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ORIGINAL PAPER

Cultivation of *Chlamydomonas reinhardtii* in Anaerobically Digested Vinasse for Bioethanol Production

Marija B. Tasic[1] · Anderson de Jesus Bonon[2] · Maria Ingrid Rocha Barbosa Schiavon[3] · Bruno Colling Klein[4] · Vlada B. Veljković[1,5] · Rubens Maciel Filho[3]

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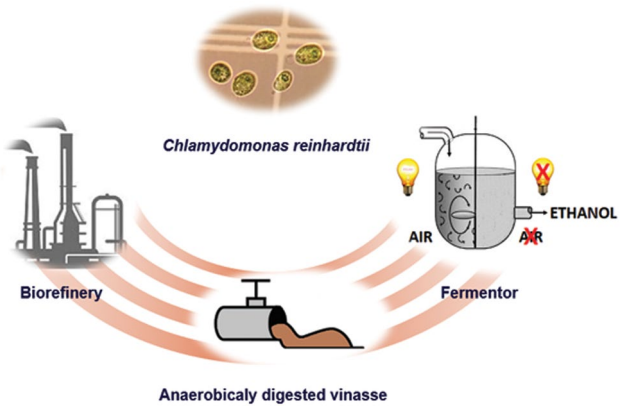
Abstract

This study describes a new algal biofuel process that integrates sugarcane biorefinery wastewater treatment by nutrient removal with algae into bioethanol. The process is free of common industrial problems, including algal contamination, nutrients and fresh water usage, carbohydrate extraction, liquefaction, and saccharification. Cultivation and fermentation were conducted in one step by turning the light-air on and off, respectively. Three series of experiments with *Chlamydomonas reinhardtii* CC-1093 cultivation and fermentation were performed in anaerobically digested vinasse. Control experiments were a reference to compare the influence of chloride and ammonium-sulfate stress conditions on ethanol yield. Experimental results showed: (1) algal biomass can be successfully cultured within biorefinery wastewater (1129 $mg \cdot L^{-1} \cdot day^{-1}$); (2) relatively high bioremediation was achieved (26.1%–83.5%); (3) obtained ethanol yield was (maximum 68.3% of the theoretical yield) in one process step; and (4) the chloride stress condition influences on algae to synthesize extracellular polysaccharides as add-in product (120 mg/L).

Graphic Abstract



Keywords *Chlamydomonas reinhardtii* · Anaerobically digested vinasse · Adsorption · Ethanol · Stress · Fermentation

Statement of Novelty

Anaerobically digested vinasse (ADV) has a negative environmental impact, which could be decreased by using microalgae as a potential biofuel feedstock. Various studies have shown that the ADV can be used in biodiesel production by cultivating microalgae on it. However, no reports show using

✉ Marija B. Tasic
marijat14@yahoo.com; mtasic@tf.ni.ac.rs

Extended author information available on the last page of the article

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ADV for microalgae bioethanol production. The microalgae strain, *Chlamydomonas reinhardtii*, can grow in very diluted raw vinasse but synthesizes ethanol in the buffer. *Chlamydomonas reinhardtii*'s bioethanol industrialization has not yet been developed. The crucial reasons are implementing synthetic media for cultivation and high pretreatment costs. The present study provides an optimized design process relating to *Chlamydomonas reinhardtii*—based ethanol production. Furthermore, the co-production of polysaccharides as a side-product and eco-fertilizer from the ferment residuals significantly improved ADV's ecological potential.

Introduction

The frequency of natural disasters is currently growing as a dangerous consequence of anthropic global warming. Increased atmospheric CO_2 concentrations and ever-higher quantities of wastewaters are two global warning enablers that require solutions. One of them is the production of biofuels, such as bioethanol and biodiesel. However, their production also creates additional quantities of CO_2 and wastewaters. Therefore, scientists are proposing biofuels facilities with less CO_2 emission and cleaner wastewater [1, 2].

