Microalgae (Diatom) Production - The Aquaculture and Biofuel Nexus

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Abstract – As fishing has become more industrialized and wild fish stocks increasingly depleted, aquaculture production has grown rapidly to address the shortfalls in capture fisheries and limitations to long-term aquaculture success. One such shortfall is the need to produce a suitable, sustainable, substitute for the capture fishery derived fish meal and oil based fish feeds currently in use, while maintaining the human protein requirements and health benefits of Long Chain (LC) omega-3 oils in farmed fish products. Fish derive the LC omega-3 oils from the food they consume, which ultimately comes from lower trophic level primary producers like microalgae. Using Integrated Aquaculture System (IAS) principles and practices, microalgae (diatoms) can be raised and processed directly for their Algal/Single Cell Oils (SCO), protein, and nutrients.

Besides the use as an aquaculture feedstock, microalgae have been investigated for biofuel production because of higher photosynthetic efficiency, higher biomass production, and faster growth compared to other terrestrial energy crops. SCO based carbon-neutral renewable liquid biofuel solutions are currently under investigation but suffer from high production costs. Liquid biofuels have been considered to displace non-renewable, petroleum-derived transport fuels of limited availability which contribute to climate change via greenhouse gas (GHG) emissions. The current high cost constraint of SCO production could be alleviated through explored water-energy-food nexus synergies between the aquaculture and biofuels sector with a concentration on innovations in microalgae/SCO production, harvesting, and processing technologies. Interdisciplinary collaborations between engineers, biologists and chemists are essential for their successful development.

Keywords – Algae Biofuel Production, Aquaculture, Diatoms, Microalgae cultivation, Water-energy-nexus, Renewable energy

I. FISHERIES SUMMARY

Historically, the oceans were considered limitless and thought to harbor enough fish to feed an ever-increasing human population. Total landings from capture fisheries increased ~5-fold in the 40 year period from 1950 to 1990 [1]. More recently, however, capture fisheries have not been able to keep pace with growing demand, and many fisheries have already been over-fished. Nearly half of the known ocean fisheries are completely exploited and 70% are in need of urgent management [2]. As fisheries become depleted and fish harder to catch, many fisherman and governments have responded with investments in equipment and technology to fish longer, harder, and farther away from their home ports.

According to the latest publication from the Food and Agriculture Organization (FAO) of the United Nations, global fish production (fish, crustaceans, mollusk, and other aquatic invertebrates, but excluding aquatic plants [seaweeds] and marine mammals) has grown steadily in the last five decades, at an average annual rate of 3.2%, outpacing world population growth (1.6%). World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012. The proportion of fisheries production used for direct human consumption increased from about 71 % in the 1980s to more than 86% (136 million tonnes [MT]) in 2012, with the remaining 14% (21.7 MT) destined to non-food uses, of which 75% (16.3 MT) was reduced to fishmeal and fish oil [3].

To meet this ever-increasing demand for fish, aquaculture has expanded rapidly and is now the fastest growing food producing industry in the world. In the 10-year period 2003-2012, the world total aquaculture production (excluding aquatic plants [macro algae (seaweed)]) increased by 71% (~7%/year) from 38.9 to 66.6 MT (~2.7 MT/year), while the world capture fisheries total increased by only 3% from 88.3 to 91.3 MT (~0.3 MT/year). A summary of the world fisheries and aquaculture production and utilization data for the most recent 5-year period available (2008–2012) is presented in Table 1 [3].

PRODUCTION	2008	2009 (million	2010 tonnes)	2011	2012
Wild Capture					
Inland	10.3	10.5	11.3	11.1	11.6
Marine	79.9	79.6	77.8	82.6	79.7
Total Capture	90.1	90.1	89.1	93.7	91.3
Aquaculture					
Inland	32.4	34.3	36.8	38.7	41.9
Marine	20.5	21.4	22.3	23.3	24.7
Total Aquaculture	52.9	55.7	59.0	62.0	66.6
Total World Fisheries	**143.1**	**145.8**	**148.1**	**155.7**	**158.0**
UTILIZATION					
Human Consumption	120.9	123.7	128.2	131.2	136.2
Non-Food Uses	22.2	22.1	19.9	24.5	21.7
Population (billions)	6.8	6.8	6.9	7.0	7.1
Per capita fish consumption (kg/yr.)	17.9	18.1	18.5	18.7	19.2

Table 1. World fisheries and aquaculture production and utilization. Adapted from [3] - totals may not match due to rounding.

