EVES ENERGY (SG) PTE LTD

JUSTIFICATION OF MICROALGAE GROWTH RATES & OIL YIELDS in SEAWATER TANKS & MORE

Key Highlights:

- Microalgae contain at least **50% oil** content: https://petrowiki.spe.org/Producing_crude_oil_from_algae
- **Chlorella sorokiniana** and other microalgae are observed to have **double timings in the range of two hours** under certain conditions: https://www.e-education.psu.edu/egee439/node/694.
- **1 MT of microalgae** absorb at least **1.8 MT of CO2**: https://link.springer.com/article/10.1007/s00253-012-4362-z
- Algae biofuel cuts **lifecycle CO2 by 68%** vs petroleum, this reduction **includes all emissions from production to use**: https://biodieselmagazine.com/articles/9336/new-study-algae-biofuel-cuts-co2-emissions-by-up-to-68-percent

Our calculations based on the above studies and our pilot project:

- **1 MT of microalgae biomass yields 0.5 MT of Crude Algae Oil (CAO). Growing and producing 1 MT of biomass absorbs 1.8 MT of atmospheric CO2 verified in publications.**
- **Hence, 1 MT of CAO produced sequesters (1.8*2) or 3.6 MT of CO2 without accounting for CO2 emissions in production to use.**
- **From previous studies, a reduction of 50-70% or about 68% of lifecycle CO2 of algae biofuel vs petroleum.**
- **From our pilot, we adjust CO2 emission by -40% to account for lifecycle CO2 from production to use, which results in 1 MT of produced CAO offsetting (3.6*60%) or 2.2 MT of CO2.**
- **We further adjust -10% and utilize 1 MT of CAO offsets 2 MT of lifecycle CO2 from CAO production to use in our calculations as a fair estimate.**

More details as follows:

The realistic amount of #microalgae #biomass that can be produced depends on several factors, including the species of microalgae, the specific growth conditions, and the cultivation method.

Under optimal growth conditions, most species have a doubling time of a few hours, and cultures can reach as much as **10 g L −1** of heterotrophic dry weight (DW) biomass and **6 g L −1** of photoautotrophic DW biomass[1].

Assuming that the tank is filled with seawater to a depth of 3 meters, the volume of the tank is `3m * 3m * 3m = 27 cubic meters`. Since 1 cubic meter is equivalent to 1000 liters, the total volume of the tank is `27 * 1000 = 27000 liters`.

Therefore, under optimal conditions, the maximum biomass that could be produced in one day in this tank could be as much as `27000 L * 10 g/L = 270 kg` for heterotrophic growth or `27000 L * 6 g/L = 162 kg` for photoautotrophic growth.

However, actual productivity can vary based on many factors including light intensity, temperature, nutrient availability, and specific characteristics of the microalgae species being cultivated.

Sources:
(1) Algal growth phases including determination of the growth rate and .... https://lnkd.in/gxv_xRHf.
(2) . https://lnkd.in/gntfWYPP.
(3) Evaluation of Growth Rate and Biomass Productivity of. https://lnkd.in/g4mQEbCz.
(4) Microalgae Biomass Production: An Overview of Dynamic ... - Springer. https://lnkd.in/gYf-8E9W.
(5) Frontiers | Biotechnological Applications of Microalgal Oleaginous .... https://lnkd.in/g9uj59Bt.
(6) Enhancing biomass and lipid productivity of a green microalga. https://lnkd.in/ggaWwRcN.
(7) undefined. https://lnkd.in/gxtQBtJm.
(8) undefined. https://lnkd.in/gvxsW5wA.

Photoautotrophic and **heterotrophic** growth refer to two different ways in which organisms, including microalgae, obtain energy and carbon for growth:

1. **Photoautotrophic Growth**: In this mode, organisms use sunlight as their energy source and carbon dioxide as their primary carbon source[1]. These organisms, also known as photoautotrophs, can fix carbon[1]. They convert inorganic materials into organic materials for use in cellular functions such as biosynthesis and respiration[1]. The biomass productivity of microalgae using photosynthesis is higher compared to terrestrial plants producing useful materials such as fatty acids and pigments[4].