The sugarcane ethanol industry produces raw vinasse as a byproduct of ethanol separation in distillation columns. Raw vinasse has a very high potential of bringing negative environmental impacts due to: (i) being generated at ratios of 6–15 L raw vinasse/L ethanol [3–5]; and (ii) being used for sugarcane field fertigation [4, 6]. The two most abounded scientific approaches for the treatment of raw vinasse are anaerobic digestion and microalgae biomass cultivation [7], but only the former currently has an industrial application. Anaerobic digestion is a first approach for the treatment of raw vinasse for biogas production, which highly contributes to biorefinery integration via power generation. However, the effluent from anaerobic digestion, known as ADV has still relatively high nutrients concentrations with a strong, unpleasant odor. Thus, ADV is more prone to becoming an environmental concern.

A possible solution to this problem can be the use of ADV as a medium for microalgae cultivation. Being dark and toxic for microalgae growth, ADV needs purification before use. The most often used purifying treatments are dilution, centrifugation, and adsorption by smectite clay (SC) and activated charcoal (AC) [8–10]. Dilution utilizes large volumes of already limited water resources, centrifugation is energetically unfavorable while adsorption generates solid waste. Adsorption decreases the concentrations of some components, thus making the stress conditions in cultivation media. Adsorbent materials must be sustainable and cheap (i.e., SC is widespread while AC can be obtained via slow biomass pyrolysis). SC can minimize chloride (Cl) [11] while AC reduces ammonium (N) and sulfate (S) concentration [12], providing N, S- and Cl- stress cultivation conditions, respectively. Although ADV has relatively high carbon concentrations, the biorefinery's CO_2 is a possible additional carbon source for the cultivation [13].

ADV has already been used as a medium for microalgae cultivation in biodiesel production. During this production, algae perform bioremediation of ADV and synthesize lipids [10, 14], which are then extracted and converted into biodiesel [15, 16]. In addition to the problems of cultivation followed by the need for sterilization, pH regulation of ADV, and the use of artificial fertilizers, research shows that biodiesel production destroys algae cells [17] while transesterification creates surpluses of glycerol in the market.

The use of ADV to produce bioethanol from microalgae has not been reported yet. Well-known algal species *Ch. reinhardtii* can produce extracellular ethanol during intracellular fermentation under dark and anaerobic conditions [18–21]. Under stress conditions, this species synthesizes high carbohydrate or lipid contents [22] and extracellular polysaccharides [23]. This alga can grow in the synthetic medium [18, 19, 24–27] and very diluted raw vinasse [28]. However, the growth in these media was accompanied by the same cultivation problems related to the production of biodiesel, the extraction of complex carbohydrates, their conversion into sugars [24–27] and fermentation using buffers [18].

This study aimed at evaluating the use of *Ch. reinhardtii* CC-1093 for ADV bioremediation and ethanol fermentation, under stress conditions. This process was developed to reduce environmental pollution caused by ADV, as well as to contribute to bioethanol, polysaccharides, and eco-fertilizer production alternatives. To the author's knowledge, this work represents the first use of *Ch. reinhardtii* in ADV for ethanol synthesis.

Material and Methods

Purification of ADV

ADV was collected from a large-scale plant for anaerobic digestion of raw vinasse installed in a sugarcane mill in the State of Sao Paulo, Brazil. After collection, the ADV was frozen to avoid the degradation of the organic components.

For the Cl-stress studies, the ADV was treated with the modified procedure described elsewhere [29, 30]. In a 1000 mL Erlenmeyer flask, 600 mL of the ADV (pH 7.06) was heated to 72 °C for 18 min. Then, AC (powder, Synth-Labsynth, Sao Paulo, Brazil) in the concentration of 8% (w/v) was added, and the mixture was magnetically stirred (750 rpm) and heated at 78 °C for 2 min. Finally, the hot

solution was filtered through a qualitative filter paper Grade 1 into a 1000 mL Erlenmeyer flask for 30 min.

For the N, S-stress studies, the ADV was treated with another modified procedure [31]. The ADV (1000 mL, pH 7.06) and SC (Sepiolite powder, Merck KGaA, Darmstadt, Germany) in a concentration of 0.52% (w/v) were poured in a 2000 mL Erlenmeyer flask, stirred magnetically (750 rpm) and heated at 70 °C for 20 min. The mixture was let to cool for phase separation for 60 min. The liquid phase (840 mL) was decanted into another 2000 mL Erlenmeyer flask and treated following the procedure for the Cl-stress studies. Samples for analysis were taken for ADV and after both purified treatments.

