



Production and characterization of algae extract from *Chlamydomonas reinhardtii*



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ABSTRACT

Background: Algae offer many advantages as biofuel sources including: high growth rates, high lipid content, the ability to grow on non-agricultural land, and the genetic versatility to improve strains rapidly and produce co-products. Research is ongoing to make algae biofuels a more financially attractive energy option; however, it is becoming evident that the economic viability of algae-based fuels may hinge upon high-value co-products. This work evaluated the feasibility of using a co-product, algae extract, as a nutrient source in cell culture media. **Results:** Algae extract prepared from autolysed *Chlamydomonas reinhardtii* was found to contain 3.0% protein, 9.2% total carbohydrate, and 3.9% free α -amino acid which is similar to the nutrient content of commercially available yeast extract. The effects of algae extract on the growth and metabolism of laboratory strains of *Escherichia coli* and *Saccharomyces cerevisiae* were tested by substituting algae extract for yeast extract in LB and YPAD growth media recipes. Complex laboratory media supplemented with algae extract instead of yeast extract showed markedly improved effects on the growth and metabolism of common laboratory microorganisms in all cases except ethanol production rates in yeast.

Conclusions: This study showed that algae extract derived from *C. reinhardtii* is similar, if not superior, to commercially available yeast extract in nutrient content and effects on the growth and metabolism of *E. coli* and *S. cerevisiae*. Bacto™ yeast extract is valued at USD \$0.15–0.35 per gram, if algae extract was sold at similar prices, it would serve as a high-value co-product in algae-based fuel processes.

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1. Introduction

Algae-based fuels and other algae derived products have recently received growing interest from the energy, food, and pharmaceutical industries [1,2]. Most algae are capable of producing energy-rich oils, and many microalgae species have been isolated that naturally accumulate high oil levels [3]. Additionally, microalgae offer several advantages over terrestrial plants as a source of transportation biofuels, including high growth rates, high lipid content, the ability to grow large cultures on non-agricultural land, and the ability to rapidly improve strains and produce co-products [4].

Despite these promising characteristics, the economic viability of algae-based biofuels is still uncertain. Recent estimates place a barrel of algae-based oil at US \$450–\$2300, compared with US \$80–110 for crude oil (2012) [1,5]. Efforts to lower the cost of algae oil production

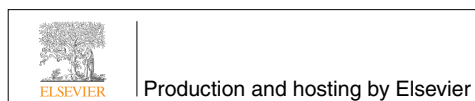
are currently focused on nutrient sourcing and usage, harvesting, strain isolation, production management, fuel extraction, co-product development, and residual biomass sourcing [6]. After the desired hydrocarbons have been extracted from harvested algae, a significant portion of residual biomass remains, with several options currently being explored for their usage. These options include anaerobic digestion of biomass to produce methane [7], pyrolysis of dry biomass to produce bio-oil [8], use as an animal or aquaculture feed [9], or use as a fertilizer [10]. This study suggests another alternative, specifically using a portion of the processed biomass to prepare a cell culture media supplement.

The main nutritional supplement used in most laboratory microbial cell cultures today is yeast extract [11], which is typically isolated from spent brewer's yeast generated as a by-product from beer production [12]. It serves as a rich source of amino acids, vitamins, nitrogen, and carbon for fungal, bacterial, mammalian, and insect cell cultures. Yeast extract is composed of the water soluble components of *Saccharomyces cerevisiae* cells and is normally produced by autolysis (the digestion of the cell by its own enzymes), although plasmolysis and hydrolysis have been used [12]. During autolysis, hydrolytic enzymes located in the cellular matrix degrade the proteins and nucleic acids of the cell. The proteins in the cell are broken down into peptides by proteases, whereas the nucleic acids, DNA and RNA, are split into nucleotides [13]. Yeast extract has also been used as a flavoring agent in soup,

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stews, gravies, canned food and snack food, and to increase the nutritional value of various foods [14]. The current cost of molecular biology grade yeast extract ranges from US \$0.15 to \$0.35 per gram (Sigma Aldrich) and thus yeast extract serves as a valuable co-product in the commercial production of bioethanol and beer.

The literature contains several applications of crude algae extracts, such as their effects on various cancer cells [15,16], antimicrobial properties [17], and for use in cosmetics [18]; however, no reports focused on using algae extract as a cell culture media supplement could be found. The aim of this work is to investigate the potential of using autolysed algae extract from the microalgae species *Chlamydomonas reinhardtii* as a supplement in microbiological media.

