EVALUATING THE POTENTIAL OF USING FOOD-GRADE WASTES TO ENHANCE PRODUCTIVITY OF MIXOTROPHIC SPECIES OF ALGAE.

by

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LIST OF ABBREVIATIONS USED

Abbreviation	Meaning
μ	specific growth rate (d^{-1})
ATP	adenosine triphosphate
chl-a	chlorophyll a
fish-HTL	hydrothermal liquification of fish remains
F_0	initial fluorescence
FL3	cell fluorescence of flow cytometer
F_m	maximum fluorescence
FSC	forward angle scatter
F_v	variable fluorescence
F_v/F_m	ratio of variable to maximum fluorescence
F[t]	fluorescence at time t
HAB	harmful algal bloom
HL	High Light Level; 190 μmol photons m^{-2} s^{-1}
LL	Low Light Level; 20 μmol photon m^{-2} s^{-1}
ML	Medium Light Level ; 40 μmol photons m^{-2} s^{-1}
PSII	photosystem II
RMSE	root mean square error
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
t	time
t_{lag}	length of the lag phase
t_{sat}	length of the exponential phase

ABSTRACT

Microalgae industries can produce a large variety of valuable products but are limited by high production costs. Mixotrophic growth, when paired with high-nutrient waste products, provides a solution which can reduce costs and increase the productivity of these industries. This study screened six microalgae species which are typically used in aquaculture, with the goal of optimizing mixotrophic growth when supported by food-grade wastes. While all three wastes could support some algae growth, only whey permeate enhanced growth rates and yield. To evaluate changes in biomass and cell quotas, *Tetraselmis suecica* was grown on a larger scale with 1% whey permeate. Mixotrophic growth of *T. suecica* resulted in down-regulation of photosynthesis. This down-regulation did not occur with the addition of 1g/L of bicarbonate. Increasing the phosphorous and carbon available to *T. suecica* through bicarbonate and whey permeate additions significantly increased growth rates and biomass yields.

CHAPTER 1 - INTRODUCTION

1.1 Background

The impact of harmful algal blooms (HABs) is a burgeoning global issue. The economic damage can be measured in billions of dollars, while toxins produced by the algae cause numerous significant health issues (Hoagland *et al.,* 2002). Eutrophication, a major cause of HABs around the world, is caused by human activity, including industrial runoff (Heisler *et al.,* 2008). Many industries may struggle to dispose of by-products because they could result in eutrophication if released into the environment.

Finding a cost-effective means of remediating these wastes would benefit the industries producing them and could subsidize the production of high-value products. Industrial-scale production of microalgae could fill this role, contributing to environmental protection while also benefitting industry. Algae are already produced on a large scale for many applications, including aquaculture feeds and health supplements such as antioxidants, carotenoids, xanthophylls, and omega-3 fatty acids (Abreu et al., 2012). Production cost is the main limiting factor in large scale production of algae. High costs are associated with supplying sufficient light and nutrients to grow the algae and with harvesting algal products and biomass (Lee, 2001). Nutrient expense could be reduced by utilizing high-nutrient waste products. Using algae as a part of a mass culturing system would be advantageous for the industries producing the high-nutrient wastes by reducing the disposal costs, and for the grower by mitigating acquisition cost.

Algae typically grow phototrophically, using light as an energy source, and many are also capable of heterotrophic growth using organic substrates. Certain species can

also grow mixotrophically by combining photo- and heterotrophy. Mixotrophy is particularly interesting to mass culturing industries, as the cultures often have higher growth rates than photo- or heterotrophic cultures (Abreu et al., 2012; Mohammad et al., 2014; Bashir et al., 2019). The use of mixotrophy could enhance cost savings.

Phototrophically grown algae will be dependent on light availability, therefore the optical density of a culture will limit productivity. If a culture becomes too optically dense, self-shading from the algae will limit light penetration (Jung et al., 2014). When grown mixotrophically, *Chlorella vulgaris* had reduced pigment quotas compared to phototrophic cultures (Abreu. et al., 2012). This downregulation of pigments under mixotrophic conditions could allow increased biomass concentrations in the reactor, compared to phototrophic conditions. Therefore, mixotrophy may provide a method to significantly increase productivity of a phototrophic culture by increasing both the growth rates and the yield of the culture.

Growing algae mixotrophically still requires the operator to provide light, nutrients, and organic substrates, which can have high costs (Bhatnaga et al., 2011). Cost mitigation may be realized with waste products containing high organic carbon in addition to other nutrients needed for microalgae growth. These products could qualify as both an inexpensive supply of nutrients and an energy source to fuel mixotrophic growth.

Phytoplankton are highly diverse and have significant inter- and intra-species variability in their responses to growth conditions (Bashir et al., 2019). This variability is significant because changing growth conditions can affect production of a target compound or biomass. Commercial production depends on maximizing productivity by finding the ideal growth conditions for the desired product. The Redfield ratio, 106:16:1

molar C:N:P, an average nutrient composition of algae in the ocean (Redfield, 1934), is used as a baseline to estimate the nutrient requirements of algae. However, not all algae have compositions that match the Redfield ratio (Quigg *et al.,* 2003), and the composition is influenced strongly by internal storage of excess phosphorous (Liefer *et al.,* 2019), which means they require different concentrations of nutrients.

Waste streams with complex compositions need careful evaluation to determine the percentage of nutrients accessible for algal growth for a given organism (Lowrey *et al.,* 2015). A challenge with amendments is balancing the supply of nutrients and organic substrate with potential toxicity and substrate inhibition which can occur at high amendment concentrations (Lowrey et al., 2015; Pang et al., 2019).

1.2 Objectives:

This study evaluates the potential for food-grade wastes to act as substrates for microalgae and to enhance their growth rates, biomass yields or cell quotas of desirable products. First, an extensive screening experiment evaluated the potential of algae to grow on the waste products. The screening tested for the optimal light intensity and waste product concentrations for each species of algae and amendment. The results of the culture screening informed species choice and culture conditions used for the later experiments on photosynthetic competence and biomass composition in the chlorophyte *Tetraselmis suecica. T. suecica* was chosen as the candidate species because it was capable of growth comparable to control cultures using 1% whey permeate as a source of phosphorous. Following the biomass composition experiment, the effect of an addition of inorganic carbon on *T. suecica*'s investment in light harvesting was evaluated.

CHAPTER 2 – SCREENING MICROALGAL GROWTH ON FOOD-GRADE WASTE STREAMS

2.1 Background

To grow, phytoplankton require sources of energy and nutrients (e.g., carbon, nitrogen, and phosphorous), vitamins, and trace metals. The specific requirements of algae can vary significantly between different species (Ho *et al*., 2003). Phytoplankton up- or down-regulate their internal quotas for proteins, lipids, and light harvesting apparatuses in response to changing growth conditions (e.g., Geider *et al*., 1996). In addition, further variability in growth rate, cell quotas, and nutrient and energy requirements can depend on photosynthesis, heterotrophy or mixotrophy (Abreu *et al*., 2012; Candido & Lombardi, 2017; Pagnanelli *et al*., 2014). Regardless of their mode of nutrition, there will be a either a limit on the rate of growth (Blackman limitation) or yield (Liebig limitation). To grow algae on a large scale it is essential to first understand the limiting effects of different conditions and modes of nutrition.

Since algal growth integrates multiple metabolic reactions, Blackman limitation reflects the slowest of these reactions (Falkowski *et al.,* 1992). In the case of a phototrophically growing species, the rate-limiting step in photosynthesis could be in light harvesting or the Calvin cycle. If an increase in the light level boosted growth rate, it suggests the lack of light limited the growth rate. However, if the increased light failed to result in increased growth rate (assuming the light is below levels that could cause photoinhibition), this could indicate that Calvin cycle activity is limiting.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is typically believed to be the rate-limiting step in the dark reactions of photosynthesis (Young *et al*., 2015).

The catalytic rate of Rubisco is determined in part by the availability of carbon dioxide relative to oxygen as both are substrates for the enzyme (Raven *et al.,* 2014). Increasing the availability of carbon dioxide relative to oxygen can increase the rate of the dark reactions because carboxylation is favoured over oxygenation.

Heterotrophic growth oxidizes organic substrates, releasing carbon dioxide, which could increase the catalytic efficiency of Rubisco. Therefore, mixotrophic cultures could achieve higher growth rates than photoautotrophic ones. That can be achieved both by directly using the organic substrates in heterotrophic metabolism, and by increasing the efficiency of the dark reactions (Martinez *et al.,* 1991). There is an alternative response, though, by which growth on an organic substrate causes a down-regulation of photosynthesis (Roth et al., 2019). For phytoplankton that show this response, cultures grown in the light with organic carbon sources are effectively heterotrophic rather than mixotrophic.

While Blackman limitation addresses growth rate, Liebig limitation of yield describes the limits to algae production from a given growth medium (Falkowski *et al.,* 1992). Specifically, yield is limited by the nutrient with the lowest concentration relative to the needs of the organism. An algal cell's nutrient requirements vary significantly between species (Ho *et al*., 2003) from changes in the internal cell quotas for proteins, light harvesting apparatus, or energy-storage compounds resulting from different growth conditions. Increasing the availability of the limiting nutrient in the media increases the yield but results in higher production costs (Bhatnagar *et al.,* 2011).

Food-grade waste products are a potential source of inexpensive nutrients that could fuel algal growth. Potentially high in nutrients and organic carbon, they are safe for

humans. Biomass or bio-products produced with them should be safe for human consumption. Three food-grade wastes were used in this study: whey permeate, a by-product of cheese making; vinasse, a by-product of alcohol distillation; and fishery waste, specifically, the aqueous phase from hydrothermal liquification of fish remains (fish-HTL). Whey permeate, a well-studied waste product, contains high concentrations of lactose (46-52 g L^{-1}) and phosphate (1-3 g L^{-1}). Both could support algal growth (Yadav *et al.,* 2015). While the organic carbon and phosphorous are readily available to algae, the nitrogen in whey permeate is generally in complex compounds which are likely inaccessible (Yadav *et al.,* 2015). Aqueous-phase products from hydrothermal liquification of biomass can contain organic carbon, phosphorous, and nitrogen which are likely accessible to algae. But the products can also contain growth suppressing phenols (Biller *et al.,* 2012). Similarly, vinasse derived from sugarcane contains organic carbon and nutrients to potentially support algal growth, but also toxins (Candido & Lombardi, 2017).

The green alga *Chlorella vulgaris* can grow mixotrophically supported partially or completely by the nutrients available with these waste products. Studies have found *C. vulgaris* grown with whey permeate showed increases in growth rate, final biomass yields, and yields of lipid, protein, and starch (Abreu *et al.,* 2012). Higher growth rates of *C. vulgaris* could be achieved with diluted vinasse as a culture medium (Candido & Lombardi, 2017). While *C. vulgaris* grown with the aqueous phase from HTL of algal biomass does not show higher algae yields or growth rates, it was found to reduce the total organic carbon in the waste (Barreiro *et al.,* 2015). The fish-HTL and vinasse used in this study are made from different products than those in the above studies but were

processed in similar ways. While their exact nutrient compositions vary from those reported in the literature, they are still expected to contain sufficient nutrients to support algal growth.

As the goal of these experiments was to determine if mixotrophic growth with food-grade wastes can enhance the growth rate and yield for practical applications, species with potential or those already utilized in algae industries were chosen as the species to examine. The species chosen included *Nannochloropsis oculata, Pavlova lutheri, Phaeodactylum tricornutum, Tetraselmis suecica, Thalassiosira pseudonana,* and *Rhodomonas salina. N. oculata, P. lutheri, T. suecica, T. pseudonana,* and *R. salina* have value as animal feeds, especially in the aquaculture industry. *N. oculata, P. lutheri,* and *P. tricornutum* produce significant quantities of eicosapentaenoic acid (EPA), an omega-3 fatty acid with health benefits for humans (Swanson *et al.,* 2012).

Optimal algae production depends on the species and conditions, specifically if they are grown heterotrophically, photoautotrophically, or mixotrophically. Not all algae can grow mixotrophically, nor can all algae grow effectively with food-grade waste amendments. Species capable of mixotrophic growth include *N. oculata* (Pagnanelli *et al.,* 2014)*, P. lutheri* (Bashir *et al.,* 2019)*, T. suecica* (Mohammad *et al.,* 2014)*, T. pseudonana* (Baldisserotto *et al.,* 2021) and*, R. salina* (Kitano *et al.,* 2019*)*. Some strains of *P. tricornutum* are capable of mixotrophic growth (Garcia *et al.,* 2005) but not all strains naturally have the necessary transporters needed to develop with glucose as a substrate (Zaslavskaia et al., 2001).

A large-scale screening experiment is required to determine the optimal candidate species and growth conditions for further analysis. There is a trade-off in human effort between quality and quantity when collecting data for an experiment. Quality refers to the depth of characterization (and so, here, the volume of culture required); quantity refers to the number of tests. As a result of the large number of species and treatment conditions necessary to identify a candidate species and optimal conditions, the latter was chosen. For this experiment, there were three main hypotheses to test. The first hypothesis is that higher concentrations of whey would increase yield and growth rate. Adding nutrients should increase algal yield, assuming the nutrients were previously limiting. The organic carbon should provide fuel for heterotrophic growth. The second hypothesis is that the highest concentrations of whey will inhibit the growth of algae. That situation has been observed in previous studies with high concentrations of organic carbon (Pang *et al.,* 2019). The third hypothesis is that increasing the light level would increase growth rates, as is expected with a photoautotrophic culture (MacIntyre *et al.,* 2002). The results from this study were used to determine a candidate species and conditions for larger-scale experiments (Chapter 3).

While experiments were performed with all three food-grade wastes, the analysis focuses on whey permeate, the best-performing waste. For a more thorough analysis of the fish-HTL treatment, see Chen (2021).

2.2 Methods

2.2.1 Phosphate content of the food-grade wastes

Previous experiments have shown that whey permeate, vinasse, and HTL products of biomass can contain enough phosphorous to support significant algal growth (Barreiro *et al.,* 2015; Candido & Lombardi, 2017; Yadav *et al.,* 2015). The total dissolved phosphorous (TDP; sum of dissolved organic phosphorous and orthophosphate) in the food-grade wastes was determined by the MAPEL lab in November 2020, prior to the screening experiment. The analysis used the molybdate blue colorimetric assay for orthophosphate (Strickland *et al.,* 1972), after oxidation of the sample to convert organic phosphate to inorganic orthophosphate (Solozano *et al.,* 1980). This assay used triplicate KH_2PO_4 standards that were prepared at 0, 1, 2, 5, 8, and 10 µM to cover the range of concentrations, although the standard curve can exhibit non-linearity in this range.

The TDP in vinasse, whey permeate, and fish-HTL was assayed at dilutions of 0.03%, 0.1%, 0.3%, and 1% V:V in seawater. Three replicates of each sample were placed in scintillation vials that had been baked at 400 $^{\circ}$C for at least two hours to remove organic contaminants. The liquid samples were dried at 95 $^{\circ}$C until all liquid had evaporated. They were then muffled at 450 $^{\circ}$C for 2 hours and, after cooling, were hydrolyzed with 0.75 M HCl at 80 $^{\circ}$C for 20 minutes. Following addition of the colorimetric mixed reagent, absorption by the phosphor-molybdenum blue product was measured three times at 885nm in a 1-cm cuvette with a Cary 4000 spectrophotometer.

The standard curve was fit with a linear model. For all the food-grades wastes, absorbance in the 1% and 3% samples was outside the range of the standard curve.

Concentrations in these samples were estimated by extrapolating from the concentration in the 0.03%, 0.1%, and 0.3% samples.

A second phosphate analysis was performed as part of this thesis in November 2021. The standard curve used a higher range of standards (0, 5, 12, 20, 23, 26, 30, 35, and 40 μM). A set of whey permeate samples was assayed at concentrations of 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, and 1.6% to confirm the trends observed in the previous phosphate analysis.

2.2.2 Algae Culturing and Maintenance

Nannochloropsis oculata, P. lutheri, P. tricornutum, T. suecica, T. pseudonana, and *R. salina* were the species chosen for the culture screening experiment (Table 2.1). Prior to the experiment, these cultures of algae were grown in nutrient-replete, semi-continuous conditions (MacIntyre and Cullen 2005) under continuous illumination at either low light (LL; 20 μmol photon m^{-2} s^{-1}), medium light (ML; 40 μmol photon m^{-2} s^{-1}), or high light (HL; 190 μmol photon m^{-2} s^{-1}) on custom-built light tables illuminated by warm white LED strips. Cultures were maintained at low optical density in 6-mL borosilicate tubes. They were grown in either L1 seawater media or f/2 seawater media to meet the nutrient requirements of each species (Table 2.1). The growth media were prepared according to the National Center for Marine algae and Microbiota (https://ncma.bigelow.org/algae-media-recipes). The main differences between the two media types are in the chelation and concentrations of trace metals added; the concentrations of nitrogen and phosphorous are the same.

Table 2.1 Microalgal cultures used in the screening experiment. Clonal designations are from the source collections, the National Center for Marine Algae (NCMA) in Boothbay, ME, USA, and University of Texas at Austin Culture Collection of Algae (UTEX) in Austin, TX, USA. The cultures were maintained in f/2 (Guillard, 1975) or L1 (Guillard and Hargraves, 1993) media, as indicated.

Species	**Clone**	**Medium Used**
Nannochloropsis oculata	CCMP525	L1
Pavlova lutheri	CCMP 1335	L1
Phaeodactylum tricornutum	CCMP1327	f/2
Tetraselmis suecica	UTEX2286	L1
Thalassiosira pseudonana	CCMP1335	f/2
Rhodomonas salina	CCMP1319	L1

Prior to the experiment, cultures were monitored daily by measuring chlorophyll-a (chl-a) fluorescence as a proxy for biomass (Brand *et al.,* 1981) using a Turner 10-005R or 10AU fluorometer (Turner Designs, USA) and a FIRe fluorometer (Satlantic, Canada). Chlorophyll-a fluorescence is only a reliable proxy for biomass when the cultures is in balanced growth (discussed in more detail below) but provides a rapid, non-destructive, and non-invasive method to monitor growth. The potential for algae to downregulate fluorescence while reaching balanced growth was not a significant concern because the cultures were maintained in balanced growth (MacIntyre and Cullen*,* 2005) by diluting them into fresh media when the fluorescence reached a threshold equivalent to 30% of the maximum value observed in stationary phase. Cultures were dark acclimated for 30 minutes before reading to allow photosynthetic and non-photosynthetic quenching of fluorescence to relax. The specific growth rate of the phytoplankton was estimated using the change in fluorescence from the Turner 10AU (Wood *et al.,* 2005):

$$\mu = \frac{ln\left(\frac{F_2}{F_1}\right)}{t_2 - t_1} \qquad \text{(Eq. 1)}$$

where μ is the specific growth rate (d^{-1}), F_1 and F_2 are blank-corrected fluorescence (Arb.) at t_1 and t_2 (d).

The variable fluorescence ratio (F_v/F_m), which is an estimate of the proportion of functional reaction centers, was measured with single-turnover induction curves 100 μs in duration with the FIRe fluorometer. The estimate of F_v/F_m was obtained by non-linear fitting (Gorbunov *et al.,* 2004) using Fireworx software (Audrey Barnett, http://sourceforge.net/projects/fireworx/). Cultures were used to inoculate experimental treatments only after reaching balanced growth. Cultures were considered to be in balanced growth after ten generations in which there was <10% variation in the daily growth rate and F_v/F_m (MacIntyre and Cullen, 2005).

For the screening experiment, cultures were grown as batch cultures in sterile 24-well plates with 1 mL of media/culture in each well, with a thin semi-permeable membrane across all wells to maintain sterility. and with a hard plastic cover to prevent evaporation. Cultures were grown in f/2 or L1 media or modified media amended with the wastes. The amended media omitted the $NaH_2PO_4 \bullet H_2O$ used in the full media and contained an addition of one of the three food-grade wastes. As there was no added phosphate in the amended media recipe, any phosphorus used for algal growth would be coming from one of the added food-grade wastes. Standard L1 or f/2 media has a molar C:N:P ratio of approximately 56:24:1 while the amendments would have similar levels of inorganic carbon and nitrogen but varying levels of phosphorous and organic carbon (Table 2.2). The standard L1 and f/2 media and the media with food-grade wastes were all prepared in the same way. All the required nutrients and/or amendments were added into filtered seawater and autoclaved to sterilize them. Amended media were prepared at

Table 2.3 Estimated stoichiometric composition of control and whey-amended media. The DOC concentration is made assuming whey permeate is 85% by mass lactose and assuming all organic carbon in the form of lactose. The estimated DIC is based on historical values for the seawater used to prepare media. Estimates of nitrate+nitrite are phosphate from analysis of 1% whey permeate at CERC lab (Natasha Rondon Vivas, pers. comm.).

	Dissolved Organic Carbon (DOC, µM)	Dissolved Inorganic Carbon (DIC; µM)	Nitrate+Nitrite (N; µM)	Phosphate or TDP (P; µM)	DOC:DIC:N:P
L1/f/2	0	2000	880	36	0:56:24:1
Whey 0.1%	29800	2000	881.4	6.7	4432:297:131:1
Whey 0.3%	89400	2000	884.2	18.6	4812:107:48:1
Whey 1%	298000	2000	894	62	4806:32:14:1
Whey 3%	894000	2000	922	175	5095:11:5:1

the concentration needed so there were four sets of amended media (0.1%, 0.3%, 1%, and 3%) of each food-grade waste amended media. There may be variations between two different batches of food-grade waste but all the plates for a species were always grown on the same batch of food-grade waste to reduce this variability within treatments. Plates were grown with a media blank, control culture with f/2 or L1 media, and media with 0.1%, 0.3%, 1%, and 3% waste amendments. These four concentrations were chosen because for whey permeate, fish-HTL, and vinasse, the media should range from phosphorous limited at 0.1% and 0.3% to limited by another nutrient at 1% or 3%. There were four replicate wells for each control and treatment on each plate, and a plate for every amendment was grown at low, medium, and high light.

Only the outer wells of the plates (those on the edges of the plate) were used for analysis. This meant there were only 2 replicates for the control, 0.1%, 0.3%, and 1% treatments and 4 replicates for the 3% treatment. In some cases, one of more or these replicates had to be discarded due to an anomalous lack of growth (see 2.2.8 Statistics for more information on the criteria used for removing outliers). Independent experiments (unpublished data) in the MAPEL lab found that inner wells were carbon-limited, significantly reducing algal growth in the inner wells ($p < 0.05$). The carbon limitation was confirmed when plates were grown without a lid in sealed Tupperware containers with an internal CO_2 supply. With this experimental setup, there was no longer a significant reduction in growth in the inner wells. Growing a plate without a lid outside of a sealed container was not a viable option as evaporation would lead to a well completely drying out.

In the screening experiment, fluorescence was a proxy for biomass. The fluorescence was monitored daily using a Bio Teck Synergy 4 plate reader with excitation at 485/20 nm (central wavelength/half-height band width) and emission at 680/30 nm, read from the bottom. Plates were dark acclimated for 30 minutes before reading. Daily fluorescence data from the plate reader was used to generate a growth curve and fitted to a modification of Blackman's bilinear model (see below). Wells were harvested once they had been in the stationary phase for a time equivalent to 3 generations during exponential growth. The harvested samples were stored in cryovials with 0.4 µL of glutaraldehyde and flash-frozen with liquid nitrogen to analyze the cell counts and size later with a BD Accuri C6 flow cytometer (cf. Gasol and Del Giorgio, 2000).

2.2.3 Monitoring Growth Rates

Many models have been used to fit growth curves (reviewed by Zwietering et al., 1990). One of the most commonly used is the Gompertz model (Zwietering *et al.,* 1990). A version of Blackman's bilinear model (Jones et al., 2014), modified to include a lag phase (Equation 2), was used instead, as it could fit the experimental data with comparable accuracy but has defined transitions between the lag, exponential, and stationary growth phases (Figure 2.1).

$$F_t = F_{\text{init}} \text{ for } t < t_{\text{lag}} \quad \text{(Eq. 2a)}$$

$$F_t = (F_{\text{final}} - F_{\text{init}}) \frac{(t - t_{\text{lag}}) + t_{\text{exp}} - |(t - t_{\text{lag}}) - t_{\text{exp}}|}{2 \cdot t_{\text{exp}}} + F_{\text{init}} \quad \text{(Eq. 2b)}$$

where F_{init}, F_{final}, and F_{t} are the initial and final (i.e., stationary-phase) fluorescence intensities (Arb.) and the intensity at time t (d), respectively; and t_{lag} and t_{exp} are the durations of the lag and exponential growth phases (d), respectively (Figure 2.1). The specific growth rate during exponential phase, μ (d^{-1}, see Equation 1), is equivalent to $(F_{\text{final}} - F_{\text{init}})/t_{\text{exp}}$ and the generation time (d) during exponential growth is equivalent to $\ln(2)/\mu$.

The model was fit using the Solver add-in in Microsoft Excel; iteratively adjusting the fit coefficients to minimize the root mean square error (RMSE). The lag phase (Figure 2.1) can reflect a period of adjustment when algae acclimate to new media, or when the fluorescence of the culture was under the lower limit of detection for the instrument (Miller and Miller, 2005).

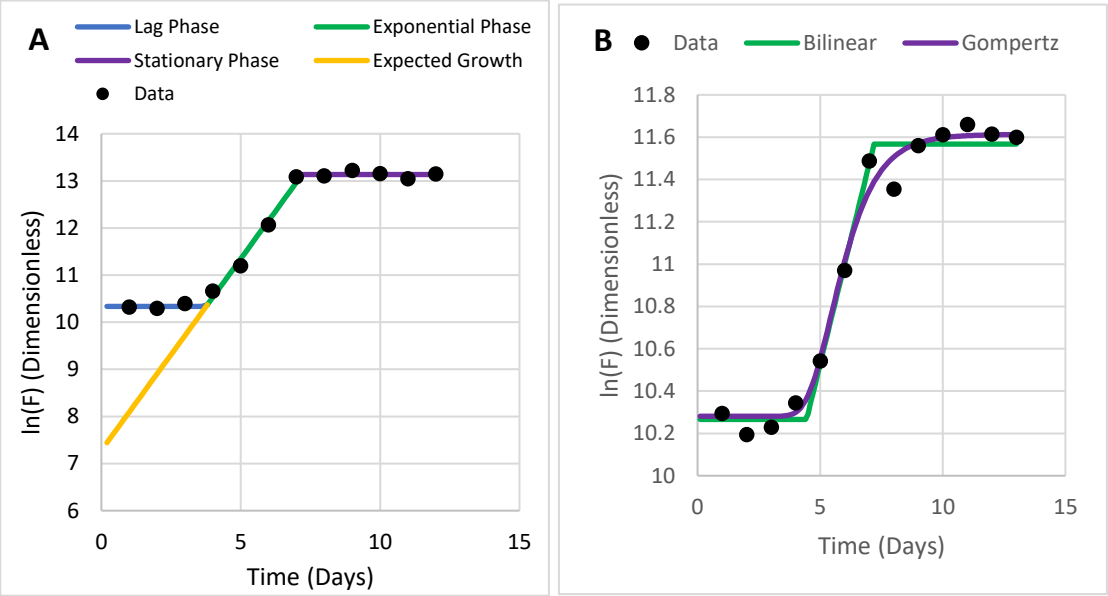

Figure 2.1 *(A)* Equation 2 fit to data for a control cultures of *T. suecica* grown at medium light. The Lag, Exponential and Stationary phases were determined based on fit results of the modified bilinear model. The expected growth is an extrapolation of the exponential phase growth rate on the assumption that the initial fluorescence reflects the instrument's lower limit of detection rather than the algae. *(B)* A comparison of how well the modified Blackman Bilinear (RMSE = 0.075) and Gompertz (RMSE = 0.068) models fit the daily measurements of fluorescence in a control culture of *P. lutheri* grown at high light. Note the clearly-defined transitions between the different growth phases with the Bilinear model.