2. **Heterotrophic Growth**: Heterotrophs depend on organic matter already produced by other organisms for their nourishment[1]. In the context of microalgae, heterotrophic growth may result in higher biomass productivity by avoiding light limitations[6]. It decreases area footprint and installation costs[6]. The heterotrophic metabolism of some model microalgae is also described as well as the productivities that could be encountered in heterotrophy[6].

In addition to these two modes, there is also **mixotrophic growth**, where organisms utilize both organic and inorganic carbon sources, such as glucose[4]. This can enhance biomass yield and the content of useful substances in microalgae[4].

Please note that the actual productivity can vary based on many factors including light intensity, temperature, nutrient availability, and specific characteristics of the microalgae species being cultivated.

Source:
(1) 5.1A: Photoautotrophs and Photohetrotrophs - Biology LibreTexts. https://lnkd.in/gH_sxF26.
(2) Frontiers | Effect of Different Cultivation Modes (Photoautotrophic .... https://lnkd.in/gc6KzjmH.
(3) undefined. https://lnkd.in/gmXTczqV.
(4) . https://lnkd.in/gbjRHYEy.
(5) Impacts of photoautotrophic, photomixotrophic, and heterotrophic .... https://lnkd.in/gADyWXxy.

(6) Photoheterotroph Vs Photoautotroph: What’s The Difference?. https://lnkd.in/gJBEgQYu.
(7) undefined. https://lnkd.in/gyhjaM9r.

Microalgae, like other photosynthetic organisms, primarily use light energy to grow and produce biomass. They convert light energy into chemical energy through the process of photosynthesis, which involves the conversion of carbon dioxide into organic compounds[1,2].

However, **apart from light energy, microalgae also utilize other sources for energy and growth**. These include:

1. ****Carbon Dioxide** (CO2)**: Microalgae use CO2 for photosynthesis, converting it into organic compounds. This process also helps in carbon sequestration[1,3].
2. ****Nutrients****: Microalgae require various nutrients for growth, including nitrogen, phosphorus, and other trace elements[2].
3. ****Water****: Water is a crucial component for the growth of microalgae and is involved in the photosynthesis process[4].

It's important to note that the efficiency of these processes can be influenced by environmental conditions such as light intensity, temperature, and nutrient availability[1,2]. Therefore, optimizing these conditions is key to maximizing microalgal biomass production[1,2].

Source:
(1) Biomass from microalgae: the potential of domestication towards ....
https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-018-1019-3.
(2) High-value biomass from microalgae production platforms: strategies and ....
https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-018-1225-6.
(3) The Role of Microalgae in Renewable Energy Production ... - IntechOpen.
https://www.intechopen.com/chapters/61371.
(4) Algal biomass valorization for biofuel production and carbon ... - Springer.
https://link.springer.com/article/10.1007/s10311-022-01458-1.

Oil energy is derived from light energy through the process of photosynthesis. During photosynthesis, algae convert light energy into chemical energy, which is stored in the bonds that connect atoms and molecules together. This chemical energy is then transformed into other forms of energy such as heat, kinetic, and electrical energy.

Indonesia daily sunlight 3.0 – 5.6 kWh/m2
1 kWh = 3.6 MJ. Hence, assume 5 kWh/m2 = 5 * 3.6 = 18 MJ/m2 daily

Our tank surface area (top side) is 3x3m = 9m2
Hence, our tank receives 18*9=**162 MJ daily (not including light energy received by microalgae due to aeration circulation for even light distribution. If we include a multiplying factor to account for circulation, the daily energy will be much more).** *Note that the effective energy received and converted by the microalgae would be different depending on strain and wavelength.*

Our tank of 11MT contains 30kg of biomass and 15kg of biomass is harvested every 24H. The biomass doubles **every 24H on average**. In reality, the biomass can double every few hours and obtain greater than 15kg biomass daily. **Hence using a doubling time of 24H is conservative**. *Note that our actual production data is among the leading if not leading in the industry.*

