Optical behavior of algae particles in photobioreactors
Master Thesis Project

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Abstract

Algae have been shown to be a promising source of potential biofuel, as well as other beneficial by-products. The cultivation process of algae is still open to refinement, however; bioreactor design can still be optimized. One of the factors that affect light penetration through a bioreactor growing algae is cell shape. This study endeavored to find (i) if a shape factor could be determined for different cell shapes that could be applied to existing or future photobioreactor models and (ii) if algae cells could be modeled as titanium dioxide particles, in order to make use of existing, extensive work done on their optical properties and behavior. The specific scattering, absorption, and extinction coefficients for six different species of algae (of three different cell shapes) and P25 Degussa powder were determined using an integrating sphere set-up. Their optical properties and behavior were analyzed and compared. Cells from the same genus behaved similarly and smaller particles, regardless of shape, generally inhibited light penetration into the reactor more than larger particles. Therefore, algae species with larger cells can be grown in higher concentrations than those with small cells before light extinction poses an issue. Cell chemical make-up is also known to affect light penetration, and in this study it appeared that more protein-heavy cells block more light than cells which are mostly lipids. Furthermore, the algae particles behaved very differently from those of titania; specifically, they are strong absorbers and titania particles are not. These initial results would suggest that it is inadvisable to model algae particles as agglomerates of titanium dioxide.
Contents

List of Figures .................................................................................................................. ii
List of Tables ....................................................................................................................... ii
Introduction ......................................................................................................................... 1
  Background Information ................................................................................................. 1
  Biofuel from algae ........................................................................................................... 1
  Other useful by-products from algae ............................................................................... 1
Goal of study ....................................................................................................................... 2
  Reasons for study .......................................................................................................... 2
Literature study .................................................................................................................. 2
Optical properties .............................................................................................................. 2
  Algae ............................................................................................................................... 2
  Titania particles .............................................................................................................. 4
Integrating Sphere ............................................................................................................. 4
Radiative transfer ............................................................................................................... 6
  Lambert-Beer .................................................................................................................. 6
Two-flux model .................................................................................................................. 6
Six-flux model ................................................................................................................... 7
Modiﬁed two-ﬂux model ................................................................................................. 8
Methods .............................................................................................................................. 9
Verification .......................................................................................................................... 10
Experiments ....................................................................................................................... 11
Samples .............................................................................................................................. 12
Results and Discussion ..................................................................................................... 14
  Implications ..................................................................................................................... 20
Conclusion .......................................................................................................................... 22
Recommendations ............................................................................................................. 23
List of Terms Used ............................................................................................................ 24
Works Cited ......................................................................................................................... 25
Appendix A: Useful algae by-products ........................................................................... 30
Appendix B: Additional information about samples ......................................................... 31
Appendix C: Additional plots ............................................................................................ 33
List of Figures

Figure 1 – Extinction, absorption, and scattering of algal cells .................................................. 2
Figure 2 – Scattering contribution ratios of phytoplankton vs. NAPs ........................................ 3
Figure 3 – Normalized flux vs. path length for different scattering albedos ......................... 3
Figure 4 – Experimental set-up for measurements using an IS ................................................... 5
Figure 5 – Visual representation of the Lambert-Beer law ......................................................... 6
Figure 6 – Visual representation of the two-flux model ............................................................... 7
Figure 7 – Visual representation of the six-flux model ............................................................... 7
Figure 8 – IS set-up for measuring $k \lambda$ .................................................................................. 9
Figure 9 – Set-up for measuring scattering and absorption with an IS ..................................... 10
Figure 10 – Scattering and absorption coefficient as determined by Gaigalas et al .................. 11
Figure 11 – Scattering and absorption coefficient verified at TU Delft .................................. 11
Figure 12 – Algae growth media: freshwater and saltwater ...................................................... 12
Figure 13 – A. falcatus sample and dilutions ........................................................................... 12
Figure 14 – Titanium dioxide suspension .................................................................................. 14
Figure 15 – Absorption spectra for C. vulgaris ............................................................................ 14
Figure 16 – Percentage of incoming light scattered, absorbed and reflected for C. vulgaris .... 15
Figure 17 – Optical coefficients for A. falcatus ......................................................................... 15
Figure 18 – Light penetration through A. falcatus ..................................................................... 16
Figure 19 – Comparison of 3 radiative transfer models for A. falcatus ..................................... 16
Figure 20 – Backward light intensity from a sample of C. vulgaris .......................................... 17
Figure 21 – Normalized backward light intensity vs. light backscattered .................................. 17
Figure 22 – Light penetration through various sample types ..................................................... 18
Figure 23 – Free space between cells of A. falcatus and S. obliquus .......................................... 21
Figure 24 – Specific optical coefficients for P25 Degussa powder ............................................ 22

List of Tables

Table 1 – Average extinction coefficients for all samples tested ................................................. 18
Table 2 – Order in which shapes block light, from most (left) to least (right) ......................... 19
Table 3 – Chemical composition of the six algae species considered ........................................ 19
Table 4 – Concentration required for desired light penetration at 10 cm depth ...................... 20
Introduction
Globally, humans make use of a staggering amount of energy. This energy comes predominantly from fossil fuels. Some estimates indicate that, at current consumption rates, there are 36, 58, and 200 years worth of oil, natural gas, and coal remaining, respectively (1). However, the International Energy Agency (IEA) predicts that the world’s energy consumption will increase 66% by 2030. More specifically, and worryingly, the global oil demand is expected to double, on its own (2). A chilling result from Jefferson’s 2006 study showed the global demand for oil being unmet by 2023, in a mid-case scenario (3).

In order to meet this increasing energy demand—and turn away from the current, unsustainable carbon-based economy—it is important to seek out and develop alternative, renewable energy supplies. One possible source is biofuel; specifically of interest to this study is biofuel derived from algae.

Background Information
Biofuels, in general, are any (renewable) energy source produced from natural materials (4). Under this definition, humans have been using biofuel since the ‘invention’ of fire, burning biomass (wood) for cooking and heating. However, biofuel from algae is a much more recent development.

Biofuel from algae
The first mention of algal biofuel comes from an early 1950s project report on a mass culture of microalgae grown on the roof of the Massachusetts Institute of Technology (MIT) in the United States (5). The fuel crisis of the 1970s led to a renewed surge in the study of microalgae biofuels in the United States; the Department of Energy (DOE) and the Aquatic Species Program (ASP), run by the National Renewable Energy Laboratory (NREL), looked into the production of oil from microalgae from 1980 to 1995. Their study paid particular attention to whether to use open ponds or closed photobioreactors, resulting ultimately in an open pond pilot plant in Roswell, New Mexico (5). However, as the initial panic from the oil crisis faded, funding was eventually cut and the ASP was shut down in 1996 so remaining programs could focus on bioethanol, mainly from corn.

Still, biofuel from algae offers distinct advantages over bioethanol from corn (or other food crops). Use of food crops for bioethanol production can cause food scarcity, while over-cultivating crops for energy can lead to reduced biodiversity of crops and soil erosion, to name just a few negative consequences (6). The cultivation of algae takes up less land area and uses far less water with respect to crop yield.

Other useful by-products from algae
An interesting and important point is raised by Rene Wijffels of Wageningen University in the Netherlands, among others (7) (8) (9). He explains that biofuel is actually the least valuable (and often least abundant) product that can be harvested from algae (8). The problem isn’t so much securing investors interested in algal biofuel as finding companies willing to use the other by-products that are grown in addition to the oil. Indeed, a wide range of useful (and profitable) substances can be garnered from algae, including, but not limited to those shown in the list in Appendix A (7). These products have a variety of uses, ranging from wastewater treatment, to food
colorants, to medicinal compounds. Furthermore, algae can also be used for CO₂ capture, which presents an interesting and attractive tactic for fighting global warming (10). The wide variety of useful products that can be obtained from algae makes its efficient and optimized growth an even more worthwhile point of research.

