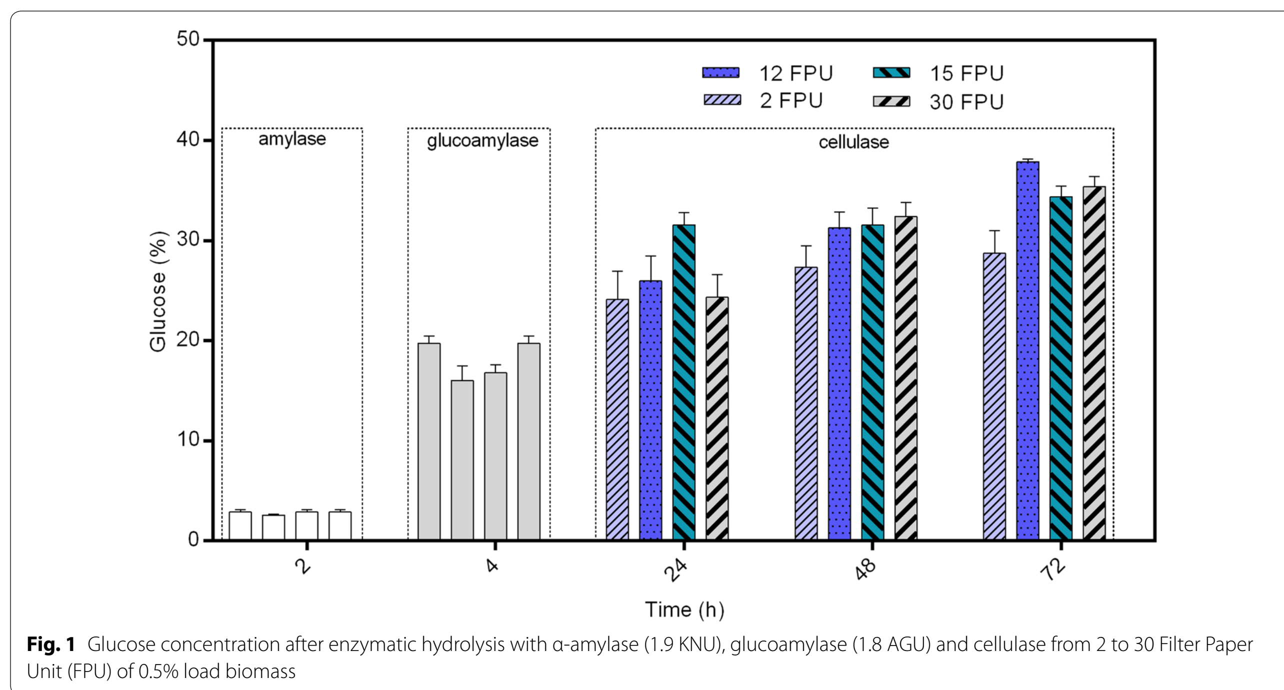

Fig. 1 Glucose concentration after enzymatic hydrolysis with α-amylase (1.9 KNU), glucoamylase (1.8 AGU) and cellulase from 2 to 30 Filter Paper Unit (FPU) of 0.5% load biomass

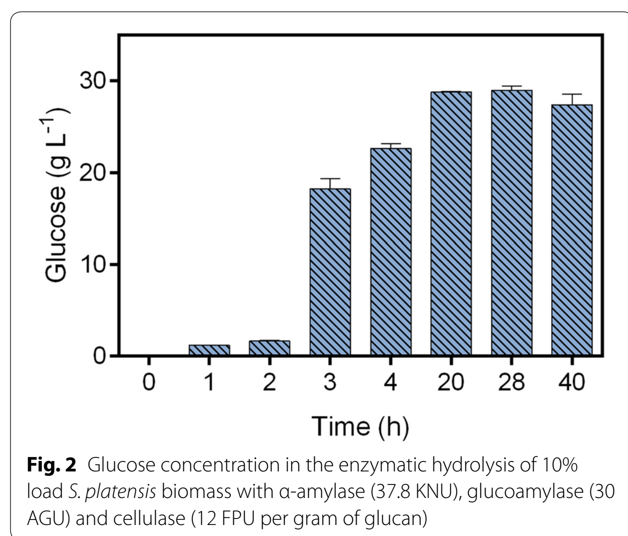

Fig. 2 Glucose concentration in the enzymatic hydrolysis of 10% load *S. platensis* biomass with α-amylase (37.8 KNU), glucoamylase (30 AGU) and cellulase (12 FPU per gram of glucan)