Based on 10% moisture dry algae cake, 15kg daily biomass (weight is based on at least 80% water removed) *0.9 (to approximate the removal of additional 10% of water) *0.5 (CAO extraction efficiency is approx 50% of dry biomass) = **6.75kg of CAO per 9m2 of land area or 3035kg/acre or 7500kg/ha of CAO produced daily**. **Note**: **This translates to approx 1 million MT of CAO per year from 400,000 tanks. If we assume 18kg daily biomass, we will obtain 1.2 million MT of CAO per year as reported.**

3035kg/acre/d = 1107775 kg/acre/year or 1108 MT/acre/year, which translates to 2216 MT/acre/year of biomass since our CAO ratio to DAC (dry algae cake) is 1:1, which is close to 2248 MT/acre/year as reported in our deck.

Higher heating value (HHV) of microalgae biomass is around 22 MJ/kg. Hence 6.75 kg CAO would contain approx **148.5 MJ** of energy daily vs **162 MJ** from Indonesia's daily sunlight. **Therefore, there is more than enough light energy from Indonesia's daily sunlight to support our daily CAO biomass produced (without even taking into account aeration circulation which will increase the amount of energy from sunlight to produce even more biomass. Note that the remaining dry algae cake from the biomass is made up of mostly proteins, fibre, 10% water, carbohydrates, phenols and minerals).**

References:

https://en.wikipedia.org/wiki/Photosynthetic_efficiency
https://www.frontiersin.org/articles/10.3389/fbioe.2021.804608/full
https://www.mdpi.com/1996-1073/16/1/531
https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-018-1019-3
https://link.springer.com/article/10.1007/s11101-022-09819-y
https://www.ijsr.net/archive/v9i5/SR20510190145.pdf
https://www.worldbank.org/en/topic/energy/publication/solar-photovoltaic-power-potential-by-country
https://opus.lib.uts.edu.au/bitstream/10453/156695/2/Microalgae%20carbon%20capture_Manuscript%20clean%20version.pdf

Further Notes:

Microalgae can convert the **nutrients** present in wastewater such as CO_2, nitrogen, phosphorous, and other elements into huge biomass, proteins, and carbohydrates to energy-rich complex organic lipids[3]. Microalgae can be cultured by different methods and under different conditions. They need light as an energy source to convert the absorbed water and CO_2 into biomass through photosynthesis[1]. Photosynthetic products accumulate in various forms, such as cell components or storage materials, and vary from 20 to 50% of total biomass[1].

(1) Bioremediation of dairy industry wastewater and assessment of nutrient ....
https://link.springer.com/article/10.1007/s13399-022-03068-x

(2) Use of microalgae for food feed and bioproducts - BioMed Central.
https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-018-0879-x

(3) Sustainable microalgal biomass production in food industry ... - Springer.
https://link.springer.com/article/10.1007/s11101-022-09814-3

(4) Methods to Increase Microalgae Carbohydrates for Bioethanol Production ....
https://ojs.sampoernauniversity.ac.id/index.php/IJOCED/article/view/301

Picochlorum renovo is a fast-growing, resilient algal strain that has shown great potential in both scientific inquiries into its biology and in the development of broad applications in sustainable fuel, food, and chemical production[1]. Chlorella sp. is also a food-grade strain that has been evaluated for its folate content as an alternative algae-based food production strain[3].

(1) Research Reveals Fast-Growing, Resilient Algal Strain.
https://www.energy.gov/eere/bioenergy/articles/research-reveals-fast-growing-resilient-algal-strain

(2) Frontiers | Microalgae a Superior Source of Folates: Quantification of ....
https://www.frontiersin.org/articles/10.3389/fbioe.2019.00481/full

(3) Development of a high-productivity, halophilic, thermotolerant ... - Nature.
https://www.nature.com/articles/s42003-019-0620-2.pdf

(4) undefined. https://doi.org/10.1038/s42003-019-0620-2

The fastest-growing microalgae species under optimal conditions for photoautotrophic growth (energy from photosynthesis only) include Nannochloropsis and Ankistrodesmus[1]. Other fast-growing species include Chlorella miniata, Coelastrella sp., Desmodesmus quadricauda, Neochloris oleoabundans, and Verrucodesmus verrucosus[2].