**Goal of study**
This study seeks to answer two interconnected sets of questions regarding the optical properties of algae. Firstly, studies have shown that the shape of the algae particles can have a significant effect on the (error in) scattering measurements (11). This study will endeavor to show more explicitly what the exact effect of particle shape on light penetration into the photobioreactor is. Secondly, this study will evaluate whether algae particles scatter light in a similar manner as titania (titanium dioxide) particles and, if so, whether they could be modeled accordingly. There has been much work done on modeling the optical behavior TiO₂, so it may be possible to benefit from pre-existing work.

**Reasons for study**
This study seeks to answer some issues which may lead to better photobioreactor design. To be able to design or model the best photobioreactor set-up, all the complex processes taking place within must be understood. Currently, there are no fully comprehensive models that account for all the complex phenomena taking place with which to simulate a photobioreactor (12). For this to happen, the effect of all these factors—and how they interact with each other—must be fully understood. One aspect which needs further study is the light scattering which takes place within the reactor, since light penetration depth affects the growth of algae.

**Literature study**

**Optical properties**
When modeling a photobioreactor (like one used for growing algae, for example), it is important to have a full understanding of the processes taking place inside. In particular, it is worth considering the scattering from all sources, not just algae particles, such as, inter alia, bubbles, dissolved nutrients (11)(13).

**Algae**
First of all, it has been shown theoretically and experimentally that “spectral variations of scattering resemble the inverse variations of absorption...” (14)(15). See Figure 1, right.

Pilon et al. (16) state that “although scattering becomes the dominant phenomenon contributing to the overall extinction of light, it is mainly in the forward direction so light penetrates within the reactor” (13). However, measuring the coefficients of this process poses technical challenges and not often easily or accurately predictable (17).
Sun et al. (2010) found that, for in situ conditions, for both scattering and backscattering, “... the mean contribution ratios of the algae are all low, usually below 10% ...” meaning that most (up to or above 90%) of scattering is due to non-algal particles (NAPs) (14). This can be seen from Figure 2, where the scattering contribution ratio of non-algal particles is compared to phytoplankton scattering contribution ratios. Furthermore, unlike phytoplankton, these NAPs show uniform scattering properties (14). However, this study was done in a lake, rather than with a small, highly concentrated sample in a lab or in a photobioreactor (14). While the issue of most scattering coming from NAPs is unlikely to be the case in a lab or reactor situation, such as we are concerned with, it does show that significant scattering can occur from other sources.

Berberoğlu et al. (2006) developed a model for a photobioreactor containing gas bubbles and cyanobacteria which accounts for the scattering by both (18). They propose a radiative transfer equation as an energy balance on radiative energy travelling in the direction $\hat{s}$, as well as a formula for the average single scattering albedo $\omega_{\text{eff}}$ (ratio of scattering efficiency to total extinction efficiency). The albedo is influenced by the amount of bubbles and adsorption cross section of these bubbles in the reactor.

They concluded that “scattering is unimportant when the single scattering albedo is less than 0.5 and scattering is strongly in the forward direction”, as shown in Figure 3, below. Conversely, scattering is important when the scattering albedo is greater than 0.5, indicating that scattering is the dominant method of light extinction. Also, as scattering plays more of a role, the choice of phase function used in interpreting the results becomes more important. As can be seen from Figure 3, the curves differ greatly depending on this choice, the greater the scattering albedo.
The implication here is that choosing the ‘wrong’ function could result in poor reactor design. For example, in the case below, where $\omega_{\text{eff}} = 0.91$, choosing for either the Henyey-Greenstein or truncated phase functions would indicate that light penetrates at least 10 cm into the reactor. However, if the ‘no in-scattering’ assumption is more accurate for the algae strain in use, then maximum light penetration actually occurs at 2 cm and the remaining 8 cm depth of algae would not be illuminated, resulting in a poorly functioning reactor. Of course, the amount of bubbles is also different between the two cases, which does account for some of this effect. In this study, however the effect of bubbles will be neglected, since it is purely the effect of algae particle shape that are of interest here.

Several known factors can influence the light (back)scattering of algae cells. These include, but are not limited to, size (19), shape (20) (19) (21), morphology (11), cell core location (21), cell-to-cell-core size ratio (21), intracellular gas vacuoles (11) (20) (22), incident light wavelength (22), absorption (11) (14), cell membrane or wall (11). Many of these characteristics, in turn, are dependent on taxonomic group (11).

Shape is particularly important as shown in a study done by Mugnai and Wiscombe’s in 1989 which suggests that spheres are “the most unrepresentative shape possible—almost a singularity if you will”, as cited by Gordon and Du (20). If a spherical shape is assumed for an algae particle which is actually disk-like, the result can be up to 50% more scattering per particle than predicted (20). Kroon et al., (1996) and Yurkin et al., (2007) have studied the theoretical study of light scattering by disk-shaped particles (23) (24). Whitmire et al. (2010) found that “assuming sphericity and homogeneity resulted in underestimates of the backscattering coefficient by up to an order of magnitude.”

Titania particles
Titanium dioxide is often used as the catalyst for light-induced breakdown of water-borne pollutants (25). However, solving the radiation transfer equation that governs its optical properties is a difficult task, even for simple geometries, “particularly for the presence of the scattering-in contributions.” (25). Cabera et al. (1996) advocate experimental measurements over theoretical approaches to calculating relevant coefficients, as once either the absorption or scattering coefficient is known, the other can be found by subtracting the known coefficient from the extinction coefficient. They found that the scattering coefficient is always a very significant part (always more than two-thirds, and generally a much larger fraction) of the extinction coefficient and consequently, under normal conditions, scattering effects in photocatalytic reactions cannot be neglected.”

In contrast, Satuf et al. (2005) found that the absorption coefficient can be on par with or greater than the scattering coefficient for short wavelengths of light (26). They used the method proposed by Cabrera et al., but with a different (isotropic) phase function ($p=1$) for parameter estimation. This again highlights the sometimes pronounced effect of phase function choice on results.

**Integrating Sphere**
To meet the goals set out earlier, several experiments were run using an integrating sphere (IS). These goals, to reiterate, are (i) to determine, if possible, the explicit effect of particle shape on light
penetration through the sample and (ii) to see if algae particles can be modeled like titania particles, to make use of existing, extensive models. The IS was used to measure the absorption and reflectance of light through: (i) algae particles (six different species); and (ii) titanium particles. More specifically, three different shapes of algae particles were tested. Currently, models or theories exist for “homogeneous spheres (Mie theory), two-layered spheres (Aden–Kerker theory), and homogeneous and two-layered spheroids (Extended Boundary Condition Method, EBCM)” (27). While there aren’t any algae strains known to have perfectly homogeneous, spherical shaped particles, it is possible to test particles of many different shapes, as well as an increasingly popular shape in use, at least locally, the ‘banana’ (28).

An IS is a device for measuring optical radiation (12). It does this by collecting and spatially integrating radiant flux, either directly or after having passed through a sample. It can be used to measure the total reflectance and transmittance of a scattering material such as a cuvette of algae. In its simplest form, an integrating sphere is a hollow sphere with two small holes, an entrance and an exit, and a diffuse, white, reflective interior. A light source is placed at the entrance and a detector at the exit, and a sample as desired (29). Figure 4 shows one theoretical set-up for measuring such qualities.

