

Article

Evaluation of Marine *Synechococcus* for an Algal Biorefinery in Arid Regions

Tomasz Bochenski ¹, Tanmay Chaturvedi ^{2,*}, Mette Hedegaard Thomsen ² and Jens Ejbye Schmidt ³

¹ Department of Chemistry, Khalifa University of Science and Technology, Masdar Campus, P.O. Box 54224 Abu Dhabi, UAE; tt.bochenski@gmail.com

² Department of Energy Technology, Aalborg University, Niels Bohrvej 8, DK-6700 Esbjerg, Denmark; mht@et.aau.dk

³ Institute of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark; jesc@kbm.sdu.dk

* Correspondence: tac@et.aau.dk; Tel.: +45-714-416-74

Received: 2 May 2019; Accepted: 10 June 2019; Published: 12 June 2019



Abstract: Implementing microalgae biorefinery in arid environments requires utilization of strains that can grow at high temperatures (above 28 °C) and salinity levels (above 30 ppt). In this study, we investigate the newly isolated seawater strain, *Synechococcus*, native to the United Arab Emirates, and evaluate its value as a perspective organism for cultivation (for fuel and bio-products) in regions with freshwater scarcity. The strain displayed tolerance to a wide range of temperature (22–37 °C) and salinity (20–41 ppt), with maximum biomass concentration of 0.72 g L⁻¹ and a maximum growth rate of 82 mg L⁻¹ d⁻¹ at 25 °C and 33 ppt salinity. Lipids accumulation reached up to 26% of dry weight in nitrogen-depleted conditions (with 1.8 mM of nitrates addition to the media), whereas protein content exceeded 50% dry weight. In this study, harvesting is investigated using three chemical agents: Ferric chloride, sodium hydroxide, and chitosan. Cell disruption is analyzed for four distinct treatments: Enzymatic, alkaline, ultrasonic, and hydrothermal. Among tested methods, flocculation with sodium hydroxide and ultrasonication were found to be the most efficient techniques for harvesting and cell disruption, respectively. The growth characteristics of the local strain and the potential to derive protein and lipids from it makes it a promising biomass in a biorefinery context.

Keywords: microalgae; biorefinery; cell disruption

1. Introduction

Some of the most urgent issues in the world today include combating climate change, rapidly reducing dependence on fossil fuel resources, and discovering new sustainable energy sources [1]. Countries of the Middle East, such as the United Arab Emirates (UAE), are certainly among the most notable examples of countries that have been heavily dependent on fossil fuels in the past, and are nonetheless now diversifying their energy mix and turning their attention towards renewable energy sources [2]. Current research in alternative liquid fuels categorizes biomass derived fuels into three types. Based on the widely agreed definitions, first-generation bioethanol is derived from edible starch-rich feedstocks (such as corn or wheat) [3]. Bioethanol of this type are particularly widespread in the United States and in Brazil, with annual production in 2016 reaching 60 billion liters [4] and over 30 billion liters [5], respectively. However, first-generation bioethanol have gained a lot of criticism as the biomass directly competes with food and feed, placing sustainability of the process into question [6]. Second-generation bioethanol use non-edible lignocellulosic waste feedstock from a variety of sources (forestry, municipal, and agricultural waste, etc.) to produce the bioethanol [7].

The technology is not yet fully mature and very often cited as economically or energetically unfeasible due to high energy inputs required for lignocellulosic biomass pretreatment [8]. Third-generation biofuels are derived from photosynthetic microorganisms (microalgae), and are widely regarded as a very promising source of future biofuels due to their ability to produce high amounts of lipids per area (more than 50% dry cell weight), relatively low nutrient requirements, ability to grow in arid regions, and possible carbon dioxide sequestration [9]. Maintaining low processing costs and producing novel bio-based products holds the key to successful commercialization of this technology [10]. Biorefinery concept, which considers utilization of other components of algae biomass, is one of the promised solutions [11,12]. Among possible marketable by-products, algal proteins appear to be an interesting fraction due to their high biomass weight contribution and high nutritional quality, as benchmarked against conventional, protein-rich food items [13,14]. Additionally, the possibility of extracting non-fuel products such as pigments, biomaterials, carbohydrates, etc., have led to an increased interest in exploring a variety of algae species [15]. The opportunity to extract a diverse range of products and precursors, with an emphasis on incorporating protein streams in the biorefinery, can significantly improve process economics and justify producing algal biofuels [16].

Governments of several oil-rich countries, e.g., the UAE, have pledged their contribution to fight climate change by reducing carbon dioxide emissions and setting up targets for renewable energy generation. In the case of the UAE, the goal was set at 50% of domestic energy consumption to be supplied from renewable sources by 2050 [17]. Significant investments have been made in the field of solar energy, as sunlight is abundant in the region and hence the most promising source of renewable energy [18]. Meanwhile, liquid fuels (which are almost exclusively derived from fossils) also require substitution in the future and cultivating microalgae for biofuels could potentially serve as a solution as long as automobiles continue to rely on liquid fuels. Abundance of sunlight throughout the year, as well as the presence of large, non-utilized desert areas are some of the advantages that make implementation of large-scale (seawater) algae projects more viable. However, in addition to typical challenges that are related to commercialization of algal fuels, there are several characteristics unique to the region that need to be taken into account while designing a prospective, algae-based, biofuels-producing facility. Technological challenges include high temperatures, high evaporation rates, water scarcity, dust and sand accumulation, and high water salinity. Average monthly temperature ranges from 19 °C in January to 34.5 °C in July [19]. However, the monthly average high temperature exceeds 35 °C in all months from May to October, with its peak in July and August (41 °C in each) [20]. This constraint is highly significant as most of the algal strains would significantly decrease their growth rate or even die when exposed to extreme temperature levels, with most of the commonly investigated strains having their optimal growth temperatures between 20 °C to 30 °C [21]. High temperatures also lead to elevated evaporation rates, which then results in increased seawater salinity. The UAE is also a country with one of the highest water scarcity indices [22], and therefore extensive utilization of freshwater resources for algal biomass production would raise questions on the sustainability of the process. Dust and sand accumulation is also an important issue on the cultivation sites and may result in strain contamination or deterioration of the growth [23]. It is hypothesized that some of these challenges can be resolved by utilizing a locally obtained strain that displays stable and better growth characteristics at high temperatures and elevated salinity levels than typically non local strain.