As for the optimal pond depth for microalgae cultivation, the most common values are between **0.2 and 0.5 meters**, with **0.3 meters** being the most used value[6,7]. However, the optimal depth can depend on various factors including the type of algae being cultivated, the local climate, and specific production goals. For instance, in Taiwanese shrimp hatcheries where Skeletonema costatum, for biofuel and feed, is produced successfully, rectangular outdoor concrete ponds of 10-40 tons of water volume and a water depth of **1.5-2 meters** are commonly applied[1].

Source:
(1) Which algae grow fastest under optimal conditions?. https://biology.stackexchange.com/questions/40866/which-algae-grow-fastest-under-optimal-conditions
(2) The cultivation of five microalgae species and their potential for .... https://energsustainsoc.biomedcentral.com/articles/10.1186/s13705-022-00337-5.
(3) Frontiers | Dynamic Modeling of the Microalgae Cultivation Phase for .... https://www.frontiersin.org/articles/10.3389/fenrg.2015.00041/full.
(4) Meteorological Data-Based Optimal Control Strategy for Microalgae .... https://www.hindawi.com/journals/complexity/2019/4363895/.
(5) Frontiers | Selection of Tropical Microalgae Species for Mass .... https://www.frontiersin.org/articles/10.3389/fenrg.2022.912904/full.
(6) A concise review on the cultivation of microalgal biofilms for biofuel .... https://link.springer.com/article/10.1007/s13399-022-02783-9.
(7) Large Scale Cultivation of Microalgae: Open and Closed Systems - Springer. https://link.springer.com/protocol/10.1007/7651_2018_130.
(8) Variables Governing Photosynthesis and Growth in Microalgae Mass ... - MDPI. https://www.mdpi.com/2227-9717/9/5/820.

The **fastest-growing microalgae** are those that can thrive under optimal conditions for photoautotrophic growth (energy from photosynthesis only). The growth rate of algae can be influenced by several factors:

1. **Temperature**: The optimal temperature range for phytoplankton cultures is 20-30°C[5]. Temperatures higher than 35°C can be lethal for a number of algal species, especially green microalgae[5]. Conversely, temperatures lower than 16°C will slow down the growth of algae[5].

2. **Light**: Algae require light for photosynthesis, and thus, their growth rate can be influenced by the intensity and duration of light exposure.

3. **Nutrients**: Algae need various nutrients to grow, including nitrogen, phosphorus, and other trace elements.

4. **pH**: The optimum pH for most algae species is 8.2 to 8.7[6]. If the pH level is too low, it can slow down growth[6].

5. **Salinity**: Some algae strains can grow quickly and thrive in harsh conditions, such as intense heat, sunlight, and hyper-saline waters[4].

In terms of specific species, **Chlorella sorokiniana** has been observed to have **double timings in the range of two hours** under certain conditions[1]. However, this was in an acetate-containing medium and it would not have relied a lot on photosynthesis to grow[1]. In terms of photoautotrophic algae, consistent quick growth has been observed for **Dunaliella** species[1].

Sources (some repeated):
(1) 9.3 Algae Growth and Reaction Conditions | EGEE 439: Alternative Fuels .... https://www.e-education.psu.edu/egee439/node/694.
(2) How Fast Does Algae Grow? Growth Rate Factors! - Outdoor Moss. https://outdoormoss.com/how-fast-does-algae-grow.
(3) Potential of a Fast-Growing Algae Strain Revealed Through NREL Research. https://www.nrel.gov/news/program/2019/potential-of-a-fast-growing-algae-strain-revealed-through-nrel-research.html.
(4) Which algae grow fastest under optimal conditions?. https://biology.stackexchange.com/questions/40866/which-algae-grow-fastest-under-optimal-conditions
(5) Microalgae: Cultivation, Biotechnological, Environmental, and .... https://link.springer.com/chapter/10.1007/978-981-15-8999-7_23.
(6) Potential of a Fast-Growing Algae Strain Revealed Through NREL Research. https://bing.com/search?q=optimum+conditions+for+the+fastest+growing+microalgae.