2. Materials and methods

2.1. Microorganisms and culture conditions

C. reinhardtii wild-type 137c (mt⁺) strain was purchased from the Chlamydomonas Center (Duke University, Durham, NC) and grown in Tris-acetate-phosphate (TAP) medium at 25°C under constant light. *Escherichia coli* strain DH5 α was grown aerobically at 37°C with constant shaking in modified Luria-Bertani (LB) medium containing either yeast or algae extract. *S. cerevisiae* strain YSG50 [19] was grown at 30°C under anaerobic conditions with constant shaking in modified yeast extract-peptone-dextrose plus adenine (YPAD) medium containing either yeast or algae extract. All component concentrations in standard LB and YPAD media recipes were kept constant, the substitution of yeast or algae extract was the only modification.

2.2. Preparation of algae extract

C. reinhardtii was grown in 3-liter batches in a 5-liter B. Braun Biostat B benchtop fermentor (Sartorius, Goettingen, Germany) under constant fluorescent light (65 $\mu\text{m m}^{-2} \text{s}^{-1}$ as measured by a Field Scout Quantum Meter; Plainfield, IL). The culture temperature was maintained at 25°C using a circulating water bath. Cultures were mixed with six agitating vanes at 200 rpm. Humidified filtered air was bubbled into the growing culture at a nominal rate of 2 mL/s. Cultures were typically grown for six days to stationary phase and then harvested. The harvested culture was centrifuged at 4000 $\times g$ for 5 min, pelletized, washed and resuspended twice with tap water. After washing, the supernatant was discarded and 40 mL of distilled water was added to the pelletized algae. The resuspended algae cells were then placed in a 50°C water bath for 24 h for autolysis. The autolysed cells were centrifuged at 10,000 $\times g$ for 10 min and the supernatant was collected and dried in a rotary evaporator under vacuum. The resulting product was then dried overnight using a vacuum pump and ground to create an algae extract powder.

2.3. Characterization of the algae extract

The α -amino nitrogen content was measured by the ninhydrin colorimetric method, using glycine as a standard [20]. Total protein was measured by using a Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA). Carbohydrate content was measured using the phenol-sulfuric acid colorimetric method, using glucose as a standard [21].

2.4. *E. coli* growth and protein production

Cultures of *E. coli* were grown aerobically in the presence of standard LB ingredients: 10 g/L Bacto™ tryptone, 10 g/L NaCl, and 5 g/L of either Bacto™ yeast extract or the algae extract produced in this study. All cultures were inoculated (1:100) from overnight cultures (which were propagated aerobically in the same modified LB media) and grown for 12 h at 37°C with constant shaking at 250 rpm. Cell growth was monitored by measuring the optical density at a wavelength of 600 nm using a NanoDrop 2000c spectrophotometer (Thermo Scientific,

Waltham, MA). Total protein production was measured using Bradford assay with bovine serum albumin as a standard [22]. Assays were performed following 3 rapid freeze thaw cycles to disrupt cells and centrifugation for 10 min at 10,000 $\times g$ to clear away membrane debris.

2.5. *S. cerevisiae* growth and ethanol production

Cultures of *S. cerevisiae* were grown under anaerobic conditions in the presence of standard YPAD ingredients: 20 g/L Bacto™ peptone, 20 g/L α -D-glucose, 0.040 g/L adenine hemisulfate, and 10 g/L of either Bacto™ yeast extract or the algae extract produced in this study. Cultures were inoculated (1:100) from overnight cultures (which were propagated anaerobically in the same modified YPAD media) and grown for 24 h at 30°C with constant shaking at 250 rpm. Cell growth was monitored by measuring the optical density at 600 nm using a NanoDrop 2000c spectrophotometer. To measure ethanol production, samples were analyzed by high-performance liquid chromatography (HPLC, Agilent 1260), with a refractive index detector (RID, 35°C, Agilent G1362A). Samples were centrifuged and filtered using a 0.2 μm sterile syringe filter, and 5 μL of the filtered sample was injected into the HPLC. An Aminex HPX-87H (300 \times 7.8 mm) ion exchange column (Bio-Rad, Hercules, CA, USA) was operated at 45°C, and an eluent of 5 mM aqueous sulfuric acid flowing at 0.6 mL/min was applied for product separation. Products were identified by comparison with authentic samples.

3. Results and discussion

3.1. Preparation of algae extract

Batch cultures of *C. reinhardtii* were grown in a small-scale photobioreactor. Cultures were harvested when the algae reached stationary phase, usually after 6 days of cell growth. Autolysis was used to breakdown cellular components of the *C. reinhardtii* cells and was conducted by placing the harvested algae into a water bath at 50°C for 24 h. The supernatant of the autolysed algae was dried using a rotary evaporator and the final dry powder product was weighed. Using this method, 0.15 g of dry algae extract powder was obtained per liter of algae culture.