Inoculum Preparation, Cultivation, and Self-Fermentation

Inoculum Preparation

The *Ch. reinhardtii* CC-1093 starter culture was purchased from the Chlamydomonas Resource Center of the University of Minnesota (Saint Paul, MN, USA) and grown in the YA-agar medium [32]. A pre-growth liquid culture was made by inoculating approximately 300 mL of *tris*–acetate–phosphate (TAP) medium [32] in a 500 mL Erlenmeyer flask with a pea-sized cells pellet taken from the agar culture. Table 1 gives the conditions used for inoculum preparation. The cells grew aerobically under white fluorescent constant illumination on an orbital shaker. The pH of the medium was adjusted by adding 1.0 M NaOH. The cultivation took about two days to reach the mid-log phase (5 10^6 cells/mL). An appropriate aliquot (10%) was then taken to inoculate new experimental cultures.

Cultivation and Self-Fermentation

This stage included two consecutive phases, i.e., aerobic-light cultivation and anaerobic-dark intracellular fermentation, called here cultivation and self-fermentation, respectively. The *Ch. reinhardtii* CC-1093 strain was grown in the sterile TAP synthetic (Control), Cl- and N, S-stress ADV media. Conditions applied to the cultivations were the same as those described in Table 1. The aerobic cultures grew in 500 mL Erlenmeyer flasks containing 300 mL of the medium. The 2.5% CO_2-air mixture (0.75 L/min) was delivered to the flask cultures containing the N, S-stress ADV medium by a peristaltic air pump (LT15, Whitewater). There was no need for dilution, extra nutrients, and pH adjusting. Samples for analysis were taken immediately after resuspension (0^{th} day), daily through the four days of fermentation, and after a 24 h dark anaerobic period. The cell-free supernatants were obtained through centrifugation (Eppendorf, model 5810R) at 3500 rpm, 15 °C for 15 min.

Table 1 Culture conditions for *Ch. reinhardtii* growth

Parameter	Temperature	Stirring speed	Illumination	pH	Aeration
Units	°C	rpm	Lux		L/min
Value	23	125	8140	7.3	1
St dev	±2	±1	±2	±1	±0.2

Analytical Methods

ADV Composition

The samples of the treated ADV and cell-free supernatants taken after the cultivation and self-fermentation stages were analyzed for total carbon (TC), total nitrogen (TN), ions, and total reducing sugars. Before analysis, all samples were filtered through a 0.45 µm membrane filters (Millex FG1, Billerica-MA, USA). TC and TN were analyzed using a Shimadzu TOC-VCSN equipment (50 mL injection, replicate injections made if standard error of peak area > 0.2%). Cation and anion analyses were carried by Laboratório de Recursos Analíticos e de Calibração (LRAC, FEQ, UNICAMP, Campinas/SP, Brazil) and Instituto de Química (IQ, UNICAMP, Campinas/SP, Brazil), respectively. Polysaccharides, represented as total reducing sugars, were measured by the Dubois's phenol–sulfuric acid method [33].

Algae Growth

The biomass density was measured gravimetrically (mg/L, dry weight) and by direct cell counting with the Neubauer hemocytometer at a microscope (Olympus, model CX21). For determining the dry cell weight (DCW), the wet algae biomass, collected by filtration through an appropriate filter paper (GC/F, City, England), was dried in an oven at 105 °C to constant weight.

The specific algae growth rate µ (day^{-1}) in the exponential growth phase and the volumetric productivity of a batch culture P (mg/(L·day)) in the logarithmic growth phase were calculated by the following equations:

$$\mu = \left(\frac{ln\left(N_{t_2}/N_{t_1}\right)}{t_2 - t_1} \right) \Big/ ln2 \quad (1)$$

where N_{t1} and N_{t2} are DCW at times t_1 and t_2, respectively; and

$$P = \mu \cdot Y \quad (2)$$

where Y (mg/L) is the yield of the culture.