Figure 4 - Experimental set-up for measurements using an IS (29)

Nelson and Prézelin (30) describe how to calibrate an IS for determining the absorption coefficient of scattering suspensions. However, this method requires the sample to be placed inside the IS, which is not possible for this study. Gaigalas et al. (31) describe how to measure and interpret the absorption and scattering of microalgae with an IS set-up. Pilon et al. (16) build on this and describe various methods for solving the radiative transfer equation (RTE) using determined values for the absorption and scattering coefficients of microalgae experimentally determined with an IS set-up (16). They state that “in order to simulate light transfer in photobioreactors and use any of the above-mentioned light transfer models, the spectral radiative characteristics, namely, $k_\lambda$, $\sigma_\lambda$, and $\phi_\lambda(\mathbf{s}_i,\mathbf{s})$, [absorption coefficient, scattering coefficient, and scattering phase function, respectively] of the microorganisms are required ... ” and can be determined through either experimental measurements or theoretically (16).
Radiative transfer

The radiative transfer equation (RTE) describes what happens to a beam of light (radiation) as it travels; the equation accounts for energy losses (due to absorption), gains (due to emission), and redistributions (due to scattering). The steady-state expression of the RTE for collimated light as defined by Pilon et al. (2011) is given below:

$$\mathbf{s} \cdot \nabla I_\lambda(\mathbf{r},\mathbf{s}) = -\beta_{\text{eff},\lambda} I_\lambda(\mathbf{r},\mathbf{s})$$

Where $I_\lambda$ is the intensity of the light at location $\mathbf{r}$ travelling in direction $\mathbf{s}$ and $\beta_{\text{eff},\lambda}$ is the effective extinction coefficient, expressed as the sum of the scattering ($\sigma_\lambda$) and absorption ($k_\lambda$) coefficients.

There are various methods and models available for solving the RTE in order to describe the optical behavior of spherical particles under various conditions. An overview of these is given in the following sections.

Lambert-Beer

One simplistic solution is the Lambert-Beer Law (LB). This method assumes that incoming light is either absorbed or scattered in the forward direction (transmitted), see Figure 5 below.

In this case, the amount of light absorbed ($A$) or transmitted ($T$) can be calculated as shown below:

$$A = \log \left( \frac{q_0}{q^+} \right) = \varepsilon \cdot C \cdot l$$

$$T = \frac{q_0}{q^+} = 10^{-A}$$

where $A$ is the absorbance (dimensionless), $q_0$ is the initial light intensity in $W \cdot m^{-2}$, $q^+$ is the light intensity after passing through the solution of interest ($W \cdot m^{-2}$), $\varepsilon$ is the wavelength-dependent molar absorptivity or extinction coefficient in $M^{-1} \cdot cm^{-1}$, $C$ is the concentration of the solution through which the light passes in $M$, $l$ is the path length in $cm$, and $T$ is the transmittance (dimensionless).

This model is too simple, as it ignores backscattered light.

Two-flux model

Another simple solution which reduces calculation times considerably is the two-flux model (TFM). The generalized two-flux approximation was presented by Mengüç and Viskanta, 1983, and has been used to model photobioreactors by Cornet et al., 1998, and Cornet and Albiol, 2001, for example.
This approach assumes that any incoming radiation is scattered purely in the forward and/or backward directions, and not absorbed (32).

\[ -\frac{dq^-}{dx} = -(k_{\lambda} + \sigma_{\lambda})q^- + \sigma_{\lambda}q^+ \]

\[ \frac{dq^+}{dx} = -(k_{\lambda} + \sigma_{\lambda})q^+ + \sigma_{\lambda}q^- \]

where \( q^\pm \) is the radiation flux in the backward (\(-\)) or forward (\(+\)) hemisphere in W\(\cdot\)m\(^{-2}\), \(k_{\lambda}\) is the wavelength-specific absorption coefficient, \(\sigma_{\lambda}\) is the wavelength-specific scattering coefficient, and \(\frac{dq^\pm}{dx}\) is the change in the radiation flux as a function of distance in the x-direction in W\(\cdot\)m\(^{-3}\).

This model is also too simple, as it ignores absorbed light. Also, in the case of TiO2, light is only scattered backward.

Six-flux model
The six-flux model (SFM) is a significantly more complicated model. In this case, however, in addition to being scattered forward or backward, incoming light can also be counted as being scattered upwards, downwards, right or left (see Figure 7, below), or absorbed.
Equations for the SFM, as provided by Brucato et al. (33) for a flat, heterogeneous photoreactor are shown below (33):

\[
\frac{dq^+}{dx} = \frac{q_0}{(1-\gamma)} \exp \left(-\frac{x \cdot a\sqrt{1-(b/a)^2}}{\lambda_0}\right) \left[1 - \gamma \exp \left(-\frac{2x \cdot a\sqrt{1-(a/b)^2}}{\lambda_0}\right)\right]
\]

\[
-\frac{dq^-}{dx} = \frac{q_0}{(1-\gamma)} a \left(1 - \sqrt{1-(b/a)^2}\right) \exp \left(-\frac{x \cdot a\sqrt{1-(b/a)^2}}{\lambda_0}\right) \\
- \gamma \left(1 + \sqrt{1-(b/a)^2}\right) \exp \left(\frac{x \cdot a\sqrt{1-(a/b)^2}}{\lambda_0}\right)
\]

with:

\[
\gamma = \frac{1 - \sqrt{1-(b/a)^2}}{1 + \sqrt{1-(b/a)^2}} \exp \left(\frac{2l \cdot a\sqrt{1-(b/a)^2}}{\lambda_0}\right)
\]

\[
a = 1 - \omega p_f - \frac{4\omega^2 p_s^2}{(1 - \omega p_f - \omega p_b - 2\omega p_s)}
\]

\[
b = \omega p_b - \frac{4\omega^2 p_s^2}{(1 - \omega p_f - \omega p_b - 2\omega p_s)}
\]

where \(a\), \(b\), and \(\gamma\) are dimensionless parameters, \(q^\pm\) is the radiation flux in the backward (-) or forward (+) hemispheres in W·m\(^{-2}\), \(l\) is the path length in cm, \(p_{f,b,s}\) are the probabilities of light being scattered in the forward, backward or sideward directions, \(\omega\) is the scattering albedo and \(\lambda_0\) is a length constant in m.

This model is more complicated than what is required for this study, but nonetheless noteworthy.

**Modified two-flux model**

The modified two-flux model (M-TFM) presented by Motegh et al. (34) combines the optimum characteristics of Lambert-Beer and the TFM. This model more accurately describes light scattering by algae particles, without getting as complicated as the six-flux model (see below). That is, all incoming radiation is counted as being scattered forward and/or backward, and/or absorbed.

The model given in Motegh et al.’s paper that is most applicable for this study is titled ‘bidirectional scattering by photocatalyst particles in a non-absorbing liquid’ and is shown below:

\[
I_f(x) = \frac{e^{-\tau_{xx} l_0} + e^{\tau_{xx} l_0} \eta}{1 + \eta}
\]

where \(I_f(x)\) is the forward light intensity in the \(x\) direction, \(\tau_{xx}\) is the dimensionless local optical thickness, \(l_0\) is the incident light intensity and \(\eta\) is a dimensionless parameter given by:
where \( P_{bs} \) is the probability of backscattering, \( \omega_s \) is the scattering albedo, \( \tau_s \) is the total optical thickness, and \( a \) is given by:

\[
a = [(1 + \omega_s)(1 - \omega_s + 2P_{bs}\omega_s)]^{1/2}
\]

**Methods**

**Absorption coefficient**
The absorption coefficient can be measured by placing the sample between the light beam and the entrance hole of the integrating sphere, as shown in Figure 8. An IS is limited in that it can only measure radiation scattered forwards or backwards, not angularly, meaning any light not coming through is counted as lost due to adsorption, rather than in-scattering. However, because microalgae are generally strongly forward-scattering, an IS can still be used. To ensure that this is the case, the reflectance of the sample can also be measured by placing the sample on the opposite side of the sphere from which the light enters, to measure what reflects back into the sphere. If this is very low, then the sample is indeed strongly forward scattering. Still, the absorption coefficient measured this way will be affected by scattering error and need to be corrected (16).