The objective of this study is to determine the potential of a newly isolated strain of *Synechococcus* native to the UAE as a candidate microorganism for a microalgae biorefinery in an arid region. Growth characterization together with biomass composition analysis was performed in addition to an investigation of the two most crucial steps for the economics of the process: Harvesting and cell breakage [24]; moreover, efficiency of different techniques used at these stages is often cited as highly strain-dependent [25,26]. The selection for each processing stage is dictated by number of factors, such as technology costs, its efficiency, strain used, and targeted products [27,28]. In this study, harvesting is investigated using three chemical agents: Ferric chloride, sodium hydroxide, and chitosan. Ferric chloride contains multivalent ions which take part in the process of coagulation [9], sodium

hydroxide is an auto-flocculating agent, whereas chitosan induces the process of flocculation [26]. Cell disruption is analyzed for four distinct treatments: Enzymatic, alkaline, ultrasonic, and hydrothermal. These technologies have distinct mechanisms of action: Catalyzed cell wall/membrane degradation for enzymatic hydrolysis [29], saponification of membrane-bound lipids for alkaline treatment [30], physical effects due to high shear forces for ultrasonication [31], and cell deformation and compaction under heat treatment [32].

Based on the results, processing of proteins from this algae strain can yield precursors for bio-based products. Furthermore, the value of utilizing proteins in a biorefinery can provide an additional revenue stream while opening a pathway for the valuable and underutilized biomass fraction of proteins (which can certainly be obtained from algae and other biomass).

2. Materials and Methods

2.1. Bioprospecting for New Species, Isolation, and Identification

Bioprospecting for new, robust species local to UAE was performed in the spring of 2014 during algal bloom in the Eastern Mangroves Park in Abu Dhabi (24°27'N 54°25'E). Selection of the location was dictated by the half-closed nature of the reservoir, abundance of halophytic plants, and the close proximity of nutrition originating from drainage systems of the city. Water was collected approximately 10 m from the coast at a depth of at least 50 cm. Twenty liters of seawater was transferred to the plastic container and allowed to incubate for 7 days in the open space, with the addition of commercially obtained, concentrated solution of macroalgae food (bought off-the-shelf). Fresh water was added regularly to account for evaporation. At the end of the 7th day, subsamples were collected and transferred to the laboratory bench. From this point, the enrichment/isolation procedure was carried out in an indoor environment. Three-step strain purification was performed in order to select for a strain with high adaptability to nutrient concentration (by applying varying nitrogen and phosphate levels), elevated temperature (up to 40 °C), and increased salinity (over 50 ppt). In order to fulfill the objectives, three subsequent growth conditions were established. In the final stage, the algae solution was diluted in F/2 medium [33] and spread over solid agar containing F/2 medium (15 g of agar per 1L medium). After incubation for 7 days in room temperature, single colonies were selected and reinoculated or re-streaked for further experiments.

Strain identification was carried out externally by AccuVis Bio through 18S rRNA sequencing. Algal DNA was extracted using AccuVis Bio Bacterial Genomic DNA Isolation Kit (AV1003) and amplified through PCR using a set of universal primers, including forward primer 5'-TTTCATGGAG AGTTTGATCCTGGCTCAGG-3' as well as the reverse primer with a sequence 5'-AGAGTGCTTTCGC CTTTGGTGTTCCTCC-3'. The primers were selected by the ISO 9001:2015 certified AccuVis Bio laboratory and the protocol designed by Norgen Biotek Corp. was followed. Sequencing was conducted using BigDye® Terminator v1.1 Cycle Sequencing Kit for both forward and reverse reactions, and the results were analyzed using Sequencing Analysis Software v5.2. Final identification was achieved through pairwise alignment of the obtained sequence using LALIGN software and NCBI BLAST search.

2.2. Culture Growth Media

Applicability of the isolated strain for the prospective arid-environment biorefinery was investigated by measuring algae growth in a variety of different temperature conditions (22 °C, 27 °C, 32 °C, and 37 °C), salinity levels (20 ppt, 27 ppt, 34 ppt, and 41 ppt), and medium formulations (F/2, ASW [34], ASW/BG11 [35]). Algal growth media are nutrient formulations that are added to the culture in order to enhance growth, and are primarily composed of nitrogen and phosphorus sources, as well as microelements and vitamins, according to the specified composition. F/2 and ASW (artificial seawater) are some of the most commonly used and relatively simple media, containing 1.2 mM and 1.8 mM of nitrate ions, as well as 45 µM and 86 µM of hydrophosphate ions, respectively. ASW/BG11, which is a common medium used to grow cyanobacteria, consists of higher concentrations of nitrate (13 mM) and

hydrophosphate ions (251 μM). The pH of each sample was maintained constant through the growth phase and any pH shifts were corrected to sustain the levels indicated for each of the growth media.

2.3. Bioreactor Experimental Setup

Experimental runs were performed in duplicates. All growth experiments were carried out in the volume of 1 L under light illuminance of 1200 lux, in light-to-dark cycles of 16 h to 8 h, with constant aeration to ensure proper mixing of each culture. Unless stated otherwise, cultures were grown at a room temperature of 22–25 °C under salinity of 33 ppt in F/2 medium. Each sample was prepared by mixing 100 mL of inoculum with 800 mL of distilled water containing appropriate medium and salt concentration and further adjustment to 1 L so that the optical density of the starting culture is no higher than 0.100 at $\alpha = 680$ nm. Temperature tolerance experiments were carried out in a water bath. Cell concentration was measured through optical density using UV-VIS Spectrophotometer scanning 670 SP/25610, with wavelength $\alpha = 680$ nm against the deionized (DI) water of relevant salinity as a blank. Absorbance was correlated to volatile solids (VS) using a previously prepared conversion curve. Measurements were conducted every 24 h until the stationary phase was reached for all samples.

2.4. Biomass Characterization

The isolated strain was analyzed in terms of its biomass distribution into lipids, proteins, and carbohydrates. Algal biomass was retrieved by centrifugation for 40 min at 4000 rpm and washed initially with water, followed by ethanol (in a soxhlet apparatus). Harvested cells were lyophilized and stored at 4 °C before analysis. Prior to composition analysis, ash content was determined and, as such, all further values are referred to as percentage of volatile solids, instead of total algae biomass. Determination of total lipids was performed using chloroform/methanol extraction [36]. Total proteins were quantified through elemental analysis [37] using Flash Analyzer. The resulting amount of nitrogen was multiplied by generally accepted coefficient (4.78) for nitrogen-to-protein conversion in microalgae [38]. Total monomeric carbohydrate (such as glucose, xylose, galactose, mannose, arabinose) were quantified by two-step hydrolysis using sulphuric acid, followed by an analysis using HPLC on Agilent 1260 Infinity [39]. Total solids were quantified by gravimetric determination after drying the samples at 105 °C for 10 h, whereas the ash content was measured by further ashing the samples in the furnace at 575 °C for 12 h, according to the standard protocol [40].