Cell counts were assessed using an ImageJ® Cell and Particle Counter (The National Institute of Health, Maryland, USA). Background and cellular debris were excluded in all cellular count. The control of contamination was performed by streaking suspension samples onto a plate solid YA medium [32].

Monitoring of Algae Composition

The biomass was withdrawn from the culture flask and immediately analyzed for carbohydrates, starch, proteins, and lipids to avoid intracellular metabolites being rapidly degraded or metabolized. Carbohydrates, proteins, and lipids were analyzed following the standard methods for measuring the growth of algae and their composition [34]. The method of Khona et al. [23] was used for starch extraction and estimation.

Ethanol Content

The ethanol concentration was measured in the samples of the purified ADV and the cell-free supernatants after cultivation and self-fermentation. All samples were filtered through a 45 µm membrane before gas chromatography (GC) analysis using a GC-FID (Agilent-6850) equipped with a DB-FFAP–J&W column (nitroterephthalic-acid-modified polyethylene glycol: 20 m × 250 µm × 0.25 µm). GC conditions: injection volume: 2 µL; inlet (Split/Splitless): 250 °C, 5.5 psi with split set at 20:1; detector (FID): 300 °C, H_2 flow—35 mL/min, air flow—300 mL/min and make-up gas (N_2) flow ≈ 30 mL/min (make up + column flow); oven program: 55 °C hold 3 min; up to 250 °C/min at 40 °C/min, 2.13 min hold; carrier gas program (H_2): 1.0 mL/min, hold 3 min; up to 5 mL/min at 6 mL/min, until the end of run. The quantification was performed with the external calibration curves prepared with the standard solutions in ethanol (> 99.9% analytical, commercial standards, Sigma-Aldrich).

Statistical Analysis

The statistical analysis was performed using OriginPro8 (OriginLab Corporation, Northampton, MA, USA) to determine the means and the student's t-test needed for comparison between treatments ($\alpha = 0.05$).

Results and Discussion

The Efficiency of ADV Purification by Adsorption

In the present work, the *Ch. reinhardtii* microalga was cultivated in the ADV at a laboratory scale under atmospheric, non-axenic conditions. However, the attempts to cultivate this microalga using dark ADV (pH 7.42) failed due to an outgrowth of fungi and bacteria (data not shown). Therefore, to achieve high algal biomass productivity, the light transmittance through the culture medium was increased by adsorption using AC and SC. These two adsorbents were selected as being easily available, relatively cheap, and highly effective towards removing the dark impurities. SC bonds chloride, but much more ammonium and sulfate [11]. Therefore, SC provides N,S-stress conditions. On the other hand, AC reduces ammonium and sulfate [12] but does not impact chloride and thus provides high salt concentration as a stress factor. Also, both materials raised pH, thus eliminating the pH adjusting step. Finally, ADV autoclaving was also avoided since AC and SC act against bacteria and viruses [35–38]. The use of AC and SC disclosed two main results. First, during adsorption, 33%(v/v) and 22%(v/v) of the ADV were lost in the Cl- and N, S-stress media, respectively, which agreed with a previous report [9]. Second, the adsorption procedures needed less total run time (50 min), compared to the previously proposed one of 700 min [31]. Also, in the applied N, S-stress purifying procedure, a cheaper decantation step replaced the filtration step used by Lombardi et al. [31].