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II. INTRODUCTION – ISSUE RECOGNITION

Many populations depend on fish as part of their daily diet, with this dependence usually higher in developing countries than developed ones. Fish, being a concentrated source of protein, essential fatty acids and micronutrients, can have a significant positive nutritional impact even in small quantities. According to [3], in 2012 fish provided more than 2.9 billion people with almost 20% of their average per capita intake of animal protein, and 4.3 billion people with almost 15% of their protein requirement.

Examination of Table 1 reveals several important trends:

- Aquaculture fisheries accounted for ~42% of the world's 2012 total fish production output and increased at ~5.2% per year;
- Wild capture fisheries accounted for ~58% of the world's 2012 total fish production output and was nearly constant at ~0.3% per year;
- Total world fisheries used for human consumption increased at ~2.5% per year; and
- World per capita fish consumption increased at a rate of ~1.5% kg/year and use for non-food uses (e.g. fishmeal and fish oil) decreased at ~2.3% per year.

World population in 2012 was estimated at 7.1 billion, and is projected to reach 9.6 billion in 2050, with the majority of the population growth occurring in developing regions [4]. If one simply considers maintaining the 2012 global average for per capita fish at 19.2 kg, this would result in an additional 48 MT of fish required per year by 2050. If one then considers the average 1.5% annual consumption rate increase, this would result in an additional 83.2 MT of fish required per year by 2050. In either scenario, if the static levels of wild capture continue, it is clear that only through aquaculture can this increase in fish production be met.

Presuming these production trends persist, the demands of a growing population, will far outstrip the useable yield of the seas. Global aquaculture production has no choice but to grow in order to ensure a sufficient supply of protein to the world's population. However, to do so sustainably, the industry will need to become less dependent on whole wild fish for feeds and either identify alternate naturally occurring feedstock or modify culture species and practices, which will require influencing consumer preferences.

III. AQUAFEEDS – FISHMEAL AND FISH OILS

Omega-3 (ω-3 or n-3) long-carbon chain (≥C20) polyunsaturated fatty acids (LC-PUFAs) have received considerable interest because of the clear evidence of their human dietary health benefits and the concurrent decline of their traditional global wild fisheries source (e.g. fishmeal and fish oil) [5]. A significant, but declining, proportion of world capture fisheries non-food production is processed into fishmeal (mainly for high-protein feed) and fish oil (as a feed additive in aquaculture and also for human nutritional supplements). In natural systems, wild fish derive the LC-PUFA omega-3 oils and necessary nutrients from the lower trophic species they consume. Forage fish play an important role here in converting primary producers like microalgae into food for higher trophic species including humans, larger fish, marine mammals, and seabirds [6]. Overexploitation of forage fisheries (e.g. anchoveta [*Engraulis ringens*] fisheries) can lead to local stress on higher trophic species, particularly during El Nino - Southern Oscillation events [7, 8]. In aquaculture, in order to produce healthy fish comparable to their wild counterparts, farmed fish obtain the LC PUFA omega-3 oils and necessary nutrients from the aquafeed (fishmeal and oil) made from the forage fish, processed fish bones and offal, or other fish by-products.

The aquaculture sector currently consumes about 75% of the global fish-oil production produced from non-food wild capture fisheries (Table 1). This percentage is declining because of the increasing demand for fish oil for human nutritional supplements. LC-PUFAs omega-3 oils are perhaps the most commercially successful marine lipids derived from fish oils. The increased focus on the benefits of fish oils has boosted the demand for fish oil for direct human consumption, with an annual growth rate of 15–20% [8]. Despite starting slowly around 2000, the market for omega-3 oils for human nutritional supplements has grown considerably. According to market studies, global demand in 2010 for LC-PUFA omega-3 ingredients was US $1.6 billion [10].