2.5. Harvesting

Three different agents—ferric chloride, chitosan, and sodium hydroxide—were analyzed for their efficiency in separating microalgal cells from the water medium. Culture of *Synechococcus* was grown in 20 L volume until stationary growth phase was reached. All of the harvesting experiments were conducted in a volume consisting of 20 mL of culture per sample and a relevant added amount of flocculating or coagulating agent. For each flocculating agent, the time in which cells aggregated was different. Two points of measurement were defined: First, when there is a clear phase separation, and second, when it can be assumed that the process has reached its full extent.

Sodium hydroxide solution was prepared by dissolving 20 g of 99.8% solid sodium hydroxide in 500 mL of distilled water and further pH adjustment to reach 1 M solution. Ferric chloride was prepared by the dissolution of 2.7 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 100 mL of distilled water. Chitosan preparation was performed by dissolving 1 g of deacetylated chitosan in 100 mL of 1% acetic acid for 30 min with constant stirring. All chemicals were purchased from Sigma Aldrich. Harvesting efficiency was calculated according to Equation (1).

$$\text{Harvesting efficiency} = [(\text{OD}_{680}(\text{initial}) - \text{OD}_{680}(\text{water phase})) / \text{OD}_{680}(\text{initial})] \times 100\% \quad (1)$$

The percentage recovery is defined as the difference between the initial light optical density (at 680 nm) of the culture, and the optical of the water phase after a given time, divided by the initial absorbance.

2.6. Cell Disruption

Four well-studied methods were tested in order to evaluate their qualitative effect on cell disruption for the local *Synechococcus* strain. Each method has been evaluated qualitatively and aimed at distinguishing whether the strain is sensitive or resistant to the given treatment. All procedures were performed for biomass concentration of 1% to assure similar conditions. Cell disruption efficiency was defined as a percentage of VS from the initial biomass weight that are present in the liquid phase, as given in the Equation (2). After each procedure, samples were centrifuged in 50 mL Eppendorf tubes at 4000 rpm for 45 min to separate liquids and solids. Samples were lyophilized and weighed, and the percentage recovery values were calculated.

$$\text{Cell disruption efficiency} = [m_{\text{liquid phase}} / (m_{\text{liquid phase}} + m_{\text{solid phase}})] \times 100\% \quad (2)$$

Ultrasonication was performed at a 125W power output using Branson 450 Digital Sonifier for 10 min and 30 min in cycles of 5:2 s, with samples located in an ice bucket, in order to reduce the heat impact. Enzymatic hydrolysis was performed by adding 1% cellulase, hemicellulase, or both to the sample, using enzymes purchased from Novozymes. The reactions were carried out for 12 h at a temperature of 40 °C to ensure the period was long enough to efficiently disrupt the cell structure. Hydrothermal pretreatment was performed at 121 °C for 30 min in the autoclave. Chemical treatment with alkali was conducted at pH 12 for 2 and 6 h at a temperature of 40 °C. Additionally, the control sample consisting of only algae in water at 1% dry cell weight (dcw) was included to account for self-dissolution. For the best method, a protein release was measured using Bradford assay with bovine serum albumin as a reference.

3. Results

3.1. Strain Identification and Growth Characteristics

Environmental sampling was performed in the Eastern Mangrove Park in Abu Dhabi, UAE, and resulted in isolating axenic culture of *Synechococcus* strain using a combination of serial dilutions, elevated growth conditions, and solid agar plating. The isolated strain was identified as *Synechococcus*, using 18S rRNA sequencing combined with its pairwise alignment against the sequence database, as described in Section 2.1. *Synechococcus* belong to cyanobacteria and is one of the most widespread organisms on the planet [41]. Cells of the local strain are characterized by their round shape and tendency to form spore-like communities. Cell dimensions are in the range of 1–10 µm, with the peak of the curve at approximately 4 µm.

Growth characteristics of this strain are presented in Figure 1. The locally isolated *Synechococcus* strain displayed relatively uniform growth throughout the entire tested range of temperature and salinity values. Several differences in the early stage of exponential growth phase can be explained by adaptation to new conditions. In the salinity tolerance study (Figure 1a), average daily growth rates were generally in the same range for all experimental runs. Maximum biomass concentration fell in the range from 0.2 g L⁻¹ at salinity of 20 ppt to 0.27 g L⁻¹ at salinity of 41 ppt. Within the values tested, the higher salinity conditions allowed slightly higher maximum (final) concentration of algal cells as compared to lower salinity environments. However, the average differential growth rate in the exponential phase was relatively uniform and ranged from 28 mg L⁻¹ d⁻¹ (at 34 ppt) to 30 mg L⁻¹ d⁻¹ (at 27 ppt). Temperature tolerance tests (Figure 1b) show even lesser disproportions between experimental runs. Higher growth rates and maximum cell concentrations were obtained for the middle values (27 °C and 32 °C) as compared to the more extreme ones (22 °C and 37 °C). Biomass concentration at the end of the exponential phase was equal to 0.275 g L⁻¹ for 27 °C and

32 °C and 0.25 g L⁻¹ for 22 °C and 37 °C, whereas the highest average differential growth rate in the exponential phase was observed for 27 °C at 37 mg L⁻¹ d⁻¹, and the lowest for the extreme values at 34 mg L⁻¹ d⁻¹. Based on this study, it can be concluded that this strain favors higher salinity environments (34 or 41 ppt), and its optimal temperature should fall within the 27–32 °C. Moreover, the algal growth is only slightly inhibited with the increase of temperature to 37 °C, and no significantly negative effect is observed when salinity is elevated to 41 ppt. These results may indicate that the strain can possibly be considered as the suitable algal specie to be used in a micro algal biorefinery in arid regions. The low biomass growth of *Synechococcus* strain is a concern at this stage; however, the utility of biomass utilization is maximized when it is combined alongside other feedstock in a biorefinery. Multiple products emanating from diverse feedstock allows for the development of a resilient biorefinery. Additionally, the fact that this particular strain can grow in high salinity water and high ambient temperatures presents opportunistic synergies to tap into whereby integrated aquaponics systems can be designed such that fish or shrimp farming effluents can serve as irrigation sources for growing halophytes and algae [42,43]. Theoretical knowledge of aquaponic system involving fish and vegetables is available, however the novelty lies in adding salt tolerant algae to this system while trying to utilize saline water [44–46].