galactose and arabinose, which have been reported for *A. platensis,* were absent (Depraetere et al. 2015). It is noteworthy that after the addition of α-amylase (0–2 h) and glucoamylase (2–4 h), the glucose concentration reached approximately 23 g L^{-1}, subsequently increasing by only 4 g L^{-1} after the addition of the cellulase cocktail (Fig. 2), which means the carbohydrate fraction of the *Spirulina* biomass is composed mainly of glycogen, as has been reported previously (Phélippé et al. 2019). The lower saccharification yield, compared with that of the 0.5% biomass load, could be due to the reaction time of α-amylase and glucoamylase since the amount of glycogen to be hydrolyzed was higher than in the bioreaction with a 0.5% biomass load, and therefore a longer reaction time would be needed as well some disruption method can be used (Keris-Sen and Gurol 2017). On the other hand, the remaining 4 g L^{-1} of glucose released after the cellulase addition could be derived from the unspecific activity of cellulases over α-1,4 bonds, despite the low efficiency of this reaction. In this sense, it is worth mentioning that the cell wall of *A. platensis* does not contain hemicellulose or cellulose in its structure, unlike other vegetal materials (Tornabene et al. 1985).

Although the yield of the saccharification process could be higher, the enzymatic hydrolysis of biomass is a promising approach due to the absence of toxic molecules such as acetate, formic acid and phenolic compounds that affect the production of metabolites during fermentation (Phwan et al. 2018).

Fermentation of hydrolysates from *A. platensis* by *E. coli* MS04

The saccharified *A. platensis* biomass was fermented with the ethanologenic *E. coli* strain MS04 in mini-bioreactors according to the process described by (Vargas-Tah et al. 2015). The glucose consumption and ethanol production kinetics are shown in Fig. 3. Ethanol reached a final concentration of 12.7 g L^{-1} after 9 h of fermentation when the glucose was depleted. A

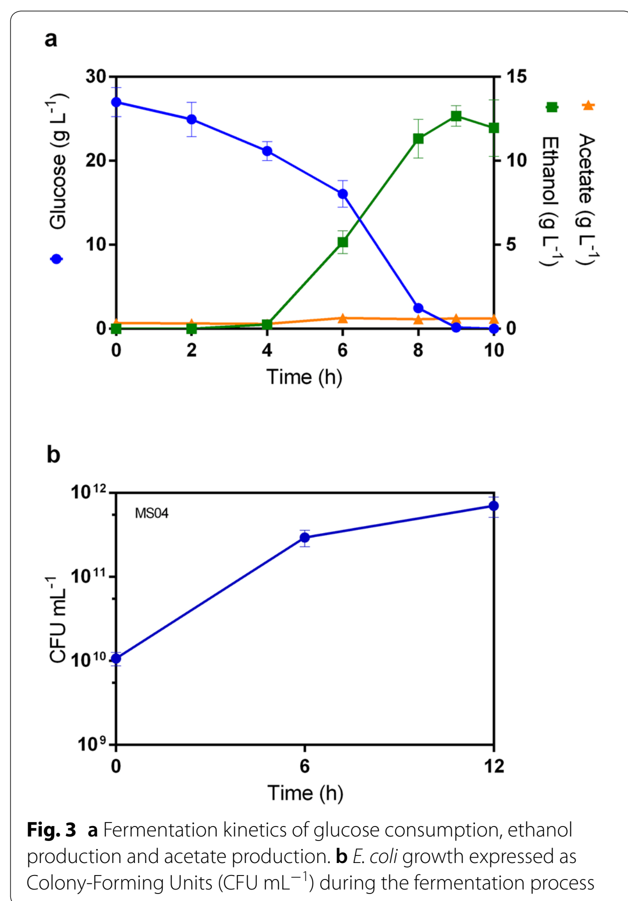

Fig. 3 **a** Fermentation kinetics of glucose consumption, ethanol production and acetate production. **b** *E. coli* growth expressed as Colony-Forming Units (CFU mL^{-1}) during the fermentation process