Comparing the two models (e.g., Figure 2.1) across different species and conditions found no significant difference in the RMSE values ($p>0.05$; $n = 60$). In some growth curves, the late stationary phase showed a significant decline in fluorescence; these values were removed from the model fitting to get more accurate estimates of the stationary-phase fluorescence. These data points were only removed in cases where there were already multiple (minimum 3) similar data points in the stationary phase and the removal resulted in a smaller RMSE value.

Growth rates could not be determined unambiguously due to the down-regulation of fluorescence in cultures with amendments (see below). A reduction in chl-a and

fluorescence per cell was observed when *T. suecica* was grown with 1% whey during the exponential growth phase (See. Chapter 3, Figure 3.1). If this is true for other species grown mixotrophically, an estimate of growth rates based solely on fluorescence would likely be an underestimation of a culture's growth rate. The initial biomass, final biomass, and length of growth period are needed to estimate growth rates for amended cultures. The final biomass Is known from the biovolume estimates calculated from the flow cytometer data. It can be assumed that there will be no significant increase in biomass during the stationary phase, so the growth period can be estimated as the sum of the lag phase and exponential phase of the plate reader fluorescence data (Figure 2.1). Growth rates estimated with fluorescence from this method will be referred to as apparent growth rates to make explicit that they may not accurately reflect the true growth rates. In the event of down-regulation of chlorophyll a, the apparent growth rate will be lower than the actual rate. Although the fluorescence may not represent an accurate measure of biomass or growth rate, it does show when algae are growing, based on increasing fluorescence. It is assumed that the algae are growing or acclimating to the amendments during the lag phase. However, it cannot be observed because the fluorescence is below the lower limit of detection of the instrument. Therefore, adding the lag and exponential phase should estimate the time it took to reach the final biomass.

2.2.4 Evaluating biovolume of mixotrophic cultures

Fluorescence is not the most accurate proxy for biomass so, to evaluate the final yield of the treatments, a different method is needed. An estimate of the concentration and size of cells could be used as a more accurate measure of yield. A BD Accuri C6

Flow Cytometer was used to determine cell concentrations in the harvested culture, and proxies of cell size (Forward angle light scatter, FSC; 488 nm), and chlorophyll a quota (red fluorescence, FL3; fluorescence, excited at 488 nm and detected at >670 nm). Cultures were dark acclimated before reading and diluted if needed to get events/second below 200. Samples were read until 10,000 events or 4 minutes had passed, whichever happened first. The flow cytometer counted all events, including bacteria or other non-algae particles. To distinguish between algae and other events, FL3 fluorescence was used to estimate which events could be confidently counted as containing algae cells. Data from the BD Accuri software was exported and processed in Python to classify events based on FL3 and to estimate cell volume from FSC.

The relationship between size and FSC was determined using Spherotech polystyrene particle standards (0.88, 1.35, 2.0, 3.2, 5.17, 7.49, 10.1, and 16.5 μm diameter). The relationship between log[FSC] and log[volume] of the standard beads was linear ($R^2 = 0.92$; $p < 0.001$), so a linear regression was used to estimate equivalent spherical volume (ESV) from FSC (Appendix - Figure A.2). From this relationship, an estimate of the final yield as biovolume (total ESV)/μL of algae and all other events was determined for each sample. As the flow cytometer could not distinguish between an individual cell or an aggregate of cells, biovolume was selected as the measure of total yield as it would give a better estimate of yield than cell concentration.

Algae (and, likely, algae/bacterial aggregates) were discriminated from other events based on FL3 fluorescence in the control cultures (Figure 2.2). Typically, there was a clear division between these subpopulations, based on FL3 but also distinct in FSC. The high-fluorescence population represented algae and the low-fluorescence one

represented bacteria and other low-fluorescence material such as algae with completely down-regulated pigments, or colloidal material from the wastes. These will be referred to as “Other”. This classification is imperfect because if algae down-regulated fluorescence they might fall below the FL3 threshold and because, in some cases of cultures grown with food-grade wastes, there was not a clear distinction between two subpopulations. While the method does provide a method to identify events which are confidently determined to be algae, it may underestimation of the concentration number of algal cells. This method does make it challenging to determine if algae fluorescence per cell is being down-regulated with mixotrophic growth. If an algal cell down-regulates pigments below the threshold it may not longer be detected as an algae cell. While microscopy could be used for a more accurate measure of algal cells, it was not a viable option considering the number of samples to process. Due to technical issues with the flow cytometer, not all samples from the screening experiment could be counted. In cases where the samples have not been processed, the tables including data from the flow cytometer list the data as “N/A”.

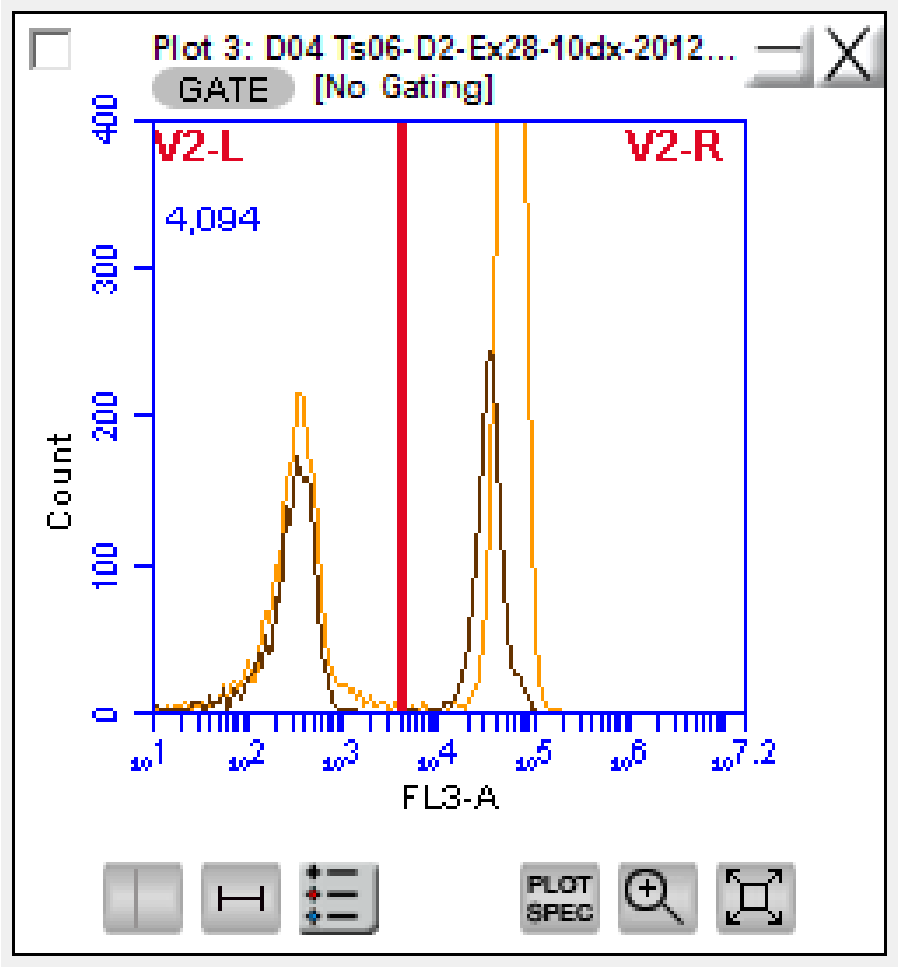

Figure 3.2 Frequency distribution of red fluorescence (FL3) for two control cultures of *T. suecica* from a plate grown at ML. Note log scale. The threshold (red line) indicates the cut-off between events classified as algae (FL3 > threshold) and "Other" particles (bacteria, completely depigmented algal cells, colloids etc.; FL3 < threshold).

2.2.5 Evaluation of Abiotic Factors

The food-grades wastes can have fluorescence which is detectable by the fluorometers. It is important to understand how this fluorescence changes over time when evaluating the algal growth curves. In typical (control) media, only the algae contribute to changes in the fluorescence of the culture. With the amended media, where there is a potential for the amendments to contribute to the fluorescence, it is essential to evaluate

the background fluorescence with the amendments. To better understand this, an abiotic plate was prepared, consisting of a sterile media blank and sterile modified L1 media with 1% whey permeate, 1% fish-HTL, or 1% vinasse. Six replicate wells for each treatment and control were prepared, and the plate was placed on a light table at intermediate light (80 μmol photons m^{-2} s^{-1}). The fluorescence of the plate was measured daily for seven days, using the plate reader as described above (Chapter 2.2.2 Algae culturing and maintenance), to test for any variation in background fluorescence.

2.2.6 Statistical Analysis

One-way ANOVA was used with Tukey-Kramer post-hoc analysis to determine if treatments significantly varied from the control ($p < 0.05$). In some cases, a well would have an outlier of no or very low growth, uncharacteristic for the responses in the replicate and at other concentrations. For example, there would be observed growth at 0.3% whey permeate and 3% whey permeate but a well at 1% whey permeate had nearly no growth while one grew as expected. In this case, the outlier well would be removed from the analysis and statistical analysis could not be performed for that treatment. In some cases, these outliers occurred in the control wells. In these cases, the control wells from other plates with the same species at the same light level were used to identify the outlier wells and these other control wells were included to statistical analyses could still be performed. (Note that one plate per species per light intensity was tested for each food-grade waste and that all of these were inoculated from the same parent culture and were grown at the same time.) If a well was removed or added to the analysis it is noted in the tables under the number of samples used. The expected number of samples for the

control, 0.1%, 0.3%, and 1% treatments is 2, while the 3% treatment should have 4 samples. Any deviations from these sample numbers are the result of additions or removals with the methods described here.

2.3 Results

2.3.1 Phosphate Analysis

Previous experiments with phosphate analyses showed evidence of absorption saturation at high concentrations of food-grade wastes. A secondary experiment with a larger range of controls and whey permeate was measured to confirm this trend of absorption saturation. The standards went up to a phosphate concentration of 10 μM and a maximum 1% concentration of whey (Figure 2.3. A, B) and to a phosphate concentration of 40 μM and 1.6% whey permeate for the second experiment (Figure 2.3. C, D). Figure 2.3C shows that absorbance saturation appears to occur past phosphate concentrations of 20 μM. Both experiments show absorbance saturation in whey permeate at concentrations higher than 0.3% whey permeate. In the first experiment (Figure 2.3 B) it is not clear exactly when the absorption saturation occurs past 0.3% but figure 2.3 D shows it likely occurs around 0.4% whey. This is consistent with the expectations based on the standards as 0.4% whey has an estimated phosphate concentration of 20.6±1.3 μM. Therefore, any concentrations of whey above 0.3% will have to be estimated based on the fits from Figure 2.3 A or 2.3 C. The fit from 2.3 A has been used to estimate the phosphate concentration of the food-grade wastes as all three wastes were tested on the same day as this measurement was taken. This data is used to

create estimates of media composition for whey permeate (Table 2.2) and in the appendix (Table A.2) for fish-HTL and vinasse.

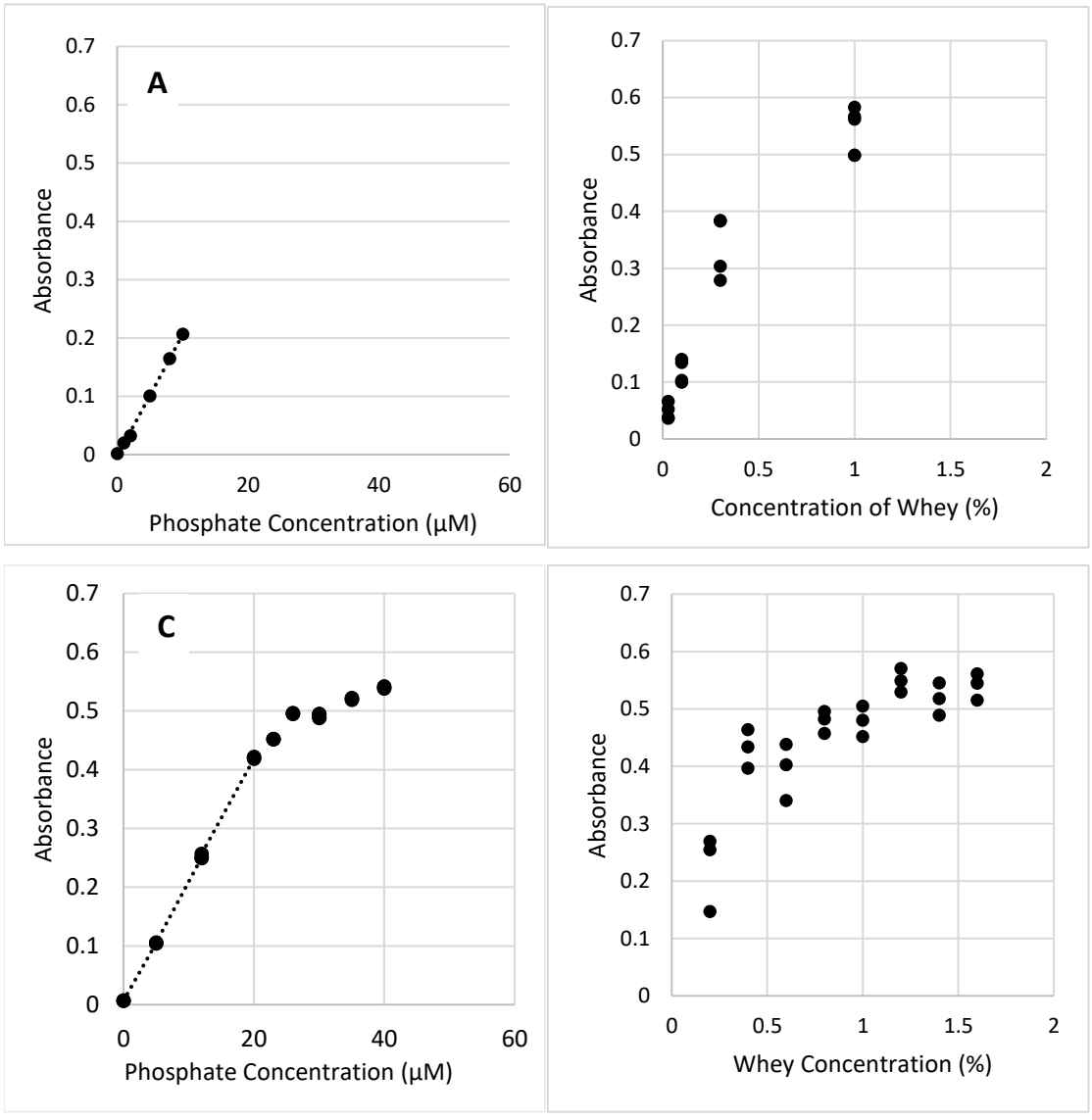

Figure 2.5. Phosphate concentration and absorbances of standards (left) and whey permeate (right) measured with the spectrophotometer in November 2020 (A,B) and November 2022 (C,D). The dashed lines are the model fits used to determine the relationship between phosphate concentration and absorbance, $y = 0.0208x - 0.0027$ (A) and $y = 0.0207x + 0.0042$ (C). A polynomial instead of a linear fit was attempted to be fit to the data in but the higher whey concentrations were outside the range of the standard curve.

All three amendments exceed the phosphate concentration in L1 media at a 3% addition (Figure 2.4): 48 μM in 3% fish-HTL and 175 μM and 141 μM in 3% whey permeate and vinasse, respectively, compared to 36 μM in the control media. Quigg et al. (2003) showed significant variation in the N:P ratio between different phyla and superfamilies, but all algae required more nitrogen than phosphorous. The control L1 and f/2 media have an N:P ratio of 24:1 mol mol^{-1}. In contrast, the 3% whey permeate, vinasse, and fish-HTL have an N:P ratio of 5:1, 6:1, and 18:1, respectively, when added to the control media. Based on the Redfield Ratio (16:1), this suggests that whey permeate and vinasse treatments have an excess of phosphorous at 3%, while fish-HTL has an excess of nitrogen. In all three cases, there is a higher amount of phosphorous relative to nitrogen in all amendments compared to the control media, so phosphorous is less likely to be a limiting nutrient than it would be in f/2 or L1 media.

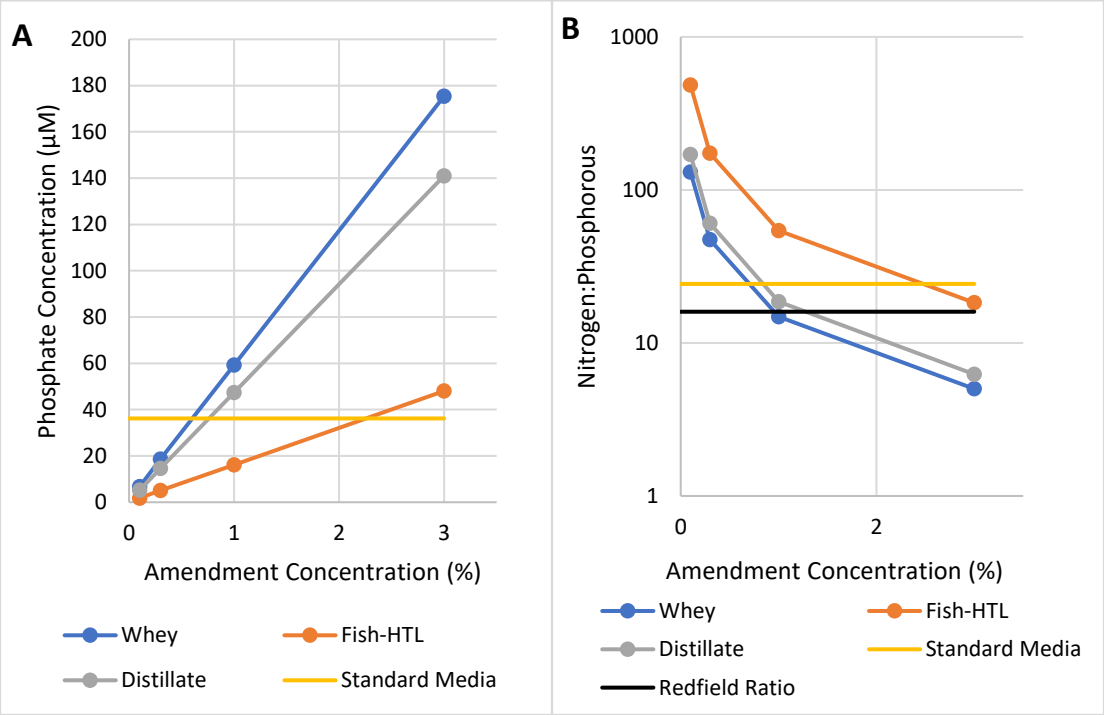

Figure 2.7 **(**A) Average phosphate concentrations of modified f/2 or L1 media amended with 0.1%, 0.3%, 1%, and 3% waste additions; the horizontal line is the phosphate concentration in control f2/ or L1 medium. (B) The molar nitrogen to phosphorus ratio of modified f/2 or L1 media amended with 0.1%, 0.3%, 1%, and 3% waste additions; the horizontal lines are the ratio in control media and the Redfield Ratio (16:1).

2.3.2 Growth Curves and Model Fits

The Bilinear model (Equation 2) was fit to data from each of the wells of every plate. This model provided estimates of the length of the lag and exponential phases, initial fluorescence, and maximum fluorescence. This data provided a simple criterion to determine when the algae had entered stationary phase. The generation time during exponential phase is equivalent to ln(2)/µ. A sample was assumed to be in stationary phase at the equivalent of 3 generation times after the end of the exponential growth phase, t_{exp} (Equation 2). Once algae were determined to be in the stationary phase, the

well was harvested for cell counts. In some cases, there was a sharp reduction in the fluorescence in the stationary phase that could not be captured by the bilinear model. In these cases, the phase in which fluorescence declined was not included in the model fit. An example of this can be seen in Figure 2.5 B where the data points for the final 3 days of the experiment were removed from the model fit as including them would have resulted in an underestimation of the maximum fluorescence of about 50%.

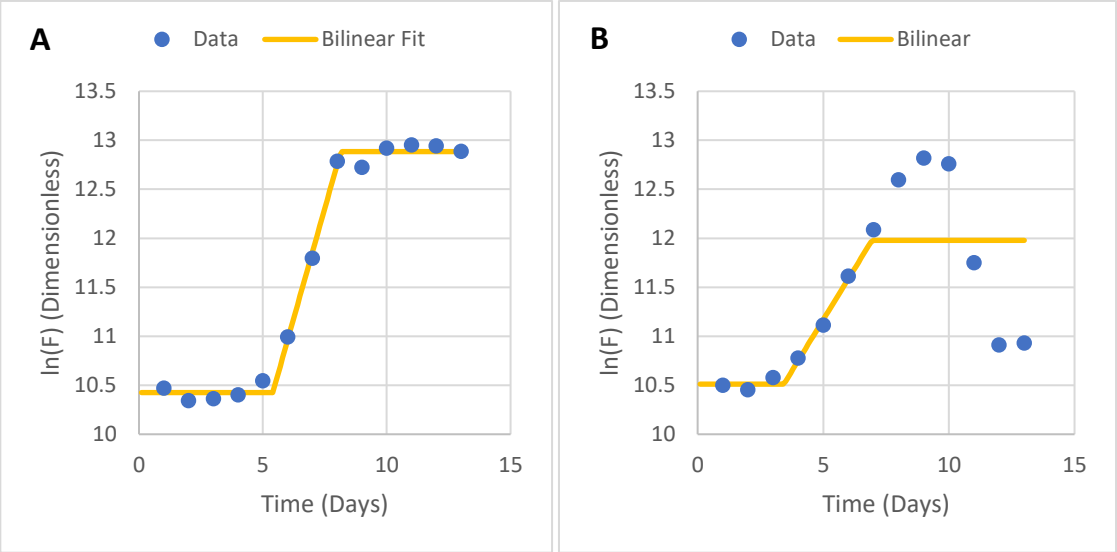

Figure 2.9 Example of a growth curve from a single well taken from a plate growing (A) *Pavlova lutheri* at high light with 1% whey permeate, and (B) Thalassiosira pseudonana at low light with 1% whey permeate. The modified bilinear model fits to the data are overlaid on the data. Note that the last 3 points in (B) were not included in the curve fit to avoid biasing the estimate of fluorescence during stationary phase.

Generally, there was no significant difference ($p > 0.05$) in the stationary-phase fluorescence of the whey-amended cultures compared with the controls (Table 2.3). In 4 of the 6 species tested (*P. lutheri, P. tricornutum, N. oculata,* and *R. salina*), the control cultures with the lowest maximum fluorescence occurred at high light and the highest fluorescence occurred at medium or low light (Table 2.3). Of these species, *T.*

pseudonana was the only species examined that showed a significant ($p < 0.05$) increase in the stationary-phase fluorescence from low to high light (Figure 2.6 A). In *T. suecica,* there was no significant difference in stationary-phase fluorescence in the control cultures between light levels (Figure 2.6 B).

Table 2.6 Stationary-phase fluorescence, F_{final} (Equation 2) in control and whey permeate-amended cultures. Data are presented as mean ± the standard deviation of ln-transformed, with the number of samples (wells) averaged for each estimate. Significant differences between amended cultures and the control ($p \leq 0.05$; ANOVA and Tukey-Kramer tests are noted with an "*" in the table. In cases there are more or fewer samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details). The data are ordered by species and growth light level (low, LL; medium, ML; and high, HL).

		Whey Treatment - Maximum Fluorescence (Dimensionless)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
T. pseudonana	HL	12.31 ± 0.41	2	11.21 ± 0.04	2	11.37 ± 0.31	2	11.09 ± 1.08*	2	11.18 ± 0.52	4
	ML	12.50 ± 0.21	2	11.92 ± 0.52	2	11.42 ± 0.94	2	11.43 ± 1.21	2	11.48 ± 1.20	4
	LL	12.95 ± 0.19	2	11.92 ± 0.01*	2	12.54 ± 0.07	2	12.81 ± 0.03	2	11.22 ± 0.30*	4
T. suecica	HL	15.15 ± 0.02	2	12.60 ± 0.2	2	14.04 ± 0.02	2	15.01 ± 0.02	2	14.12 ± 1.72	4
	ML	14.8 ± 1.45	3	12.61 ± 0.26	2	14.20 ± 0.88	2	15.73 ± N/A	1	14.5 ± 0.30	4
	LL	15.15 ± 1.12	2	12.91 ± 0.05	2	15.46 ± 0.01	2	14.98 ± 0.22	2	14.6 ± 0.37	4
P. lutheri	HL	12.61 ± 1.40	2	12.54 ± 0.63	2	11.99 ± 0.32	2	13.06 ± 0.25	2	12.95 ± 0.12	4
	ML	14.22 ± 1.23	5	14.04 ± 0.19	2	14.65 ± 0.07	2	13.39 ± 1.70	2	14.17 ± 0.11	4
	LL	14.75 ± 0.59	2	14.60 ± 0.22	2	15.08 ± 0.11	2	14.89 ± 0.01	2	13.58 ± 0.10	4
P. tricornutum	HL	13.89 ± 0.47	2	11.48 ± 0.37	2	12.79 ± 0.31	2	13.34 ± 0.07	2	14.07 ± 0.08	4
	ML	14.98 ± 0.72	2	12.29 ± 0.09	2	13.78 ± 0.35	2	13.84 ± N/A	1	13.35 ± 0.13*	4
	LL	14.98 ± 0.26	4	12.60 ± 0.27	2	13.95 ± 0.1*	2	14.47 ± 0.18	2	13.45 ± 0.38*	3
N. oculata	HL	13.45 ± 0.06	4	12.36 ± 1.14	2	13.32 ± 0.38	2	13.32 ± 0.38	2	9.03 ± 6.17	4
	ML	13.57 ± 0.87	2	12.11 ± 0.27	2	13.92 ± 0.70	2	14.00 ± 0.21	2	13.21 ± 0.67	4
	LL	14.53 ± 1.01	2	11.99 ± 1.71	2	14.94 ± 0.28	2	14.98 ± 0.08	2	14.45 ± 0.36	4
R. salina	HL	11.83 ± 0.36	2	10.46 ± 0.11*	2	10.54 ± 0.25*	2	10.21 ± 0.13*	2	11.25 ± 0.27	4
	ML	12.27 ± 0.14	2	11.12 ± 0.12*	2	11.98 ± 0.18	2	12.17 ± 0.02	2	11.66 ± 0.09*	4
	LL	12.68 ± 0.02	2	11.08 ± 0.24*	2	12.48 ± 0.12	2	12.44 ± 0.09	2	11.98 ± 0.03*	3

If there was a significant difference in stationary-phase fluorescence between whey permeate-amended cultures and controls, it was always a significant reduction in the amended cultures (Table 2.3). A similar trend of decreased stationary-phase fluorescence is observed in cultures grown with the fish-HTL and vinasse amendments. When cultures were grown with fish-HTL or vinasse, there were more cases of significantly lower maximum fluorescence with these amendments at all concentrations compared with whey treatments. For example, when *T. suecica* was grown with whey permeate, fish-HTL and vinasse only the whey permeate had fluorescence similar to the control treatments (Figure 2.7). Although in other species and light levels there were cases of growth with vinasse or fish-HTL, the majority of treatments resulted in significantly lower growth ($p<0.05$) (Appendix - Table A4, A5).