**Scattering coefficient**
Measuring scattering is a more complicated task, due to the fact that incoming light can be scattered in any specific direction. Light which is scattered directly backward is measured as shown in Figure 7, using the set-up for reflectance. However, as mentioned previously, algae particles are strongly forward scattering. How, then, do we separate forward-scattered light from light which has simply been transmitted? A method has been suggested by Gaigalas et al. (2009) using the set-up shown in Figure 9, below (neglecting position 3). Here, the absorption of the light (including full forward-scattering) is measured first, in the typical set-up (position 2). Next, the cuvette holder is placed at such a distance from the IS entrance port (position 1) that it is assumed that only transmitted light enters the IS, with forward-scattered light falling outside the small entrance port.

This way, light which normally would have been counted as transmitted can be separated into a measure for scattered light. The effective scattering and absorption coefficients can be derived from these measurements, as will be described later.
**Scattering phase function**
Simply enough, “the scattering phase function ... of microorganisms can be measured using a nephelometer” (16). In most cases, the function is measured as a function of the polar angle, since “for spherical or randomly oriented particles ... the phase function does not change as a function of the azimuthal angle” (16). Furthermore, Pilon et al. state that measurements taken at 632.8 nm can be used for modeling (as a first order approximation), since the particles are strongly forward scattering. For the purpose of this study, the phase function was not measured.

**Verification**
Firstly, to ensure the proper set-up of cuvette holders 1 and 2 (see Figure 11, previously), the results of Gaigalas et al. must be replicated. In this case a Perkin Elmer Lambda 900 machine rather than Perkin Elmer Lambda 850 was used, but this should not make a significant difference. Similar carboxyl modified polystyrene spheres were also used, albeit from a different supplier.

Gaigalas et al. state that “the microspheres were diluted by $10^4$ and suspended in a solution containing a 50-fold dilution of SRM 1932 (fluorescein solution) in acetate buffer” (31). However, in a cuvette with a volume of 3 ml, this resulted in such a miniscule concentration of spheres that they could not be detected by the spectrometer. The dilution of microspheres in the fluorescein-buffer solution was decreased to $10^3$ and ultimately $10^2$ before useable results were achieved. Other than this deviation, the method from Gaigalas et al. was followed accordingly. The results from this test are shown in Figure 11, alongside the results achieved by Gaigalas et al., in Figure 10.

The difference in scale between the two sets of results can most likely be accounted for by the much larger concentration of microspheres in the second case. However, these results are satisfactory to show that the altered set-up with the integrating sphere works as desired and can be use to determine the scattering and absorption coefficients for microalgae samples.
Due to the pronounced effect of shape on the optic properties of algae, five different strains with four different shapes were tested. These are outlined in Table B-1 in Appendix B. Before they could be tested in the spectrometer, all samples had to be diluted in order to allow sufficient light to pass through. This was done by mixing 1 ml of the algae with 10 ml of water and, in all but one case, mixing 1 ml of this dilution with another 10 ml of water. Each sample is described in more detail in the following section.

Experiments

Each (diluted) sample was measured in the spectrometer for a range of path lengths (cuvette widths), 1, 2, 5, and 10 mm, and dilutions (halved, and quartered). Each arrangement was measured both in holder 1 and 2. The full arrangement of readings to be taken is shown in Table B-2 in Appendix B.

Blue cells in this table indicate the size of the cuvette to be used, whereas green cells indicate the concentration of solution to be used. There are 6 runs to be done for each solution (except water and TiO2), or 46 runs in total.
In addition to the algae samples and distilled water, the two growth media were also tested to see if they exhibited substantially different absorbing or scattering behavior as compared to water. These were not diluted. The growth media were prepared according to the method described by Salim et al. and compromised primarily salts (35).

**Ankistrodesmus Falcatus**

The figure above shows bottles containing the original sample as well as progressive dilutions of it; the starting concentration of the sample was 5.65 kg/m³. The second sample from the left was used as the ‘maximum’ concentration; this sample was halved to make the next dilution, and so on. The notes from the falcon tube containing the original sample are detailed below:

<table>
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<tr>
<th>A.F.</th>
<th>Sample name (<em>ankistrodesmus falcatus</em>)</th>
</tr>
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<tr>
<td>SAG 202.9</td>
<td>Culture collection of algae origin (Goettingen University, Germany)</td>
</tr>
<tr>
<td>17-6-2012</td>
<td>Date of creation</td>
</tr>
<tr>
<td>M-8a</td>
<td>Freshwater growth medium</td>
</tr>
</tbody>
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Chlorella Vulgaris

Figures showing the dilutions for samples of *C. vulgaris*, and all other algae strains, can be found in Appendix C. The un-doctored sample had a starting concentration of 7.30 kg/m³ dry weight, and the ‘maximum’ concentration used for measurements was a 50-fold dilution of that. The notes from the falcon tube of the original sample are detailed below:

<table>
<thead>
<tr>
<th>Sample name (chlorella vulgaris)</th>
<th>Date of creation</th>
<th>Freshwater growth medium</th>
</tr>
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<tbody>
<tr>
<td><strong>C.V.</strong></td>
<td><strong>25-5-2012</strong></td>
<td><strong>M-8a</strong></td>
</tr>
</tbody>
</table>

Ettlia Texensis

The starting concentration of the un-doctored sample of *E. texensis* was 11.03 kg/m³, dry weight, and a 20-fold dilution of that was taken as the ‘maximum’ concentration. The notes from the falcon tube of the original sample are detailed below:

<table>
<thead>
<tr>
<th>Sample name (ettlia texensis)</th>
<th>Date of creation</th>
<th>Freshwater growth medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E.T.</strong></td>
<td><strong>M-8a</strong></td>
<td><strong>SAG 79.80</strong></td>
</tr>
</tbody>
</table>

Neochloris Oleoabundans

The original sample of *N. oleoabundans* had a concentration of 2.12 kg/m³, dry weight, and the ‘maximum’ concentration was taken as a 5-fold dilution of that. The notes from the falcon tube of the original sample are detailed below:

<table>
<thead>
<tr>
<th>Sample name (neochloris oleoabundans)</th>
<th>Date of creation</th>
<th>Freshwater growth medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N.O.</strong></td>
<td><strong>Zou</strong></td>
<td><strong>SAG 276-3a</strong></td>
</tr>
</tbody>
</table>

Scenedesmus Obliquus

The concentration of the original sample of *S. obliquus* was 7.90 kg/m³, dry weight, and a 40-fold dilution of that was taken as the ‘maximum’ concentration for measurements. The notes from the falcon tube of the original sample are detailed below:

<table>
<thead>
<tr>
<th>Sample name (scenedesmus obliquus)</th>
<th>Date of creation</th>
<th>Freshwater growth medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S.O.</strong></td>
<td><strong>18-7-2012</strong></td>
<td><strong>M8a</strong></td>
</tr>
</tbody>
</table>
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**Tetraselmis suecica**

The concentration of the original sample of *T. suecica* was 3.59 kg/m³, dry weight, and the ‘maximum’ concentration for measurement purposes was a 5-fold dilution of that. The notes from the falcon tube of the original sample are detailed below:

<table>
<thead>
<tr>
<th>T.S.</th>
<th>Sample name (<em>tetraselmis suecica</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ccap 66122a</td>
<td>Culture collection of algae origin (European Centre for Marine Biotechnology, UK)</td>
</tr>
<tr>
<td>24-7-2012</td>
<td>Date of creation</td>
</tr>
<tr>
<td>Zou</td>
<td>Saltwater growth medium</td>
</tr>
</tbody>
</table>

**Titanium dioxide**

A 50mL solution of 1g/L titanium dioxide (P25, Degussa) powder in distilled water was prepared for testing. This proved to be too opaque, and so 5 ml of this solution was diluted again with a further 5ml of ultrapure water to be used as the ‘maximum’ concentration during testing. **Figure 14** depicts an image of a visually identical suspension of TiO₂ found in work by Shirai et al. (36).