Table 3 Ethanol yield, volumetric productivity and product concentration for ethanol production from *A. platensis* hydrolyzed biomass and mineral media

A. platensis hydrolyzed biomass	
$Y_{ethanol\ 9h}$ ($g_{ethanol}$ g_{TS}^{-1}) [a]	92.12 (3.64)
$Q_{ethanol\ 9h}$ ($g_{ethanol}$ L^{-1} h^{-1})	1.41 (0.10)
Product concentration $_{9h}$ ($g_{ethanol}$ L^{-1})	12.66 (0.61)
Control (mineral media)	
$Y_{ethanol\ 28h}$ ($g_{ethanol}$ g_{TS}^{-1}) [a]	74.20
$Q_{ethanol\ 28h}$ ($g_{ethanol}$ L^{-1} h^{-1})	0.38
Product concentration $_{28h}$ ($g_{ethanol}$ L^{-1})	10.18
μ (h^{-1}) Specific growth rate	0.22

Values in parenthesis indicate the standard deviation from three independent experiments

$Y_{ethanol}$, yield of ethanol on glucose consumed

$Q_{ethanol}$, volumetric rate of ethanol production

μ, specific growth rate

[a] Product yield on glucose as percentage of the maximum theoretical (0.51 g_{EtOH}/g_{Glc})

high ethanol production yield of 92% from glucose (as a percentage of the maximum theoretical glucose concentration) was obtained (Table 3). This result demonstrates the effectiveness of the *A. platensis* hydrolysates as culture medium for ethanologenic *E. coli*. In studies implementing the use of other hydrolysates, such as those from corn stover, fermented with the same ethanologenic strain under similar fermentation conditions, lower ethanol yields were reported (Vargas-Tah et al. 2015). The ethanol volumetric productivity, obtained under non-aerated conditions, 1.41 g L^{-1} h^{-1} (Table 3), is the highest reported for strain MS04, being 42%, 25% and 370% higher when compared to those of mineral media under microaerobic conditions (Fernández-Sandoval et al. 2016), the fermentation of corn stover hydrolysates (Vargas-Tah et al. 2015), and mineral media under non-aerated conditions (control fermentation of this work; Table 3), respectively.

These results show that *A. platensis* biomass, cultivated under the conditions mentioned above, contains a significant amount of nutrients such as proteins, vitamins and minerals resulting in a rich culture media for *E. coli*, which favors ethanol productivity and yield. Furthermore, the global process yield of 160 $L_{ethanol}$ $ton^{-1}_{Aplatensis}$, calculated as the volume of ethanol obtained from the total *A. platensis* biomass (approximately 130 $kg_{ethanol}$ $ton^{-1}_{Aplatensis}$), was an excellent result. As shown in Table 4, the values reported for ethanol yield and productivity vary among the microalgae, carbohydrate hydrolysis methodology and ethanologenic microorganism used. Further, the ethanol productivity can be easily increased by increasing the *A. platensis* load in the hydrolysis steps, using biomass with a higher carbohydrate content and pretreating the microalga with thermochemical or physical methods to improve glucose release.

The colony-forming units from strain MS04 (CFU; determined in agar rich media with kanamycin) showed that the bacteria maintained their metabolism with total sugar consumption even in the last hours of ethanol production. The ethanol accumulation without losing the microorganism viability and consequently reaching a higher ethanol yield is an important aspect of increasing the scale of production (Mukhopadhyay 2015). Remarkably, a positive fermentation control experiment performed in mineral media containing 25 g L^{-1} of glucose (Table 3) reached glucose depletion at 28 h and had significantly lower values for ethanol yield and volumetric productivity when compared to those of the fermentations with *A. platensis* hydrolysates (Table 3). These results confirm the relevance of *A. platensis* hydrolysates as culture media for ethanologenic *E. coli*, and the effectiveness of reserve carbohydrates as substrates to efficiently generate ethanol and probably, in the future, to deploy biorefineries based on third-generation biofuel technologies.