3.2. Chemical characteristics of algae extract

The chemical characteristics of the algae extract isolated from autolysed *C. reinhardtii* cells are summarized in Table 1. For comparison, the chemical properties of commercially available Bacto™ yeast extract (BD Biosciences, San Jose, CA) were also determined. The α -amino acid nitrogen and total carbohydrate content of the algae extract were found to be 3.86% and 92.2 mg/g, respectively. These values are similar to the α -amino acid nitrogen and total carbohydrate content of yeast extract, which were 4.12% and 90.2 mg/g, respectively.

Algae extract differed from the yeast extract in total protein content as measured by Bradford assays [22]. A total protein content of 3% was measured for the algae extract, whereas no protein was detected for the yeast extract. This discrepancy can be explained by noting that the Coomassie blue dye in the Bradford assay does not bind to free amino acids (specifically arginine or lysine), or to proteins smaller than about

Table 1
Chemical properties of yeast extract and algae extract.

Property	Yeast extract ^a	Algae extract
α -Amino nitrogen (%)	4.12 \pm 0.16	3.86 \pm 0.05
Total protein (%) ^b	ND ^c	2.99 \pm 0.06
Total carbohydrate (mg/g)	90.2 \pm 0.2	92.2 \pm 0.1

^a Commercially available Bacto™ Yeast Extract from BD Biosciences.

^b Total protein measured by Bradford Assay.

^c Not detected.

3000 Da [23,24]. Bacto™ yeast extract consists mainly of proteins 250 Da or less (>60%) and has very few proteins that are 3000 Da or greater (<5%) [25]. Thus the Bradford assay did not indicate any protein in the yeast extract due to the small percentage of proteins 3 kDa or larger present in the extract.

A total protein content of 3% for the algae extract indicates that the method used to breakdown the algae extract likely resulted in incomplete autolysis. Temperature and reaction time have been found to be critical parameters in optimizing the autolysis properties of yeast [12]. Typically, autolysis is carried out at 50°C for 24 h to obtain yeast extract [26], and the same conditions were used in this study to prepare the algae extract. Further studies are therefore needed to determine the optimum autolysis parameters for algae in order to maximize the nutritional content and yield of the algae extract. Besides changing the temperature and/or reaction time, another option is to treat the algae with exogenous hydrolytic enzymes. Enzymatic treatments on dried brewer's yeast cells have been shown to improve the nutritional content and flavor characteristics of yeast extract [27]. Another option is to use a different processing technique such as plasmolysis. Plasmolysis involves the addition of inorganic salts or organic solvents to an autolysis process to accelerate the degradation process and has been successfully used to improve RNA extraction of brewery's yeast and yeast extract production [28].

3.3. Effects of algae extract on bacterial metabolism

The effects of algae extract on the growth and metabolism of the bacterium *E. coli* were analyzed by substituting algae extract for yeast extract in the LB medium of *E. coli* cultures. A concentration of 5 g/L for algae and yeast extract was chosen because this is the most common concentration used in laboratory media preparations. The maximum OD₆₀₀ for *E. coli* was 20% greater in the presence of algae extract compared to cell cultures grown in the absence of yeast extract (Fig. 1). It should be noted that this higher saturation density was not achieved by higher specific growth rates, but rather by sustaining growth over a longer period of time (Fig. 2). The initial specific growth rate of the *E. coli* culture was actually 14% lower when grown in algae extract instead of yeast extract; however, *E. coli* cultures grown in algae extract were able to sustain positive growth and protein production for 11.5 h whereas cultures grown in yeast extract reached stationary phase within 9 h. These trends may be explained by the whole protein present in algae extract (3% w/w) that is absent in yeast

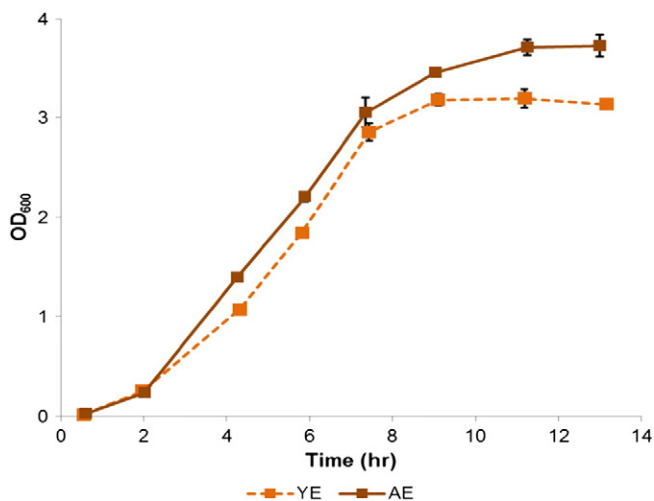


Fig. 1. Aerobic growth of *E. coli* on LB medium containing 5 g/L of either yeast extract (YE) or algae extract (AE). Each measurement represents the mean of triplicate experiments \pm standard deviations.