Aerobic *Ch. reinhardtii* Cultivation

Since the used *Ch. reinhardtii* CC-1093 strain did not grow on nitrate, the treated ADV composition (Table 2) was shown to be an ideal medium for its cultivation. This strain grew in all the media tested, with the maximum cell growth in the Cl-stress medium (Fig. 1). Although CO_2 was introduced in the N, S-stress medium to reduce the pH and provide an additional carbon source, the Cl-stress medium provided the better growth conditions for *Ch. reinhardtii* due to the higher $NH4^+$ concentrations (Table 2). With the specific growth rate of 1.48 day^{-1}, the biomass productivity of 1129 mg/(L·day) was obtained in the Cl-stress cultivation. In the synthetic media, the production rates were lower, i.e., from 5.61 mg/(L·day) for the laboratory scale photobioreactor [39] to 483.33 mg/(L·day) for the 100 L capacity outdoor cultivation [40]. Higher productivity rates of about 2000 mg/(L·day) can be reached by *Ch. reinhardtii* growing on sewer wastewater in a biocoil reactor (9 L) [41]. However, the sewer wastewater medium needs pH control and CO_2 as an additional source of carbon, leading to higher cultivation costs. In the medium with 0.2% ADV, *Chlorella vulgaris* grew slower (0.76 day^{-1}) [14]. The same species grew faster in the ADV (1.2 day^{-1}) [9] than *Ch. reinhardtii* (0.92 day^{-1}, Table 3) under the N, S-stress conditions. However, the former species required dilution of the ADV (80%) while *Ch. reinhardtii* did not. Generally, the ADV supported a better growth of *Ch. reinhardtii* (Table 3) than raw vinasse 108.72 ± 4.77 mg/(L·day) [28].

Table 2 Variations in ADV composition (mg/L) during aerobic *Ch. reinhardtii* cultivation

Parameter	ADV	Cl-stress		N, S-stress		ADV [9]	N, S-stress [9]	
		Before	After	Before	After		Before	After
pH	7.42	8.30	8.96	8.62	7.08	7.93	8.31	8.43
TC	4460	2994	1137	3235	2391	5342	2417	1214
TN	210.0	189.9	69.2	40.7	6.7	455.8	117.6	10.1
K^+	3412	2664	1356	2113	1240	3504	2637	1660
Na^+	362.0	144.4	135.6	116.3	100.3	74.9	57.4	10.6
NH_4^+	–	161.0	32.0	15.0	1.7	134.6	47.6	1.0
PO_4^{3-}	82	< 1	–	< 1	–	75.8	–	–
NO_3^-	–	< 2	< 2	< 2	< 2	–	–	–
NO_2^-	–	< 3	< 3	< 3	< 3	–	–	–
SO_4^{2-}	–	101	60	52	41	936	–	–
Cl^-	–	1482	1430	654	623	–	–	–

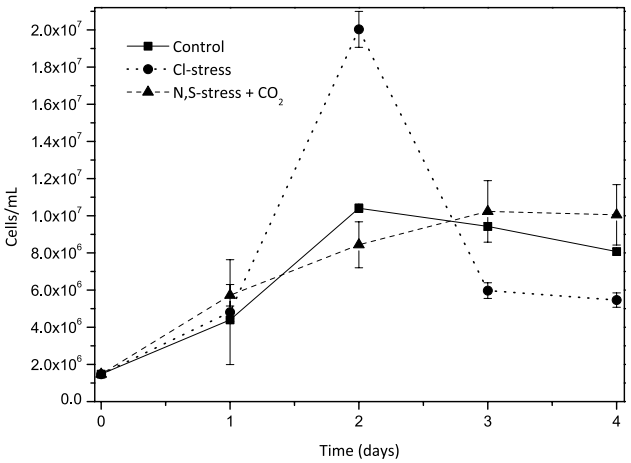

Fig. 1 Aerobic growth of *Ch. reinhardtii* in various ADV media

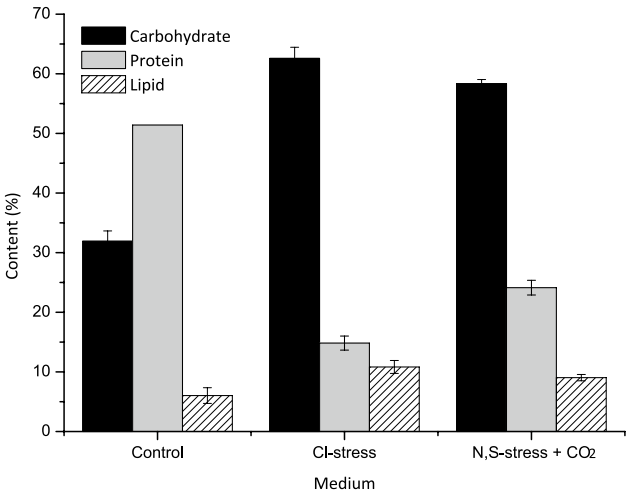

Fig. 2 Biochemical composition of *Ch. reinhardtii*, at maximum biomass growth, cultivated in various ADV media under aerobic conditions