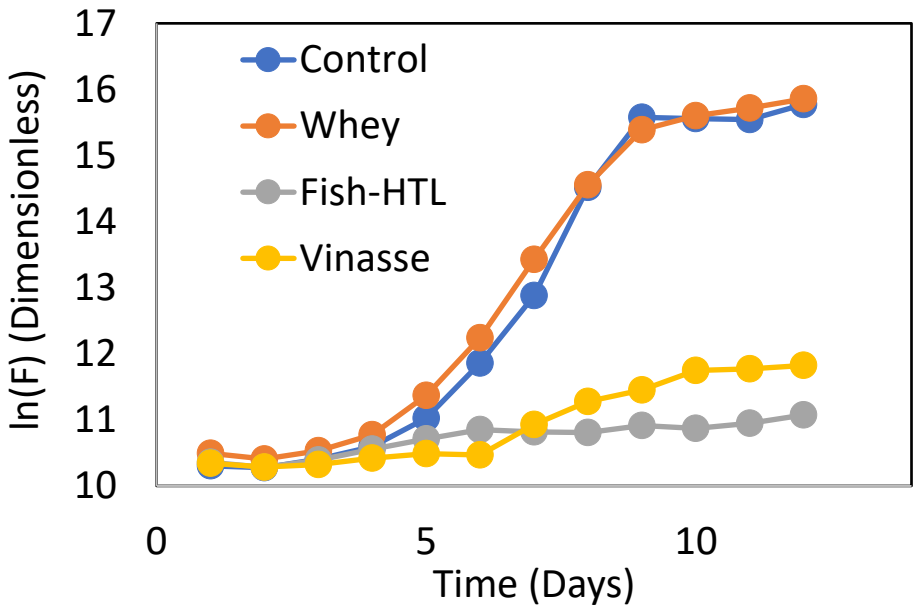

Figure 2.13 Daily fluorescence data of a single well taken from a plate growing *T. suecica* at medium light with no waste products (Control), 1% whey permeate, 1% fish-HTL and 1% vinasse.

There are no clear trends in the apparent growth rates of waste-amended cultures in the screening experiment. With the control cultures, the highest apparent growth rates occurred with the medium or high light cultures. In the case of *T. pseudonana*, the apparent growth rate was lower in high-light cultures than medium-light cultures, but the high-light fits are compromised by the very short growth interval (< 48 hours) and may not reflect the true growth rate. With all the species except for *N. 30culate* and *P. lutheri*, there was at least one light level with a growth rate that was significantly higher ($p<0.05$) than the other light levels. For some species, such as with *P. tricornutum* (Figure 2.8 A) , this trend in the apparent growth rate was maintained when grown with whey permeate. In these cases, the apparent growth rate in the treatments was consistent with the control,

where the highest growth rate occurred at higher light levels and the lowest at low light. In contrast, *T. pseudonana* at 3% whey permeate (Figure 2.8 B) had all three growth rates which were similar and not significantly different ($p<0.05$). There were a few treatments which have a significantly reduced apparent growth rate but no cases where apparent growth rates significantly increased. Similar trends were observed in the Fish-HTL and vinasse treatments except there were more cases of significantly lower apparent growth rates and evidence of inhibition of growth (Appendix - Table A6, A7).

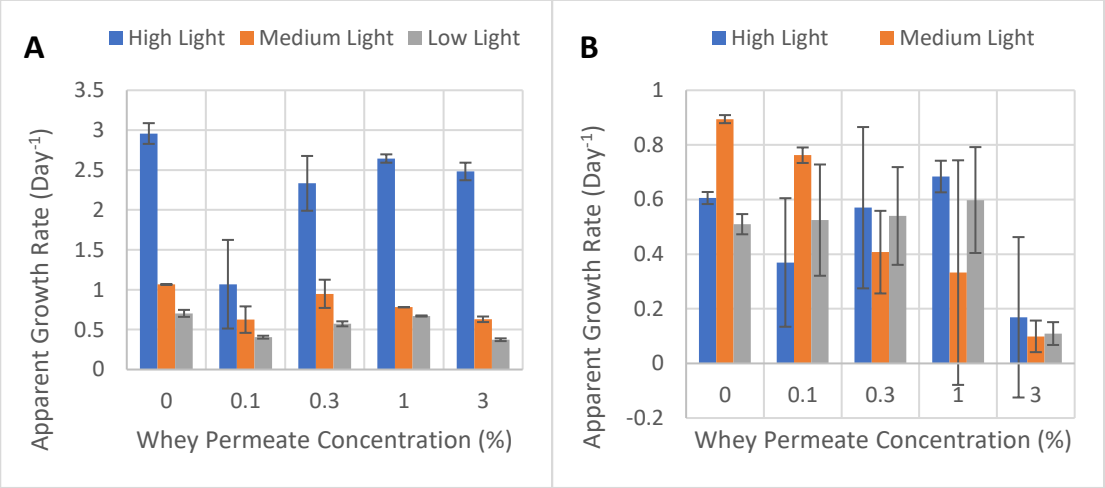

Figure 2.15 Apparent growth rate, μ (Equation 1), presented as mean ± standard deviation at high, medium and low light for (A) *P. tricornutum*; and (B) *T. pseudonana*.

Table 2.4 The average apparent growth rates plus or minus the standard deviation of the data from the culture screening experiments. The apparent growth rates are taken from the model fits of Equation 1. The number of wells used to calculate th mean fluorescence is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significa and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with ar in the table. In some cases there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

		Whey Treatment - Apparent Growth (days)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	0.61 ± 0.02	2	0.37 ± 0.24	2	0.57 ± 0.30	2	0.17 ± 0.29	1	0.17 ± 0.29	4
	ML	0.89 ± 0.01	2	0.76 ± 0.03	2	0.41 ± 0.15	2	0.33 ± 0.41	2	0.1 ± 0.06	4
	LL	0.51 ± 0.04	2	0.52 ± 0.20	2	0.54 ± 0.18	2	0.6 ± 0.19	2	0.11 ± 0.04	4
T. suecica	HL	1.24 ± 0.002	2	0.86 ± 0.26	2	1.18 ± 0.03	2	1.37 ± 0.60	2	1.37 ± 0.60	4
	ML	1.2 ± 0.03	3	0.54 ± 0.08	2	0.9 ± 0.07	2	1.03 ± N/A	1	0.92 ± 0.03	4
	LL	0.48 ± 0.02	2	0.18 ± 0004	2	0.47 ± 0.01	2	0.34 ± 0.02	2	0.4 ± 0.03	4
P. lutheri	HL	0.53 ± 0.23	2	0.47 ± 0.12	2	0.7 ± 0.13	2	0.61 ± 0.11	2	0.61 ± 0.11	4
	ML	0.69 ± 0.11	5	0.59 ± 0.01	2	0.63 ± 0.02	2	0.31 ± 0.19	2	0.6 ± 0.06	4
	LL	0.43 ± 0.03	2	0.37 ± 0.07	2	0.44 ± 0003	2	0.42 ± 0004	2	0.32 ± 0.03	4
P. tricornutum	HL	2.96 ± 0.13	4	1.07 ± 0.56	2	2.33 ± 0.34	2	2.48 ± 0.11	2	2.48 ± 0.11	4
	ML	1.07 ± 0003	2	0.63 ± 0.17	2	0.95 ± 0.18	2	0.78 ± N/A	1	0.63 ± 0.03	4
	LL	0.7 ± 0.04	4	0.41 ± 0.02	2	0.57 ± 0.03	2	0.67 ± 0.01	2	0.37 ± 0.02	4
N. oculata	HL	0.89 ± 0.10	4	0.48 ± 0.35	2	0.68 ± 0.11	2	0.69 ± 0.004	2	0.69 ± 0.004	4
	ML	0.67 ± 0.13	2	0.36 ± 0.06	2	0.61 ± 0.06	2	0.64 ± 0.04	2	0.45 ± 0.11	4
	LL	0.41 ± 0.01	2	0.16 ± 0.15	2	0.37 ± 0.01	2	0.3 ± 0.004	2	0.25 ± 0.03	4
R. salina	HL	0.14 ± 0.001	2	0.03 ± 0.03	2	0.05 ± 0.04	2	0.2 ± 0.08	2	0.2 ± 0.08	4
	ML	0.4 ± 0.01	2	0.14 ± 0.09	2	0.3 ± 0.08	2	0.38 ± 0.003	2	0.28 ± 0.03	4
	LL	0.31 ± 0.003	2	0.11 ± 0.03	2	0.25 ± 0.05	2	0.19 ± 0.004	2	0.17 ± 0.001	3

2.3.3 Flow Cytometric Analysis of Algal Populations

Cell counts performed with the flow cytometer were used to estimate the concentration of algal cells in the culture. The chlorophyll fluorescence measured by the flow cytometer distinguished events that contained algae from all other events which could include bacteria or algae with heavily down-regulated pigments. Figure 2.9 A, B and C clearly distinguishes between the algae (upper population) and other events (lower population) when grown in control media; however, with amended media, the populations were less clearly defined (Figure 2.9 D). In some cases, there was no separation between them. In these, the threshold chlorophyll fluorescence for the control culture was used to classify algae vs everything else (Figure 2.9).

As can be seen by comparing whey-amended vs control cultures in Figure 10, there was an increase in forward angle scatter, consistent with larger size, in the amended cultures. Whey-amended cultures often had a long "tail" at the upper end of the distribution, where there was an increase in both forward angle scatter and chlorophyll fluorescence. This was likely due to formation of cell aggregates, which was confirmed when cultures were examined under a microscope (see Appendix, Figure. A4-A7). This was most pronounced for cultures grown in high concentrations of an amendment, especially at higher concentrations of 1% and 3% whey permeate. The difficulty in confidently identifying algae and uncertainties in the relationship between forward angle scatter and volume are sources of potential error for estimates of biovolume and growth rate in later analyses.

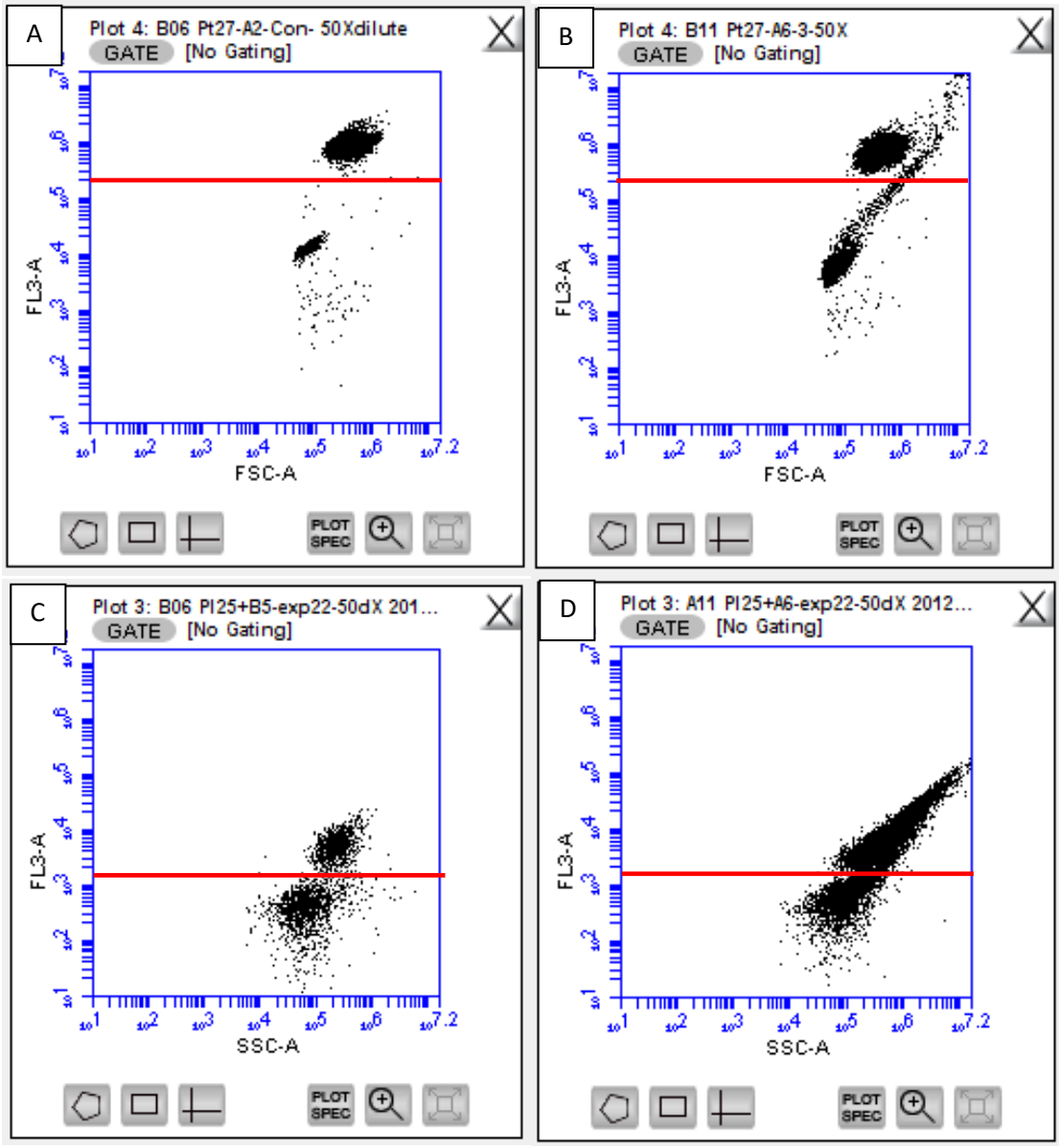

Figure 2.16 Biplots of Forward Angle Scatter (FSC) and Chlorophyll Fluorescence (FL3) for cultures of *Phaeodactylum triconutum* (A,B) and *Pavlova lutheri* (C,D) grown without (A,C) and with whey permeate (B,D). The red lines mark the fluorescence thresholds used to distinguish algae from other events at 200,000 for A,B and 1200 for C, D. See section 2.2.4 for details on choosing the threshold values.

With the control cultures, there appears to be no effect of growth light intensity on the total biovolume accumulated over the course of the experiment. In almost every species there was no significant difference in biovolume with changing light levels. The only exception to this was *R. salina* grown without whey permeate which had a significantly higher biovolume at medium light compared to low light (Figure 2.10 A). It was difficult to identify trends in the biovolume data between light levels as dataset was incomplete, due to technical issues with the flow cytometer. There was a clear trend of biovolume increasing with the concentration of whey permeate increase in most species. In the case of *P. lutheri* and *R. salina* at high light and low light, respectively, this increase resulted in a significant increase of biovolume compared to the control (Table 2.5). Otherwise, while this trend occurs across treatments was consistent, it did not result in a significant enhancement of biovolume compared to the control ($p>0.05$). This trend could generally be seen with all screened species grown with whey permeate except *T. pseudonana* where high variability in the data obscures any clear trends (Table 2.5).

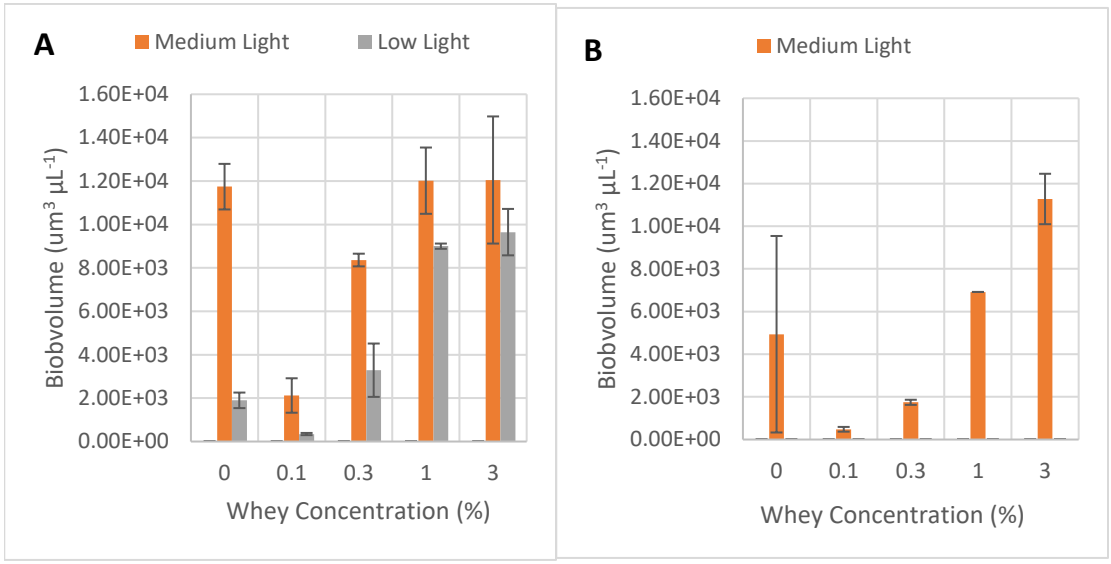

Figure 2.18 Total biovolume ($\mu m^3\ \mu L^{-1}$) of particles classified as algal (mean ± standard deviation) at medium and low light for (A) *R. salina* and (B) medium light for *T. suecica.*

Table 2.9 Total biovolume ($\mu m^3\ \mu L^{-1}$) of particles classified as algal (mean ± standard deviation) from the culture screening experiments. The number of wells used to calculate the mean fluorescence is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significance and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with an "*" in the table. In some cases, there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

		Biovolume (um3 µL-1)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	2682 ± 148	2	1349 ± 1010	2	2127 ± 1286	2	1555 ± 1593	1	4413 ± 1075	4
	ML	3706 ± 195	2	1416 ± 977	2	2109 ± 1368	2	2796 ± 1577	2	2686 ± 1097	4
	LL	2803 ± 1053	2	1635 ± N/A	1	2991 ± N/A	1	5340 ± N/A	1	6417 ± N/A	1
T. suecica	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	3032 ± 1544	3	203 ± 47	2	741 ± 51	2	2936 ± N/A	1	4786 ± 502	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	1137 ± 1506	2	407 ± 305	2	1094 ± 247	2	10517 ± 1437	2	15036 ± 1749	4
	ML	13261 ± 15260	5	1568 ± 856	2	9214 ± 4999	2	3997 ± 3799	2	13482 ± 652	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. tricornutum	HL	16478 ± 6549	4	2806 ± N/A	1	10750 ± N/A	1	13182 ± N/A	1	15056 ± 1100	4
	ML	13047 ± 1562	2	3188 ± 456	2	11965 ± 10711	2	27302 ± N/A	1	19574 ± 4488	4
	LL	18258 ± 2719	4	4352 ± 1084	2	15923 ± 5169	2	18228 ± 2283	2	14037 ± 1409	4
N. oculata	HL	29117 ± 23674	4	N/A	0	N/A	0	N/A	0	N/A	0
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	42633 ± 42909	2	15695 ± 20522	2	49077 ± 15820	2	57155 ± 1297	2	59609 ± 12125	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	4984 ± 446	2	900 ± 337	2	3549 ± 124	2	5101 ± 648	2	5114 ± 1243	4
	LL	1897 ± 358	2	344 ± 51	2	3286 ± 1229	2	9000 ± 121	2	9648 ± 1069	3

While there was evidence of an increase in biovolume with certain species and concentrations of whey permeate, it was unclear if the increase in biovolume was the result of an increase in the number of algal cells, an increase in cell size, or an increase in aggregates of cells. The flow cytometer measures each event but does not distinguish between a single cell and an aggregate of cells. Looking at the changes in event biovolume size for each treatment at the 10th, 50th, and 95th percentile there was evidence of a potential shift towards larger cells or aggregates forming when *T. suecica, R. salina,* and *P. lutheri* are grown with whey permeate (Appendix – Figure A.4). However, in all three of these species, there was only a significant increase in biovolume with *P. lutheri* at medium light with 0.3 and 3% whey permeate.

In the case where mixotrophy results in down-regulation of pigments (Abreu et al., 2012), it was expected that the biovolume-specific fluorescence (FL3/ESV) of a mixotrophic culture will decrease compared to a phototrophic culture. A significant reduction in the biovolume-specific fluorescence of algal cells can be seen in at least one light level and amendment concentration for *T. pseudonana, P. lutheri, P. tricornutum,* and *R. salina* (Appendix – Table A.5). This reduction was observed across the frequency distributions at the 10^{th}, 50^{th} and 95^{th} percentiles in most of the amended cultures in *T. pseudonana* and *P. lutheri*. In *P. tricornutum* and *R. salina,* significant reductions were found at all amendment concentrations. There were no significant changes in *T. suecica* and *N. oculata*.

2.3.4 Flow Cytometric Analysis of Other (Non-Algal) Populations

Because the flow cytometer used in this study was not capable of sorting samples based on their scattering and fluorescence characteristics, it was not possible to determine if the events classified as "other" were algal cells with heavily down-regulated pigments, bacteria, or colloids. In general, the total biovolume for the "other" data followed a similar trend to the algal biovolume, with the mean values increasing with concentrations of whey permeate (Table 2.6). However, because of high variability between replicates, the differences were only significant ($p < 0.05$) in two cases. In *T. suecica* at medium light with 3%, there was a significant increase with whey concentration, and in *T. pseudonana* at high light there was a significant decrease.

The relationship between total biovolume of algae and the other material varied greatly between species (Figure 2.11). In some cases, the total other biovolume was significantly ($p<0.05$) lower than algal biovolume (Figure 2.11 A) but in others, it was comparable to the algae biovolume at most treatments (Figure 2.11 B).

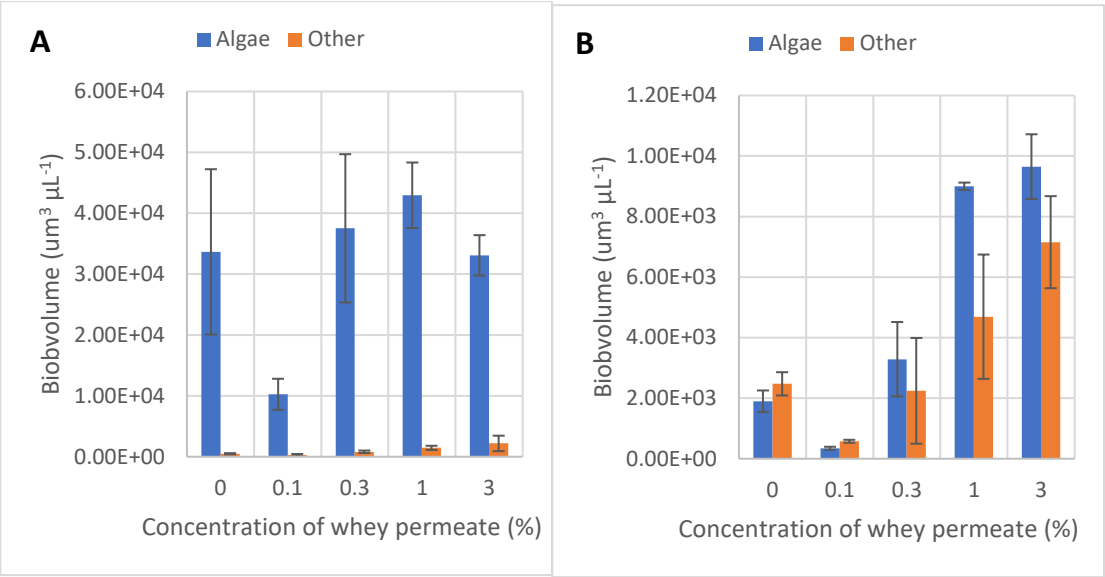

Figure 2.20 Variation in total biovolume with the concentration of whey permeate (mean ± standard deviation) for events identified as algae and "other" by flow cytometry for (A) *P. tricornutum* at low light, and (B) *R. salina* at low light.

Table 2.12 The average biovolume (um^3 μL^{-1}) of other events plus or minus the standard deviation of the data from the culture screening experiments. The number of wells used to calculate the mean biovolume is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significance and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with an "*" in the table. In some cases, there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

Whey Permeate - Other Biovolume (um3 µL-1)											
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	1537 ± 62	2	444 ± 198	2	424 ± 88	2	333 ± 181	1	639 ± 122	4
	ML	2575 ± 1036	2	650 ± 306	2	2977 ± 786	2	5261 ± 2526	1	4930 ± 2863	4
	LL	1409 ± 1228	2	128 ± 0	1	128 ± 0	1	1504 ± 0	1	334 ± 0	1
T. suecica	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	55 ± 28	3	74 ± 55	2	227 ± 24	2	599 ± 0	1	2687 ± 396	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	783 ± 683	2	486 ± 271	2	679 ± 52	2	470 ± 35	2	643 ± 444	4
	ML	843 ± 620	5	174 ± 114	2	254 ± 148	2	258 ± 180	2	1639 ± 199	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. tricornutum	HL	338 ± 89	2	123 ± 0	1	262 ± 0	1	136 ± 0	1	118 ± 0	4
	ML	1908 ± 1590	2	232 ± 33	2	356 ± 260	2	409 ± 79	2	1365 ± 313	4
	LL	253 ± 33	4	142 ± 44	2	142 ± 44	2	891 ± 50	2	1110 ± 427	3
N. oculata	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	6557 ± 4590	2	807 ± 667	2	807 ± 667	2	5546 ± 64	2	15959 ± 4347	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	946 ± 115	2	180 ± 21	2	896 ± 143	2	2746 ± 278	2	3442 ± 1104	4
	LL	2474 ± 385	2	576 ± 49	2	576 ± 49	2	4692 ± 2053	2	7154 ± 1520	3

2.3.5 Abiotic Fluorescence

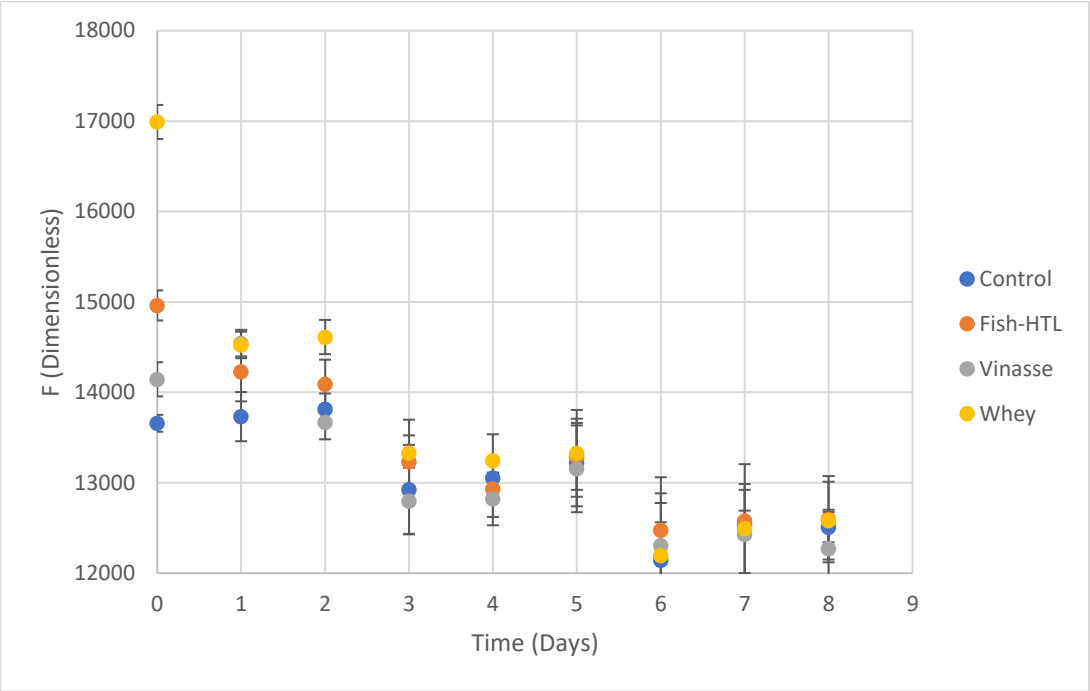

Figure 2.22. Average fluorescence of abiotic plate over time for L1 medium (Control) and L1 medium amended with 1% whey permeate, vinasse or fish-HTL. Data are shown as mean ± standard deviation of 6 replicates.

Understanding the potential influence of abiotic factors on the background fluorescence of media was essential, considering the significance of fluorescence in the analysis. Figure 2.13 shows that all amended media had a higher initial fluorescence than the control, with whey permeate-amended media significantly higher than all other amendments ($p < 0.05$). Fluorescence dropped rapidly in the first 24 h in the whey permeate-amended media to become much closer to the other amendment types. There was a drop in all media on days three and six.