Dr Lanz Chan is humbled to have had the privilege of being a part of the team involved in the Malaysian **pilot project** of circa 500 tanks, which successfully achieved ISCC certification (https://iscc-system.org/) in 2022 following over eight years of rigorous testing and dedication.

It is with great respect and admiration that he acknowledges three other distinguished groups in Malaysia that are also pioneering similar cutting-edge technologies:

1. The innovative minds at https://myroil.com/, in partnership with Boustead, among others.
2. The visionary team at http://www.mykoglobal.com/, collaborating with Techna-X, and more.
3. The groundbreaking efforts of Petronas, SEDC (Sarawak Economic Development Corporation), Chitose, the Japanese Government, Eneos, Honda, and others, as detailed in the noteworthy articles linked here. They are testing their photobioreactor system alongside the tank system to prove weather conditions which I believe have been proven.
https://www.petronas.com/media/media-releases/petronas-sedc-energy-develop-microalgae-oil-sustainable-aviation-fuel / https://chitose-bio.com/news/5312/ / https://www.bloomberg.com/news/articles/2022-01-23/algae-market-potentially-worth-320-billion-draws-honda-eneos

We are excited to share that we have embarked on a new journey by establishing our team in Singapore and Indonesia. Together, we have developed our own groundbreaking system known as the **Proprietary & Copyrighted Disaster-Resistant ALGAE MATRIX BIOREACTOR TANK SYSTEM with IoT-Automation**, abbreviated as AMTS. You can find more information about this system as published at https://finamatrix.net/news/. Our project is set to unfold on Seram Island, near Bengoi, covering a vast expanse of 3000km2.

One of the hallmarks of our project is its estimated cost-effectiveness at similar efficiencies/productivities, with projected savings of approximately 20% compared to similar endeavors in Malaysia, all thanks to our innovative enhancements and streamlined processes.

As we embark on this exciting journey, we are actively engaged in discussions with energy funds, etc exploring potential investments / partnerships in exchange for shares on Nasdaq. As always, we welcome any inquiries you may have regarding our project.

There are several strains of microalgae that are known for their rapid growth rates. Here are a few examples:

1. **Stichococcus bacillaris ACUF158**: This strain has been reported for its ability to use glucose[3].
2. **Chlamydomonas acidophila SAG 2045**: This strain is also known for its ability to use glucose[3].
3. **Chlamydomonas pitschmannii ACUF238**: This strain is another example that can use glucose[3].

Please note that the actual rate of cell division can vary greatly depending on the species and environmental conditions such as light, temperature, and nutrient availability.

#naturalresources #scienceandenvironment #renewableenergy

Sources:
(1) Acid Tolerant and Acidophilic Microalgae: An Underexplored World of .... https://lnkd.in/gvTcNeTC.
(2) Microalgae strain catalogue. https://lnkd.in/gWfKppTd.
(3) Microalgae strain catalogue. https://lnkd.in/gNA9yE_w.
(4) The cultivation of five microalgae species and their potential for .... https://lnkd.in/gm9JsAnn.
(5) Enhancing biomass and lipid productivity of a green microalga. https://lnkd.in/ggaWwRcN.

Microalgae are known for their rapid growth rates, and some species can indeed divide very quickly under optimal conditions. However, the specific rate of division can vary greatly depending on the species and environmental conditions such as light, temperature, and nutrient availability. While some microalgae like Chlorella can divide approximately **every 2-3 hours** under optimal conditions[3], it's important to note that these are general guidelines and actual results may vary depending on the specific strain of microalgae and the conditions in which it is grown.