Table 3 Kinetic growth parameters of *Ch. reinhardtii* cultivation

Media	Control	Cl-stress	N, S-stress
DCW, mg/L	901 ± 82	763 ± 403	634 ± 103
μ, day^{-1}	1.41 ± 0.02	1.48 ± 0.42	0.92 ± 0.08
P, mg/(L·day)	1279	1129	583

Composition of *Ch. reinhardtii* Biomass

The high algal carbohydrate content is essential for ethanol synthesis. The stress conditions in the ADV influenced the microalgal biomass composition, as can be seen in Fig. 2. The highest carbohydrate content (starch of 48%, w/w) was produced in the Cl-stress medium. Also, all the tested media fostered higher carbohydrate concentrations than the control medium could provide. The starch content of 80% using a mutant strain of *Ch. reinhardtii* cw15 under the N-depletion conditions in the TAP medium is reported [26]. The same carbohydrate content (80%) is achieved using the ADV as a cultivation medium and a consortium of various algae (*Chlorella* sp., *Pseudanbaena* sp., *Chloromonas* sp.) and a bacterium [42]. The stressed conditions implemented in the present study also increased the lipid level. The percentages of lipids varied from 6.0% in the control medium to 10.8% in the Cl-stress medium (Fig. 2). Similar strain *Ch. reinhardtii* CC125 also manifests the elevated levels of starch and lipid in the Cl [23] and the N-stress medium [43]. When *Ch. reinhardtii* was nitrogen-starved, then carbon was stored as starch rather than used for growth. However, if the storage of starch is blocked by mutation, then the lipid accumulation increases [22, 44, 45]. Generally, sulfur is required for aerobic algae growth. However, in the absence of sulfur and at the pH lower than 6.9, *Ch. reinhardtii* stops cell division and thus, accumulates significant protein concentrations [20]. The N, S-stress conditions and the lower pH used in

the present study were also responsible for higher protein content compared to the Cl-stress medium.

Ch. reinhardtii is stressed upon the exposure to NaCl [23]. With 1482 mg/L of chloride in the ADV medium (Table 2), *Ch. reinhardtii* grew in the palmelloid form (Fig. 3a). In response to the Cl-stress, extracellular polysaccharides were also accumulated, as can be seen in Fig. 3b. The extracellular polysaccharides form a gel (often referred to as mucilage), which is mainly consisted of acidic polysaccharides, and already used as the soil conditioner [23, 46]. Due to the presence of extracellular polysaccharides in the Cl-stress medium and the dissolution of CO_2 in the N, S-stress medium, TC was apparently removed by 62.02% and 26.09%, respectively (Table 2). The TN removal in the Cl- and N, S-stress media was 63.56% and 83.48%, respectively.

Anaerobic *Ch. reinhardtii* Growth

The high levels of carbohydrates in the algal biomass indicated that these organisms could be used for ethanol synthesis. The main anaerobic product of carbohydrate degradation in the dark by *Ch. reinhardtii* cultures is ethanol. In this study, the ethanol yields were 0.347 g/g and 0.382 g/g of starch, corresponding to 61.9% and 68.3% of the theoretical yield, or approximately 166.4 mg/g and 172.1 mg/g of dry algal biomass, for the Cl- and N, S-stress ADV media, respectively. A low sulfate concentration and a pH of 7.08, in the N, S-stress ADV medium (Table 2), provided higher starch conversion rates, thus favoring the ethanol fermentation. Although the Cl-stress ADV had a higher starch content, *Ch. reinhardtii* did not synthesize more ethanol. This trend is expected since high pH [18, 20] and sulfur deprivation [20] promote the synthesis of by-products, such as H_2 and organic acids. The ethanol yields achieved in this study were the highest among the self-fermentation microalgae species (Table 4).