Comparing the results of the abiotic plate to a biotic plate (Table 2.7, Figure 2.13, 2.14) showed that among the control, fish-HTL and vinasse blanks, the difference in fluorescence over 8 days (1200 – 2400 counts) was small (4-7%) relative to the initial

fluorescence of *T. pseudonana* grown in the same media (mean 31100 counts). However, the significantly higher initial fluorescence of the abiotic whey permeate-amended medium was equivalent to 12.5% of the initial fluorescence in the culture. The decline in background fluorescence in the whey permeate-amended medium (4400 counts) was equivalent to 24% of the increase in the culture through the growth phase (18100 counts). Even so, it was unlikely that this higher initial fluorescence significantly impacted estimates of growth and biovolume, as the first few days of growth (when the background fluorescence is falling) were typically in lag phase of growth (see Figure 2.8). The reduction in fluorescence over the first 24 hours of growth was visible but has little effect on the curve fitting. A few species had a lag-phase shorter than three days; in these cases, the higher fluorescence might have influenced growth rate calculations. In these cases, the increased fluorescence values in the cultures were often an order of magnitude larger than the reductions in background fluorescence. Overall, it was unlikely that the amendment fluorescence was likely to have significant impacts on observed fluorescence beyond the first 24 hours.

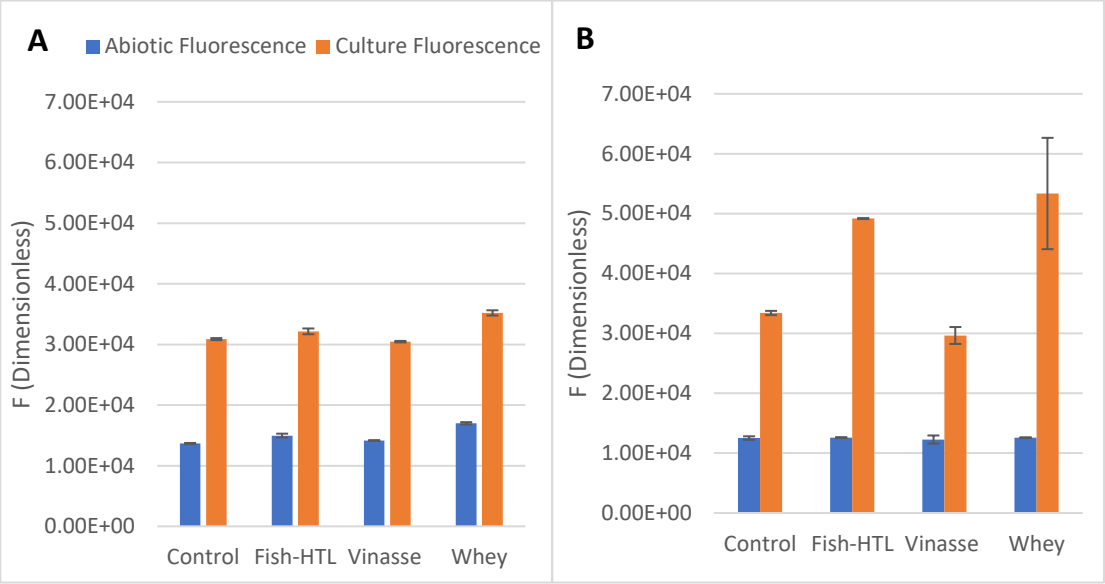

Figure 2.23 The initial (A) and final fluorescence (B) of the abiotic plates and plates of *T. pseudonana* grown with 1% amendments at medium light presented as mean ± standard deviation. Fluorescence data was taken from the plate reader at day 0 (A) and day seven for the abiotic plate and day six (B) for *T. pseudonana*.

2.4 Discussion

The ability of microalgae to grow mixotrophically appears to be a favorable trait. There's a wide range of microalgae species and strains capable of mixotrophic growth in the oceans. Mixotrophy provides a significant ecological advantage, allowing an alga to grow on both light and organic substrates and with lower reliance on light availability.

In the screening experiment, whey permeate, fish-HTL, and vinasse supported algae growth in at least one treatment condition, in all the species tested. However, there were often reduced growth rates or biovolume yields compared to the control. Since there is no phosphorous in the media beyond the waste amendments, this suggests that the algae can utilize the amendments as a source of phosphorous. These results are consistent with previous studies, which have found that certain species and strains of algae can grow

on whey permeate, vinasse, and the aqueous phase of hydrothermal liquification of biomass (Abreu et al., 2012; Biller et al., 2012; Canido & Lombardi, 2017).

Of the three amendments, whey permeate proved to be the best option. It consistently supported the growth of all species of algae and often resulted in yields similar to the control at certain concentrations. In contrast, the treatments of vinasse and fish-HTL showed a significant reduction in biomass — to the point of little to no growth, with some concentrations. Low yields at low amendment concentrations of 0.1% and 0.3% could be due to phosphorous limitation (Figure 2.4). However, yields were also low at higher concentrations, which have sufficient phosphorous levels to support growth.

Considering that whey permeate supported growth, while the fish-HTL or vinasse could not, it suggests something in these other amendments inhibit algae growth. This trend is consistent with literature that suggests the aqueous phase of HTL of biomass and vinasse can contain toxic compounds which inhibit algal growth (Canido & Lombardi, 2017; Leng et al., 2018). Evidence shows waste amendments can support growth rate enhancement and biovolume if the growth is mixotrophic (Mohammad et al., 2014; Bashir et al., 2019; Zanette et al., 2019). This was the expectation for the experiment. Algae have more phosphorous available with at least one concentration of food-grade waste than in the control culture and the wastes contained organic compounds with a potential to enhance productivity. However, more commonly, there was no enhancement or a reduction in growth rate or biovolume. These observations may have resulted from nutrient limitation, inhibitory effects of amendments, or an unexpected consequence of mixotrophic growth.

Only whey permeate produced biovolume yields similar to the control treatments, although all wastes contained sufficient phosphate to support algal growth. (Phosphate was the only nutrient in the control media omitted from the waste-amended ones.) The addition of whey permeate significantly reduced stationary-phase fluorescence, apparent growth rates, and biovolume-specific fluorescence in multiple species at phosphate concentrations higher than the control media.

The excess phosphate is not expected to result in a down-regulation of fluorescence. And these lower fluorescence measurements are not generally associated with reduced biovolume. In addition, the whey permeate cultures do not appear to show the inhibition of the biovolume yields seen with fish-HTL and vinasse. Therefore, the most likely explanation is that many of these algae species were growing mixotrophically. Although the expected enhancement to growth rates and yields were not observed, this may be due to limitations in the methodology of the screening experiment.

2.4.1 Increasing Growth Rates with Whey Permeate

Under Blackman limitation, it's expected that algal growth rates will increase with light availability, until they become limited by dark reactions or photoinhibition. The highest light level used in the screening experiment, 190 µmol photon m^{-2} s^{-1}, is below the intensity at which photoinhibition of growth occurs in most cultures (MacIntyre et al., 2002), although inhibition has been documented at 100-250 µmol photon m^{-2} s^{-1} in deep-water isolates of *Prochlorococcus* adapted to low light (Moore et al., 1995).

The isolates in this study came from surface, near-shore waters and should be adapted to relatively high light. All but two species, *T. pseudonana* and *R. salina*, followed the expected trend of growth rates increasing or saturating with light level in the control treatments. In *T. pseudonana*, the exponential growth phase lasted less than three days. The short duration could result in significant underestimation of growth rate. It is not clear why the growth rate was so low in the high-light culture of *R. salina*. In the tubes of acclimated culture used to inoculate the plates, growth rates were significantly higher: 0.56 d^{-1} over 80 days vs 0.14 d^{-1} in the plate cultures.

Multiple studies have found enhancement of algal growth rates when grown mixotrophically (Mohammad et al., 2014; Bashir et al., 2019; Zanette et al., 2019). However, no combination of species, light, and concentration of whey permeate in the screening experiment showed growth-rate increases. Instead, significant reduction in the apparent growth rate occurred in multiple cases. The apparent growth rate is based on the change in fluorescence which will be reduced in a mixotrophic culture that down-regulates pigments, as frequently observed in mixotrophic cultures (Abreu. et al., 2012), so may underestimate growth rates where down-regulation occurs.

Down-regulation of fluorescence can be seen in the flow cytometer data. Multiple species showed a significant reduction in biovolume-specific fluorescence. Unfortunately, the small volume of cultures in the plates made it impossible to sample through the growth phase, so it is unknown when this down-regulation occurred in the experiment. A reduction in fluorescence in the exponential phase could have masked the growth of algae. That would explain the lower apparent growth rates. The down-regulation could also have occurred during the stationary phase, when phototrophic

cultures of algae may have affected pigments in response to nutrient limitation. If mixotrophic pigment down-regulation did occur in early growth of mixotrophic cultures, it could result in a similar biovolume-specific fluorescence as a control culture that had down-regulated pigments in stationary phase when both cultures were harvested.

At various concentrations of whey permeate, there was a significant ($p<0.05$) reduction in the stationary-phase fluorescence of the cultures from the plate reader, across multiple species and light levels. However, being a measure of total fluorescence, the product of biovolume and biovolume-specific fluorescence, this did not necessarily translate to significant down-regulation in pigments. Therefore, the stationary phase fluorescence is unlikely to be a reliable method of determining whether cultures grew mixotrophically. A more reliable method would be to measure the biovolume and biovolume-specific fluorescence throughout the experiment. Adopting this methodology in the screening experiment was not possible with the small volumes of the culture used. However, growing larger volumes to allow daily sampling of cell counts would have allowed testing of fewer species and wastes.

Fluorescent material in the whey permeate may also have caused underestimates of growth rates. While fluorescence largely decreased within 24 hours and appeared similar to control media over 1-2 days, it was significantly different from the control media on the first day. There was consistent but not always significant evidence of higher initial fluorescence with increasing concentrations of whey (Appendix – Table A.3). The decrease in the background fluorescence during the early stages of growth could result in underestimations of algae growth based on fluorescence.

While methodology may have reduced reliability, it is also possible that the apparent growth rates reflected changes resulting from the whey permeate additions. Whey permeate is capable of stimulating mixotrophic growth in some species of microalgae (Yadav *et al.,* 2015). Multiple studies have found that mixotrophically grown phytoplankton have higher lipid quotas than photoautotrophic cultures (Abreu *et al.,* 2012; Cheirsilp & Torpee, 2012; Baldisserotto *et al.,* 2021). Lipids, energy-dense molecules, could slow the cell division rate. Data from Rodolfi *et al.* (2009) show a negative correlation between lipid quota and specific growth rate between species (Appendix Figure A.1). Where algae produce higher lipid quotas under mixotrophic growth, there may be a corresponding reduction in growth rates compared to a photoautotrophic culture.

Growth inhibition was evident in the reduced stationary-phase biovolumes in cultures grown with fish-HTL and vinasse. Whey permeate does not contain the toxins or inhibitory compounds found in fish-HTL and vinasse. However, it does contain high concentrations of organic carbon in the form of lactose. Evidence shows that substrate inhibition can occur with high concentrations of organic carbon, which can reduce the activity of some enzymes essential for growth (Pang et al., 2019). Therefore, it's possible that the treatments that show reduced growth at high concentrations of whey permeate may have resulted from substrate inhibition with the high concentration of organic carbon.

Due to limitations on the methods used and variability in results, there are no clear or consistent trends across all species or light levels. While it is clear growth is possible

in all examined species with mixotrophic growth, the exact response of a species to a specific concentration of whey can be highly variable.

2.4.2 Increasing Yield with Whey Permeate

According to Liebig limitation, increasing limiting nutrient(s) will increase the final yield of algae. In the control cultures, the expectation is that carbon is the limiting nutrient as the molar C:N:P ratio in the medium is 56:41:1, which is deficient in carbon compared to the Redfield Ratio (106:16:1). All three food-grade wastes contained enough phosphorous to exceed the control phosphorous concentrations at an addition of 1% or 3% but only the whey permeate consistently supported growth at 1% and 3% treatments. In the whey permeate treatments, the limiting nutrient is likely to change as the concentration of whey permeate is increased. Because inorganic carbon and nitrogen in the whey permeate are low, their availability will not differ much from the control with increasing concentrations of the amendment. In contrast, phosphorous is likely to change from being limiting at low whey permeate additions to being in excess at higher concentrations. Therefore, it was expected that there will be a lower yield of biovolume at lower concentrations and an increase in the yield at high concentrations of whey permeate. In the 0.1% and 0.3% whey permeate treatments, there is only 6.7 μM and 18.6 μM of phosphorous compared to the 35 μmol of phosphorous of the control medium. In these treatments, there was lower biovolume on average, although the differences were not significant because of the high variability between replicates. In the 1% and 3% treatments, the phosphorous concentrations were 62 μM and 175 μM respectively, much higher than what was available in the control. There was a significant increase in the

biovolume only at some light levels in *P. lutheri* and *R. salina*. Differences from the controls at other light levels and in other species were not significant because of high variability between replicates.

The flow cytometric analyses consistently show a trend in which there was a change in the frequency distributions of forward angle scatter, a proxy of cell size, and chlorophyll fluorescence in whey permeate-amended cultures. In these treatments, there is no longer the clear distinction between bacteria and algae that is seen in the control cultures but a single large population that stretches to larger sizes and higher fluorescence. One possible reason for this is the formation of aggregates, possibly facilitated by algae-bacterial interactions (Powell and Hill, 2014). In some cases, the events which confidently are identified as containing algae based on fluorescence can be an order of magnitude brighter or larger than the control populations. These are more likely to be aggregates than single cells, though their exact composition is unknown.

Algae may be forming aggregates with bacteria resulting in larger aggregates. But it is also possible that the bacteria are competing with the algae for the resources in the food-grade wastes. Competition or even inhibition from bacteria could explain the low yields of algae in some high concentrations of food-grade wastes. However, because algae may be down-regulating pigments it can be difficult to distinguish between algae and bacteria in the data. In addition, an increase in bacteria may not inhibit algae yield but could even increase it. The relationships between algae and bacteria are diverse and can range from positive to negative. It seems unlikely that bacteria have a significant impact on most treatments. In most cases, even if the increase in other events below the threshold to be algae are assumed to be bacteria, this population is generally smaller than

the population of algae. The influence of bacteria on algae growth is important, especially in larger-scale algae industries, but examining that relationship with food-grade wastes is beyond the scope of this study.

Overall, there was little impact of varying light levels on the final yields of culture, as biovolume. This makes sense as the factor determining yield, through Liebig limitation, is nutrient availability and this did not vary between light levels. There was no evidence for mixotrophic growth on the organic-rich wastes reducing Blackman limitation by accelerating growth, as would be expected at low light levels where the photoautotrophic growth rate is reduced. This may be because the limitations of the experimental approach prevented it from being observed.

2.4.3 Limitations of Experiment

Previous studies with the species used in the screening experiment have found evidence of mixotrophy enhancing growth rates or biomass yields (Wang *et al.,* 2012; Mohammad *et al.,* 2014; Shene *et al.,* 2016; Bashir *et al.,* 2019; Zanette *et al.,* 2019; Baldarrissio *et al.,* 2021). This trend was not consistently seen in the screening experiment. This may be the result of underestimations of growth rates and the number of cells due to using fluorescence to monitor and identity algae.

Fluorescence was used throughout the screening experiment for monitoring and identifying algal cells. Fluorescence has the advantage of being fast, sensitive, and non-destructive but is not an accurate method for assessing biomass, especially with mixotrophic cultures. Screening multiple species of algae under different conditions with replication requires that a very large number of samples be monitored. This meant in

order to complete the experiments within a reasonable time-frame, the focus was on reducing the scale (volume) of cultures and maximizing efficiency, rather than cultivating larger volumes of culture that would be assessed with different more time- and volume-intensive methods. However, the culture screening experiment allowed the identification candidate species and conditions of interest to examine in more detail in later experiments.

The plate reader used in the experiment was very efficient as it allowed for multiple cultures to be read in a small fraction of the time it would require with a Turner 10AU fluorometer and FIRe fluorometer, as was used to maintain the parent cultures in balanced growth. The latter could have provided useful information to try to better understand how light harvesting and pigment quotas might have changed during the experiment. Unfortunately, as carbon limitation of the initial plate method was found midway through the culture screening experiment, some treatments had only half the number of desired replicates. As the plates could not all be inoculated and monitored at the same time because of limited space on the light table, the approach was continued so that all the plates were grown with the same methods. The limited number of replicates does make drawing conclusions more difficult as there were cases where two replicates were very different, but there were limited other data to compare the results against. The plate reader is a very effective tool for screening a large number of cultures but it would be better to ensure all the wells of a plate obtain sufficient carbon dioxide to support growth to increase replication.

As it was known that fluorescence may be an unreliable proxy for biomass, a flow cytometer was used to evaluate the final biovolume of the cultures. Unfortunately, this

instrument also relies partially on fluorescence for the identification of events. While the distinction between algae and bacteria is clear with the control cultures, it was not in food-grade waste-amended cultures. In these cases, it was decided to classify the events which were most likely to be algae using the same threshold as the controls. However, there may be an underestimation of the algal biovolume as algal cells in which pigments have been down-regulated may be not counted and bacteria may be counted as algae if they are a part of an algal/bacterial aggregate. These limitations could be avoided by using an imaging plate reader. This would give more reliable classification and more accurate estimates of biovolume than using forward-angle scatter as a proxy. Unfortunately, none was not available during the screening experiments.

2.4.4 Discussion Summary

Whey permeate was the most effective food-grade waste in supporting the growth of algae across multiple concentrations and light levels. This suggests that at a minimum, the phosphorous in whey permeate is accessible to algae. Increasing the concentration of whey did often increase the yield but its effect on growth rate is difficult to discern, so Hypothesis 1, that whey permeate would increase growth rate and yield, can be rejected. There is a possibility that the growth rate and yield both increased significantly, but the changes couldn't be detected due to limitations in the methods used for the screening. Contrary to expectations with Hypothesis 2, that the highest concentrations of whey permeate would inhibit growth, 3% whey permeate did not inhibit the growth of algae with any observed species as there was no significant reduction in biovolume compared to the control cultures. Although there are cases of significantly reduced apparent growth

rates at 3% whey permeate, this is likely the result of reduced biovolume-specific fluorescence, not necessarily a reduced growth rate. Finally, Hypothesis 3, that increasing light levels would increase growth rates in mixotrophic cultures in the mixotrophic cultures, was also rejected.

The goal of the culture screening experiment was to evaluate multiple species of microalgae grown at different light levels and with different concentrations of food-grade wastes. This resulted in a very large number of samples: over 1260 cultures were grown to evaluate all the conditions. The experiment focused on gathering data on a large range of cultures as opposed to looking at a single culture in more detail. While the approach clearly demonstrated that whey permeate was a better growth substrate for all species, broader conclusions about possible stimulation of growth rate and/or yield were difficult to reach because of limitations in the methods. There are other instruments and techniques which could have been used to gather better data, but these are more time intensive and would not have allowed the large range of cultures and conditions to be evaluated. However, the approach did allow promising candidate species and wastes to be identified which could then be evaluated in greater detail. *T. suecica* was chosen as the candidate species because it is fast-growing and widely used in the aquaculture industry. *T. suecica* did not significantly reduce growth rates at medium and high light and had biovolume yields comparable to the control cultures with 1 and 3% whey permeate enrichment.

CHAPTER 3 –BIOMASS AMD COMPOSITION OF *TETRASELMIS SUECICA* GROWN WITH WHEY PERMEATE

3.1 Background

Algae are highly effective at adapting to a wide variety of different conditions and up- or down-regulate their metabolism and composition in response to variations in light, nutrients, or temperature. To grow algae on a large scale, it is important to understand how they respond, as they could increase or decrease a valuable compound in response to the growth conditions. The Dynamic Balance model (Geider et al., 1996, 1998) can be used to predict changes in the chl-a/carbon ratio and growth rates of phytoplankton, through resource allocation to light-harvesting, catalytic and structural components, or carbon storage. The model is based on the redox state of the electron transport chain. Under high-irradiance conditions, electrons will become available faster than can be used by downstream metabolism. In this case, the algae would down-regulate light-harvesting to match the rate of the Calvin cycle and direct resources elsewhere. Conversely, if there is lower light availability and electrons cannot be produced fast enough to keep up with the Calvin cycle, light-harvesting will be increased to better meet the demands of the algae. While the model does not directly consider carbon availability, the model assumes Rubsico is the slowest step of the dark reactions and thus, the downstream demand is set by the Rubisco pool size.

The Dynamic Balance model does not include mixotrophy, but it can be considered in this framework. The addition of whey permeate may increase CO_2 availability as algae use the organic carbon for heterotrophic growth. If this occurs in the

light, it could increase the catalytic rate of Rubisco if CO_2 is rate-limiting, which the model predicts would drive up-regulation of light harvesting (chlorophyll a). However, when algae are grown mixotrophically, there is often a down-regulation in light-harvesting (Roth et al., 2019). The Dynamic Balance model would suggest the light-harvesting is down-regulated to match the slower down-stream reactions (Geider et al., 1996). Instead, it may reflect down-regulation of both Calvin Cycle enzymes and pigments by high internal glucose (Roth et al., 2019). The first step in lactose metabolism is hydrolysis to produce glucose and galactose.

A study by Ho *et al*. (2003) indicates that *T. suecica* does not follow the Redfield ratio but instead has a C:N:P ratio of 199:26:1. The media used in the experiments were made with tangential-flow-filtered seawater from the NRC facility in Ketch Harbour, Nova Scotia, Canada. Shadwick et al. (2011) estimated the dissolved inorganic carbon to be between 1900-2000 μmol L^{-1} depending on the time of year and direct measurement on the seawater are consistent with this (Brenan Duhamel, unpublished). Based on this, the molar DIC:DIN:DIP ratio of the unmodified L1 media used in the experiments is estimated to be between 53-56:24:1. Consequently, carbon is likely to be limiting to growth of *T. suecica* even with the supply of additional phosphorous. The addition of inorganic carbon to enhance algal growth has been well studied (Mohemiani, 2013, White et al. 2013, Qi et al., 2019) with additions of either carbon dioxide or bicarbonate. Bicarbonate is an inorganic carbon available to algae through natural dissociation to CO_2 or more effectively through facilitated dissociation by the enzyme carbonic anhydrase, possibly with directly uptake through a carbon concentrating mechanism (CCM; Raven et al., 2014, White et al., 2013). Additions of bicarbonate take advantage of algae's existing

CCMs to increase the internal CO_2, notably around the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), to reduce the likelihood of its oxygenase activity (photorespiration).

The addition of bicarbonate will increase the carbon available to the algae, ideally increasing it to an amount that better fits the requirements of *T. suecica*. White et al. (2013) found that 1g L^{-1} (0.0119 M) of bicarbonate was the optimal addition to enhance the productivity of *T. suecica*. The addition of 1 g L^{-1} would be expected to raise the molar DIC:DIN:DIP ratio to 330:24:1, bringing carbon in excess of the biomass demand determined by Ho et al. (2013). If the yield of *T. suecica* increases with such a carbon addition, it would demonstrate that carbon was the limiting resource according to Liebig's Law. Further, it is also possible that increasing carbon availability could also alleviate Blackman limitation and increase the growth rate of the algae. If the bicarbonate addition increases the yield or growth rate of *T. suecica* even when there was a 1% whey permeate addition, it would suggest that algae are not processing the organic carbon in the whey permeate (primarily lactose) in the same way it uses CO_2 and bicarbonate in the DIC pool. Instead, it is possible that the glucose derived from lactose is being used directly in storage molecules or cell synthesis (Perez-Garcia 2015, Roth *et al*., 2019), rather than being respired to CO_2 and used in photosynthesis.

The screening experiments showed that whey permeate can support growth of *T. suecica* and at a 1% concentration supports yields similar to the control treatments. However, it is not clear how mixotrophic growth may change the investment in light harvesting and internal cell quotas. The goal of the larger-scale experiments with the biomass harvest and bicarbonate enrichment experiments is to evaluate how the whey

permeate addition may change how *T. suecica* allocates resources as it grows and is in the stationary phase. The first hypothesis is that whey permeate will support *T. suecica* yields and growth rates similar to the control cultures in tubes or flasks as has been previously seen in plates (Chapter 2). These differ in whether they allow diffusional gas exchange (plates and flasks) or don't (capped tubes). The second hypothesis is that *T. suecica* grown with whey permeate will reduce investment in the light harvesting apparatus as it shifts towards more heterotrophic growth. The experiments were designed to get a better understanding of how resources are allocated in algal cells and how mixotrophy and increased nutrients may impact an algal cell.

3.2 Methods

3.2.1 Biomass Composition Experiment

Based on the screening experiment (Chapter 2), *Tetraselmis suecica* was selected as an ideal candidate species for the harvest experiment. A larger final volume of culture was required to evaluate the effect of 1% whey permeate on the growth rate and protein, lipid, and chlorophyll quotas. *T. suecica* was grown semi-continuously at 80 μmol photons m^{-2} s^{-1}, a light level between the medium and high light treatments in the screening tests. A parent culture used to inoculate the experimental treatments was grown in L1 media until in balanced growth, using the methods described in Chapter 2.2.2. Intermediate light was chosen as, in the screening experiment (Chapter 2), there was no significant difference in apparent growth rate between the medium and high light in controls cultures of *T. suecica*. Using high light would not be cost-effective at commercial scale if it did not result in faster biomass production. Cultures were

considered to be in balanced growth where daily growth rate and F_v /F_m had a difference of less than 10% (coefficient of variation) for ten generations. Once in balanced growth, cultures were grown in three 40-mL tubes to increase the culture volume. Each was then used to inoculate 500 mL of media in 2-L flasks. The tops of the flasks were covered with a cheesecloth and cotton bung to allow for gas exchange (Guillard, 1975). Four sets of 3-replicate flasks were prepared during the experiment at different times as only three flasks could fit on the light table at a time. Two sets of flasks were prepared for the control and two sets were prepared for the whey permeate treatment. For the control and whey permeate treatment one flask was grown to the stationary phase and the other flask was grown into the exponential phase. Flasks were well mixed daily by carefully swirling the media before taking a 4-mL subsample in a sterile Laminar flow hood. The subsample was placed in a 6mL borosilicate tube which could be read on a fluorometer and used for chl-a analysis.

Three control and three whey permeate flasks were each grown to the stationary phase first to determine an expected final yield of *T. suecica* growth in a flask. Once a flask was determined to be in stationary phase (See Chapter 3.2.3), it was harvested. Once the stationary phase was determined for the control and 1% whey permeate flask, a new set of flasks was grown to be harvested in the exponential phase. These were prepared in the same way as the stationary-phase c but were harvested when the fluorescence of the culture was around 60% of the value in the stationary phase. Upon harvesting the stationary and exponential phase flasks, 1 mL of culture was flash-frozen in liquid nitrogen in cryovials with 4 µL of glutaraldehyde for cell counts (Gasol and De Giorgio, 2000). Each flask was filtered to obtain three samples for chl-a, total dissolved

phosphate, and protein analysis. Total dissolved phosphate samples were measured using the same methods described in Chapter 2.2. Monitoring the pH of the flasks during growth was considered but the pH probe of the lab was not functioning during this phase of the experiments.

3.2.2 Bicarbonate Enrichment Experiment

A smaller-scale mixotrophic experiment was performed after the flasks to evaluate how whey permeate and bicarbonate additions influenced the biomass, photosynthetic apparatus, and Chl-a quota of *T. suecica*. Tubes with gas-impermeable caps were used instead of flasks to minimize gas exchange and so allow for greater control of carbon availability throughout the experiment. By preventing significant gas exchange, the effect of the additional carbon supplied through whey permeate and bicarbonate could be better observed. In addition, the smaller volume tubes allowed for multiple replicates and treatments to be run simultaneously, as available space only allowed for a maximum of three flasks to be grown at the same time A culture in balanced growth at intermediate light was used as an inoculant for the treatment tubes. Three replicates were prepared for each treatment, which consisted of different media compositions: L1 media as the control (L1); L1 media with 1 g L^{-1} (11,900 µmol L^{-1}) of sodium bicarbonate (L1-B); L1 media with 1% whey permeate (L1-W); and L1 media with 1% whey permeate and 1g L^{-1} of bicarbonate (L1-W&B) (Table 3.1). The culture tubes were grown at intermediate light and subsampled daily for cell counts and measurements of fluorescence and chl-a. Cultures were well mixed prior to subsampling by inverting each tube at least five times. This experiment continued until cultures were

determined to have been in the stationary phase for the equivalent of three generations in the exponential growth phase (See Chapter 2.2.3). On each day of the experiment, subsamples for cell counts and chl-a were taken to help characterize how yield and chl-a changes each day when grown mixotrophically. There was not a sufficient volume of culture from this experiment to evaluate the biomass composition for proteins or phosphates, as was done in the biomass composition experiment.