Source:
(1) Diatom - Wikipedia. https://lnkd.in/gUq44A4q.
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Creating a seawater tank for microalgae cultivation involves several steps. Here's a general guide on how you can set up a 3m x 3m x 3m tank for growing Chlorella under natural sunlight:

1. **Tank Construction**: Construct a tank with dimensions 3m x 3m x 3m. The tank can be made from a variety of materials, but it should be durable, non-toxic, and resistant to corrosion from seawater[1]. Glass or food-grade plastic are commonly used materials[1].
2. **Location**: Choose a location that receives ample sunlight, as Chlorella requires light for photosynthesis[1]. However, be aware that too much direct sunlight can lead to overheating and harm the algae[1].
3. **Water Source**: Fill the tank with seawater. If you're not near the sea, synthetic seawater can be made by mixing sea salts with freshwater[1].
4. **Nutrient Addition**: Algal cultures must be enriched with nutrients to make up for the deficiencies in the seawater[2]. Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate[2]. Micronutrients consist of various trace metals and vitamins[2].
5. **Aeration/Mixing**: Install an aeration system to provide the algae with carbon dioxide for photosynthesis and to keep the water moving[2]. This prevents the algae from settling and ensures that nutrients are evenly distributed[2].
6. **Algae Inoculation**: Once your tank is set up and the conditions are right (temperature around 20-24°C, salinity around 20-24 g.l -1, light intensity around 2,500-5,000 lux), you can add your Chlorella culture[2].

7. **Monitoring**: Regularly check the pH, temperature, and nutrient levels in your tank[2]. Adjust as necessary to maintain optimal growth conditions[2].
8. **Harvesting**: Once the algae reach a certain density, you can harvest them. This is typically done by filtration or centrifugation[2].

Source:
(1) An overview of cultivation methods and the reasons for cultivating ....
https://marine-aquaculture.extension.org/an-overview-of-cultivation-methods-and-the-reasons-for-cultivating-microalgae/.
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http://site.iugaza.edu.ps/mwhindi/files/Lab4-Algal-Culture-Methods-batch-and-continuos.pdf.
(4) Fish, Algae, and Oysters: The Winning Trio in Aquaculture.
https://kids.frontiersin.org/articles/10.3389/frym.2019.00131.
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https://www.eufic.org/en/food-production/article/microalgae-what-are-they-and-how-to-grow-and-use-them.

Optimizing the growth of Chlorella involves several factors:

1. **Water Quality and Temperature**: Chlorella can grow in seawater or freshwater. The water should be free from contaminants such as heavy metals, pesticides, and chlorine[4]. The ideal temperature for chlorella cultivation is around 25-30°C (77-86°F)[4].

2. **Light Intensity and Photoperiod**: Chlorella is a photosynthetic organism, which means it requires light for growth. The intensity and duration of light play a crucial role in determining the growth rate of chlorella[4]. Ideally, chlorella should be exposed to a light intensity of around 5,000-10,000 lux for approximately 12-16 hours a day[4].

3. **Nutrient Availability**: Chlorella requires a balanced supply of nutrients for optimal growth. The primary nutrients needed for chlorella cultivation are carbon, nitrogen, and phosphorus[4]. These nutrients can be provided through the use of chemical fertilizers or organic matter such as compost[4].

4. **Carbon Dioxide Supply**: Chlorella requires carbon dioxide (CO_2) for photosynthesis[4]. You can use a CO_2-generator, such as tablets or aerosol systems, to keep CO_2 in the water[8].

5. **Harvest Time**: Chlorella can be harvested daily.

6. **Growth Rate**: Chlorella has a rapid growth rate[4] doubling in mass every few hours.

7. **Culture Density**: The culture density should be 1-2 g/L[4].

Remember that these are general guidelines and actual results may vary depending on specific conditions.

Source:
(1) Mastering Chlorella Cultivation: A Guide To Successful Growth. https://shuncy.com/article/grow-chlorella.
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