In the processes where microalgae biomass was the feedstock for yeast fermentation, the ethanol yields were 1.7 times higher than that obtained in the self-fermentation. However, the self-fermentation bypassed the pretreatment and saccharification steps and might be economically and energetically preferable. Also, the self-fermentation presented herein was conducted in the same medium used for the cultivation. In the other studies, the biomass is separated from the cultivation medium and then it is used for the self-fermentation in a puffer, which increase the production costs. In comparison to different ethanol biomass sources, the ethanol yields reported here were in the range of cassava and cane bagasse [48, 49], higher than sugar cane [49], beat [50] and sweet sorghum [48], but lower than corn [48], wheat [48] and corn stover [51]. After self-fermentation, all the media kept high content of potassium ions (Table 2), which meant that the treated ADV could be further used as an environmentally friendly fertilizer.

Conclusion

We defined initial, simple and competitive processes to synthesize microalgae ethanol, utilizing ADV. Although the processes dismissed dilution, supplementing of nutrients, pH adjusting and autoclaving, ADV required purification under low temperatures conditions. Two purifying treatments were proposed using adsorbent materials. The adsorption made stress conditions to the *Ch. reinhardtii* growth. The Cl-stress

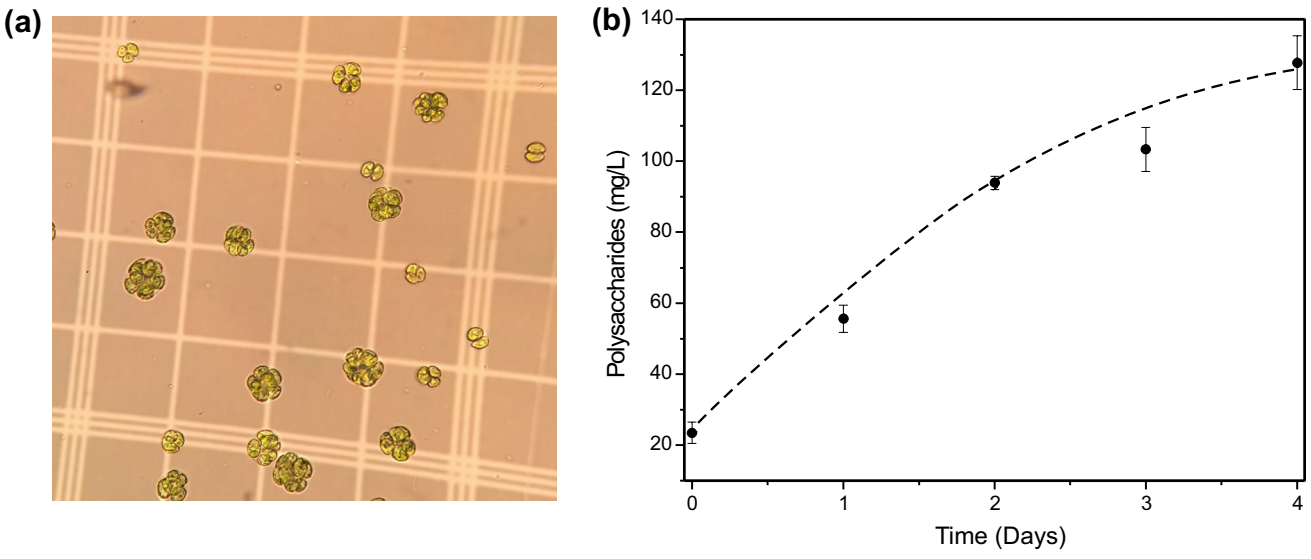

Fig. 3 *Ch. reinhardtii* exposed to Cl-stress in ADV medium: (**a**) growth of palmelloids cells; and (**b**) kinetics of extracellular polysaccharides synthesis