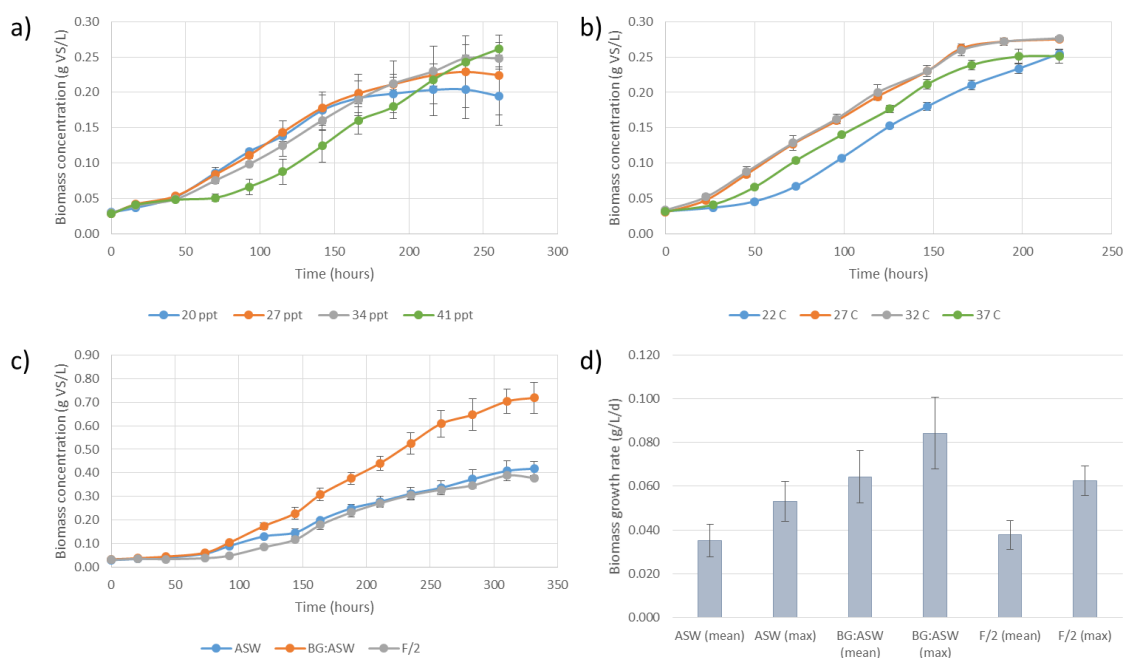


Figure 1. Growth characterization for a *Synechococcus* strain local to Abu Dhabi: (a) Salinity tolerance assay, (b) temperature tolerance assay, (c) media composition growth assay, and (d) comparison of daily biomass growth rates for different media formulations (with maximum and minimum readings).

Growth of the local *Synechococcus* strain in several nutrient media compositions was also investigated. The isolation was primarily performed using an F/2 medium as a source of nutrition. However, it is the ASW/BG11 medium that is the generally used for cultivation of blue-green algae, including cyanobacteria [35]. Three growth media—F/2, ASW, and ASW/BG11—were analyzed and the results are presented in the Figure 1c. The maximum concentration was significantly higher (0.72 g L⁻¹) for the strain grown in the ASW/BG11 medium as compared to F/2 (0.38 g L⁻¹) and ASW (0.41 g L⁻¹). In addition, the daily biomass growth rate reached, on average, above 62 mg L⁻¹ d⁻¹ compared to 38 mg L⁻¹ d⁻¹ for F/2 and 36 mg L⁻¹ d⁻¹ for ASW (Figure 1d). Moreover, the maximum daily growth rate reported for ASW/BG11 medium was 84 mg L⁻¹ d⁻¹. Based on the composition of the media, it is apparent that ASW/BG11 is a complex nutritional medium, with eight times higher nitrate content, which suggests that nitrogen availability is a limiting factor in the growth of *Synechococcus*.

3.2. Biomass Composition Characterization

The local *Synechococcus* strain was characterized for the percentage content of lipids, proteins, and carbohydrates at the late exponential phase of growth. Biomass characterization was carried out for algae grown in the same medium that was used for isolation (F/2), at room temperature and with 33 ppt salinity. Lipids were found to represent 26% of the cell dry weight in the nitrogen-depleted conditions, whereas proteins and carbohydrates contributed to 30% and 34%, respectively (Table 1). The remaining 10% were allocated to ash (4%) and unidentified residuals (6%). High amounts and relatively proportional distribution of three main constituents—lipids, proteins, and carbohydrates—are considered an advantage from the perspective of a biorefinery.

Table 1. Biomass composition of *Synechococcus* cultivated in F/2 medium.

Biomass Compositional Analysis	
Component	Percentage
Proteins	30
Lipids	26
Carbohydrates	34
Ash	4
Residuals	6

Composition analysis with regard to proteins and lipids was also carried out for algae grown in ASW/BG11 medium. Table 2 presents the percentage contribution of the two constituents for *Synechococcus* strain cultured in low-nitrogen F/2 medium and high-nitrogen ASW/BG11 medium. In different cultivation conditions, the distribution varies. Cells cultivated in nitrogen-sufficient conditions accumulate the majority of their biomass as proteins at the expense of lower lipid content, and conversely, nitrogen-deficient conditions lead to lower protein content and higher lipid contribution. Cultivation of the local *Synechococcus* strain in ASW/BG11 is more advantageous when proteins are targeted—not only are biomass growth parameters higher than for the F/2 medium, but protein content also increases significantly to 54% dry weight as compared to 28% in nitrogen-depleted conditions. However, high nitrogen content in the medium is undesirable when lipids are targeted as the main product. Lipid concentration is increased almost nine-fold from 3% when cultivated in ASW/BG11 to 26% when the local *Synechococcus* strain is grown in the F/2 medium.

Table 2. Accumulation of proteins and lipids in *Synechococcus* grown in F/2 and ASW/BG media.

Type of Media	Weight Percentage of Biomass	
	Proteins	Lipids
F/2	28	26
ASW/BG	54	3

3.3. Harvesting Evaluation

Three different agents were tested for their ability to agglomerate cells of local *Synechococcus* strain. Significant differences in performance were observed as it is presented in Figure 2. The graphs show the cell agglomeration as the percentage of cells remaining in the water phase, for the different amounts of chemicals added, in the given time period. Autoflocculation of *Synechococcus* cells using sodium hydroxide (Figure 2a) was the fastest occurring reaction, with as little as 10 min required to separate the cells from water. Efficiency of above 90% was obtained at the final solution with pH 12, and maximum recovery was observed for samples treated at pH 13 (94% recovery). In all experiments, agglomerated cells underwent gravitational sedimentation, leaving the biomass layer floating on the surface. Flocculation with ferric chloride (Figure 2b) was another efficient method, yet the time required for phase separation was higher and equal to 30 min. Depending on the volume of coagulant

added, the aggregated cells would either float on the surface (above 0.05 g L^{-1}) or sediment (below 0.05 g L^{-1}). In contrast to sodium hydroxide, ferric chloride can significantly deteriorate algal biomass, hence a maximum dosage of 0.1 g L^{-1} is suggested based on observations. Cell aggregation efficiency was improved after an additional 3.5 h of treatment, though only for high-concentration samples, which, as explained, are not recommended. The last of the tested chemicals, chitosan (Figure 2c), was found to be effective in a relatively narrow range of concentrations. Moreover, the settling time is higher than for NaOH and FeCl_3 , with lower maximum efficiency.