Table 4 Comparative productivities (kg ton^{-1}) from microalgae with potentiality to bioethanol production

Microalgae	Hydrolysis	Ethalogenic microorganism	Theorical yield (%)	Productivity (kg ton^{-1})	References
Chlorella (73.58% total sugars)	Chemical	*S. cerevisiae*	91	240	Zhou et al. (2011)
A. platensis (60–65% carbohydrate)	Chemical	*S. cerevisiae*	53.3	163	Markou et al. (2013)
Chlorella	Chemical pretreatment/enzymatic	*S. cerevisiae*	82.1	230	Phwan et al. (2019)
A. platensis (40% carbohydrate)	enzymatic	*E. coli* MS04	92	130	Our study

Future perspectives

Technoeconomic analysis to use microalgae as feedstocks for biofuel production has been investigated by Rajesh Banu et al. (2020) based on several options of integrated biorefineries. Such study shows that a system can be integrated with several bioproducts, thus improving the economic gains and thus assisting in the effective implementation at commercial scale. De Souza et al. (2018) demonstrated that bioethanol production is relevant product to reinforce microalgal technologies for agricultural, commercial and industrial development. According to Abomohra et al. (2016) 5000–7500 tons of microalgae are already worldwide produced, with an average annual income of US $ 1.25 billion, and taking into account that the production of *A. platensis* is already established at industrial level, the use of ethanologenic bacteria, such as the one evaluated in this study, could allow to deploy third-generation biorefineries as it has been reported with lignocellulosic feedstocks.

It has been reported that second-generation ethanol can be more cost-effective than first generation but the economic feasibility will be demonstrated in the long term with reliable technologies and feedstock supply (Gyekye 2017; Karagoz et al. 2019). This comparison is important for third-generation bioethanol from microalgae, which likewise has excellent future prospects (Chowdhury and Loganathan 2019), if associated with biodiesel production from lipids (Dasan et al. 2019). High ethanol yields and volumetric productivities, as those obtained in our work, encourage the development of pilot-scale tests, to evaluate the technical and economic feasibility to generate bioethanol, and other products, from hydrolyzed *A. platensis* biomass using ethanologenic bacteria.

Conclusion

The enzymatic hydrolysis of 10% w/w *A. platensis* biomass, using a specific sequence of enzymes, produced a fermentable syrup with 27 g L^{-1} glucose and a high amount of nutrients, which favors ethanol production with high yield and productivity: 92% conversion yield from the glucose consumed and a volumetric productivity of 1.4 g of ethanol L^{-1} h^{-1}, which is at least 40% higher than productivity values reported in previous studies from *A. platensis* hydrolysates. In the experiments, the production of ethanol was 12.6 g L^{-1} and showed a yield of 160 $L_{ethanol}$ ton^{-1} dried biomass.

Abbreviations

AGU: Amiloglucosydase Units; ANOVA: Analysis of Variance; CAH: Concentrated Acid Hydrolysis; CFU: Colony Forming Units; FAME: Fatty Acid Methyl Ester; FPU: Filter Paper Unit; GC/MS: Gas Chromatograph equipped with a Mass Spectrometer; HPLC: High-pressure Liquid Chromatography; KNU: Kilo Novo Units; NREL: National Renewable Energy Laboratory's; OD_{600}: Optical Density at 600 nm.

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Authors' contributions

EW, JJ and ES-I worked in the experiments with hydrolysis and fermentation. A M and ES-I produced the recombinant bacteria. MVGM, AM and RCSS designed the experiments and supervision the research. FFN produced the microalgae. All authors read approved the final manuscript.

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