Table 4 A review of microalgae used in laboratory-scale ethanol synthesis

Medium	Microflora	Pretreatment	Biomass productivity, mg/(L·day)	Starch, %	Fermentation		Reference
					Maximum ethanol yield, mg/g[a]	Time, h	
TAP	*Ch. reinhardtii* (CC-1093)	–	1279.4	17	24.2[b]	24	This study
ADV	*Ch. reinhardtii* (CC-1093)	Cl–stress	1129.2	48	166.4[b]	24	This study
ADV	*Ch. reinhardtii* (CC-1093)	N, S-stress, 2.5% CO_2	583.3	45	172.1[b]	24	This study
P49	*Ch. reinhardtii* (UTEX 2247)	0.5% CO_2	40[c]	45	66.7[b]	46	[18]
P49	*Chlamydomonas* sp. (YA-SH-1)	0.5% CO_2	109.1[c]	30	82[b]	44	[19]
MC	*Chlorococcum littorale*	5% CO_2	1647.1	15	20.6[b]	24	[47]
TAP	*Ch. reinhardtii* (UTEX 90)	1 M CH_3COOH feed-batch	600	35	292[d]	24	[24]
TAP	*Ch. reinhardtii* (UTEX 90)	1 M CH_3COOH feed-batch	3100	44	235[d]	40	[25]
HSM	*Ch. reinhardtii* cw15 (CC-4349) mutant	N-stress	6122.2[e]	30	142.1[d]	24	[26]
Synthetic	*Chlamydomonas fasciata* Ettl (NIES-437)	CO_2	122.88	43.5	194[d]	24	[27]

[a]Based on biomass weight

[b]Ethanol during self-fermentation

[c]Calculated on average pond's depth

[d]Algal biomass is a feedstock for yeast ethanol fermentation

[e]Calculated on suggested average cultivating medium volume of 0.45 L in a 1L flask for 4 days

conditions provided the highest biomass productivity, better ADV bioremediation, and extracellular polysaccharides as an add-in product. On the other hand, the N, S-stress medium provided the highest ethanol yield, utilized CO_2 and therefore could contribute to less CO_2 emission from biorefineries. Due to the unchanged potassium ions concentrations after self-fermentation, ADV could be further used for fertigation with positive environmental impact.

Both Cl stress- and N, S stress-based processes showed to be promising for possible use in industry. Processes mimicked natural microalgae life-cycle phenomena because they used species from the sugarcane raw vinasse pond and produced ethanol without damage to the cells. The purification of ADV was simple and used cheap and natural adsorbent materials, while cultivation and self-fermentation might be conducted in the same equipment.

In order to meet energy efficiency criteria, the conversion was conducted in one unit by switching of light-air, which mimicked a sequential batch process, and therefore the continuous-flow operation was not feasible. However, compared to a continuous process, this one-step batch process could lower total capital investment costs and ethanol selling price, bring high flexibility in changing products (from ADV remediation to ethanol, polysaccharides or eco-fertilizer production), and avoid single fault to stop the entire course of batch production at an industrial scale.

The only disadvantage was the generation of waste adsorbent residues. Therefore, before industrial utilization, optimization, environmental and economic feasibility analysis are needed.

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Author Contributions MBT performed the fermentations, acquisition, analysis, and interpretation of data, design conception and drafting of the article. AJB was involved in the acquisition, analysis, and interpretation of GC data. MIRBS was involved in the acquisition and interpretation of absorbents data. BCK was involved in the acquisition, analysis, and interpretation of ADV data. VBV participated in design conception and drafting of the article. RMF participated in the design of this study as well as coordination and supervision of the work. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

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Affiliations

Marija B. Tasic[1] · Anderson de Jesus Bonon[2] · Maria Ingrid Rocha Barbosa Schiavon[3] · Bruno Colling Klein[4] · Vlada B. Veljković[1,5] · Rubens Maciel Filho[3]

1 Faculty of Technology, University of Nis, Bulevar oslobodjenja 124, 16000 Leskovac, Serbia

2 HORIBA Scientific, HORIBA Instruments, Inc, 9755 Research Dr, Irvine, CA 92618, USA

3 Faculty of Chemical Engineering, University of Campinas, University Town Zeferino Vaz, Avenue Albert Einstein 500, Campinas, Sao Paulo CEP 13083-852, Brazil

4 National Renewable Energy Laboratory (NREL), 15013 Denver W Pkwy, Golden, CO 80401, USA

5 The Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11000 Belgrade, Serbia