Table 3.1 Estimates of phosphorous and nitrogen from the control (L1), 1% whey media (L1-W) with (L1-W&B) and without 1 g L^{-1} bicarbonate additions (L1-B). Phosphorous content here is in the form of phosphate and nitrogen is the form of nitrate and nitrite. Organic carbon estimates are made assuming whey permeate is 85% lactose and assuming all organic carbon in the form of lactose is available to algae.

	Dissolved Organic Carbon (DOC; µM)	Dissolved Inorganic Carbon (DIC, µM)	Dissolved Inorganic Nitrogen (DIN, µM)	Dissolved Inorganic Phosphorus (DIP, µM)	Molar ratio of DOC:DIC:DIN:DIP
L1	0	2000	880	36	0:56:24:1
L1-W	298000	2000	894	62	4806:32:14:1
L1-B	0	14000	880	36	0:389:24:1
L1-W&B	298000	14000	894	62	4806:226:14:1

3.2.3 Fluorescence Monitoring

Chlorophyll a fluorescence was monitored for the duration of the biomass composition and bicarbonate enrichment experiments using a Turner 10AU fluorometer (Turner Designs, USA) and a FIRe fluorometer (Satlantic, Canada). The fluorescence from the Turner fluorometer was used to monitor growth using fluorescence as a proxy for biomass. Using fluorescence as a proxy for biomass has limitations, as discussed in Chapter 2; however, as cell counts were used for a more accurate measure of yield, the

fluorescence provides a rapid method to track when cultures reach stationary phase. It was determined that the culture had reached the stationary phase based on fitting the data to the modified Blackman's bilinear model (Chapter 2, Equation 2). While the Turner fluorometer was useful for the monitoring of chl-a fluorescence and growth, the FIRe fluorometer provided further insights into the investment in light harvesting. Fitting the fluorescence induction curve (Gorbunov and Falkowski, 2004; Oxborough et al., 2012) gives estimates of the concentration of functional reaction centers (variable fluorescence, F_v, relative units), the proportion of functional reaction centers (variable fluorescence ratio F_v/F_m, dimensionless), the size of the Photosystem II antenna (σ, $Å^2$) and photosynthesis turnover time ($1/\tau$, ms).

3.2.4 Chlorophyll a Analysis

Chlorophyll a measurements were performed based on the methods described by Welschmeyer (1994). Chlorophyll analysis was performed on either a directly pipetted sample (MacIntyre & Cullen, 2005) or a culture collected on a Whatman GF/F glass-fibre filter (effective pore size of 0.7 μm) with a vacuum pump at low pressure (<17 kPa) under green light. Filters were placed in scintillation vials and covered with 6 mL of a 3:2 (V:V) mixture of DMSO: 90% acetone (Shoaf and Lium, 1976) to extract chlorophyll a. Samples were frozen at -20 °C if they could not be measured immediately. Blank-corrected fluorescence was converted to an estimate of chlorophyll a concentration using a standard curve measured with purified chlorophyll a (Sigma C6144).

3.2.3 Protein analysis

For the protein samples, 3 or 6 mL of culture from stationary and exponential phases, respectively, was filtered onto a pre-combusted 25-mm Whatman GF/F glass filter with a vacuum pump at a low pressure (<17 kPa) using a funnel that was rinsed with 10 mL of 0.22 μm-filtered seawater. A 10-mL filtered seawater sample was also collected as a blank. Both the filtered culture and blank were frozen in Petri dishes until they could be analyzed.

Protein concentrations were estimated in protein samples and standards using a modified Bradford assay with purified bovine serum albumin (Sigma-Aldrich P0914) as a standard (Appendix- Figure A.3). Methods were modified from the user guide associated with the Pierce Detergent-Compatible Bradford Assay Reagent (Thermo Scientific, catalog number: 23246). Samples were incubated in a water bath at 95 °C for 30 minutes in 15-mL HDPE centrifuge tubes with 2 mL of 0.1% Triton X-100 lysis buffer. The contents were then sonicated on ice for 1 minute with alternating periods of 15 seconds of sonication and 15 seconds of rest. They were left to react at room temperature for 30 minutes, after which they were centrifuged at 15,000 x g for 30 minutes. The supernatant was pipetted out and transferred to clean Falcon tubes for analysis. An equal volume of Pierce Detergent-Compatible Bradford Assay Reagent was added to each Falcon tube and allowed to incubate for 10 minutes before reading absorption on a spectrophotometer at 595 nm. The addition of the reagent was staggered to minimize the time between 10-minute incubation and absorbance reading on the spectrophotometer. Filtered Milli-Q

water was used to zero the instrument and Milli-Q with the reagent blank was read as a blank at the start, middle and end of the readings on the spectrophotometer.

3.2.4 Cell Counts

Cell counts for the flask and tube experiments were performed using a hemocytometer. In addition, some samples were analyzed using fluorescence microscopy for chl-a fluorescence and DNA staining (Gasol and Del Giorgio, 2000) with SYBR green (Sigma S7567). Fluorescence microscopy was used to determine if aggregates were algal cells, bacterial aggregates, or algae and bacteria aggregates. Images of the cells were taken with a digital camera, with complete squares of the hemocytometer in view to provide a scale. The program ImageJ (Institute of National Health of the United States and Laboratory for Optical and Computational Instrumentation, University of Wisconsin) was used to determine the size of cells by drawing outlines around them and using the program to estimate the area of the cell. The radius from the equivalent spherical diameter was then used to calculate the equivalent spherical volume of the cell.

3.3 Results

3.3.1 Biomass Harvest Experiment – Fluorescence Analysis

There was no clear down-regulation in fluorescence with the whey permeate addition in *T. suecica*, which was consistent with the screening experiments in Chapter 2. There was an increase in ln[fluorescence] and F_v in both, although initial values were

lower in the control and values reached an asymptotic value after 6 days. Thereafter, F_v declined in the control flasks but did not in the whey-amended cultures. Trends in F_v/F_m differed significantly between treatments during the growth phase and then converged, declining in both treatments. This can be seen in Figure 3.1 where, within the first day of the experiment, the addition of whey permeate to *T. suecica* appeared to significantly ($p<0.05$) change F_v/F_m. However, F_v, which is not affected by the blank, was similar in the control and whey permeate treatments. A higher F_m in the whey permeate treatment on the first day of the experiment with no change in F_v would explain the trend in F_v/F_m. The higher fluorescence detected could be the result of the L1 media blank used with the FIRe fluorometer to make the measurements. As seen in the abiotic experiments in Chapter 2, whey permeate-amended L1 media initially had significantly higher fluorescence than L1. This suggests that using an L1 media blank may not result in accurate results for ln[Fluorescence] and F_v/F_m, both of which can be biased by an inappropriate blank (Cullen and Davis, 2003). It is possible that the apparent differences in ln[Fluorescence] and F_v/F_m in the first few days of the experiment were the result of biases resulting from the media blank used. The optimal solution to this would be to measure algal fluorescence, then filter out any algae from the culture and use the filtered media as a blank. However, this was not practical due to the small subsamples taken from the flasks each day.

The photosynthetic antenna size (σ) increased in the control treatments as the culture grew more concentrated but there was no significant trend in the whey permeate-amended flasks except for a sharp decrease on the final day of the experiment. There was no obvious change in the photosynthetic turnover rate (τ) and no difference between the

treatments. There was no clear evidence for whey permeate addition having an impact on the light harvesting apparatus of mixotrophically growing *T. suecica*.

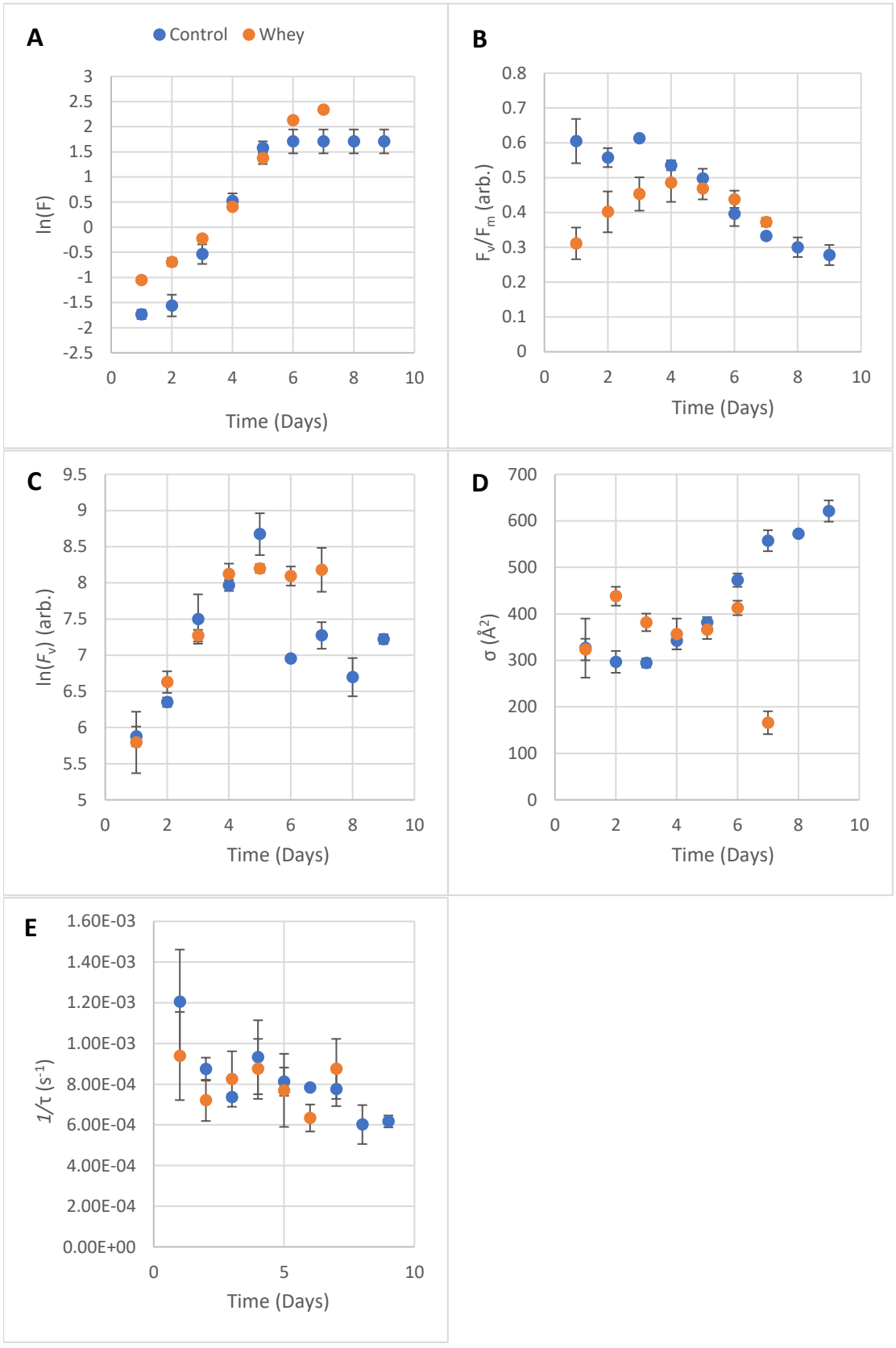
A
Control
Whey
ln(F)
Time (Days)
B
F_v/F_m (arb.)
Time (Days)
C
$\ln(F_v)$ (arb.)
Time (Days)
D
σ (Å²)
Time (Days)
E
1/τ (s⁻¹)
Time (Days)

Figure 3.1 Daily plots of the (A) natural logarithm of fluorescence, (B) the proportion of functional reaction centers (F_v/F_m), (C) the number of functional reaction centers (F_v), (D) the antennae size (σ) and (E) the photosynthetic turnover rate ($1/\tau$), for the control and 1% whey permeate flasks grown into stationary phase.

3.3.2 Biomass Composition Experiment – Final Yields

Previous experiments have shown that there are limitations when using fluorescence to estimate the final yields of a culture, so cell counts were used instead in the flask experiments. There was not a significant difference in final cell concentrations between treatments in stationary phase (Figure 3.2). The control cultures had significantly higher chl-a cell^{-1} content than the whey permeate cultures but there were no significant differences in the phosphate quotas nor the protein quotas. In the stationary phase, the protein quotas were not significantly different in the control and whey flasks. The optical density of the protein samples from the flask experiment ranged between 0.461-0.679 O.D.. Most of these samples were above the lower limit of detection which was at an optical density of 0.524 O.D. (Appendix – Figure A.3). The whey permeate flasks harvested in exponential phase were all below this lower limit of detection and therefore could not be accurately measured. The phosphorous quotas per cell also showed no significant differences between control and whey flasks in both the exponential and stationary phases.

None of these measurements were found to be completely consistent with the literature. The chl-a cell^{-1} was closest as the control ranged between 1.26-2.73 pg cell^{-1} which is slightly lower than the literature which has values closer to 2.03-3.80 pg cell^{-1} depending on the nutrient availability (Fabregas *et al.,* 1985). But the highest average

protein quota was 4.7 pg cell^{-1} which was lower than the lowest protein quotas for *T. suecica* in literature (Fabregas *et al.,* 1985; Cid *et al.,* 1991). In the study by Cid *et al.,* the control culture contained 27.5 pg protein cell^{-1} but could reach 88.61 pg protein cell^{-1} with an addition of glucose and other organic compounds.

The exponential cultures were harvested when the cell concentration was 60% of the stationary-phase concentration. There was a significant reduction in the chlorophyll quota in the whey permeate-amended cultures but no difference in the phosphorous quota. The protein quota in the exponential-phase, whey permeate-amended culture was below the limit of detection.

Both the chlorophyll quota and the phosphorous quota were significantly lower in stationary phase than exponential phase for the control and whey permeate cultures. There was no significant difference in protein quota between the two phases in the control culture.

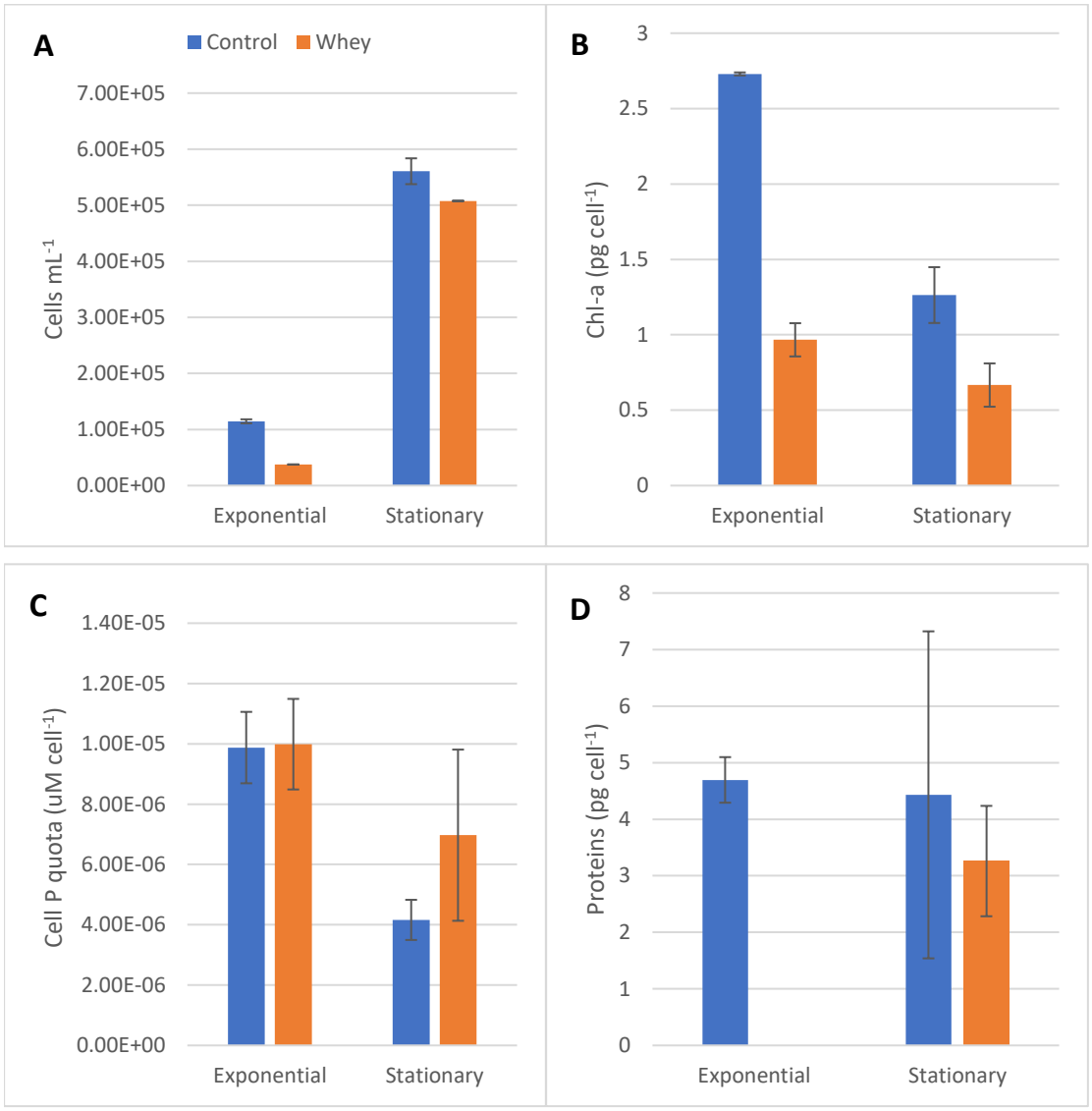

Figure 3.2 The results from the flask harvest experiment of *T. suecica* with the mean of replicates ± standard deviation of cells mL^{-1} (A), cell concentrations; (B), the cell Chl-a quota (pg $cell^{-1}$); (C), the cell phosphorous quota (µM $cell^{-1}$), and (D) the cell protein quota (pg $cell^{-1}$ mL^{-1}).

3.3.3 Bicarbonate Enrichment Experiment – Fluorescence Analysis

The bicarbonate enrichment experiment was designed to examine if an addition of DIC would change how *T. suecica* grew with whey permeate. In the L1-W treatment the fluorescence (Figure 3.3 A) and chl-a/cell (Figure 3.3 D) decreased significantly ($p<0.05$) compared to the other treatments. In addition, there was a decrease in F_v/F_m and a decrease in the variable fluorescence (F_v) with the whey permeate amendment (Figure 3.3 C, D). The maximum fluorescence (F_m) (Figure 3.3 A) is relatively constant for the L1-W treatment throughout the experiment. This suggests that not only are the algae not producing any more photosystem II reaction centers (PSII RC) as indicated by F_v (Figure 3.3 C) but over time there was a net loss of functional PSII RCs. The same reduction in fluorescence, PSII reaction centers, and chl-a does not occur with the addition of bicarbonate to whey permeate-amended media there. Instead, the L1-W&B and L1-B treatments follow very similar trends (Figure 3.3 C– F). The L1 treatment showed a decline in F_v/F_m (the proportion of functional PSII reaction centers) in the early stationary phase (Figure 3.3 D); treatments with a bicarbonate addition did not show this decline until later in the stationary phase.

T. suecica grown with a 1% whey permeate amendment showed a significantly reduced cell quota of chl-a (Figure 3.3 D) from the exponential phase onwards. In contrast, the L1-W&B and L1-B treatments had a significantly increased cell quota compared with the L1 treatment in the late exponential and early stationary phase before decreasing below the value of the control in the late stationary phase (Day 5). Despite differences in the cell quotas of chl-a and reaction centers (Figure 3.3 D, C), the ratios of these (i.e., the amount of chl-a per functional reaction center, chl-a/F_v) was similar in the

L1-W, L1-B and L1-W&B treatments and higher than in the controls in late exponential growth and early stationary phase (Day 3 & 4, Figure 3.3 F). In the late stationary phase (Day 5), the down-regulation in chl-a and maintenance of F_v in the two bicarbonate-enriched treatments resulted in a rapid reduction in chl-a/F_v (Figure 3.3 F) compared to the control and whey permeate-enriched cultures.

The photosynthetic cross-section (σ) is the size of the PSII antenna. There was no significant variation in the size of the antennae through stationary phase in the L1, L1-B, and L1-W&B cultures (Figure 3.3 F). The lack of trend was distinct from the increase in chl-a/F_v, although both parameters represent ratios of photosynthetic pigments to PSII RCs. The difference is that σ only accounts for pigments associated with PSIIs, while chl-a/F_v includes both PSII and PSI. In contrast to the other treatments, the L1-W treatment showed a progressive increase in antenna size with time, in parallel with the progressive loss of functional RCs.

The photosynthetic turnover rate time at PSII, 1/τ, is determined by the rate of the downstream reactions (e.g., the Calvin Cycle). The treatments with a bicarbonate addition initially were much higher on Day 1 but fell to values lower than the control until the final day when they returned to similar values as the control. This may be the result of the high error associated with the first day of the sample due to the low signal:noise ratio at low initial biomass. In contrast, 1/τ decreased in the whey permeate treatment over time, indicating a reduction in Calvin Cycle activity, and remained significantly higher than the other treatments throughout the experiment. The addition of bicarbonate to whey permeate amendment prevented down-regulation of the Calvin Cycle (i.e., an increase in 1/τ, Figure 3.3 G), in parallel with the lack of down-regulation of light-harvesting (Figure

3.3 B–F); both parameters showed a response very similar to the addition of bicarbonate alone.

In the L1, L1-B and L1-W&B treatments, there is a reduction in F_v, F_v/F_m, chl-a/cell, or chl-a/F_v on the fifth day of the experiment. The L1 treatment has a sharp decline in variable fluorescence while the bicarbonate-enriched treatments show a decline in F_v, F_v/F_m, and chl-a/F_v. The most likely explanation for this trend was that these stationary phase cultures were reallocating resources in response to a limiting nutrient. In previous experiments, a 1% whey permeate addition resulted in changes to growth or fluorescence but generally did not result in significant down-regulation in fluorescence. However, in the bicarbonate enrichment experiment, there was a significant difference between the 1% whey permeate tubes and all other treatments in most measurements. The primary difference between these experiments was that the plate-based screening and the flask experiments allowed gas exchange, while the tubes were closed with an impermeable cap and did not.

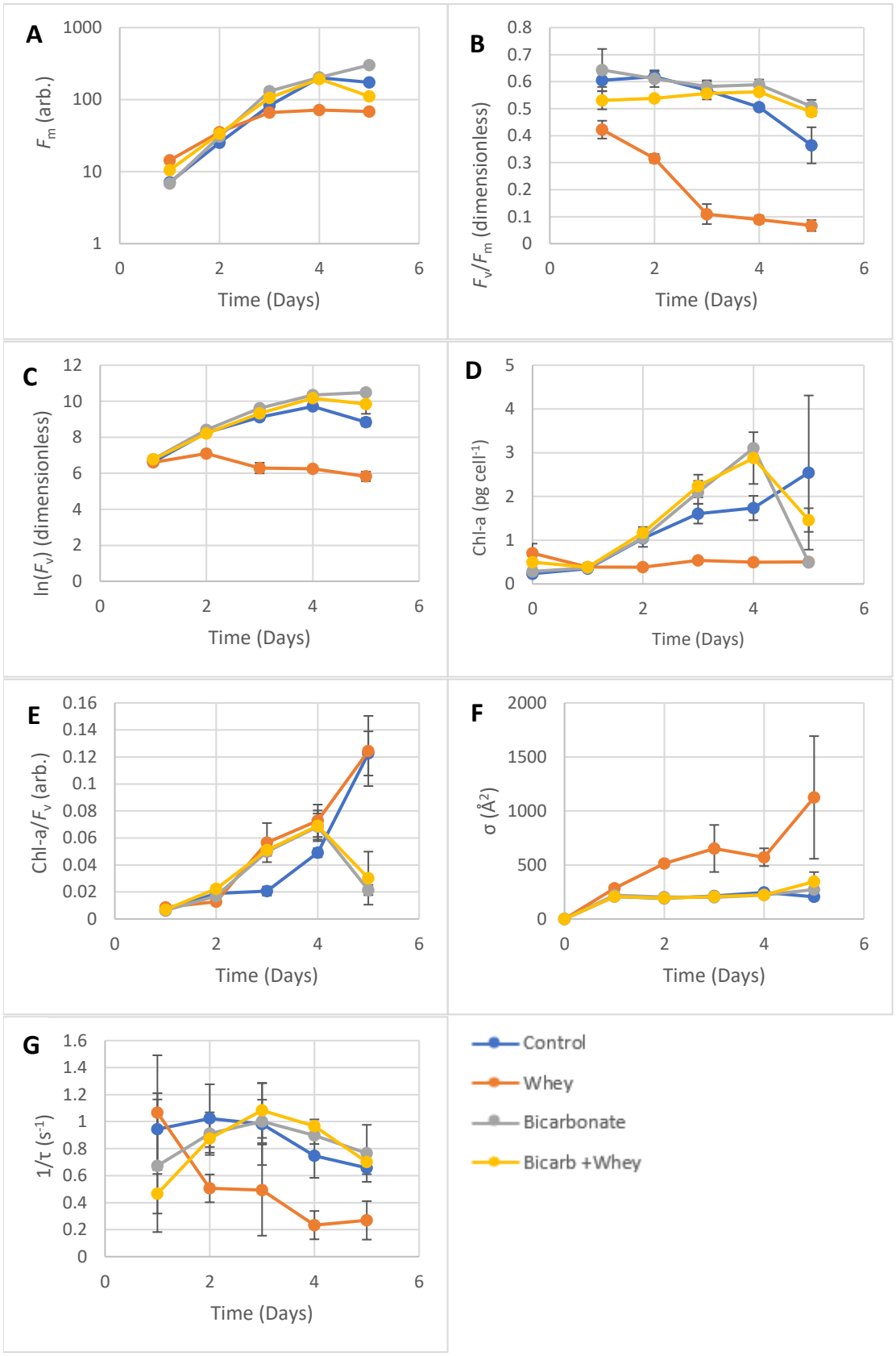
A
1000
100
10
1
F_m (arb.)
0
2
4
6
Time (Days)
B
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
F_v/F_m (dimensionless)
Time (Days)
C
12
10
8
6
4
2
0
ln(F_v) (dimensionless)
Time (Days)
D
5
4
3
2
1
0
Chl-a (pg cell^{-1})
Time (Days)
E
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Chl-a/F_v (arb.)
Time (Days)
F
2000
1500
1000
500
0
σ (Å^2)
Time (Days)
G
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/τ (s^{-1})
Time (Days)
Control
Whey
Bicarbonate
Bicarb +Whey

Figure 3.3 Variations in metrics of abundance and physiological status in *T. suecica* grown in L1 medium ± whey permeate and ± bicarbonate. Cultures were inoculated from a parent in balanced growth on Day 0. (A) the average maximum fluorescence (F_m), (B) the ratio of variable fluorescence to maximum fluorescence (F_v/F_m), (C) variable fluorescence (F_v), (D) the cell chlorophyll a quota, (E) the ratio of cell chlorophyll a quota to variable fluorescence, (F) the average photosynthetic cross section, σ, and (G) the photosynthetic turnover time, 1/τ,.