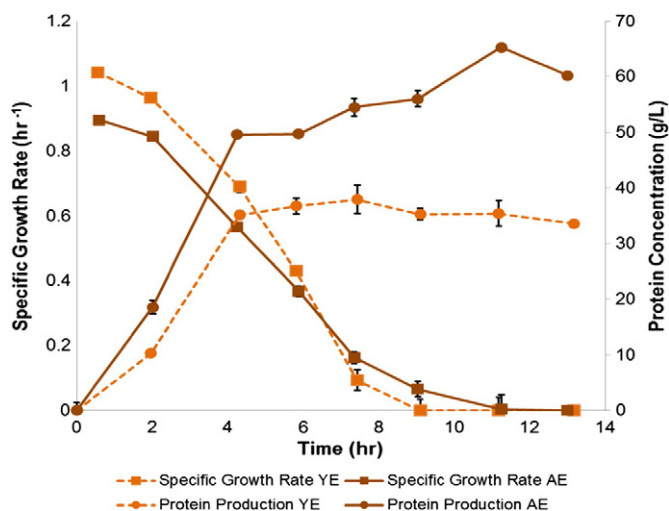


Fig. 2. Specific growth rates and protein production from *E. coli* grown on LB medium containing 5 g/L of either yeast extract (YE) or algae extract (AE). Each measurement represents the mean of triplicate experiments \pm standard deviations.

extract (Table 1). In algae extract, nutrients remained “locked” in whole proteins that were not immediately metabolized by *E. coli* as they were in yeast extract cultures. This resulted in lower immediate specific growth rates, but sustained growth and delayed stationary phase.

The effects of the algae extract on industrially relevant *E. coli* metabolism were evaluated by measuring total protein accumulation by Bradford assay. *E. coli* cultures grown in algae extract exhibited a 17% greater peak protein density when compared with cultures grown in the presence of yeast extract (Fig. 2). Interestingly, protein production was not linked directly to cell concentration and most protein production for both cultures took place early in the exponential phase. It appears that the highest protein production rates occurred simultaneously with highest specific growth rates when substrate levels were greatest (substrate levels have already begun to drop off by the middle of the exponential phase). As was the case in growth measurements, protein production for algae extract continued longer than in yeast extract. Cultures grown in algae extract maintained positive protein production for 11.5 h after inoculation (Fig. 2). Cultures grown in yeast extract exhibited a higher degree of self-digestion as early as 9 h when protein concentrations began to fall. These trends in prolonged protein production and the delay of self-digestion may also be explained by the additional nutrients found in whole proteins that were made available later in the growth process. This characteristic of algae extract could prove very useful in some industrial applications where prolonged protein expression is necessary to achieve maximum production from batch cultures before encountering stationary phase, especially when producing enzymes or secondary metabolites where cells must remain viable for longer periods of time.

3.4. Effects of algae extract on yeast metabolism

The effects of algae extract on the growth and metabolism of the yeast *S. cerevisiae* were assessed by cultivating *S. cerevisiae* in the standard YPAD media recipe made with 10 g/L of algae extract instead of 10 g/L of yeast extract. The maximum OD₆₀₀ for *S. cerevisiae* was 120% greater when grown in the presence of algae extract compared to cultures grown in the presence of yeast extract (Fig. 3). As was the case with *E. coli*, higher cell density at saturation was due to prolonged growth rather than greater specific growth rates (Fig. 4). The same explanation of whole protein content resulting in delayed nutrient availability in algae extract may also apply in this situation; however, due to the great disparity between the saturation cell culture densities, one may consider the possibility that yeast extract contains a metabolite