At this pH (neutral), the powder tends to form large agglomerates, around 8-16 μm, which is in line with the average algae particle size tested (37). However, this leads to the agglomerates settling out quite quickly (within minutes). This meant that the contents of each cuvette had to be shaken or stirred between each reading with the IS set-up. After each reading was taken, the cuvette was immediately inspected to ensure that the TiO₂ particles were still sufficiently suspended that the readings could be useable.

**Results and Discussion**

For each sample (and dilution) the absorption spectra was measured for holder 1 and holder 2. An example of the resulting plot for *chlorella vulgaris* at maximum concentration is shown in **Figure 15** to the right. As was shown in **Figure 9**, the holder 2 position is directly adjacent to the IS; this means that both transmitted light and forward scattered light is read by the detector. Hence, the absorption appears lower because there is a lot of light coming through. In the holder 1 position the cuvette is placed at a distance from the IS; in this position only transmitted light is read by the
detector, as forward scattered light falls outside the inlet. Therefore, subtracting the readings from holder 2 (absorption) from the holder 1 readings (absorption + scattering) we can see how much light is scattered rather than just transmitted.

Once the reflectance measurements are taken, the data can be arranged to show the percentage of light which is absorbed and scattered, forward and backward, shown in Figure 16. Therefore, a certain percentage (the amount backscattered) must be taken in to account and added to the final scattering coefficient determined by Gaigalas’ method. Furthermore, the reflectance measured by the IS has inherent errors that must also be accounted for. The machine cannot reliably measure reflectance below 20%; this 20% appears as a baseline, even when no sample is in the sample holder, and therefore 20% should be removed from measurements (38). This has already been taken into account in Figure 16 (meaning that the reflectance measured was actually 29%, not 9% as shown here). 9% appeared to be the average reflectance across all species of algae, independent of path length or concentration. The only outlier was A. falcatus, which had an average measured reflectance of 1%.

Another oddity, given the strong apparent dependence of absorbance and forward scattering on the wavelength of the incident light, is the flatness of the reflectance profile. It is not clear what causes this uniformity (across all species, no less), but the apparatus was re-calibrated and yielded the same results upon further trials.

Using the method utilized by Gaigalas et al. (31), the scattering, absorption, and extinction coefficients can be determined. For each sample the specific extinction coefficient for each run was determined, and then the path length and concentration divided out; the ultimate extinction coefficient for each sample was the average of these. A plot showing these coefficients as a function of wavelength is shown in Figure 17.
wavelength for *A. falcatus* is shown in Figure 17, with concentration and path length divided out. Similar plots for all other samples can be found in Appendix C. Other samples of algae had more reflectance, and therefore those plots show scattering slightly higher than absorption than is shown here. Unsurprisingly, absorption coefficients for all species of algae are the approximate inverse of their respective scattering coefficient.

Using the extinction coefficient determined before, the light penetration into the photobioreactor can be calculated. This is shown in the plot below for *A. falcatus* at four different concentrations. Again, similar plots for other samples can be found in the Appendix. The result is predictable, with the light penetration increasing in regular intervals each time the concentration is halved. It was also shown, unsurprisingly, that halving the path length had the same effect as halving the concentration (see Appendix C).

This plot was generated using the TFM, for ease of use. This was deemed allowable, as the TFM and the M-TFM produce very similar results (see Figure 19, below; TFM and M-TFM lines are overlapped); however, the TFM is quicker to use. As in the figure below, LB slightly overestimates the light penetration for most algae samples. This illustrates how choosing the wrong model could lead to poor reactor design.
The reason that the TFM and the M-TFM are overlapping in Figure 19 is that the true difference between the models lies in what happens to the light which does not penetrate into the reactor. As mentioned previously, in the Models section, the TFM assumes that incoming light is transmitted (forward) and/or scattered backward. The M-TFM, meanwhile, assumes that incoming light is transmitted (forward), scattered (backward), and/or absorbed. Since both models show the same amount of light penetrating forward, this indicates that the M-TFM will show much less light being scattered backward, as some of it must be absorbed. Therefore, the M-TFM accounts for the TFM overestimating light lost from backward scattering; this can be seen from Figure 20, below:

Figure 20 - Backward light intensity from a sample of C. vulgaris as described by two different models

The previous figure shows the backward light intensity resulting from incident light impacting on a sample of C. vulgaris, as calculated by the TFM and the M-TFM. Were the value for percentage of light scattered backward ($P_b$) in the M-TFM set to 1, the lines would overlap. The backward light intensity is strongly affected by the value of $P_b$, in fact. This is shown, for several different scattering albedoes, in Figure 21 below.

Figure 21 – Normalized backward light intensity versus increasing amount of light backscattered for different scattering albedoes ($\omega = 1$ indicates that all extinction is due to scattering and $\omega = 0$ corresponds to no scattering)
The average extinction coefficient was determined for all samples by dividing out the concentration and path length for all results. When the same, arbitrary concentration is used for all samples, then the light penetration in a theoretical photobioreactor through each sample is demonstrated in the plot below in Figure 22. Again, this plot was generated using the TFM for ease of use.

![Figure 22](image)

**Figure 22** - Light penetration through various sample types with the same arbitrary concentration, 3.5 g/L dry weight. This concentration was chosen as it falls within the range specified as typical for growing in photobioreactors (39). AF stands for *A. falcatus*, CV for *C. vulgaris*, ET for *E. texensis*, NO for *N. oleoabundans*, SO for *S. obliquus*, TS for *T. suecica*, and TiO2 for titanium dioxide.

The extinction coefficients as determined for each sample (and used to generate the previous plot) are shown in Table 1 below. The extinction coefficient determined for Degussa P25 TiO2 was on the same order of magnitude as that found by Mehrvar et al. (40).

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Average extinction coefficient (m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. falcatus</em></td>
<td>12.82</td>
</tr>
<tr>
<td><em>C. vulgaris</em></td>
<td>37.93</td>
</tr>
<tr>
<td><em>E. texensis</em></td>
<td>5.87</td>
</tr>
<tr>
<td><em>N. oleoabundans</em></td>
<td>5.26</td>
</tr>
<tr>
<td><em>S. obliquus</em></td>
<td>29.70</td>
</tr>
<tr>
<td><em>T. suecica</em></td>
<td>4.39</td>
</tr>
<tr>
<td>P25</td>
<td>14.05</td>
</tr>
</tbody>
</table>

In terms of shape, then, versus light penetration, from ‘thickest’ (shallowest light penetration) to ‘thinnest’ (deepest light penetration), at the same concentration, there are spheres (5 μm, *C. vulgaris*), banana-shaped (10-20 μm, *S. obliquus*), powder agglomerates (8-16 μm, P25 Degussa...
powder), banana-shaped (15-105 μm, A. falcatus), spheres (10 μm, E. texensis), spheres (6 μm, N. oleoabundans), and cordiform (10-15 μm, T. suecica). This is illustrated by Table 2, below, which shows the cell shapes in this order, and in the accurate size relative to each other:

**Table 2 – Order in which shapes block light, from most (left) to least (right)**

<table>
<thead>
<tr>
<th>C. vulgaris</th>
<th>S. obliquus</th>
<th>Degussa powder</th>
<th>A. falcatus</th>
<th>E. texensis</th>
<th>N. oleoabundans</th>
<th>T. suecica</th>
</tr>
</thead>
<tbody>
<tr>
<td>*</td>
<td>*</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*Algae cell images from (41)

** Degussa P25 agglomerate image from (42)

These results are not initially intuitive; there is no immediate trend based on either shape or size. It is surprising that the largest particles, the ‘bananas’ of A. falcatus did not impede light penetration the most—they are only fourth ‘thickest’. In fact, it is the smaller ‘banana’-shaped particles that impede light penetration more, those of S. obliquus. This might at first suggest that smaller particles in the same shape category block more light than their larger counterparts because they are able to stack together and interlock more closely, leaving smaller gaps for light to get through. However, if we look only at samples with spherical particles, for example, than we have, from most-light blocking to least-light blocking, 5 μm, 10 μm, and 6 μm diameters. This leads to the conclusion that, shape and size do not alone govern light penetration.

However, it is not so terribly surprising that the results for E. texensis and N. oleoabundans are similar, as both strains are technically part of the Neochloris genus (E. texensis is sometimes called Neochloris texensis (43)). Nor is it very surprising that so little light penetrates the P25 Degussa powder agglomerates, since TiO$_2$ tends to reflect over 80% of incident radiation in water (in the wavelength range we are interested in) (44).

Another interesting point is immediately visible just by looking at the images of the algae cells. This suggests that cell make-up may play a role; the chemical composition of all species of algae tested is shown in Table 3 below.

**Table 3 – Chemical composition of the six algae species considered**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proteins %</th>
<th>Carbohydrates %</th>
<th>Lipids %</th>
<th>Nucleic acid %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. falcatus (46)</td>
<td>~15</td>
<td>19</td>
<td>54</td>
<td>-</td>
</tr>
<tr>
<td>C. vulgaris (45)</td>
<td>51–58</td>
<td>12–17</td>
<td>14–22</td>
<td>4–5</td>
</tr>
<tr>
<td>E. texensis (47)</td>
<td>- a</td>
<td>- a</td>
<td>42–52</td>
<td>- a</td>
</tr>
<tr>
<td>N. oleoabundans (48)</td>
<td>14–22</td>
<td>14–35</td>
<td>21–60</td>
<td>-</td>
</tr>
<tr>
<td>S. obliquus (45)</td>
<td>50–56</td>
<td>10–17</td>
<td>12–14</td>
<td>3–6</td>
</tr>
<tr>
<td>T. suecica (49)</td>
<td>50–65</td>
<td>18–23</td>
<td>17–27</td>
<td>-</td>
</tr>
</tbody>
</table>

*E. texensis is not widely used so it was not possible to find detailed information on its chemical composition.
The images of *C. vulgaris* and *S. obliquus* from Table 2, aside from being the smallest, also appear much less green than the cells from other species. They do indeed share similar cell make-up: *S. obliquus* is 50–56% proteins, 10–17% carbohydrates, 12–14% lipids, and 3–6% nucleic acid, on a dry matter basis, while *C. vulgaris* is comprised of 51–58% proteins, 12–17% carbohydrates, 14–22% lipids, and 4–5% nucleic acid (45). However, *T. suecica*, which is on the complete opposite end of the spectrum from *S. obliquus*, has a similar composition, with 50–65% proteins, 18–23% carbohydrates, and 17–27% lipids. Therefore, it seems that the amount of proteins in a cell cannot be solely accountable for how opaque that cell is.

Alternatively, it can be shown what the (dry weight) concentration would need to be for each species in order to have a light intensity of 100 W/m² (the minimum light intensity needed for algae to grow is 1/10th of direct sunlight, or 30-100 W/m²) penetrating at 10 cm depth (10 cm is the depth of the ‘large’ lab-scale photobioreactor at the University of Texas (50)). The results, using LB, the TFM, and the M-TFM, are shown in Table 4 below.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Concentration (g/L)</th>
<th>LB</th>
<th>TFM</th>
<th>M-TFM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. falcatus</em></td>
<td>2.2</td>
<td>1.8</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td><em>C. vulgaris</em></td>
<td>1.0</td>
<td>0.6</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td><em>E. texensis</em></td>
<td>4.5</td>
<td>4.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td><em>N. oleoabundans</em></td>
<td>4.2</td>
<td>4.4</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td><em>S. obliquus</em></td>
<td>1.0</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td><em>T. suecica</em></td>
<td>4.9</td>
<td>5.3</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td><em>P25</em></td>
<td>1.5</td>
<td>1.6</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

Typically, in photobioreactors, algae are grown at concentrations between 1–5 g/L, which means these results are well within reason.

**Implications**

One goal for producing algae is for it to be economical. One way of looking at this would be that the more algae you can produce in a set space, the better. It can be seen from Table 4 that it is possible to ‘fit’ more of certain types of algae in and still have the light penetrate through the entire reactor sufficiently to facilitate growth. Looking at it from this standpoint, *T. suecica*, *N. oleoabundans*, and *E. texensis* are the best candidates, in that order, based purely on light penetration vs. concentration. All three of these strains of algae have been reported to have some potential for biofuel, among other products.

This order changes, however, depending on the oil content of each strain, and the top three becomes: *N. oleoabundans*, *E. texensis*, and *A. falcatus*. For instance, *N. oleoabundans* provides a greater amount of lipids (double, in fact) than *T. suecica* does (see Table 3). Therefore, even though a lesser concentration of *N. oleoabundans* than *T. suecica* is required to have the minimum light intensity penetrating at the same depth, the greater lipid payoff most likely makes it a more promising candidate.
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Another consideration would be the photo efficiency of the algae; because the light is absorbed does not mean that it is used, or used to maximum potential. A possible next step could be to investigate how exactly the light which is absorbed is used by the algae cells.

Although it is impossible to establish any kind of explicit ‘shape factor’ based on these results, another step to take could be to look at space between cells. That is, it was shown that smaller cells sometimes blocked less light and it can be theorized that this is due to their ability to pack in together more tightly. Perhaps there is some connection between how much ‘free’ space there is between the cells when they are packed together as tightly as they can and how much light is able to penetrate through them. For example, see Figure 21 below.

![Figure 23 - Free space between cells of A. falcatus (top) and S. obliquus (bottom) for different packing arrangements. The black square/rectangle indicates the total free space between A. falcatus cells; the white square, superimposed over the black square, represents the free space between S. obliquus cells.](image)

When the cells are not optimally packed (left), there is obviously much greater free space between A. falcatus cells through which light can penetrate. Although the difference is not so great, there is still more free space between A. falcatus cells even when packing is optimal (right).

Although titania exhibits similar light-blocking abilities when at concentrations and particle (agglomerate) sizes similar to those of algae species, it does not make sense to model algae as TiO2 particles. The titania agglomerates do not absorb light, in the range we are interested in (the growing range for algae), while algae does absorb quite strongly. Furthermore most of the light which impacts it is reflected (up to and greater than 80%), which is quite unlike those algae species tested (which averaged 9% reflection). Titania particles do absorb light in the UV range, however, which raises the question: can the absorbance of visible light by algae be modeled in a similar way to that of UV light by P25 particles? The results from Cabrera et al (25) for the specific extinction, scattering, and absorption coefficients for P25 Degussa powder in the range where it does absorb (275–395 nm) have been reproduced in the following plot.
It is apparent that, even in this wavelength range, titania particles are not strong absorbers. Furthermore, comparing the image above with Figure 17 (the optical coefficients for *A. falcatus*), or similar plots for other algae species in Appendix C, we can see that they are quite different. Therefore, it probably does not make sense to model algae particles after TiO$_2$ particles, even in the light range where the titania agglomerates do absorb light.