3.3.4 Bicarbonate Enrichment Experiment – Cell Growth

It was expected that the L1-W treatment would support cell yields similar to the control, as had been seen in previous experiments. Despite the increased nutrients with the L1-W treatment, there was a significant reduction in the yield of cells on day three and onwards compared to the other treatments (Figure 3.4). The growth of cells in the L1-W treatment slows after Day 2, unlike the other treatments which do not slow until after Day 3. The bicarbonate addition significantly increased the cell yield on Day 3 compared to the control for both the L1-B and L1-W&B treatments. The L1-B treatment remained significantly higher for the duration of the experiment but on the final day of growth, the L1-W&B treatment was no longer significantly larger than the control. Interestingly, despite the L1-W&B treatment having higher phosphorous and carbon than the L1-B treatment, the L1-B treatment had a significantly higher final cell yield. Overall, the biovolume yields of *T. suecica* follow a similar trend to the yield of cells.

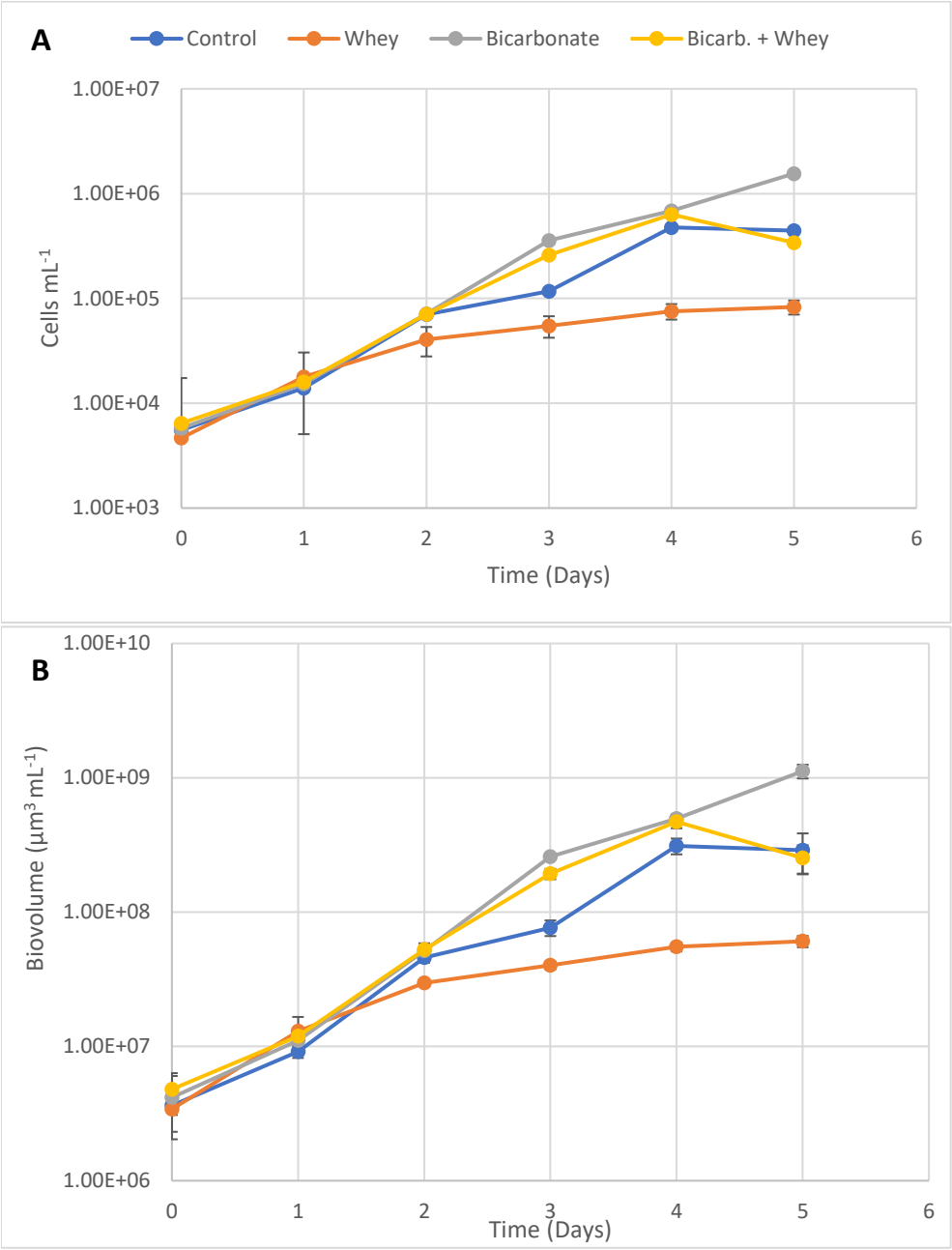

Figure 3. 4 (A) The average cell concentration and (B) the total biovolume (both as mean ± the standard deviation) of *T. suecica* grown with and without whey permeate and bicarbonate at intermediate light.

When grown in tubes without gas exchange, there was no significant difference in the growth rate of the whey permeate tube compared to the control (Figure 3.5 A). Despite the similar maximum growth rates, the L1-W culture has significantly ($p<0.05$) lower biovolume yields than the control. The bicarbonate addition did significantly increase the growth rate relative to the L1 culture but this enhancement was not observed in the L1-W&B treatment. The average volume of *T. suecica* in the L1 cultures was 634 ± 112 μm^3 which was consistent with reported sizes of 607 ± 61 μm^3 (Abiusi *et al.,* 2014). *T. suecica's* cell size increased significantly ($p<0.05$) compared to the control with 1% whey permeate with or without 1 g L^{-1} of bicarbonate (Figure 3.5 B). The L1-W, L1-B, and L1-W&B treatments all reach similar cell sizes, with none having cells significantly larger ($p<0.05$) than the others. Despite the significant increase in cell size, the low number of cells in the 1% whey permeate treatment still resulted in significantly reduced yield, as biovolume, compared to the control. However, the L1-B treatments show significantly increased biomass relative to the control, with the L1-B treatment being significantly higher than all other treatments.

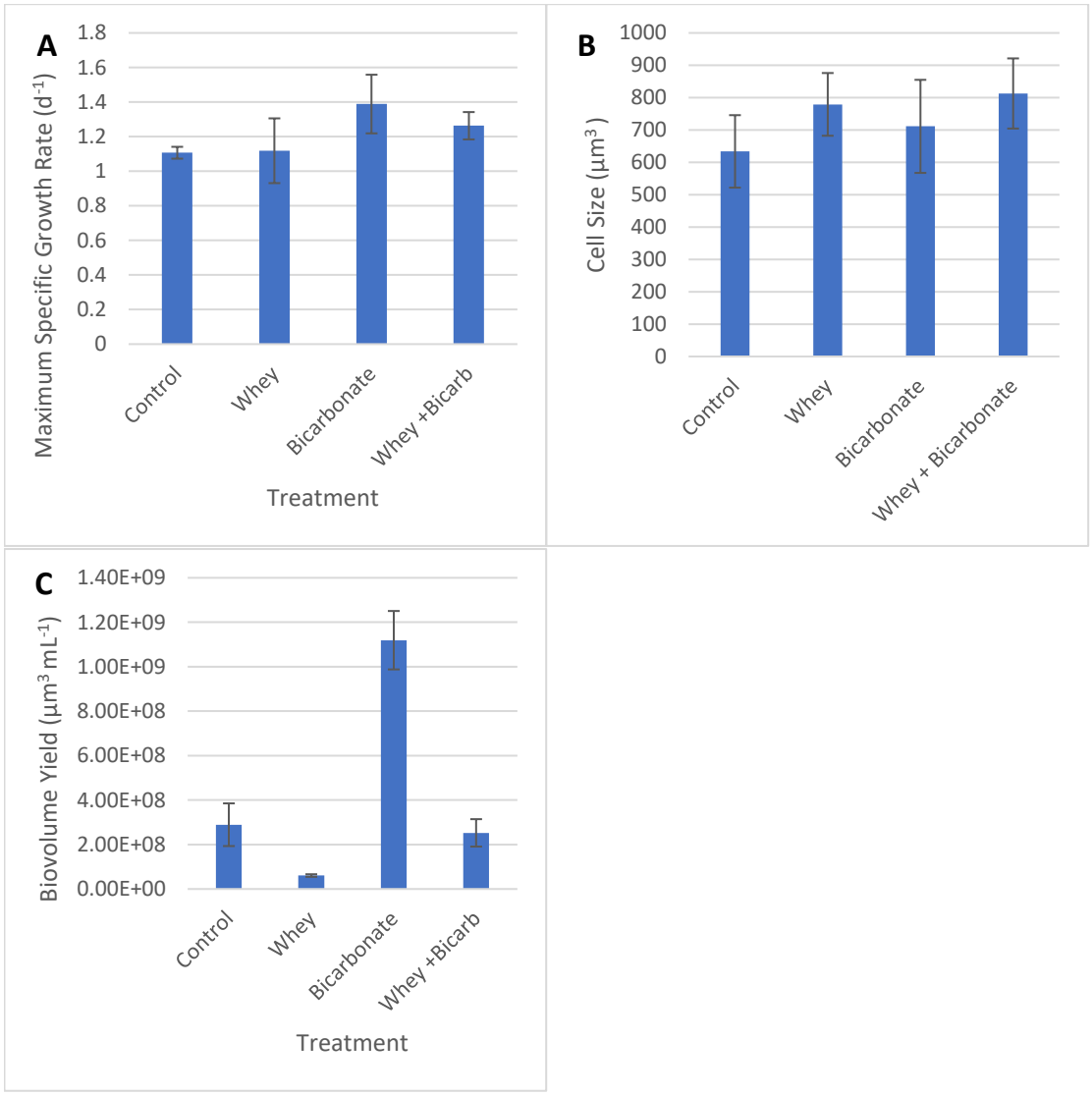

Figure 3.5 (A) the specific growth rate of all replicates, (B) cell volume and (C) average biovolume yields (all as mean ± the standard deviation) at harvest from control, 1% whey permeate, 1 g L^{-1} bicarbonate and 1% whey permeate + 1 g L^{-1} bicarbonate treatments.

3.4 Discussion

3.4.1 Investment in Light Harvesting

In every treatment in both the biomass composition analysis and bicarbonate enrichment in all treatments, there was an increase in maximum fluorescence over time and clear evidence of growth. This indicates that even if mixotrophic growth is resulting in the down-regulation of pigments, there is not a complete halt of photosynthesis. However, there does appear to be significant variation in how much algae invested in light harvesting in the different treatments.

As a phototrophic culture of algae grows, it is expected that there will be an increase in the investment in light harvesting. As cells grow and divide, the photosynthetic structures and apparatuses will be split into each cell. Over time, if energy is not invested in producing additional photosynthetic apparatuses, they will decrease per cell. In the control cultures, there is generally a trend of increasing chl-a/cell and the number of functional reaction centers until stationary phase. In stationary phase, cultures become nutrient-limited and will reallocate resources away from light harvesting (Geider et al., 1996, 1998). Over time, the control cultures reduce the proportion of functional reaction centers, but the number of functional reaction centers increases. This is most likely the result of algae not being able to produce a sufficient number of reaction centers to maintain F_v/F_m ratio as cells divide, likely due to carbon limitations as this trend is not seen in samples with bicarbonate enrichment.

As algae grow, the culture density will increase and the mean irradiance in the culture will decrease. This will cause increased resource allocation to light harvesting, i.e., pigments (Geider et al., 1996, 1998). This can be seen in the antennae size of the

flask control cultures and the chl-a/cell content of the tube control cultures. The trend is notably absent in the whey permeate treatment, in which neither antennae size nor chl-a content increased. There was an increase in the number of functional reaction centers in the whey permeate cultures grown in flasks which was not seen when they are grown in tubes. Instead, the whey permeate tube cultures do not appear to invest heavily in light harvesting and instead just maintain the same chl-a/cell content and have a slow reduction in the number of reaction centers. Overall, the whey permeate treatments show less investment in light harvesting throughout the experiments.

In contrast to the L1-W treatment, the addition of bicarbonate leads to an increase in investment in light harvesting. In both the L1-B and L1-W&B treatments, there was an increase in the number of reaction centers and chl-a/cell compared to the control cultures. Interestingly, the whey permeate culture grown with bicarbonate behaved more like the L1-B treatment than the L1-W treatment. Instead of a down-regulation in light harvesting, there was an increased investment in light harvesting. The bicarbonate-enriched cultures have additional inorganic carbon compared to the control cultures and this appears to allow *T. suecica* to increase its investment in light harvesting. Both L1-B and L1-W&B had increases in biovolume, though only the former was significant. This increased yield of biovolume means the cultures are more dense and therefore would need to have a higher investment in light harvesting in order to grow.

The whey permeate and bicarbonate-enriched treatments have different pigment quotas and biomass yields, but the amount of chl-a per functional reaction center shows a very similar trend. This suggests that while the L1-W cultures have reduced investment in light harvesting, the reaction centers they have maintained are kept functional similarly to

those in bicarbonate-enriched cells. Interestingly, all three of these treatments are more similar to each other than to the L1 culture with respect to chl-a/F_v. Throughout most of the experiment, the control had lower chl-a/F_v.

The availability of inorganic carbon plays a significant role in *T. suecica*'s investment in light harvesting. The only difference between the whey permeate and whey + bicarbonate treatments is the additional supply of inorganic carbon. Similarly, the only difference between the control and bicarbonate treatments is the inorganic carbon. In both treatments with additional bicarbonate, there is a clear increase in allocation of resources to light-harvesting.

3.4.2 Rates of the Dark Reactions

If algae are increasing their investment in light harvesting, it suggests that the rate of dark reactions is increasing. According to the Dynamic Balance model (Geider et al., 1996), if the rate of the dark reactions increases, then light harvesting will be upregulated to match the increased rates. Comparing the control to the bicarbonate-enriched cultures shows an increase in investment in light harvesting with the additional inorganic carbon. This suggests that the availability of CO_2 was limiting the rate of the dark reactions, mostly likely limiting Rubisco's catalytic rate. The addition of DIC would allow an increased rate of the dark reactions that is then matched by an increase in light harvesting. The L1-B treatments had significantly higher growth rates than the control culture. This does support the theory that by increasing CO_2 availability, the growth rates of the algae can be increased.

It was expected that by supplying algae with whey permeate, they would grow mixotrophically. This would have been optimal since the CO_2 produced through heterotrophic metabolism could help fuel photosynthesis and so increase the availability of inorganic carbon to allow for faster growth rates. However, there was no significant enhancement of growth rate when cultures were grown with whey permeate. In the bicarbonate-enriched cultures, the increased DIC resulted in increased concentrations of chl-a and reaction centers. The opposite trend was seen in the whey permeate culture, which showed little to no investment in light harvesting. If CO_2 were being produced through heterotrophic growth, it seems likely some enhancement to light harvesting would have been observed. Instead, it appears that algae are not using the glucose derived from lactose to increase photosynthetic activity but instead (presumably) directly utilizing the lactose for growth. Hydrolysis of lactose, a disaccharide, produces galactose and glucose, which is the end product of photosynthesis. In one mode of mixotrophic growth (Roth et al., 2019), addition of glucose to the green alga *Chromochloris zofingiensis* resulted in down-regulation of Calvin cycle enzymes and pigments. It is possible that lactose metabolism suppresses photosynthesis in the whey permeate cultures through glucose regulation. Evidence for this can be seen in the slower photosynthetic turnover rate and the reduction in reaction centres compared to the control and bicarbonate-enriched cultures.

3.4.3 Effects of Gas Exchange

While there were some similar trends between the biomass composition and bicarbonate enrichment experiments, there was variability in trends and results. Most

noticeably, the final yields of fluorescence and cells and the overall investment in reaction centers had the largest differences. In the biomass experiment, the final cell yield was not significantly different from the control while the bicarbonate experiment had significantly reduced final yields of cells and biovolume. The major differences between these two experiments were the different containers in which cultures were grown and the different amounts of gas exchange. In the flasks, there was gas exchange through the cheesecloth/cotton bungs but the tubes were sealed with caps, preventing gas exchange. The lack of any gas exchange would result in a much lower availability of CO_2 for photosynthesis and O_2 for respiration. Many species of algae have carbon concentration mechanisms, meaning they could increase their internal CO_2 concentrations relative to their environment (Colman *et al.,* 2002; Beardall & Raven, 2020). However, CCMs would have limited effectiveness if there is no gas exchange to replace the CO_2 the algae cells are taking up. The control and whey permeate cultures in the tubes are both carbon limited as supplying bicarbonate does increase yields. The limited growth in the whey permeate-amended culture is likely due to limited respiration of lactose due to the lack of oxygen, coupled with suppression of photosynthesis by the glucose that was produced (Roth et al., 2019).

3.4.4 Reallocation of Resources

The Dynamic Balance model (Geider et al., 1996) provides insights into how algae allocate resources under different conditions. In a typical phototrophic culture, algae will initially increase light harvesting to match an increase in the rate of dark reactions and as they become nutrient limited in stationary phase, they down-regulate

light harvesting in favor of energy storage. Unfortunately, the protein data from the biomass harvest experiment are not comparable to protein quotas expected from the literature (Fabregas *et al.,* 1985; Cid *et al.,* 1991). One potential explanation for the low protein quotas would be that the methods used were insufficient to properly break open the cells.

On day five of the bicarbonate enrichment experiment, there is a sudden change in the previously observed trends in some of the treatments. The L1-B and L1-W&B cultures both had a large reduction in chl-a/cell and chl-a/F_v. In contrast, the L1 culture had a sharp decrease in the number of functional reaction centers. The most likely explanation for this trend is Liebig limitation. As Day five was the final day of the experiments, all of the cultures were expected to be in the stationary phase. This means their growth is limited by one or more nutrients, in which case algae may reallocate resources. Bicarbonate-enriched cultures have higher carbon compared to the control but may be limited by nitrogen and reduce their pigments as a result. In contrast, the L1 cultures, which have much higher nitrogen relative to carbon, may be reducing their reaction centers while maintaining their pigments as they have excess nitrogen.

The additional carbon available in the L1-W and bicarbonate-enriched treatments (L1-B, L1-W&B) seems to result in larger cell sizes. In all three of these treatments, significantly ($p<0.05$) larger cells are observed. This could be due to an increase in cell quotas of energy storage compounds. In the case of the mixotrophic cultures. which literature suggests up-regulate lipids (Bashir *et al.,* 2019; Baldisserotto *et al.,* 2021), an increased investment in energy storage and larger cells may explain the lack of a significantly increased growth rate. Despite having the highest available nutrients, the

whey + bicarbonate treatment did not have the highest cell or biovolume yields. An explanation for this can be seen in the images of the cells from this treatment compared with the other treatments (Appendix A.2-5). In the L1-W&B treatment, there is a very large amount of extracellular material, which is likely extracellular polysaccharides. It is possible, this treatment is not showing significant enhancement of cell division rates because of this production of extracellular material.

3.5 Conclusions

It was expected that the organic carbon in the whey permeate would be used to fuel heterotrophic growth in *T. suecica*. This was expected to result in increased availability of CO_2 which could result in the increased growth seen in the bicarbonate treatments. However, it appears that the glucose derived from lactose in whey permeate is largely being utilized for growth instead of being metabolized for energy. In this case, the high availability of glucose may result in feedback inhibition of the dark reactions, which in turn results in reduced light harvesting to match the slower dark reaction rates.

When *T. suecica* is grown with just whey permeate, there is clear down-regulation of light harvesting, especially in the chl-a content. If there is sufficient CO_2 supplied through gas exchange, this does not result in a significant difference in the yields, but a sealed culture will have reduced total biovolume. By supplying additional inorganic carbon in the form of bicarbonate, it is possible to increase the investment in light harvesting and increase the overall yields of *T. suecica*. This is most likely the result of the increased CO_2 availability increasing the rate of the dark reactions and the light harvesting apparatuses being upregulated in response. Interestingly, adding bicarbonate to

a whey permeate culture also results in a similar trend however, the yields are not significantly increased.

The is a clear enhancement to the growth rate and yields of algae when grown with bicarbonate. Whether or not bicarbonate alone or bicarbonate with whey permeate is the best option is not clear as there is no data on the cell quotas for proteins and lipids. Bicarbonate is a relatively inexpensive source of inorganic carbon that could help reduce the costs associated with alternative methods of supplying CO_2 for algae growth. Using food-grade wastes has the potential to further improve large-scale algae culturing by reducing costs and increasing growth rates and yields. The lack of down-regulation in fluorescence and light harvesting seen in *T. suecica* cultures grown with whey permeate and bicarbonate has promising applications in the algal industries. The pigments of an algal cell can be very valuable, but if they are heavily downregulated by a mixotrophic culture, the enhanced growth rates may not make up for the reduced pigment quotas. However, the addition of bicarbonate prevents this pigment reduction while maintaining growth rates and yields similar to the control. This may be a promising option for ensuring high growth rates and yields with algae grown with food-grade wastes.

CHAPTER 4 – CONCLUSIONS

A focus on stoichiometry can guide optimal media composition for photo- and mixotrophic cultures and offer significant insights into potential explanations for the response of algae to mixotrophic growth. To evaluate the viability of using food-grade wastes to grow microalgae an understanding of optimal growth conditions and effects of these conditions were needed. A screening experiment involving *Nannochloropsis oculata, Pavlova lutheri, Phaeodactylum tricornutum, Tetraselmis suecica, Thalassiosira pseudonana,* and *Rhodomonas salina* tested for optimal growth conditions. The experiment grew each species with varying concentrations of whey permeate, fish HTL or vinasse in media that was prepared without phosphorous at three different light levels. This method had limitations resulting from small culture volumes and carbon limitation with the plates used. Still, it provided an efficient method of evaluating a large number of treatments.

The experiment demonstrated that these waste products would support at least some growth with certain species. This indicates that the phosphorous in these wastes was accessible to the algae. Unlike fish-HTL and vinasse, whey permeate supported growth in every species. However, while being the most effective food-grade waste, only in a few cases was there any signficant enhancement to growth rate or cell yields.

The screening determined *T. suecica* as the ideal candidate species for analysis of biomass composition in larger-scale experiments. The species had comparable growth and yield to the control when grown with 1% whey permeate. In the biomass composition experiment, *T. suecica* grew to a much larger volume than in the screening analysis, enabling harvesting of biomass to determine cell yields of chl-a and proteins -- along with

a more thorough evaluation of light harvesting apparatuses. At stationary phase, significant differences in the cell yields or protein quotas were insignificant., However, there were differences in the light harvesting of cultures grown with 1% whey permeate. Smaller volumes of *T. suecicia* were grown in a later experiment with and without bicarbonate. Adding inorganic carbon prevented the down-regulation of light harvesting, in contrast to *T. suecica* being grown with just whey permeate.

Industries are currently using *T. suecica* and the other species evaluated in this thesis for the production of various products. The most common application is for fish feeds. The major limitation for industries is the high cost of growing and harvesting algae. Although food-grade wastes did not tend to significantly enhance growth rates or yields, there is potential to improve algae industries because nutrient supply represents a major cost of large-scale algae growth.

The screening experiment demonstrated that whey permeate, a food-grade waste with high concentrations of phosphorous, can support algae growth, Accordingly, whey permeate as an inexpensive source of phosphorous could reduce the cost of growing algae. Further, bicarbonate, an alternative to bubbling carbon dioxide into cultures, shows potential to enhance algae yield more efficiently, with and without food-grade waste amendments. Further experiments are required to better understand how algae resource allocation may change when grown with and without whey permeate or bicarbonate and how this would apply to industries.

T. suecica grown with whey permeate typically had reduced pigments and light harvesting. Because reduced pigments result in less self-shading, it may be possible to grow denser cultures of algae, resulting in enhanced productivity of algae industries.

Supplying sufficient light to support algae growth can be costly. Algae grown mixotrophically on food-grade wastes could be less reliant on light but could still support similar growth to phototrophically grown cultures. Alternatively, if pigments are a desirable product, the addition of bicarbonate to *T. suecica* results in increased pigments compared to growing algae only with whey permeate.

With the societal imperative to develop sustainable green industry, microalgae provide the opportunity to recycle waste and produce long-term value. Due to the high costs presently incurred by algae growth, it is vital to better understand how different growth conditions can change the yields and resource allocation.

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APPENDIX

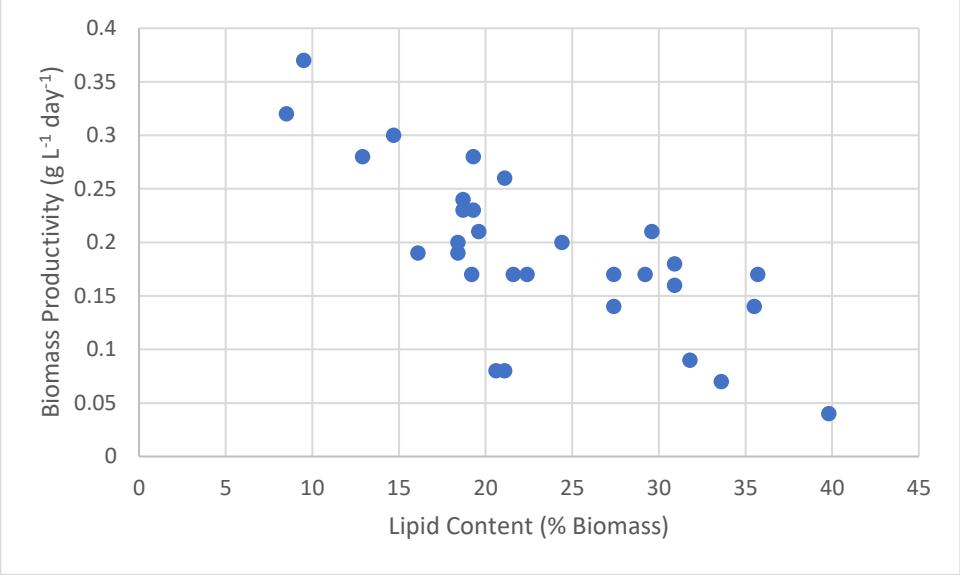

Figure A.1 Influence of lipid content on the daily biomass productivity of various species of fresh and marine algae. Data taken from Rodolfi et al. 2009.

Table A.1 Summary of mixotrophic and phototrophic growth rates and biomass of *Nannochloropsis oculata, Pavlova lutheri, Phaeodactylum triconutum, Tetraselmis suecica, Thalassiosira pseudonana*, and *Rhodomonas salina* from various studies. In cases where growth rate or biomass were not listed, they were estimated from figures displaying growth or biomass data.