Owing to the growing demand and price of fish oil, more fishmeal is being produced from fish by-products, which previously were often discarded. This can affect the composition and quality of the fishmeal with more ash (minerals), an increased level of small amino acids (such as glycine, and proline) and less protein. This in turn may affect its share in feeds used in aquaculture and livestock farming. About 35% of world fishmeal production was obtained from these fish residues in 2012 [3].

Microalgae/fish oil differs from most terrestrial vegetable oils in being quite rich in LC-PUFAs with four or more double bonds [11]. For example LC-PUFA omega-3 fatty acids eicosapentaenoic acid (20:5n-3, 5-double bonds, EPA) and docosahexaenoic acid (22:6n-3, 6-double bonds, DHA) occur commonly in microalgae/fish based oils. While many terrestrial vegetable oils provide an alternative oil source, they do not contain LC-PUFA omega-3, instead they generally contain medium-chain fatty acids (MCFA), such as oleic (18:1n-9; 1-double bond) or linoleic (18:2n-6; 2-double bonds) and in some cases the omega-3 precursor α-linolenic acid (ALA) (18:3n-3; 3-double bonds). Although the human body is capable of synthesizing DHA from dietary ALA, the conversion rate is not very efficient in the human body [12].

Currently, terrestrial plant oil and fish oil feed blends are commonly used in aquaculture diets to provide amino acids and other nutrients needed for fish growth and flesh quality; with the blending ratio determined by price, stage of production, and desired consumer outcomes [13]. However, unless carefully monitored, the reduced levels of fish oil might result in a farmed fish produced with a less-favorable fatty acid profile. Fish oil in feed should be optimized to ensure that the LC-PUFA omega-3 oils end up in the final product, and are not metabolized by the fish during growth. For example, near term strategies using minimal levels of SCOs or fish oil during grow-out followed by increased fish oil inclusion during the finishing stage are an important way to reduce the total amount of fish oil needed, while maintaining the human protein and health benefits of LC-PUFA omega-3 oils in farmed fish products.

The previously mentioned 1.5% annual fish consumption rate is well within the current 5.2% annual aquaculture total production growth rate presuming that suitable sources of "essential fatty acids" such as EPA and DHA remain available. However, the ever increasing human population and their desire/need for fish protein is adding to the growing concern as to how aquaculture will continue to produce high quality food products capable of supplying the required level of human nutrition. This is in light of:

- Increasing global per capita fish consumption;
- Decreasing amounts of wild capture derived non-food uses, specifically fish oil and fish meal; and
- Increasing the percentage of wild capture derived fish oil for direct human consumption of nutritional supplements.

Continued progress in developing aquafeed alternatives are not simply a way to reduce aquacultures' pressure on wild capture species, but rather a requirement to ultimately satisfy the sustainability and nutritional needs of our ever expanding population. Because the biggest gains in reducing forage fish in feeds will likely come from lowering inclusion of fish oils (as opposed to fish meal), there are two main avenues to future success:

1. The acceptance of genetically modified terrestrial plant LC-PUFA omega-3 oils [14]; and/or
2. Commercial development of microalgae whole-cell biomass or extracted Single Cell Oils (SCO).

Regarding avenue #1, the basic question is whether genetically modified vegetable oils that provide LC-PUFA omega-3 oils will be an acceptable substitute for fish oil. Ultimately the main constraints lie in the development of technology and costs required to produce these oils, and in the willingness of consumers and retailers to purchase farmed seafood containing genetically modified plant oils. Most terrestrial animal feeds using soy products already contain genetic modification, with the exception of organic-labeled products [13]. The controversy over genetically modified food sources is far from over, but when one considers the large numbers of people that need to be fed and potential impacts of malnutrition, this point may be mute.

Regarding avenue #2, the current high cost of SCO production precludes its use as a 100% fish oil LC-PUFA omega-3 replacement for existing commercial aquaculture aquafeeds. However, successful SCO cultivation could provide a renewable and sustainable source of essential fatty acids, in particular EPA and DHA. Single cell organisms, such as diatoms, thraustochytrids [15, 16], other microalgae and marine bacteria are the LC-PUFA omega-3 "biofactoies" of the ocean. Having access to secure sources of LC-PUFA omega-3 rich oils is vital for the continued sustainability and growth of the intensive global aquaculture industry.