**Conclusion**

The reserves of non-renewable fuel sources are diminishing while, at the same time, the demand for fuel is only increasing. There is a clear need for new sources of fuel to offset this growing deficit; a renewable source would be ideal, for obvious reasons. One such source, and a very promising one at that, is biofuel from algae. When other crops are grown, however, for food or fuel, a great deal of planning goes in to the process; seeds are not simply dropped on the ground and left to their own devices. Suffice it to say, the cultivation process for algae could stand to do with some refinement, given the usefulness and potential profitability of its products.

This study looked at the effect of particle shape on the penetration of light through a sample of algae. The purpose was to determine whether there was a discernible trend and, if so, whether some sort of shape-factor could be determined for future modeling, so that photobioreactors could be perfected and streamlined for specific particle shapes. While no conclusive trend resulted, and therefore no explicit shape-factors determined, there were still numerous useful observations to be made.

While it is not a constant, it appears possible to achieve greater optical density with smaller cells and therefore lower light penetration, although there could be other contributing factors. Also, algae
strains from the same genus would appear to behave similarly, although this is based on evidence from comparing only two members of a single genus. It is possible that chemical composition also plays a role, since more protein-heavy cells generally seem to block more light, while those that composed primarily of lipids allow more light through.

In addition, titania particles in practice behave very differently from algae, other than how much light they impede. Titania reflects light to a far greater degree (80+% vs. 9%, respectively) and does not absorb (in the wavelength range we are interested in (51)). As there has been so much work done with titania powder it is unfortunate not to be able to take advantage of it.

**Recommendations**

Out of the species of algae considered for this study, those that are capable of the highest concentrations while still letting through the minimum required light intensity for growth at 10 cm in are: *T. suecica*, *N. oleoabundans*, and *E. texensis*. Meanwhile, those that have the highest lipid content are *N. oleoabundans*, *E. texensis*, and *A. falcatus*. In terms of lipid content and best light penetration to sample concentration ratio, this means that *N. oleoabundans* and *E. texensis* appear to be clear winners as potential species for cultivation for use as biofuel. It might be worth investigating if these positive trends extend to other members of the Neochloris genus.

Furthermore, there remains a lot of work to be done in the pursuit of fully understanding all the optical properties of algae and how best to cultivate them for the desired purpose. While shape certainly plays a part, it would not appear to affect light penetration as strongly as other characteristics by any means. No obvious shape factor that could be incorporated into models could be determined by this study, simply from how they affect light penetration. In conclusion, future attempts to optimize photobioreactors should not place too heavy a dependency on the shape of the algae particles to be used, at least not without working out its interdependencies on other factors.
## List of Terms Used

### Symbols

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>a, b, c</td>
<td>Variables</td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>Increment</td>
<td></td>
</tr>
<tr>
<td>q</td>
<td>Radiation flux</td>
<td>$[\text{J}\cdot\text{m}^{-2}\cdot\text{s}^{-1}]$</td>
</tr>
<tr>
<td>s</td>
<td>Direction</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Transmission</td>
<td>$[%]$</td>
</tr>
<tr>
<td>t</td>
<td>Thickness of sample cell</td>
<td>$[\text{m}]$</td>
</tr>
<tr>
<td>X</td>
<td>Apparent absorption coefficient</td>
<td></td>
</tr>
</tbody>
</table>

### Greek symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>Extinction coefficient</td>
<td></td>
</tr>
<tr>
<td>$\theta$</td>
<td>Angle</td>
<td>$[\text{rad}]$</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>Absorption coefficient</td>
<td></td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wavelength</td>
<td>$[\text{nm}]$</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Scattering coefficient</td>
<td></td>
</tr>
<tr>
<td>$\tau$</td>
<td>Optical thickness</td>
<td>$[\text{mm}]$</td>
</tr>
<tr>
<td>$\Phi$</td>
<td>Scattering phase function</td>
<td>$[\text{sr}^{-1}]$</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Scattering albedo</td>
<td></td>
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</tbody>
</table>

### Subscripts

<table>
<thead>
<tr>
<th>Subscript</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Acceptance</td>
</tr>
<tr>
<td>eff</td>
<td>Effective</td>
</tr>
<tr>
<td>ref</td>
<td>Reference</td>
</tr>
<tr>
<td>x</td>
<td>In the x-direction</td>
</tr>
</tbody>
</table>

### Superscripts

- $+$ Forward hemisphere
- $-$ Backward hemisphere
- $\sim$ Normalized
- $^\wedge$ Vector
Works Cited


44. The Royal Society of Chemistry. Electronic Supplementary Material (ESI) for Chemical Communications. s.l.: The Royal Society of Chemistry, 2011.


64. Monteiro, C.M., Castro, P.M.L. and Malcata, F.X. *Use of the microalga Scenedesmus obliquus to remove admium cations from aqueous solutions*. s.l. : Universidade Catolica Portuguesa.


Appendix A: Useful algae by-products

All data from Janssen (2011).

- **Polyunsaturated fatty acids**
  - Eicasapentaenoic acid (EPA) – prevents coronary heart disease, hypertension, and inflammation
  - Docosahexaenoic acid (DHA) – develops brain and retinal tissues in human embryos and newborns

- **Aquaculture feedstock**

- **Carotenoids**
  - Lutein – helps avert effects of degenerative human disease, and can also be used as a food colorant in place of currently used labor- and land-intensive marigold petals

- **Oils**
  - For food

- **Pigments**
  - For food
  - Fluorescent tags – for use in diagnostics and research
  - For cosmetics

- **Protein**
  - Functional proteins – act as emulsifiers and gelling agents
  - Amino acid feedstock – used in animal feed

- **Polysaccharides**
  - Gelling agents
  - Cation chelators
  - Cosmetics
  - Skin protection – UV blockers

- **Biochemicals**
  - Starch – bioplastics
  - Polyhydroxyalkanoates (PHA) – bioplastics
  - Cyanophycin – bioplastics
  - Glycerol

- **Wastewater treatment**
  - Removal and recovery of inorganic nitrogen
  - Removal and recovery of inorganic phosphorus
  - Stimulation of aerobic wastewater treatment
  - Accumulation and recovery of heavy metals
Appendix B: Additional information about samples