Species:	**Phototrophic/ Mixotrophic**	**Organic Substrate**	**Growth Rate (days)**	**Dry Cell Weight Biomass** ($g\ L^{-1}$)	**Reference:**
P. lutheri	Phototrophic		0.116	0.318	Bashir *et al.,* 2019
	Mixotrophic	10 mmol Glucose	0.214	0.632	Bashir *et al.,* 2019
	Mixotrophic	10 mmol Glycerol	0.178	0.406	Bashir *et al.,* 2019
	Mixotrophic	10 mmol Acetate	0.147	0.252	Bashir *et al.,* 2019
	Mixotrophic	10 mmol Sucrcose	0.222	0.787	Bashir *et al.,* 2019
N. oculata					
	Mixotrophic	4 g L^{-1} glucose	0.1104		Pagnanelli *et al.,* 2013
	Mixotrophic	10 g L^{-1} glucose	0.1248		Pagnanelli *et al.,* 2013
	Phototrophic		0.14	0.14	Shene *et al.,* 2016
	Mixotrophic	2 g L^{-1} Glycerol	0.459	0.459	Shene *et al.,* 2016
	Phototrophic		0.29	0.42	Zanette *et al.,* 2019
	Mixotrophic	5 g L^{-1} Lactose	0.38	1.46	Zanette *et al.,* 2019
	Mixotrophic	5 g L^{-1} Lactose	0.38	1.46	Zanette *et al.,* 2019

Species:	Phototrophic/ Mixotrophic	Organic Substrate	Growth Rate (days)	Dry Cell Weight Biomass (g L^{-1})	Reference:
P. tricornutum	Phototrophic		0.177	0.675	Wang *et al.,* 2012
	Mixotrophic	Glucose 1 g L^{-1}	0.255	1.16	Wang *et al.,* 2012
	Mixotrophic	Sodium Acetate 5 g L^{-1}	0.277	0.625	Wang *et al.,* 2012
	Mixotrophic	Starch 1 g L^{-1}	0.173	0.611	Wang *et al.,* 2012
T. pesudonana	Phototrophic		0.755	0.483	Baldarrissio *et al.,* 2021
	Mixotrophic	1 g L^{-1} Glycerol	0.734	0.452	Baldarrissio *et al.,* 2021
	Mixotrophic	2.5 g L^{-1} Glycerol	0.783	0.748	Baldarrissio *et al.,* 2021
	Mixotrophic	5 g L^{-1} Glycerol	0.728	0.609	Baldarrissio *et al.,* 2021
T. suecica	Control		0.055	0.827	Mohammad *et al.,* 2014
	Mixotrophic	5 g L^{-1} Glucose	0.118	1.75	Mohammad *et al.,* 2014
	Mixotrophic	10 g L^{-1} Glucose	0.199	2.5	Mohammad *et al.,* 2014
	Mixotrophic	20 g L^{-1} Glucose	0.619	7.15	Mohammad *et al.,* 2014
	Mixotrophic	30 g L^{-1} Glucose	0.694	8.08	Mohammad *et al.,* 2014
	Mixotrophic	40 g L^{-1} Glucose	0.542	6.65	Mohammad *et al.,* 2014

Table A.2 Estimated stoichiometric composition of control and fish-HTL and vinasse-amended media. The phosphate estimates are based on the phosphate analysis of fish-HTL and vinasse by the MAPEL lab (Unpublished) The dissolved organic carbon (DOC) and nitrogen concentration is unknown for these amendments. The estimated DIC is based on historical values for the seawater used to prepare media.

fish-HTL concentration (%)	Dissolved Organic Carbon (DOC, µM)	Dissolved Inorganic Carbon (DIC; µM)	Nitrate+Nitrite (N; µM)	Phosphate or TDP (P; µM)	DOC:DIC:N:P
0	?	2000	880	36.2	0:55:24:1
0.1	?	2000	880 +?	1.817548	?:1100:484:1
0.3	?	2000	880 +?	5.066346	?:395:174:1
1	?	2000	880 +?	16.2045	?:123:54:1
3	?	2000	880 +?	48.0645	?:42:18:1
Vinasse Concentration (%)	Dissolved Organic Carbon (DOC, µM)	Dissolved Inorganic Carbon (DIC; µM)	Nitrate+Nitrite (N; µM)	Phosphate or TDP (P; µM)	DOC:DIC:N:P
0	?	2000	880	36.2	0:55:24:1
0.1	?	2000	880 +?	5.161298	?:388:171:1
0.3	?	2000	880 +?	14.55337	?:137:60:1
1	?	2000	880 +?	47.3394	?:42:19:1
3	?	2000	880 +?	141.0274	?:14:6:1

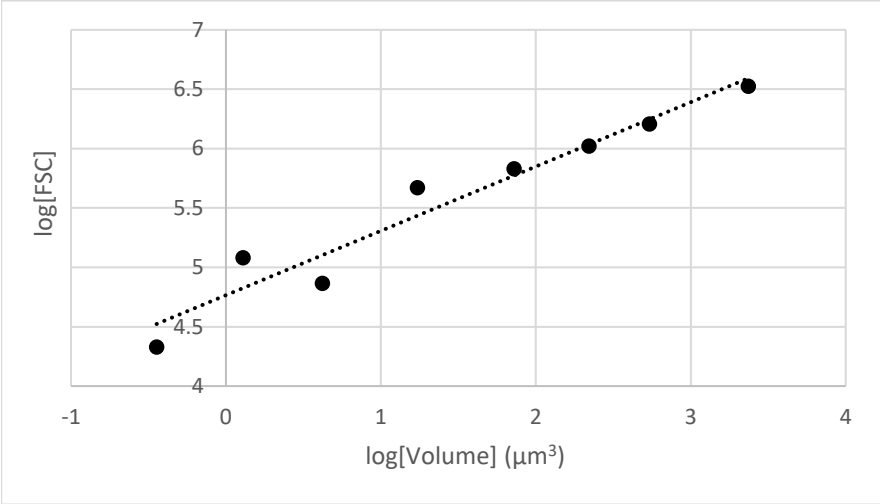

Figure A.2 A plot of the log[FSC] and log[volume] of the standard beads measured with the flow cytometer. The dashed line is the linear regression used to determine the relationship between volume and forward angle scatter, $y = 0.0490x - 4.884$.

Table A.3 Summary of the modified Blackman's Bilinear model fits including the initial fluorescence (F_0), maximum fluorescence (F_m), length of the exponential phase (tsat), length of the lag phase (tlag), the apparent growth rate (μ) and the number generations detectable in exponential phase (generations of growth) for (A) *N. oculata*, (B) *P. lutheri*, (C) *P. tricornutum*, (D) *T. suecica*, (E) *T. psuedonana*, (F) *R. salina* grown with whey permeate.

(A) *N. oculata* - Whey Permeate

Light Level	Concentration (%)	Row	Col	F_0 (Arb.)	F_m (Arb.)	tsat (days)	tlag (days)	μ (days)	Generations
HL	0	A	2	10.32	11.56	5.34	2.44	0.23	1.78
HL	0	D	2	10.47	13.09	3.59	3.53	0.73	3.79
HL	0.1	A	3	10.32	11.56	5.34	2.44	0.23	1.78
HL	0.1	D	3	10.47	13.17	3.69	3.53	0.73	3.9
HL	0.3	A	4	10.39	13.05	4.4	3.16	0.6	3.83
HL	0.3	D	4	10.6	13.59	3.96	4.3	0.76	4.31
HL	1	A	5	10.39	13.05	4.4	3.16	0.6	3.83
HL	1	D	5	10.6	13.59	3.96	4.3	0.76	4.31
HL	3	A	6	10.42	10.16	5.75	6	-0.05	-0.38
HL	3	B	6	10.57	12.92	3.13	4.18	0.75	3.38
HL	3	C	6	10.39	13.06	4.22	3.27	0.63	3.85
LL	0	A	2	10.44	15.24	11.49	6.79	0.42	6.93
LL	0	D	2	10.49	13.81	8.32	6.13	0.4	4.78
LL	0.1	A	3	10.29	10.78	8.88	1.47	0.06	0.71
LL	0.1	D	3	10.41	13.19	10.38	5.21	0.27	4.01
LL	0.3	A	4	10.44	15.13	12.87	6.39	0.37	6.78
LL	0.3	D	4	10.46	14.74	11.19	6.55	0.38	6.17
LL	1	A	5	10.5	15.04	15.21	7.39	0.3	6.54
LL	1	D	5	10.52	14.92	15.02	7.48	0.29	6.35
LL	3	A	6	10.63	14.21	15.34	8.59	0.23	5.18
LL	3	B	6	10.6	14.61	14.88	8.89	0.27	5.78
LL	3	C	6	10.63	14.87	14.93	8.52	0.28	6.12
LL	3	D	6	10.63	14.1	15.15	8.64	0.23	5.01

(B) *P. lutheri* - Whey Permeate									
Light Level	Concentration (%)	Row	Col	F_0 (Arb.)	F_m (Arb.)	tsat (days)	tlag (days)	μ (days)	Generations
HL	0	A	2	10.24	11.61	4.63	2.64	0.3	1.98
HL	0	D	2	10.35	13.6	4.26	4.18	0.76	4.69
HL	0.1	A	3	10.27	12.09	4.75	2.65	0.38	2.63
HL	0.1	D	3	10.32	12.98	4.78	2.92	0.56	3.85
HL	0.3	A	4	10.32	11.76	2.35	3.65	0.61	2.08
HL	0.3	D	4	10.34	12.21	2.35	4.28	0.8	2.7
HL	1	A	5	10.43	12.88	2.75	4.4	0.9	3.55
HL	1	D	5	10.42	13.23	3.3	4.23	0.85	4.05
HL	3	A	6	10.51	12.82	3.37	7.22	0.69	3.33
HL	3	B	6	10.49	12.92	5.51	4.79	0.44	3.51
HL	3	C	6	10.47	13.11	4.11	6.88	0.64	3.81
HL	3	D	6	10.45	12.95	3.81	6.75	0.66	3.6
ML	0	A	2	10.3	12.6	6.75	3.82	0.34	3.33
ML	0	D	2	10.38	14.85	6.11	5.61	0.73	6.44
ML	0.1	A	3	10.39	13.91	6	5.46	0.59	5.08
ML	0.1	D	3	10.37	14.17	6.3	5.26	0.6	5.49
ML	0.3	A	4	10.42	14.71	7	6.82	0.61	6.19
ML	0.3	D	4	10.41	14.6	6.53	6.69	0.64	6.04
ML	1	A	5	10.41	12.18	10.09	5.42	0.18	2.55
ML	1	D	5	10.47	14.59	9.27	6.43	0.44	5.94
ML	3	A	6	10.54	14.05	6.89	7.77	0.51	5.06
ML	3	B	6	10.52	14.17	5.78	7.68	0.63	5.26
ML	3	C	6	10.56	14.31	5.82	7.56	0.64	5.41
ML	3	D	6	10.55	14.14	5.75	7.52	0.63	5.19
HL	0	A	2	10.24	11.61	4.63	2.64	0.3	1.98
HL	0	D	2	10.35	13.6	4.26	4.18	0.76	4.69
HL	0.1	A	3	10.27	12.09	4.75	2.65	0.38	2.63
HL	0.1	D	3	10.32	12.98	4.78	2.92	0.56	3.85
HL	0.3	A	4	10.32	11.76	2.35	3.65	0.61	2.08

(C) *P. tricornutum*- Whey Permeate									
Light Level	Concentration (%)	Row	Col	F_0 (Arb.)	F_m (Arb.)	tsat (days)	tlag (days)	μ (days)	Generations
HL	0	A	2	8	13.58	1.92	0.17	2.91	8.06
HL	0.1	A	3	9.87	11.22	2	0.17	0.68	1.95
HL	0.3	A	4	8.71	12.57	1.85	0.17	2.09	5.57
HL	1	A	5	8.36	13.29	1.89	0.17	2.61	7.11
HL	3	A	6	8.62	14.12	2.12	0.17	2.59	7.94
LL	0	A	2	10.44	15.24	7.37	3.6	0.65	6.92
LL	0	D	2	10.43	14.52	6.2	3.45	0.66	5.89
LL	0.1	A	3	10.38	12.41	5.17	2.67	0.39	2.93
LL	0.1	D	3	10.36	12.79	5.82	2.64	0.42	3.51
LL	0.3	A	4	10.44	13.87	6.23	3.77	0.55	4.95
LL	0.3	D	4	10.45	14.02	6.01	3.73	0.59	5.15
LL	1	A	5	10.58	14.34	5.58	4.52	0.68	5.44
LL	1	D	5	10.53	14.61	6.12	3.88	0.67	5.88
LL	3	A	6	10.76	13.86	7.84	3.63	0.4	4.47
LL	3	B	6	10.73	13	6.11	2.6	0.37	3.28
LL	3	C	6	10.73	13.29	7.25	2.63	0.35	3.69
LL	3	D	6	10.73	13.65	7.82	3.49	0.37	4.21
ML	0	A	2	10.36	15.49	4.82	2.94	1.06	7.4
ML	0	D	2	10.48	14.47	3.73	3.27	1.07	5.75
ML	0.1	A	3	10.35	12.35	3.94	2.24	0.51	2.89
ML	0.1	D	3	10.36	12.23	2.52	2.62	0.74	2.7
ML	0.3	A	4	10.41	13.53	3.79	2.68	0.82	4.51
ML	0.3	D	4	10.46	14.03	3.33	3.22	1.07	5.15
ML	1	A	5	10.44	13.84	4.35	2.85	0.78	4.9
ML	1	D	5	10.45	11.73	6.79	2	0.19	1.84
ML	3	A	6	10.63	13.47	4.55	3.57	0.62	4.1
ML	3	B	6	10.62	13.19	4.1	3.28	0.63	3.7
ML	3	C	6	10.63	13.3	4.51	2.74	0.59	3.85
ML	3	D	6	10.66	13.45	4.14	3.61	0.68	4.03

(D) *T. suecica* - Whey Permeate									
Light Level	Concentration (%)	Row	Col	F_0 (Arb.)	F_m (Arb.)	tsat (days)	tlag (days)	μ (days)	Generations
ML	0	A	2	10.34	13.14	3.44	3.76	0.81	4.04
ML	0	D	2	10.42	15.67	4.27	4.54	1.23	7.58
ML	0.1	A	3	10.34	12.43	4.35	3.25	0.48	3.01
ML	0.1	D	3	10.37	12.79	4.09	3.16	0.59	3.49
ML	0.3	A	4	10.39	13.57	3.75	3.77	0.85	4.6
ML	0.3	D	4	10.38	14.82	4.68	3.9	0.95	6.41
ML	1	A	5	10.47	10.79	3.8	1.99	0.08	0.46
ML	3	A	6	10.74	14.68	4.26	4.81	0.93	5.68
ML	3	B	6	10.7	14.78	4.26	3.68	0.96	5.88
ML	3	C	6	10.75	14.11	3.84	3.62	0.88	4.85
ML	1	D	5	10.55	15.73	5	4.25	1.03	7.46
ML	3	D	6	10.72	14.45	3.96	4.54	0.94	5.38

(E) *T. psuedonana* - Whey Permeate									
Light Level	Concentration (%)	Row	Col	F_0 (Arb.)	F_m (Arb.)	tsat (days)	tlag (days)	μ (days)	Generations
HL	0	A	2	10.32	12.59	3.81	1	0.63	3.45
HL	0	D	2	10.25	12.02	3.04	0.96	0.58	2.56
HL	0.1	A	3	10.42	11.24	4.01	0.5	0.2	1.17
HL	0.1	D	3	10.39	11.18	1.47	1.49	0.54	1.14
HL	0.3	A	4	10.39	11.15	2.12	0.88	0.36	1.1
HL	0.3	D	4	10.44	11.58	1.46	1.63	0.78	1.64
HL	1	A	5	10.66	10.32	3.14	0	-0.11	-0.48
HL	1	D	5	10.58	11.85	1.71	2.07	0.74	1.83
HL	3	A	6	10.63	11.11	2.36	5.64	0.2	0.7
HL	3	B	6	10.57	11.63	4.12	3.89	0.26	1.53
HL	3	C	6	10.63	11.49	1.89	5.59	0.45	1.24
HL	3	D	6	10.96	10.47	2	0.47	-0.24	-0.7
LL	0	A	2	10.4	13.08	5.66	3.72	0.47	3.86
LL	0.1	A	3	10.43	11.92	2.23	3.79	0.67	2.15
LL	0.3	A	4	10.52	12.49	2.96	5.64	0.67	2.84
LL	1	A	5	10.66	12.83	2.96	5.53	0.74	3.14
LL	3	A	6	10.63	10.89	3.99	7.78	0.07	0.38
ML	0	A	2	10.34	12.65	2.54	0.91	0.91	3.34
ML	0	D	2	9.89	12.35	2.8	0.49	0.88	3.56
ML	0.1	A	3	10.29	12.29	2.56	0.91	0.78	2.89
ML	0.1	D	3	9.95	11.56	2.17	0.47	0.74	2.32
ML	0.3	A	4	10.32	10.76	1.45	0.78	0.3	0.63
ML	0.3	D	4	10.12	12.09	3.82	0.51	0.51	2.84
ML	1	A	5	10.48	12.29	2.9	1	0.62	2.61
ML	1	D	5	10.35	10.58	5.51	0.53	0.04	0.33
ML	3	A	6	10.61	13.26	23.7	3.95	0.11	3.83
ML	3	B	6	10.59	11.05	3.16	2.78	0.15	0.67
ML	3	C	6	10.58	10.96	3.1	2.97	0.12	0.55
ML	3	D	6	10.56	10.64	5.5	0.53	0.02	0.12

(F) *T. psuedonana* - Whey Permeate									
Light Level	Concentration (%)	Row	Col	F_0 (Arb.)	F_m (Arb.)	tsat (days)	tlag (days)	μ (days)	Generations
HL	0	A	2	10.33	12.08	12.4	9.5	0.14	2.53
HL	0.1	A	3	10.28	10.54	5.3	7.7	0.05	0.38
HL	0.3	A	4	10.27	10.72	5.64	7	0.08	0.65
HL	1	A	5	10.27	10.12	14.9	15.5	-0.01	-0.21
HL	3	A	6	10.55	10.85	3.33	4.47	0.09	0.43
LL	0	A	2	10.51	12.67	6.84	2.72	0.32	3.11
LL	0	D	2	10.52	12.7	6.92	2.45	0.31	3.14
LL	0.1	A	3	10.42	10.92	5.61	0.77	0.09	0.71
LL	0.1	D	3	10.37	11.25	6.77	0.78	0.13	1.28
LL	0.3	A	4	10.48	12.4	6.62	1.88	0.29	2.76
LL	0.3	D	4	10.22	12.56	11.03	0.82	0.21	3.39
LL	1	A	5	10.57	12.38	9.28	1.14	0.19	2.6
LL	1	D	5	10.32	12.5	11.51	0.83	0.19	3.14
LL	3	A	6	10.69	12.01	7.62	1.67	0.17	1.91
LL	3	B	6	10.64	11.95	7.53	1.72	0.17	1.89
LL	3	C	6	10.66	11.98	7.51	1.69	0.18	1.9
LL	3	D	6	10.66	11.99	6.62	1.8	0.2	1.92
ML	0	A	2	10.43	12.37	4.66	3.36	0.42	2.79
ML	0	D	2	10.41	12.18	4.49	2.88	0.39	2.55
ML	0.1	A	3	10.33	11.04	3.48	1.78	0.2	1.02
ML	0.1	D	3	10.39	11.21	11.34	0.1	0.07	1.18
ML	0.3	A	4	9.92	11.85	7.99	0.1	0.24	2.79
ML	0.3	D	4	10.45	12.1	4.67	3.37	0.35	2.39
ML	1	A	5	10.45	12.18	4.56	2.7	0.38	2.49
ML	1	D	5	10.49	12.15	4.43	2.7	0.37	2.4
ML	3	A	6	10.57	11.65	3.72	2.59	0.29	1.55
ML	3	B	6	10.57	11.6	3.76	2.34	0.27	1.49
ML	3	C	6	10.54	11.61	4.45	0.89	0.24	1.55
ML	3	D	6	10.57	11.79	3.74	2.57	0.33	1.76

Table A.4 Stationary-phase fluorescence, F_{final} (Equation 2) in control and fish-HTL amended cultures. Data are presented as ı ± the standard deviation of ln-transformed, with the number of samples (wells) averaged for each estimate. Significant differe between amended cultures and the control ($p \leq 0.05$; ANOVA and Tukey-Kramer tests are noted with an "*" in the table. In c there are more or fewer samples than expected, this is the result of removal of outliers or addition of control treatments (See 2 for details). The data are ordered by species and growth light level (low, LL; medium, ML; and high, HL).

		Fish-HTL Treatment - Maximum Fluorescence (Dimensionless)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	11.84 ± 0.25	2	10.81 ± N/A	1	11.12 ± 0.07*	2	11.43 ± 0.08	2	10.31 ± 0.02*	4
	ML	12.56 ± 0.03	2	10.81 ± 0.17*	2	11.30 ± N/A	1	11.88 ± 0.33*	2	10.29 ± 0.01*	4
	LL	13.07 ± 0.26	2	10.68 ± 0.28*	2	10.58 ± 0.09*	2	11.42 ± 1.02*	2	10.27 ± 0.02*	4
T. suecica	HL	14.04 ± 1.53	2	10.94 ± 0.09*	2	11.74 ± 0.37	2	12.27 ± N/A	1	13.10 ± 0.33	4
	ML	15.74 ± 0.17	2	10.50 ± 0.01*	2	10.62 ± 0.18*	2	11.15 ± 0.40*	1	11.69 ± 0.22*	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	13.31 ± 0.30	2	10.98 ± 0.58*	2	11.18 ± 0.13*	2	10.25 ± 0.01*	2	10.24 ± 0.01*	4
	ML	15.01 ± 0.11	2	11.25 ± 0.29*	2	11.71 ± 0.26*	2	10.32 ± 0.02*	2	10.23 ± 0.02*	4
	LL	15.33 ± 0.09	2	10.61 ± 0.14*	2	11.90 ± 0.42*	2	10.58 ± 0.25*	2	10.28 ± 0.02*	3
P. tricornutum	HL	13.74 ± 0.37	2	10.71 ± 0.02*	2	10.93 ± 0.11*	2	11.97 ± N/A	1	13.18 ± 0.12	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	14.98 ± 0.26	4	10.63 ± 0.10*	2	10.89 ± 0.10*	2	12.94 ± 0.02*	2	12.99 ± 0.09*	4
N. oculata	HL	13.45 ± 0.06	2	10.36 ± 0.06*	2	10.80 ± 0.18*	2	11.76 ± 0.67*	2	13.26 ± 0.26	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	15.22 ± 0.11	2	10.31 ± 0.03*	2	10.46 ± 0.17*	2	13.13 ± 0.07	2	14.36 ± 0.98	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	12.38 ± 0.001	2	10.39 ± 0.002*	2	10.52 ± 0.10*	2	10.96 ± 0.02*	2	10.26 ± 0.03*	3
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0

Table A.5 Stationary-phase fluorescence, F_{final} (Equation 2) in control and vinasse-amended cultures. Data are presented as m the standard deviation of ln-transformed, with the number of samples (wells) averaged for each estimate. Significant differenc between amended cultures and the control ($p \leq 0.05$; ANOVA and Tukey-Kramer tests are noted with an "*" in the table. In c there are more or fewer samples than expected, this is the result of removal of outliers or addition of control treatments (See 2 for details). The data are ordered by species and growth light level (low, LL; medium, ML; and high, HL).

		Vinasse Treatment - Maximum Fluorescence (Dimensionless)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	11.90 ± 0.04	2	10.93 ± 0.33	2	10.87 ± 0.2*	2	10.29 ± 0.018	2	10.27 ± 0.04*	4
	ML	12.44 ± 0.24	5	10.87 ± N/A	1	10.77 ± N/A	1	10.36 ± N/A	1	10.36 ± 0.04*	2
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
T. suecica	HL	14.04 ± 1.53	2	11.53 ± 0.02*	2	11.89 ± 0.05*	2	12.37 ± N/A	1	11.82 ± 0.20*	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	13.4 ± 0.01	2	10.92 ± 0.35*	2	12.13 ± 0.35*	2	12.3 ± 0.08*	2	11.04 ± 0.26*	4
	ML	13.11 ± 1.39	2	11.16 ± 0.97	2	11.58 ± 1.41	2	10.52 ± 0.04	2	10.36 ± 0.11*	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. tricornutum	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	14.91 ± 0.32	2	10.7 ± 0.02	2	10.47 ± 0.02	2	10.37 ± 0.0008	2	10.52 ± 0.05	4
N. oculata	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	13.71 ± 1.13	2	10.24 ± 0.02*	2	10.26 ± 0.02*	2	10.47 ± 0.02*	2	10.22 ± 0.01*	4
	LL	14.13 ± 0.97	2	10.35 ± 0.03*	2	13.75 ± 0.7	2	12.28 ± 0.16	2	11.81 ± 0.47	4
R. salina	HL	11.43 ± 0.18	2	10.35 ± 0.18*	2	10.83 ± 0.04*	2	10.70 ± 0.06*	2	10.81 ± 0.02*	4
	ML	12.82 ± 0.48	2	10.42 ± 0.01*	2	10.49 ± 0.04*	2	10.33 ± 0.06*	2	10.27 ± 0.01*	4
	LL	12.82 ± 1.2	2	10.43 ± 0.08*	2	12.21 ± 0.08	2	11.01 ± 0.06*	2	11.42 ± 0.13	4

Table A.6 The average apparent growth rates plus or minus the standard deviation of the data from the culture screening experiments with fish-HTL amendments. The apparent growth rates are taken from the model fits of Equation 1. The number of wells used to calculate the mean fluorescence is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significance and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with an "*" in the table. In some cases there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

		Fish-HTL Treatment - Apparent Growth (days)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	0.88 ± 0.09	2	0.16 ± N/A	1	0.37 ± 0.07*	2	-0.11 ± 0.05*	2	-0.11 ± 0.05*	4
	ML	1.37 ± 0.39	2	0.48 ± 0.27	2	1.07 ± N/A	1	1.71 ± 0.39	2	-0.11 ± 0.02*	4
	LL	0.49 ± 0.04	2	0.09 ± 0.06*	2	0.08 ± 0.01*	2	0.17 ± 0.16*	2	-0.03 ± 0.01*	4
T. suecica	HL	1.12 ± 0.07	2	0.50 ± 0.17	2	0.63 ± 0.19	2	0.77 ± N/A	1	0.77 ± 0.18	4
	ML	1.22 ± 0.05	2	0.09 ± 0.05*	2	0.05 ± 0.03*	2	0.14 ± N/A	1	0.25 ± 0.05*	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	0.49 ± 0.09	2	0.09 ± 0.06*	2	0.13 ± 0.02*	2	-0.04 ± 0.02*	2	-0.04 ± 0.02*	4
	ML	0.78 ± 0.04	2	0.12 ± 0.02*	2	0.13 ± 0.03*	2	0.003 ± 0.003*	2	-0.004 ± 0.02*	4
	LL	0.41 ± 0.05	2	0.03 ± 0.01*	2	0.08 ± 0.03*	2	0.02 ± 0.02*	2	-0.003 ± 0.002*	3
P. tricornutum	HL	2.95 ± 0.17	2	0.3 ± 0.01*	2	0.50 ± 0.12*	2	1.87 ± 0.088	1	1.87 ± 0.08*	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	0.70 ± 0.04	4	0.08 ± 0.02*	2	0.17 ± 0.01*	2	0.30 ± 0.02*	2	0.29 ± 0.01*	4
N. oculata	HL	0.89 ± 0.10	2	0.01 ± 0.01*	2	0.13 ± 0.02*	2	0.17 ± 0.02*	2	0.17 ± 0.02*	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	0.42 ± 0.02	2	-0.0090 ± 0.0017	2	0.0044 ± 0.000003	2	0.3 ± 0.004	2	0.28 ± 0.07	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	0.46 ± 0.04	2	0.02±0.01*	2	0.03 ± 0.01*	2	0.15 ± 0.02*	2	-0.02 ± 0.03*	3
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0

Table A.7 The average apparent growth rates plus or minus the standard deviation of the data from the culture screening experiments with vinasse-amendments. The apparent growth rates are taken from the model fits of Equation 1. The number of wells used to calculate the mean fluorescence is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significance and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with an "*" in the table. In some cases there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