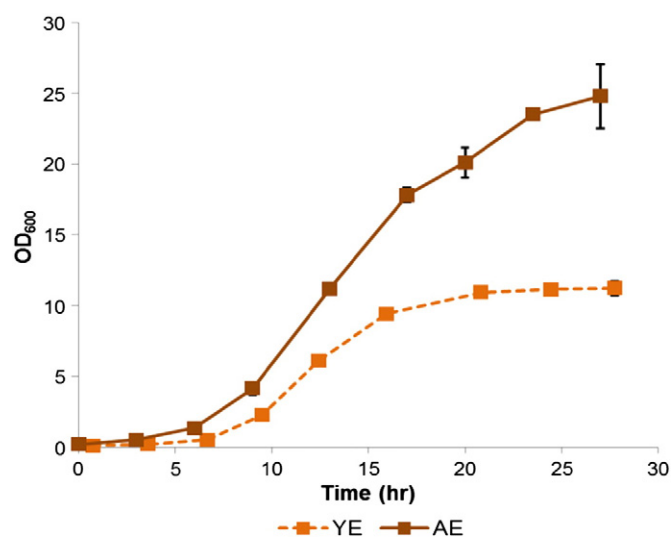


Fig. 3. Anaerobic growth of *S. cerevisiae* on YPAD medium containing 10 g/L of either yeast extract (YE) or algae extract (AE). Each measurement represents the mean of triplicate experiments \pm standard deviations.

or growth inhibitor which, when supplemented with the same metabolite from the active yeast culture produces a premature entry into stationary phase. It is also possible that algae extract contained a nutrient source that becomes necessary during the end of exponential phase and growth phase and allowed cells to continue to grow to higher densities.

The effects of algae extract on the industrially relevant metabolism of *S. cerevisiae* were investigated by measuring ethanol production over time. *S. cerevisiae* cultures that were supplemented with yeast extract produced 12% more ethanol than cultures grown in the presence of algae extract (Fig. 4). This is particularly interesting when the saturation density of cultures grown in algae extract was so much greater than their yeast extract counterparts. Ethanol production occurred when specific growth rates fell toward the end of exponential phase (Fig. 4). This means that ethanol production was highest when nutrient concentrations began to limit the growth of the yeast culture. Thus the lower concentration of ethanol in algae extract cultures may be attributed to the prolonged growth period observed in cultures grown in the presence of algae extract. The decreasing ethanol concentration observed in both algae and yeast extract cultures during late stationary was likely due to loss of ethanol to the headspace in the culture tube rather than the digestion of the ethanol by the yeast culture.

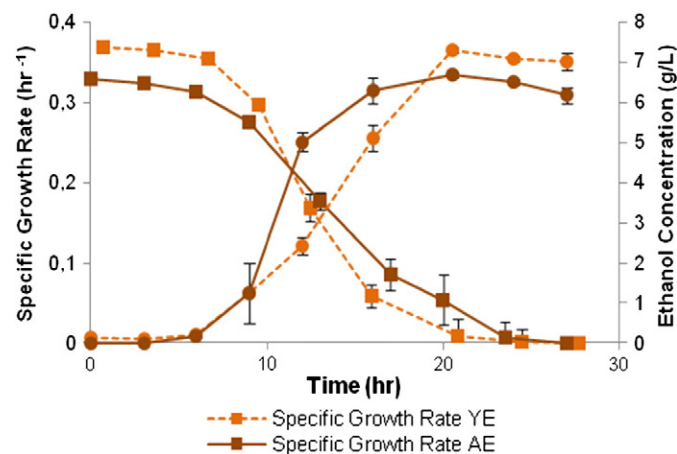


Fig. 4. Specific growth rates and ethanol production from *S. cerevisiae* grown on YPAD medium containing 10 g/L of either yeast extract (YE) or algae extract (AE). Each measurement represents the mean of triplicate experiments \pm standard deviations.

4. Concluding remarks

This study demonstrates that algae extract is a promising supplement for cell culture media. Algae extract from *C. reinhardtii* had a nutritional content similar to commercially available yeast extract and had beneficial effects on both the growth and metabolism of common laboratory strains of bacteria and yeast. Algae extract produced greater growth and metabolism saturation values as well as prolonged growth and protein production in comparison to yeast extract when used in complex media recipes for *E. coli* cultures. When used as a complex media supplement in *S. cerevisiae* cultures, algae extract produced superior growth and comparable ethanol production rates and saturation values. The prolonged growth period afforded to yeast and bacteria cultures grown in algae extract may be useful for some enzyme expression systems or secondary metabolite production. Assuming that the lipid extracted biomass from algae biofuel production is attained as a waste-stream at low costs, and that simple autolysis was performed to prepare the extract (as achieved in this study), algae extract would carry similar production costs and profit margins as yeast extract. It should be noted that yeast extract is also used as a flavor enhancer in the food industry, thus the possibility of using algae extract as a flavor enhancer should be explored in future studies. Additional studies are also needed to optimize the autolysis conditions of the algae.

Acknowledgments

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