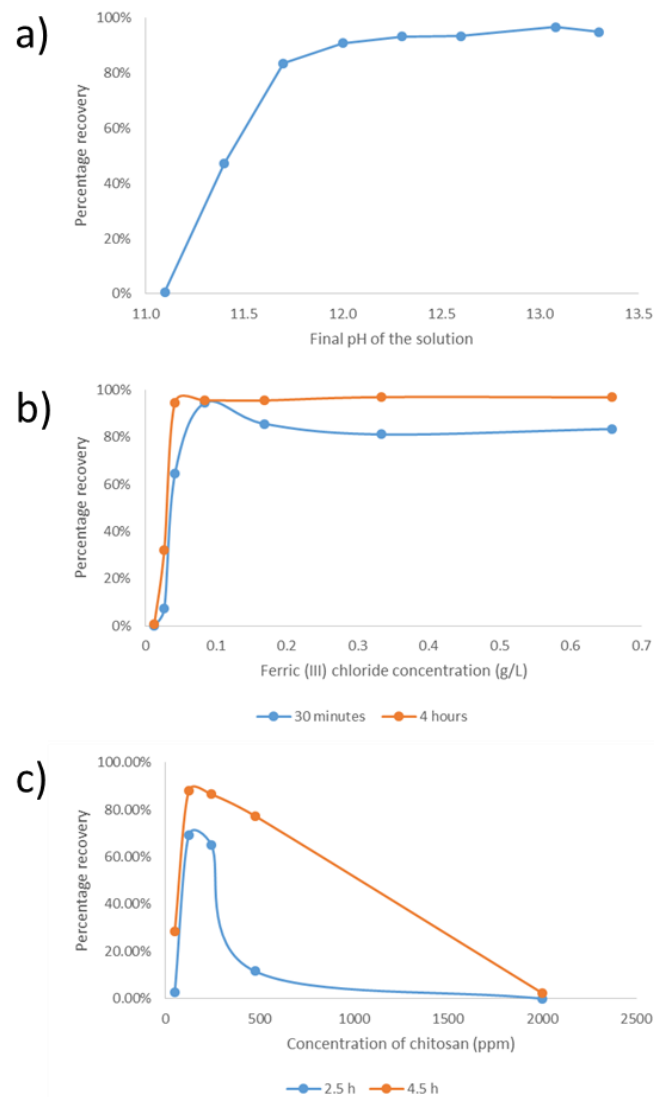


Figure 2. Harvesting efficiency: (a) Sodium hydroxide, (b) ferric chloride, (c) chitosan.

3.4. Cell Disruption and Product Recovery

Cell disruption is generally performed in order to gain access to the intracellular components of the algal biomass. Cell disruption serves as a pretreatment method, which increases extractability of the lipids, as well as allows biomass fractionation and separation for further implementation of the multi-product biorefinery. In this study, efficiency of a given technique was measured as the percentage recovery of the biomass in liquid fraction. Results are compared in Figure 3.

Ultrasonication was the only effective technique among the methods tested, with 41% of the initial dry weight of the biomass being extracted to the liquid phase after 30 min of treatment. Further, 28% of total biomass dry weight was released in the form of soluble proteins. Hydrothermal pre-treatment, the only technique that uses harsh conditions (elevated pressure and temperature), was found to be

very ineffective in breaking down the cell wall, with less than 5% recovery in liquid fraction. Chemical and enzymatic pretreatments did not display high disruption efficiency, suggesting that the cell wall is rather rigid, complex in its structure, and no membrane-bound lipids are present. Ultrasonication is regarded as a mild technique, which, if performed with proper cooling of the sample, does not lead to biomass deterioration and hence, can be used in microalgae biorefinery where protein fraction is one of the targeted products. Ultrasonication is also regarded as an energy-efficient method, requiring three times less energy input per weight of biomass than microwave treatment and four times less than high-pressure homogenization or bead beating [10]. Further research should focus on investigating other mild and highly efficient methods, such as high-pressure homogenization or bead milling.

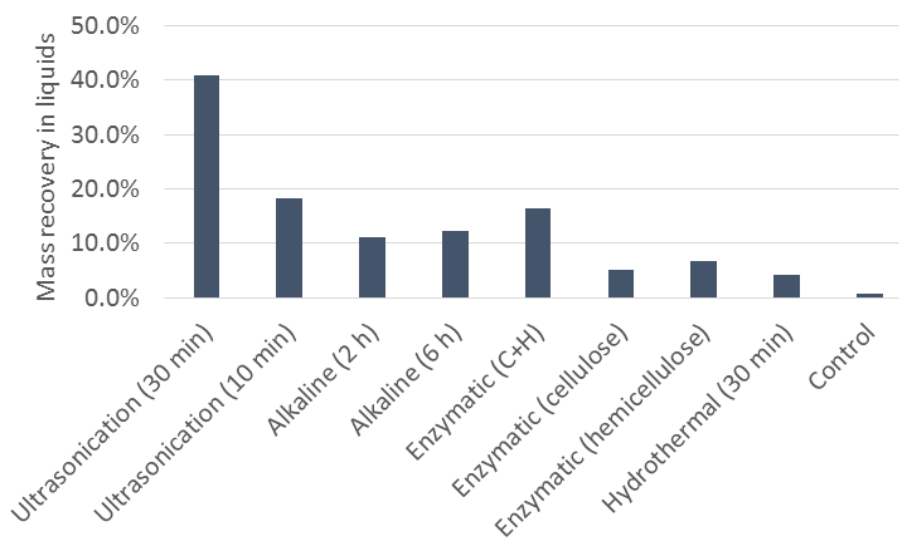


Figure 3. Comparison of several cell disruption techniques in the process of fractionation of a local *Synechococcus* strain.