Therefore, SCOs show great promise for reducing aquaculture's dependence on wild capture fisheries and thereby enabling the freeing up of the industries upward production limits by going "directly to the microalga source". Providing for a future replacement mechanism of wild capture based fishmeal and oil with a sustainable aquaculture farmed LC-PUFA omega-3 aquafeed. And if this could be done without the need of genetic modifications, so much the better since it would remove many initial investment and consumer barriers and concerns.

IV. THE AQUACULTURE AND BIOFUEL NEXUS

In November 2014, the Second International Conference on Nutrition will be held in Rome, Italy. This high-level ministerial conference will propose a flexible policy framework to address today's major nutrition challenges and identify priorities for enhanced international cooperation on nutrition. During this meeting, a paper on the role of sustainable fisheries and aquaculture for food security and nutrition is scheduled to be presented [3]. As fish products are becoming an important provider of essential nutrients, knowledge of the role that aquaculture and fisheries could play in combating malnutrition and food security seems set to be highlighted more than ever.

Although this heightened visibility of fish products is welcome, the benefits of omega-3 fatty acid-enriched fish oil or the idea of sustainable aquaculture farmed, microalga based LC-PUFA omega-3 aquafeed is not new. Reference [3] summarized findings showing that omega-3 fatty acid-enriched fish oil and/or omega-3 precursor ALA was found to provide a level of protection against many diseases and viral infections). Later reference [18] wrote on the then 60-year-old subject of microalgae as an aquaculture feed ingredient saying perhaps the most circuitous development of a microalgae aquaculture application is the use of live algal cultures as a diet for invertebrates. Difficulty arose from the need for new research information on two fronts: a better understanding of the nutritional requirements for the "livestock" species and improvements in the efficiency and effectiveness of microalgae culture methods that could be implemented by nontechnical farm personnel [18]. A review of the extensive microalgae literature reveals efforts on both fronts; however, the finish line seems no closer. Why?

The most reasonable answer is a combination of significant technical challenges coupled with insufficient research funding levels. One might say that the November 2014 Rome meeting appears to signal a formal realization that the time is at hand to reconsider the expanded role of aquaculture in meeting the world's future protein requirements. However, successful expansion will only occur with improvements in integration, automation, scalable processes that recycle unused by-products from the output streams, and utilize these by-products as resources to support other appropriate processes.

Because of the existing process and product similarities, some of the technical challenges in scaling up microalga production could be alleviated through synergies with the biofuels sector, where in both cases microalga production is the common denominator. For example, in the biofuels sector, the main product is ultimately biofuel with algae oil as a by-product used to produce biofuel and LC-PUFA nutritional supplements. The resulting algae protein and carbohydrate by-product can be used to offset and improve the overall bottom line costs. Whereas, in the existing aquaculture sector, the main product is farmed fish, with algae being used as a protein for the fish and reducing dependence on wild capture fisheries. Any residual oils not needed to meet the fish nutritional requirements will provide another by-product to offset and improve the overall bottom line costs. Improvements in microalga production technology will benefit both of these large and important industrial sectors.

The scale of global fossil fuel consumption is enormous [19]. In 2011, almost 11,000 million tons of oil equivalent were consumed in the form of oil, gas, and coal-based fossil fuels [20]. Coal and gas production rates are currently increasing faster than consumption rates. For petroleum oil however, consumption (~14% increase 2000-2011) has grown faster than oil production (~10% increase 2000-2011) in the same period largely due to the plateau in production of conventional oil; a harbinger of some major challenges and changes to the future energy mix [20]. With the need to reduce carbon emissions, and the dwindling reserves of crude oil, liquid fuels derived from renewable plant material – biofuels – are an attractive source of energy. In comparison with other forms of renewable energy, such as wind, tidal, wave, solar, and salinity gradients [21, 22, 23], which primarily target electrical energy production, liquid biofuels allow solar energy to be stored, and used in existing engines and transport infrastructure [24].