		Vinasse Treatment - Apparent Growth (days)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	0.99 ± 0.03	2	0.22 ± 0.08*	2	0.09 ± 0.03*	2	-0.05 ± 0.03*	2	-0.05 ± 0.03*	4
	ML	1.35 ± 0.55	5	0.67 ± N/A	1	0.55 ± N/A	1	0.02 ± N/A	1	-0.03 ± 0.05*	2
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
T. suecica	HL	1.12 ± 0.07	2	0.64 ± 0.15*	2	0.70 ± 0.05*	2	0.89 ± 0.10*	2	0.89 ± 0.10*	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	0.61 ± 0.14	2	0.08 ± 0.04*	2	0.23 ± 0.06*	2	0.17 ± 0.06*	2	0.17 ± 0.06*	4
	ML	0.59 ± 0.10	2	0.11 ± 0.10*	2	0.17 ± 0.18*	2	0.09 ± 0.07*	2	0.02 ± 0.03*	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. tricornutum	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	0.73 ± 0.04	2	0.10 ± 0.01*	2	0.03 ± 0.02*	2	$-6*10^{4}$ ± 0.0004*	2	0.05 ± 0.02*	4
N. oculata	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	0.61 ± 0.11	2	-0.01 ± 0.0009*	2	-0.003 ± 0.002*	2	0.10 ± 0.03*	2	-0.01 ± 0.01*	4
	LL	0.32 ± 0.04	2	0.003 ± 0.02*	2	0.26 ± 0.004	2	0.16 ± 0.02*	2	0.13 ± 0.04*	4
R. salina	HL	0.14 ± 0.01	2	0.002 ± 0.02*	2	0.14 ± 0.01	2	0.19 ± 0.03	2	0.19 ± 0.03	4
	ML	0.39 ± 0.01	2	0.02 ± 0.02*	2	0.05 ± 0.01*	2	0.01 ± 0.05*	2	-0.004 ± 0.01*	4
	LL	0.19 ±0.003	2	0.07 ± 0.01*	2	0.20 ± 0.01	2	0.08 ± 0.01*	2	0.09 ± 0.004*	4

Table A.8 Total biovolume ($\mu m^3\ \mu L^{-1}$) of particles classified as algal (mean ± standard deviation) from the culture screening experiments with fish-HTL amendments. The number of wells used to calculate the mean fluorescence is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significance and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with an "*" in the table. In some cases, there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

		Fish-HTL Treatment - Biovolume ($\mu m^3\ \mu L^{-1}$)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	3159 ± 91	2	947 ± N/A	1	1341 ± 1849	2	1330 ± 121	2	1911 ± 1014	4
	ML	1237 ± 110	2	903 ± 63	2	1436 ± N/A	1	1218 ± 326	2	1630 ± 220	4
	LL	3641 ± 53	2	223 ± 77	2	220 ± 118*	2	2194 ± 2139	2	3972 ± 640	4
T. suecica	HL	2985 ± 943	2	393 ± 371	2	991 ± 184	2	1955 ± N/A	1	5373 ± 1759	4
	ML	5881 ± 2486	2	14 ± 5*	2	21 ± 19*	2	63 ± N/A	1	674 ± 296*	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	28905 ± 19107	2	935 ± 1145*	2	1444 ± 714*	2	84 ± 51*	2	275 ± 41*	4
	ML	28604 ± 11360	2	723 ± 651*	2	1328 ± 785*	2	418 ± 449*	2	216 ± 296*	4
	LL	289 ± 26	2	925 ± 224	2	1008 ± 322	2	2453 ±460*	2	48 ± 5	3
P. tricornutum	HL	18350 ± 8047	2	2227 ± 610*	2	3746 ± 1146*	2	8351 ± N/A	1	12845 ± 956*	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	18258 ± 2719	4	320 ± 27*	2	651 ± 33*	2	3679 ± 908*	2	3307 ± 474	4
N. oculata	HL	29117 ± 23674	2	138 ± 32	2	1660 ± 387	2	3839 ± 322	2	9914 ± 3144	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	79320 ± 27524	2	111 ± 13*	2	399 ± 321*	2	11698 ± 3865*	2	56484 ± 16842	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	5049 ± 1600	2	161 ± 8	2	372 ± 141	2	1812 ± 46	2	2554 ± 2328	3
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0

Table A.9 Total biovolume ($\mu m^3\ \mu L^{-1}$) of particles classified as algal (mean ± standard deviation) from the culture screening experiments with vinasse amendments. The number of wells used to calculate the mean fluorescence is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significance and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with an "*" in the table. In some cases, there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

		Vinasse Treatment - Biovolume ($\mu m^3\ \mu L^{-1}$)									
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	2870 ± 72	2	1225 ± 159	2	1166 ± 766	2	494 ± 519*	2	1307 ± 604	4
	ML	2322 ± 1284	5	1049 ± N/A	1	532 ± N/A	1	385 ± N/A	1	67 ± 83	2
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
T. suecica	HL	2985 ± 943	2	N/A	0	N/A	0	N/A	0	N/A	0
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	36218 ± 19479	2	1591 ± 1506*	2	4971 ± 1234*	2	15902 ± 3549*	2	1486 ± 474*	4
	ML	1313 ± 65	2	317 ± 81	2	403 ± 49	2	932 ± 737	2	4561 ± 911*	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. tricornutum	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	16700 ± 2167	2	354 ± 215*	2	339 ± 338*	2	169 ± 8*	2	472 ± 124*	4
N. oculata	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	89117 ± 92434	2	755 ± 326	2	68863 ± 30821	2	37291 ± 2569	2	30461 ± 7595	4
	LL	37822 ± 39230	2	320 ± 138	2	29226 ± 13081	2	15827 ± 1090	2	12928 ± 3223	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	7567 ± 591	2	487 ± 75*	2	706 ± 36*	2	300 ± 42*	2	1243 ± 1188*	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0

Table A.10 (A) The 10th percentile, (B) the 50th percentile, and (C) the 95th percentile of the average biovolume (um^3 µL^{-1}) plus or minus the standard deviation of the data from the culture screening experiments. The number of wells used to calculate the mean fluorescence is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significance and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with an "*" in the table. In some cases, there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

(A) - 10th Percentile Biovolume (arb. /µm^3 µL^{-1})											
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	2.92 ± 2.15	2	3.58 ± 2.62	2	2.90 ± 1.97	2	2.71 ± 1.69	2	2.10 ± 0.67	4
	ML	5.47 ± 0.64	2	5.16 ± 0.17	2	6.66 ± 1.01	2	2.85 ± 0.02*	2	2.35 ± 0.17*	4
	LL	5.05 ± N/A	1	5.75 ± N/A	1	5.95 ± N/A	1	4.11 ± N/A	1	3.63 ± N/A	1
T. suecica	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	7.35 ± 1.06	3	9.06 ± 0.74	2	7.53 ± 1.22	2	9.87 ± N/A	1	9.23 ± 0.34	4
	LL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P. lutheri	HL	5.37 ± 0.29	2	5.40 ± 0.27	2	7.20 ± 0.03	2	9.35 ± 0.31	2	6.29 ± 1.51	4
	ML	6.89 ± 1.16	5	8.59 ± 1.14	2	10.92 ±0.004*	2	9.59 ± 1.57	2	10.58 ± 0.13*	4
	LL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P. tricornutum	HL	3.24 ± 0.13	3	2.45 ± N/A	1	2.88 ± N/A	1	3.52 ± N/A	1	2.78 ± N/A	1
	ML	2.15 ± 0.41	2	2.18 ± 0.64	2	2.2 ± 0.73	2	3.64 ± N/A	2	2.89 ± 0.34	4
	LL	0.83 ± 1.18	3	2.47 ± 0.17	2	2.59 ± 0.01	2	2.73 ± 0.08*	2	2.57 ± 0.09*	4
N. oculata	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LL	2.30 ± 0.22	2	4.07 ± 0.17	2	2.69 ± 0.46	2	2.19 ± 0.04	2	1.82 ± 0.13	4
R. salina	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	7.73 ± 0.07	2	6.92 ± 2.83	2	8.43 ± 0.3	2	7.28 ± 0.18	2	7.27 ± 0.06	4
	LL	3.53 ± 2.30	2	3.50 ± 1.60	2	4.45 ± 2.4	2	5.29 ± 0.14	2	5.64 ± 0.5	3

(B) - 50th Percentile Biovolume (arb. /µm³ µL⁻¹)											
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	5.33 ± 4.22	2	4.97 ± 3.28	2	10.33 ± 2.76	2	8.73 ± 6.84	2	5.03 ± 1.49	4
	ML	10.12 ± 0.24	2	9.50 ± 1.27	2	8.84 ± 1.10	2	7.28 ± 0.40*	2	4.92 ± 0.42*	4
	LL	1.62 ± N/A	1	1.62 ± N/A	1	4.91 ± N/A	1	5.22 ± N/A	1	2.06 ± N/A	1
T. suecica	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	9.77 ± 0.65	3	11.77 ± 0.75	2	10.25 ± 1.2	2	12.59 ± N/A	1	12.62 ± 0.20	4
	LL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P. lutheri	HL	7.18 ± 0.99	2	6.75 ± 0.60	2	11.08 ± 0.71	2	19.44 ± 0.57	2	14.71 ± 2.99	4
	ML	10.05 ± 2.35	5	13.55 ± 4.10	2	21.96 ± 2.38	2	19.26 ± 6.57	2	19.47 ± 0.84	4
	LL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P. tricornutum	HL	4.89 ± 0.16	3	1.88 ± N/A	1	2.20 ± N/A	1	2.59 ± N/A	1	1.19 ± N/A	1
	ML	3.42 ± 0.82	2	3.65 ± 1.26	2	3.62 ± 0.83	2	5.19 ± N/A	2	4.65 ± 0.26	4
	LL	1.11 ± 1.57	3	1.11 ± 1.57	2	3.41 ± 0.01	2	3.65 ± 0.11	2	3.70 ± 0.10	4
N. oculata	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LL	3.09 ± 0.47	2	3.09 ± 0.47	2	3.74 ± 0.36	2	3.12 ± 0.03	2	2.77 ± 0.13	4
R. salina	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	10.39 ± 0.06	2	12.28 ± 0.87	2	11.53 ± 0.47	2	11.20 ± 0.15	2	10.88 ± 0.30	4
	LL	18.23 ± 8.53	2	18.23 ± 8.53	2	18.27 ± 7.15	2	11.14 ± 0.45	2	12.31 ± 4.25	3

(C) - 95th Percentile Biovolume (arb. /µm³ µL⁻¹)											
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	18.23 ± 4.52	2	14.11 ± 2.72	2	30.83 ± 15.05	2	30.35 ± 19.05	2	13.43 ± 2.07	4
	ML	22.94 ± 2.19	2	36.46 ± 2.77	2	28.78 ± 3.39	2	21.63 ± 2.47	2	13.39 ± 1.58	4
	LL	4.72 ± N/A	1	7.35 ± N/A	1	16.16 ± N/A	1	13.12 ± N/A	1	9.88 ± N/A	1
T. suecica	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	13.36 ± 0.45	3	16.08 ± 1.67	2	20.53 ± 5.45	2	18.53 ± N/A	1	26.07 ± 4.06	4
	LL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P. lutheri	HL	13.49 ± 2.53	2	11.07 ± 1.61	2	34.39 ± 14.94	2	57.27 ± 1.23	2	39.73 ± 10.77	4
	ML	18.14 ± 7.23	5	35.97 ± 21.4	2	64.82 ± 10.16	2	50.29 ± 24.69	2	52.78 ± 6.9	4
	LL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P. tricornutum	HL	11.92 ± 0.75	3	5.14 ± N/A	1	5.23 ± N/A	1	6.66 ± N/A	1	3.14 ± N/A	1
	ML	8.18 ± 2.32	2	10.28 ± 3.9	2	11.21 ± 1.57	2	9.97 ± N/A	2	10.78 ± 1.06	4
	LL	2.45 ± 3.47	3	8.47 ± 0.29	2	7.23 ± 0.03	2	7.11 ± 0.13	2	9.34 ± 0.43	4
N. oculata	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LL	4.42 ± 0.53	2	7.08 ± 0.68	2	5.44 ± 0.36	2	4.93 ± 0.2	2	5.74 ± 1.38	4
R. salina	HL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	ML	16.91 ± 0.81	2	25.67 ± 4.9	2	31.18 ± 7.85	2	30.92 ± 2.22	2	31.09 ± 0.37	4
	LL	43.46 ± 11.86	2	74.98 ± 0	2	63.31 ± 8.22	2	52.19 ± 2.91	2	35.95 ± 27.61	3

Table A.11 (A) The 10th percentile, (B) the 50th percentile, and (C) the 95th percentile of the average fluorescence per unit biovolume (um^3 µL^{-1}) plus or minus the standard deviation of the data from the culture screening experiments. The number of wells used to calculate the mean fluorescence is included in the table under samples. ANOVA and Tukey-Kramer tests were used to determine significance and any treatment which was found significantly different ($p<0.05$) from the control of the same light level was noted with an "*" in the table. In some cases, there are more or less samples than expected, this is the result of removal of outliers or addition of control treatments (See 2.2.8 for details).

(A) - 10th Percentile Fluorescence/Biovolume (arb. /µm³ µL⁻¹)											
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	230 ± 58	2	476 ± 222	2	404 ± 49	2	483 ± 260	2	4388 ± 2332	4
	ML	7305 ± 2636	2	5742 ± 1034	2	13140 ± 5187	2	6379 ± 274	2	5556 ± 256	4
	LL	8432 ± N/A	1	19112 ± N/A	1	11098 ± N/A	1	13923 ± N/A	1	7692 ± N/A	1
T. suecica	HL	143195 ± 66087	3	182883 ± 88549	2	185174 ± 61494	2	216829 ± N/A	1	103793 ± 14258	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	8573 ± 3428	2	5681 ± 115	2	11801 ± 6143	2	18197 ± 1477	2	16386 ± 6685	4
P. lutheri	HL	22606 ± 13770	5	49306 ± 10868	2	60433 ± 3823*	2	49469 ± 6081	2	45458 ± 3216	4
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	8269 ± 217	3	3924 ± N/A	1	8230 ± N/A	1	7457 ± N/A	1	17237 ± N/A	1
P. tricornutum	HL	16233 ± 151	2	11461 ± 4794	2	13066 ± 4857	2	8683 ± N/A	2	11293 ± 1376	4
	ML	7972 ± 11274	3	18505 ± 5028	2	23116 ± 246	2	21823 ± 163	2	20492 ± 2767	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
N. oculata	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	1300 ± 174	2	1205 ± 14	2	1452 ± 6	2	1428 ± 9	2	1305 ± 42	4
	LL	143195 ± 66087	3	182883 ± 88549	2	185174 ± 61494	2	216829 ± N/A	1	103793 ± 14258	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	89494 ± 3830	2	65967 ± 5100	2	72590 ± 40034	2	46062 ± 326	2	25071 ± 466	4
	LL	40643 ± 11723	2	12674 ± 857*	2	11213 ± 2561*	2	10270 ± 1596*	2	14981 ± 1300*	3

(B) - 50th Percentile Fluorescence/Biovolume (arb. /µm³ µL⁻¹)											
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	2682 ± 148	2	1349 ± 1010	2	2127 ± 1286	2	1555 ± 1593	1	4413 ± 1075	4
	ML	3706 ± 195	2	1416 ± 977	2	2109 ± 1368	2	2796 ± 1577	2	2686 ± 1097*	4
	LL	2803 ± 1053	2	1635 ± N/A	1	2991 ± N/A	1	5340 ± N/A	1	6417 ± N/A	1
T. suecica	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	3032 ± 1544	3	203 ± 47	2	741 ± 51	2	2936 ± N/A	1	4786 ± 502	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	1137 ± 1506	2	407 ± 305	2	1094 ± 247	2	10517 ± 1437	2	15036 ± 1749*	4
	ML	13261 ± 15260	5	1568 ± 856	2	9214 ± 4999	2	3997 ± 3799	2	13482 ± 652	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. tricornutum	HL	16478 ± 6549	4	2806 ± N/A	1	10750 ± N/A	1	13182 ± N/A	1	15056 ± 1100	4
	ML	13047 ± 1562	2	3188 ± 456	2	11965 ± 10711	2	27302 ± N/A	1	19574 ± 4488	4
	LL	18258 ± 2719	4	4352 ± 1084	2	15923 ± 5169	2	18228 ± 2283	2	14037 ± 1409	4
N. oculata	HL	29117 ± 23674	4	N/A	0	N/A	0	N/A	0	N/A	0
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	42633 ± 42909	2	15695 ± 20522	2	49077 ± 15820	2	57155 ± 1297	2	59609 ± 12125	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	4984 ± 446	2	900 ± 337	2	3549 ± 124*	2	5101 ± 648	2	5114 ± 1243	4
	LL	1897 ± 358	2	344 ± 51	2	3286 ± 1229	2	9000 ± 121*	2	9648 ± 1069*	3

(C) - 95th Percentile Fluorescence/Biovolume (arb. /µm³ µL⁻¹)											
		Control		0.1% Whey		0.3% Whey		1% Whey		3% whey	
Species:	Light Level	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples	Mean	Samples
T. pseudonana	HL	64097 ± 36170	2	86626 ± 66415	2	105912 ± 10366	2	198049 ± 144851	2	53912 ± 9784	4
	ML	115508 ± 28260	2	189741 ± 78178	2	142623 ± 43757	2	92102 ± 28397*	2	45126 ± 4430*	4
	LL	15828 ± 35392	1	90016 ± 90016	1	76531 ± 76531	1	158210 ± 158210	1	13686 ± 23705	1
T. suecica	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	574292 ± 89952	3	775998 ± 77098	2	705511 ± 69384	2	658271 ± N/A	1	592691 ± 34419	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. lutheri	HL	86162 ± 15712	2	57933 ± 35453*	2	111668 ± 7633	2	213102 ± 14200	2	136159 ± 22093	4
	ML	120347 ± 58599	5	265497 ± 166966	2	339302 ± 14939	2	227223 ± 60234	2	218914 ± 25978	4
	LL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
P. tricornutum	HL	61290 ± 9849	3	10500 ± N/A	1	20729 ± N/A	1	22155 ± N/A	1	23251 ± N/A	1
	ML	115925 ± 430	2	90813 ± 55431*	2	100449 ± 41676*	2	64019 ± N/A	1	87888 ± 15398*	4
	LL	37164 ± 52558	3	131542 ± 16690	2	132589 ± 5418	2	102500 ± 5927	2	116730 ± 3495	4
N. oculata	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	LL	6870 ± 1446	2	6984 ± 1304	2	5729 ± 435	2	8316 ± 685	2	7512 ± 1179	4
R. salina	HL	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
	ML	472450 ± 16064	2	594314 ± 37913	2	455939 ± 218407	2	344563 ± 15480	2	236021 ± 18748	4
	LL	720755 ± 107466	2	399869 ± 46677*	2	561078 ± 155857*	2	244730 ± 43391*	2	251811 ± 16123*	3

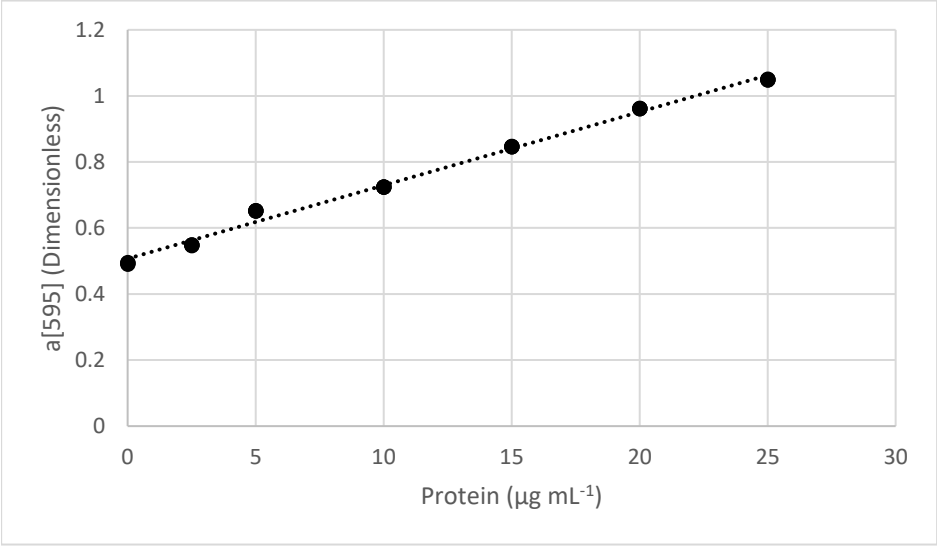

Figure A.3 The standard curve of the absorbances (595nm) of the protein concentrations of the modified Bradford assay with various concentrations of purified bovine serum albumin. The dashed line is the linear regression used to determine the relationship between phosphate concentration and absorbance, $y = 0.0318x - 0.485$.

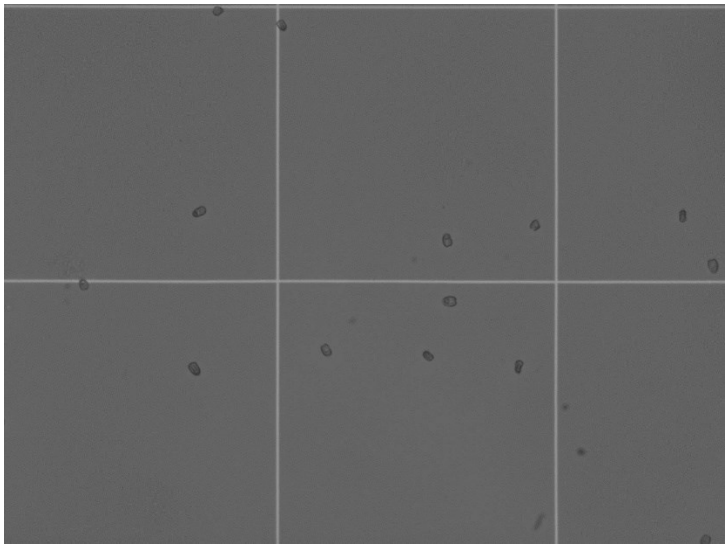

Figure A.4 Images of *T. suecica* control culture from the bicarbonate tube experiment: Grid of 0.25mm.

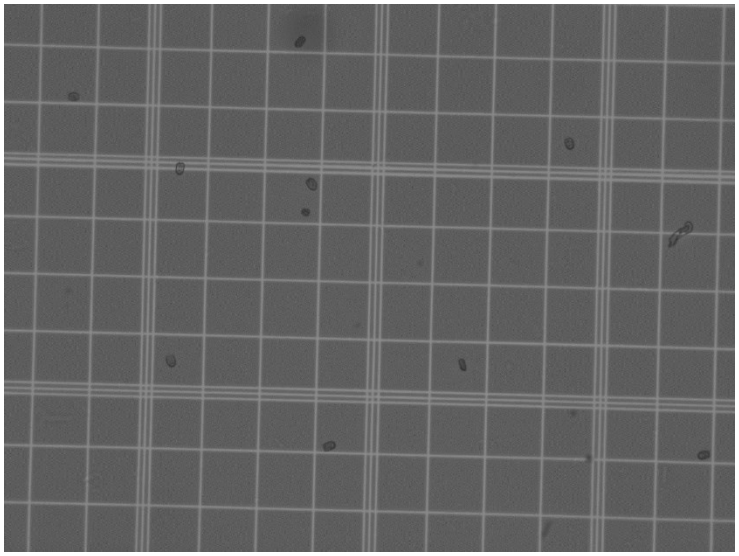

Figure A.5 Images of *T. suecica* 1% whey permeate culture from the bicarbonate tube experiment: Grid of 0.20mm.

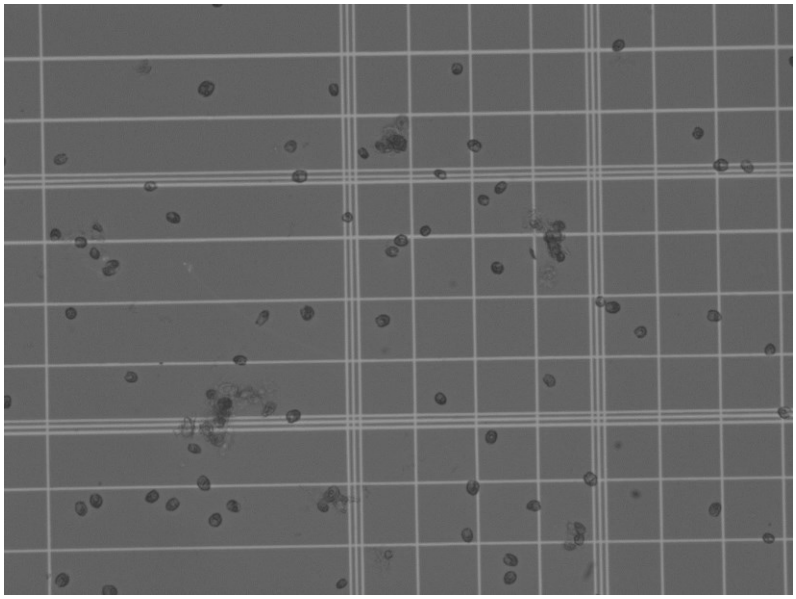

Figure A.6 Images of *T. suecica* bicarbonate culture from the bicarbonate tube experiment: Grid of 0.20mm.

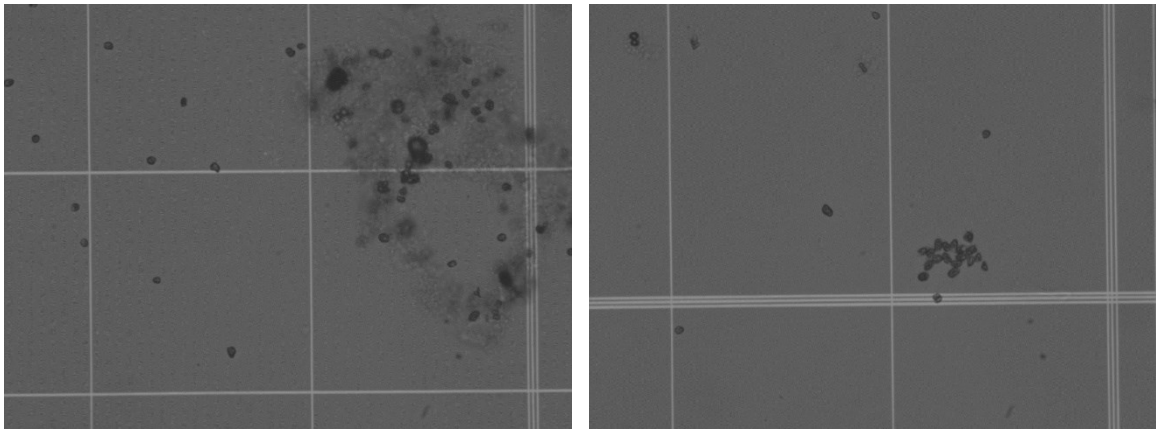

Figure A.7 Images of *T. suecica* 1% whey permeate and bicarbonate culture from the bicarbonate tube experiment: Grid of 0.25mm.

Table A.12 Overview of the experiments of this thesis. In the Screening Experiment and Biomass Composition experiment multiple sets of samples were run at different times throughout the duration of the experiment with no single sample or culture grown for the entire duration.

Experiment	Species	Replicates	Light Levels	Duration
Phosphate Analysis of Amendments (1)	None	**Total: 9 Samples** 3 replicates for each of the four concentrations of each food-grade waste	None	2020.08.09
Phosphate Analysis of Amendments (2)	None	**Total: 24 Samples** 3 replicates for eight different concentrations of whey permeate	None	2021.11.26
Screening Experiment	*Nannochloropsis oculata, Pavlova lutheri, Phaeodactylum tricornutum, Tetraselmis suecica, Thalassiosira pseudonana,* and *Rhodomonas salina*	**Total: 1080 Samples/Wells** 9, 24-well Plates/Species (1 plate/food-grade waste at each of the three light levels) 4 Replicate wells/treatment on each plate (4 wells for control and 4 wells for each amendment concentration)	Low (20 µmol photon m^{-2} s^{-1}), Medium (40 µmol photon m^{-2} s^{-1}), High (190 µmol photon m^{-2} s^{-1})	2020.12.07 - 2021.03.02
Biomass Composition Experiment	*Tetraselmis suecica*	**Total: 12 Flasks** 6 Flasks for Control and 6 Flasks for 1% Whey permeate 3 flasks of each treatment which	Intermediate (80 µmol photon m^{-2} s^{-1})	2021.07.18 – 2021.09.03

		were grown to stationary phase and 3 flasks which were grown to exponential phase.		
Bicarbonate Enrichment Experiment	*Tetraselmis suecica*	**Total: 12 Tubes** 3 tubes for each of the 3 treatments and the control.	Intermediate (80 µmol photon m^{-2} s^{-1})	2021.12.01 - 2021.12.06