3.5. Biorefinery Perspective on Product Portfolio

Several algae strains are known to be excellent producers of proteins, with many species accumulating more than 50% of their biomass in the form of proteins [9]. The existing range of algal protein products is very limited, and traditional application of algal proteins capitalizes on its nutritional value [11]. Bulk defatted algae are being sold as food or feed supplements. These products do not require protein extraction, as along as with proteins, several other nutritional components are included, such as pigments, PUFAs, vitamins, and trace elements. While purification of proteins can significantly increase the value of the product, utilizing bulk unpurified protein fraction can serve as a major component in production of bio-based materials, thus making algae-derived proteins a major feedstock in a biorefinery. Resulting polymers from unpurified proteins are somewhat similar in their properties to polyurethane elastomers. This novel product can possibly increase the value of proteins and play an important role in economics of algal production. The process design for could potentially be distributed in three sections, preparation of proteins, modification by methacrylation, and co-polymerization with commercially obtained synthetic monomer [47]. Traditional polyurethanes are produced in a polymerization reaction of isocyanides, polyols, and short diols. Isocyanides are toxic compounds and there has been much attention paid to possible replacement of them. With proteins acting as isocyanides substitute, an additional value can be established. Polyurethane sector constitutes to a relatively large market share, as it is estimated that by 2021 it will reach \$74 billion. In general, polyurethanes are a large group of materials ranging from rigid or flexible foams to coatings, adhesives, and elastomers. Their applicability can be found in variety of commodities, such as insulation panels, seals, elastomeric tires and wheels, synthetic fibers, and many others. Lipids will continue to remain of interest due to their well-studied pathway of producing fuels from lipids. The combination of

algal-derived lipids for biodiesel and proteins for novel biomaterials collectively make the microalgae biorefinery an attractive economic proposition.

4. Discussion

At this stage of research, the growth characteristics of locally isolated *Synechococcus* strain are comparable to other marine and freshwater strains [28], including other *Synechococcus* strains locally isolated in the Gulf region [48]. Accumulation of lipids as 26% of the dry weight biomass can also be considered high for a wild strain [49]. Lipid content for the strain was found to be higher than for *Arthrospira platensis* (up to 16.6%), *Scenedesmus* sp. (up to 21.1%), or *Dunaliella salina* (up to 25%), which are some of the commonly reported strains in the literature [28]. However, there is a significant capacity for improvement in terms of growth rate, as compared to some of the fast-growing strains. *Arthrospira platensis*, which is also an example of cyanobacterium, was found to grow to up to 1.6 g L⁻¹, which is twice higher than for the investigated strain [9]. Taking into account that the decrease in overall biomass concentration is less than two-fold, and F/2 medium would generally be cheaper than ASW/BG11, it would be more efficient to cultivate the local *Synechococcus* strain in F/2, if lipids production is of the main concern. However, further optimization of the growth and possible induction of lipid accumulation can lead to significant improvements. The experiments in this study were conducted at the constant pH, determined by the growth media used. In further considerations, pH levels could potentially be examined to further optimize the growth conditions. High resistance to temperature, salt concentrations, nitrogen levels, as well as stable growth indicate that this strain has the potential to be developed into a future feedstock for production of biofuels in arid environments.

In this study, cells of *Synechococcus* strain were separated by pH shift. The technique, also referred to as autoflocculation, was successfully applied (98% aggregation), to harvest *Chlorella vulgaris* cells, with pH 10.8 using NaOH or KOH [50]. Strains investigated in this study required higher pH for efficient cell accumulation—over 90% efficiency was achieved at pH higher than 12. The reason for this might be related to the salt concentration in the medium, as *Chlorella vulgaris* underwent flocculation in freshwater conditions as compared to seawater environment, in which the local strain was cultivated. The obtained results suggest that sodium hydroxide is the preferred flocculating agent among the tested ones. It is not only the fastest harvesting chemical and gives high recovery rates, but it is also the cheapest (approximately \$0.2–0.3/kg in bulk quantities). In terms of overall process economics, *Chlorella vulgaris* was most efficiently harvested with specialized poly-electrolyte, which could be used at extremely low doses [47]. Further research could possibly consider testing those on the local *Synechococcus* strain.

Ultrasonication has previously been proven to be an efficient method for cell disruption for several strains, such as *Chlorella*, *Arthrospira*, and *Porphyridium*, at the same time being unsuitable for *Scenedesmus*, *Chlorococcus*, or *Nannochloropsis*. Cell wall structure and thickness are regarded as the major factors determining the sonication efficiency [30]. No previous studies on utilization of this method on *Synechococcus* species have been performed. Therefore, the experiments performed in the course of this study show that the cells of this strain can be successfully disrupted while treated with sonication. In total, 41% of biomass was retained in the liquid phase and 28% of total biomass weight was recovered in the form of soluble proteins. This translates into 52% protein recovery into the liquid phase, which is higher than for *Arthrospira platensis* (47.1%), *Chlorella vulgaris* (18.1%), or *Haematococcus pluvialis* (8.5%), but lower than *Porphyridium cruentum* (67%) as previously reported [51]. For all strains mentioned in the previous study, high-pressure homogenization was found to be the best method and, hence, it is suggested that it be explored in future research.

Biomass has traditionally been used for the production of fuel and energy. Protein-rich biomass has served as food for cattle. Combining different types of biomass in a biorefinery opens doors to a wide range of products, of which proteins-based products are an integral part. Focusing on microalgae-derived proteins has created an opportunity for the utilization of waste protein streams as well, while simultaneously providing an alternative to toxic isocyanates. Utilization of algal proteins

for manufacturing these novel bio-materials is also beneficial from the perspective of justification of producing algal biofuels. The crucial factor drives the profitability for using microalgae as a main feedstock in biorefineries. Additional improvements in algal productivity yield will further embolden its place in a biorefinery.

Implementation of large-scale algae biorefinery based on *Synechococcus* in warm and arid environments will also require further advancements in cultivation systems. Open-pond cultivation in hot climates is difficult due to high evaporation, which leads to increased salinity of the medium. As a consequence, fresh water is still required in order to decrease the salt concentration and allow faster biomass growth. Usage of waste water might be a more sustainable alternative. In desert regions, such open ponds will also be under threat of increased dust accumulation, and preventing this from happening might significantly increase the investment costs. On the other hand, installation of photo-bioreactors and their exposure to natural light will eventually increase the temperature of the system to levels, at which the strain will no longer be growing fast. Therefore, further advancements are essential in order to fully commercialize the algae biorefinery concept in the arid regions.

5. Conclusions

Locally isolated *Synechococcus* strain grows in a stable manner across a range of temperatures (22–37 °C) and salinity levels (20–41 ppt). Highest growth parameters at 82 mg L⁻¹ d⁻¹ of maximum growth rate and 0.72 g L⁻¹ of maximum biomass concentration were observed in nitrogen-sufficient conditions. Highest productivity of lipids (26% dry weight) was achieved in nitrogen-depleted conditions. Also, 30% of the dry weight (dwt) comprised of proteins. Harvesting using sodium hydroxide and cell disruption with ultrasonication were found to be efficient processing techniques for the strain. Summarizing, the investigated strain exhibits some crucial characteristics that make it a good candidate strain for further use in research and possibly an important pathway (both as a feedstock and product) for a biorefinery.