<table>
<thead>
<tr>
<th>Name: Ankistrodesmus falcatus</th>
<th>Name: Neochloris oleoabundans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size:</strong> 15-105 μm</td>
<td><strong>Size:</strong> 6 μm</td>
</tr>
<tr>
<td><strong>Shape:</strong> Banana bundles</td>
<td><strong>Shape:</strong> Spherical</td>
</tr>
<tr>
<td><strong>Growth medium:</strong> Freshwater</td>
<td><strong>Growth medium:</strong> Saltwater</td>
</tr>
<tr>
<td><strong>Potential for:</strong> biofuel (high lipid content) (52)</td>
<td><strong>Potential for:</strong> biofuel (53), cosmetics (54), marine feedstock (55)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name: Neochloris oleoabundans</th>
<th>Name: Chlorella vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size:</strong> 6 μm</td>
<td><strong>Size:</strong> 5 μm</td>
</tr>
<tr>
<td><strong>Shape:</strong> Spherical</td>
<td><strong>Shape:</strong> Spherical</td>
</tr>
<tr>
<td><strong>Growth medium:</strong> Saltwater</td>
<td><strong>Growth medium:</strong> Freshwater</td>
</tr>
<tr>
<td><strong>Potential for:</strong> biofuel, cosmetics (54), marine feedstock (55)</td>
<td><strong>Potential for:</strong> carbon sequestration (57), heavy metal removal (58), medicine (59), bioremediation (60), marine feedstock (61)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name: Chlorella vulgaris</th>
<th>Name: Scenedesmus obliquus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size:</strong> 10-20 μm</td>
<td><strong>Size:</strong> 10-20 μm</td>
</tr>
<tr>
<td><strong>Shape:</strong> Banana bundles</td>
<td><strong>Shape:</strong> Banana bundles</td>
</tr>
<tr>
<td><strong>Growth medium:</strong> Freshwater</td>
<td><strong>Growth medium:</strong> Freshwater</td>
</tr>
<tr>
<td><strong>Potential for:</strong> biofuel (63), heavy metal removal (64), nutrition (65)</td>
<td><strong>Potential for:</strong> biofuel (63), heavy metal removal (64), nutrition (65)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name: Scenedesmus obliquus</th>
<th>Name: Tetraselmis suecica</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size:</strong> 10-15 μm</td>
<td><strong>Size:</strong> 10-15 μm</td>
</tr>
<tr>
<td><strong>Shape:</strong> Cordiform, elliptic, or ‘heart’-shaped</td>
<td><strong>Shape:</strong> Cordiform, elliptic, or ‘heart’-shaped</td>
</tr>
<tr>
<td><strong>Growth medium:</strong> Saltwater</td>
<td><strong>Growth medium:</strong> Saltwater</td>
</tr>
<tr>
<td><strong>Potential for:</strong> biofuel, marine feedstock (69)</td>
<td><strong>Potential for:</strong> biofuel, marine feedstock (69)</td>
</tr>
</tbody>
</table>

All samples were supplied courtesy of Sina Salim, PhD, Wageningen University, Wageningen, the Netherlands.
Table B-2 – Description of set-ups to be tested with integrating sphere*

<table>
<thead>
<tr>
<th>Test name</th>
<th>Cuvette size</th>
<th>Test name</th>
<th>Cuvette size</th>
<th>Test name</th>
<th>Cuvette size</th>
<th>Test name</th>
<th>Cuvette size</th>
<th>Test name</th>
<th>Cuvette size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorella vulgaris</td>
<td>CV10M</td>
<td>CV5M</td>
<td>CV2M</td>
<td>CV1M</td>
<td>CV10H</td>
<td>CV10Q</td>
<td>CV10E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ettlia texensis</td>
<td>ET10M</td>
<td>ET5M</td>
<td>ET2M</td>
<td>ET1M</td>
<td>ET10H</td>
<td>ET10Q</td>
<td>ET10E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenedesmus obliquus</td>
<td>SO10M</td>
<td>SO5M</td>
<td>SO2M</td>
<td>SO1M</td>
<td>SO10H</td>
<td>SO10Q</td>
<td>SO10E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neochloris oleoabundans</td>
<td>NO10M</td>
<td>NO5M</td>
<td>NO2M</td>
<td>NO1M</td>
<td>NO10H</td>
<td>NO10Q</td>
<td>NO10E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankistrodesmus falcatus</td>
<td>AF10M</td>
<td>AF5M</td>
<td>AF2M</td>
<td>AF1M</td>
<td>AF10H</td>
<td>AF10Q</td>
<td>AF10E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetraselmis suecica</td>
<td>TS10M</td>
<td>TS5M</td>
<td>TS2M</td>
<td>TS1M</td>
<td>TS10H</td>
<td>TS10Q</td>
<td>TS10E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>TD10</td>
<td>TD5</td>
<td>TD2</td>
<td>TD1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Water</td>
<td>DW10</td>
<td>DW5</td>
<td>DW2</td>
<td>DW1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Freshwater growth medium</td>
<td>FW10</td>
<td>FW5</td>
<td>FW2</td>
<td>FW1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltwater growth medium</td>
<td>SW10</td>
<td>SW5</td>
<td>SW2</td>
<td>SW1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Each test is run in the holders: holder 1, holder 2, and the rear holder, with which the appropriate suffix (H1, H2 or R, respectively) is added to the test name.
Appendix C: Additional plots

Ankistrodesmus falcatus

Figure C-1 – Dilutions of original sample of A. falcatus

Figure C-2: Absorption spectra for A. falcatus in holder and holder 2

Figure C-3: Percentage of incoming light scattered absorbed, and reflected by A. falcatus
Figure C-4 – Optical coefficients for *A. falcatus*

Figure C-5: Extinction of light for maximum, half, quarter and eighth concentration of *A. falcatus*

Figure C-6: Comparison of results from different models (LB, TFM and M-TFM)
Chlorella vulgaris

Figure C-6 – Dilutions of original sample of C. vulgaris

Figure C-7: Absorption spectra for C. vulgaris in holder 1 and holder 2

Figure C-8: Percentage of incoming light scattered, absorbed, and reflected by C. vulgaris
Figure C-9 – Absorption, scattering and extinction coefficients for *C. vulgaris*

Figure C-10: Extinction of light for maximum, half, quarter and eighth concentration of *C. vulgaris*

Figure C-11: Comparison of results from different models (LB, TFM and M-TFM)
Ettlia texensis

Figure C-12 – Dilutions of original sample of *E. texensis*

Figure C-13: Absorption spectra for *E. texensis* in holder 1 and holder 2

Figure C-14: Percentage of incoming light absorbed, and reflected by *E. texensis*
Figure C-15 – Absorption, scattering and extinction coefficients for *E. texensis*

Figure C-16: Extinction of light for maximum, half, quarter and eighth concentrations of *E. texensis*

Figure C-17: Comparison of results from different models (LB, TFM and M-TFM)
Figure C-18 – Dilutions of original sample of *N. oleoabundans*

Figure C-19: Absorption spectra for *N. oleoabundans* in holder 1 and holder 2

Figure C-20: Percentage of incoming light scattered, absorbed, and reflected by *N. oleoabundans*
Figure C-21 – Absorption, scattering and extinction coefficients for *N. oleoabundans*

Figure C-22: Extinction of light for maximum, half, quarter and eighth concentrations of *N. oleoabundans*

Figure C-23: Comparison of results from different models (LB, TFM and M-TFM)
**Figure C-24** – Dilutions of original sample of *S. obliquus*

**Figure C-25**: Absorption spectra for *S. obliquus* in holder 1 and holder 2

**Figure C-26**: Percentage of incoming light scattered, absorbed, and reflected by *S. obliquus*
Figure C-27 – Absorption, scattering and extinction coefficients for *S. obliquus*

Figure C-28: Extinction of light for maximum, half, quarter and eighth concentration of *S. obliquus*

Figure C-29: Comparison of results from different models (LB, TFM and M-TFM)
Tetraselmis suecica

Figure C-30 – Dilutions of original sample of *T. suecica*

Figure C-31: Absorption spectra for *T. suecica* in holder 1 and holder 2

Figure C-32: Percentage of incoming light scattered, absorbed, and reflected by *T. suecica*
Figure C-33 – Absorption, scattering and extinction coefficients for *T. suecica*

Figure C-34: Extinction of light for maximum, half, quarter and eighth concentration of *T. suecica*

Figure C-35: Comparison of results from different models (LB, TFM and M-TFM)
Titanium dioxide

Figure C-36 – Absorption spectra for titania in holder 1 and holder 2

Figure C-37 – Extinction of light for 5 mm, 2 mm, and 1 mm path lengths through titania

Figure C-38 – Comparison of results from different models (LB, TFM and M-TFM)