Author Contributions: T.B. performed the experiments and analyzed the data, M.H.T. designed the experimental plan, J.E.S. analyzed and evaluated the results and conclusions of the study. T.C. completed the writing—review and editing. All authors contributed to writing the manuscript.

Funding: This work was funded by the Cooperative Agreement between the Masdar Institute of Science and Technology (Masdar Institute), Abu Dhabi, UAE and the Massachusetts Institute of Technology (MIT), Cambridge, MA, USA. Reference BIOREFINERY 02/MI/MI/CP/11/07633/GEN/G/00 for work under the Second Five Year Agreement.

Conflicts of Interest: The authors declare no competing financial interests.

References

1. Ang, B.W.; Choong, W.L.; Ng, T.S. Energy security: Definitions, dimensions and indexes. *Renew. Sustain. Energy Rev.* **2015**, *42*, 1077–1093. [[CrossRef](#)]
2. Marafi, K. Security Concerns in The Middle East For Oil Supply: Problems And Solutions. In Proceedings of the Energy and Environmental Challenges to Security, Budapest, Hungary, 21 November 2007; Springer: Dordrecht, The Netherlands, 2009; pp. 225–235.
3. Lee, R.A.; Lavoie, J.-M. From first- to third-generation biofuels: Challenges of producing a commodity from a biomass of increasing complexity. *Anim. Front.* **2013**, *3*, 6–11. [[CrossRef](#)]
4. U.S. Fuel Ethanol Production Continues to Grow in 2017—Today in Energy—U.S. Energy Information Administration (EIA). Available online: <https://www.eia.gov/todayinenergy/detail.php?id=32152> (accessed on 30 April 2019).
5. Barros, S. *Brazil Biofuels Annual Report 2016*; USDA: Washington, DC, USA, 2016.
6. Mohr, A.; Raman, S. Lessons from first generation biofuels and implications for the sustainability appraisal of second generation biofuels. *Energy Policy* **2013**, *63*, 114–122. [[CrossRef](#)] [[PubMed](#)]
7. Naik, S.N.; Goud, V.V.; Rout, P.K.; Dalai, A.K. Production of first and second generation biofuels: A comprehensive review. *Renew. Sustain. Energy Rev.* **2010**, *14*, 578–597. [[CrossRef](#)]

8. Carriquiry, M.A.; Du, X.; Timilsina, G.R. Second generation biofuels: Economics and policies. *Energy Policy* **2011**, *39*, 4222–4234. [CrossRef]
9. Brennan, L.; Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sustain. Energy Rev.* **2010**, *14*, 557–577. [CrossRef]
10. Gong, J.; You, F. Global optimization for sustainable design and synthesis of algae processing network for CO₂ mitigation and biofuel production using life cycle optimization. *AIChE J.* **2014**, *60*, 3195–3210. [CrossRef]
11. Vanthoor-Koopmans, M.; Wijffels, R.H.; Barbosa, M.J.; Eppink, M.H.M. Biorefinery of microalgae for food and fuel. *Bioresour. Technol.* **2013**, *135*, 142–149. [CrossRef]
12. 't Lam, G.P.; Vermuë, M.H.; Eppink, M.H.M.; Wijffels, R.H.; van den Berg, C. Multi-Product Microalgae Biorefineries: From Concept Towards Reality. *Trends Biotechnol.* **2018**, *36*, 216–227. [CrossRef]
13. Becker, E.W. Micro-algae as a source of protein. *Biotechnol. Adv.* **2007**, *25*, 207–210. [CrossRef]
14. Greetham, D.; Zaky, A.; Mankanjuola, O.; Du, C. A brief review on bioethanol production using marine biomass, marine microorganism and seawater. *Curr. Opin. Green Sustain. Chem.* **2018**, *14*, 53–59. [CrossRef]
15. Chandra, R.; Iqbal, H.M.N.; Vishal, G.; Lee, H.-S.; Nagra, S. Algal biorefinery: A sustainable approach to valorize algal-based biomass towards multiple product recovery. *Bioresour. Technol.* **2019**, *278*, 346–359. [CrossRef] [PubMed]
16. Bocheński, T.; Torres, A.I.; Ashraf, M.T.; Schmidt, J.E.; Stephanopoulos, G. Evaluation of the production of lipids for fuels and proteins from microalgae by decomposition of the processing network. In Proceedings of the Computer Aided Chemical Engineering, San Diego, CA, USA, 1–5 July 2018; Elsevier: New York, NY, USA, 2016; Volume 38, pp. 1635–1640.
17. UAE Energy Strategy 2050—The Official Portal of the UAE Government. Available online: <https://government.ae/en/about-the-uae/strategies-initiatives-and-awards/federal-governments-strategies-and-plans/uae-energy-strategy-2050> (accessed on 30 April 2019).
18. Mokri, A.; Aal Ali, M.; Emziane, M. Solar energy in the United Arab Emirates: A review. *Renew. Sustain. Energy Rev.* **2013**, *28*, 340–375. [CrossRef]
19. Homepage|World Bank Climate Change Knowledge Portal. Available online: <https://climateknowledgeportal.worldbank.org/> (accessed on 30 April 2019).
20. Climate: Average Maximum Temperature °C (1977–2009). Available online: <https://services.dubaiairports.ae/dubaimet/MET/Climate.aspx> (accessed on 30 April 2019).
21. Singh, S.P.; Singh, P. Effect of temperature and light on the growth of algae species: A review. *Renew. Sustain. Energy Rev.* **2015**, *50*, 431–444. [CrossRef]
22. Dakkak Amir Irrigation Systems in the United Arab Emirates. Available online: <https://www.ecomena.org/tag/water-scarcity-in-uae/> (accessed on 30 April 2019).
23. Andersen, R.A. *Algal Culturing Techniques*; Elsevier: Burlington, MA, USA, 2005; ISBN 0080456502.
24. Richardson, J.W.; Johnson, M.D.; Lacey, R.; Oyler, J.; Capareda, S. Harvesting and extraction technology contributions to algae biofuels economic viability. *Algal Res.* **2014**, *5*, 70–78. [CrossRef]
25. Milledge, J.J.; Heaven, S. A review of the harvesting of micro-algae for biofuel production. *Rev. Environ. Sci. Bio/Technol.* **2013**, *12*, 165–178. [CrossRef]
26. Theegala, C.S. Algal Cell Disruption and Lipid Extraction: A Review on Current Technologies and Limitations. In *Algal Biorefineries*; Springer International Publishing: Cham, Switzerland, 2015; pp. 419–441.
27. Eppink, M.H.M.; Olivieri, G.; Reith, H.; van den Berg, C.; Barbosa, M.J.; Wijffels, R.H. *From Current Algae Products to Future Biorefinery Practices: A Review*; Springer: Cham, Switzerland, 2017; pp. 99–123.
28. Mata, T.M.; Martins, A.A.; Caetano, N.S. Microalgae for biodiesel production and other applications: A review. *Renew. Sustain. Energy Rev.* **2010**, *14*, 217–232. [CrossRef]
29. Harrison, S.T. Bacterial cell disruption: A key unit operation in the recovery of intracellular products. *Biotechnol. Adv.* **1991**, *9*, 217–240. [CrossRef]
30. Günerken, E.; D'Hondt, E.; Eppink, M.H.M.; Garcia-Gonzalez, L.; Elst, K.; Wijffels, R.H. Cell disruption for microalgae biorefineries. *Biotechnol. Adv.* **2015**, *33*, 243–260. [CrossRef]
31. Kurokawa, M.; King, P.M.; Wu, X.; Joyce, E.M.; Mason, T.J.; Yamamoto, K. Effect of sonication frequency on the disruption of algae. *Ultrason. Sonochem.* **2016**, *31*, 157–162. [CrossRef]
32. Du, Z.; Mohr, M.; Ma, X.; Cheng, Y.; Lin, X.; Liu, Y.; Zhou, W.; Chen, P.; Ruan, R. Hydrothermal pretreatment of microalgae for production of pyrolytic bio-oil with a low nitrogen content. *Bioresour. Technol.* **2012**, *120*, 13–18. [CrossRef] [PubMed]

33. Guillard, R.R.L. Culture of Phytoplankton for Feeding Marine Invertebrates. In *Culture of Marine Invertebrate Animals*; Springer: Boston, MA, USA, 1975; pp. 29–60.
34. McLachlan, J. Some considerations of the growth of marine algae in artificial media. *Can. J. Microbiol.* **1964**, *10*, 769–782. [[CrossRef](#)] [[PubMed](#)]
35. Stanier, R.Y.; Kunisawa, R.; Mandel, M.; Cohen-Bazire, G. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev.* **1971**, *35*, 171–205. [[PubMed](#)]
36. Axelsson, M.; Gentili, F. A Single-Step Method for Rapid Extraction of Total Lipids from Green Microalgae. *PLoS ONE* **2014**, *9*, e89643. [[CrossRef](#)] [[PubMed](#)]
37. Laurens, L.M.L. *Summative Mass Analysis of Algal Biomass—Integration of Analytical Procedures: Laboratory Analytical Procedure (LAP)*; National Renewable Energy Lab. (NREL): Golden, CO, USA, 2013.
38. Lourenço, S.O.; Barbarino, E.; Lavín, P.L.; Lanfer Marquez, U.M.; Aidar, E. Distribution of intracellular nitrogen in marine microalgae: Calculation of new nitrogen-to-protein conversion factors. *Eur. J. Phycol.* **2004**, *39*, 17–32. [[CrossRef](#)]
39. Wychen, S.V.; Laurens, L.M.L. *Determination of Total Carbohydrates in Algal Biomass: Laboratory Analytical Procedure (LAP)*; National Renewable Energy Lab. (NREL): Golden, CO, USA, 2016.
40. Wychen, S.V.; Laurens, L.M.L. *Determination of Total Solids and Ash in Algal Biomass*; National Renewable Energy Lab. (NREL): Golden, CO, USA, 2013.
41. Whitton, B.A.; Potts, M. *The Ecology of Cyanobacteria: Their Diversity in Time and Space*; Springer Science & Business Media: Berlin, Germany, 2007; ISBN 0306468557.
42. Al-Hafedh, Y.S.; Alam, A.; Beltagi, M.S. Food Production and Water Conservation in a Recirculating Aquaponic System in Saudi Arabia at Different Ratios of Fish Feed to Plants. *J. World Aquac. Soc.* **2008**, *39*, 510–520. [[CrossRef](#)]
43. Ahmed, F.; Li, Y.; Schenk, P.M. Algal Biorefinery: Sustainable Production of Biofuels and Aquaculture Feed? In *The Science of Algal Fuels*; Springer: Dordrecht, The Netherlands, 2012; pp. 21–41.
44. Gunning, D.; Maguire, J.; Burnell, G.; Gunning, D.; Maguire, J.; Burnell, G. The Development of Sustainable Saltwater-Based Food Production Systems: A Review of Established and Novel Concepts. *Water* **2016**, *8*, 598. [[CrossRef](#)]
45. Nozzi, V.; Parisi, G.; Di Crescenzo, D.; Giordano, M.; Carnevali, O.; Nozzi, V.; Parisi, G.; Di Crescenzo, D.; Giordano, M.; Carnevali, O. Evaluation of Dicentrarchus labrax Meats and the Vegetable Quality of Beta vulgaris var. cicla Farmed in Freshwater and Saltwater Aquaponic Systems. *Water* **2016**, *8*, 423. [[CrossRef](#)]
46. Shafahi, M.; Woolston, D. Aquaponics: A Sustainable Food Production System. In Proceedings of the International Mechanical Engineering Congress and Exposition IMECE2014, Montreal, QC, Canada, 14–20 November 2014; ASME: New York, NY, USA; p. V003T03A073.
47. Bochenski, T.; Chan, W.Y.; Olsen, B.D.; Schmidt, J.E. Techno-economic analysis for the production of novel, bio-derived elastomers with modified algal proteins as a reinforcing agent. *Algal Res.* **2018**, *33*, 337–344. [[CrossRef](#)]
48. Moosavi Zenooz, A.; Zokae Ashtiani, F.; Ranjbar, R.; Javadi, N. *Synechococcus* sp. (PTCC 6021) cultivation under different light irradiances—Modeling of growth rate-light response. *Prep. Biochem. Biotechnol.* **2016**, *46*, 567–574. [[CrossRef](#)]
49. Patil, P.C.; Meti, B.S. A Brief Review on Algal Lipid. *Int. J. Res. Stud. Biosci.* **2014**, *2*, 55–61.
50. Vandamme, D.; Foubert, I.; Fraeye, I.; Meesschaert, B.; Muylaert, K. Flocculation of *Chlorella vulgaris* induced by high pH: Role of magnesium and calcium and practical implications. *Bioresour. Technol.* **2012**, *105*, 114–119. [[CrossRef](#)] [[PubMed](#)]
51. Safi, C.; Ursu, A.V.; Laroche, C.; Zebib, B.; Merah, O.; Pontalier, P.-Y.; Vaca-Garcia, C. Aqueous extraction of proteins from microalgae: Effect of different cell disruption methods. *Algal Res.* **2014**, *3*, 61–65. [[CrossRef](#)]