There has been a significant amount of literature published (especially from the biofuels community) on the many facets of microalgae. Production of algal oil and its conversion to useable biofuels has been demonstrated repeatedly [25, 26, 27] but commercial production remains elusive. Algae can utilize nutrients such as nitrogen and phosphorous from a variety of waste water sources (e.g., agricultural run-off, concentrated animal feed operations, and industrial and municipal wastewater), thus providing a sustainable bioremediation of wastewater for environmental and economic benefits [28]. Another challenge for mass production of microalgae biofuels is not achieving adequate levels of production, while securing sustainable and cost effective supplies of nutrients (N, P, C and Si) to maintain continuous growth [29]. The potential to utilize the nutrients generated by a recirculating aquaculture system (RAS) together with microalgae production system can be addressed through Integrated Aquaculture Systems (IAS) [30].

V. INTEGRATED AQUACULTURE SYSTEMS

Aquaculture has a long history, originating at least in the year 475 B.C. in China [31], with renewed activity in the late 1940s through the application of aquaculture methods to restocking as a complement to natural spawning [32]. Continued expansion of this industry is expected as economic profitability and the soaring demand for seafood make land-based aquaculture an increasingly important component of the global food supply [33, 34]. However, wastewater associated with land-based commercial fish production presents an environmental challenge with respect to nutrient loading to eutrophic marine ecosystems [35]. Eutrophication is a key driver in a number of environmental problems, including reduced light penetration resulting in seagrass mortality, increases in harmful algal blooms, and hypoxic and anoxic conditions [36, 37]. IAS technology was developed based on the principals originally used in China's early polyculture systems [30], where fed species are grown together with extractive species that utilize the wastes produced by the fed species. Both the fed and extractive species components have commercial value and the overall sustainability of the production system is improved. In a microalgae/fish IAS, microalgae can be grown, harvested and processed into food resource for the fish. The algae in the IAS can also secure sustainable supplies of nutrients from the fish wastewater. While some algae species can fix nitrogen from the air in the form of NO_x, most microalgae require it in a soluble form with naturally occurring fish urea being the best and most economical source [38].

VI. MICROALGAE DISCUSSION

Microalgae have been suggested as very good candidates for biofuel and aquafeed production because of their advantages of higher photosynthetic efficiency (PE), higher biomass production and faster growth compared to other energy crops [39, 40, 41, 42]. Heterotrophic growth of some microalgae has been used for efficient production of biomass and some metabolites such as lipid [43, 44, 45], which can reduce the cost of microalga biomass production and microalga oil production. As an example, *Chlorella protothecoides* is a microalgae that can be photoautotrophically (with sunlight) or heterotrophically (without sunlight) grown under different culture conditions [46]. Heterotrophic growth of *C. protothecoides* supplied with acetate, glucose, or other organic compounds as carbon source, results in high biomass and high content of lipid in cells [47, 48]. With the addition of the organic carbon source (glucose) to the medium and the decrease of the inorganic nitrogen source in the medium, the heterotrophic *C. protothecoides* was cultivated with the crude lipid content up to 55.2%, which was about four times that in photoautotrophic *C. protothecoides* (Miao and Wu, 2004). The achieved 55.2% exceeds the "Improved Process II – Long Term Goal" of 50% presented in Table III.D.S of NREL TP-580-24190 [49].

More recently, algae have been identified and considered as renewable fuel sources with algal biomass cultivation for various products transitioning to commercial systems [50]. Certain algal strains can use agricultural, industrial, and municipal wastewater, brackish water, or saline water as a growth medium rather than freshwater, thereby lowering water stress [51]. These algae absorb nutrients and may remove heavy metals, chemicals, and pathogens that exist in urban wastewater. The economic feasibility of algae production will vary depending on the location of algal cultivation facilities, the target market for biomass sales, and the quality of the input water that may need to be treated prior to use, if the level of compounds (e.g., heavy metals) is too high [52]. Reference [53] favors the multi-species approach over industrial-scale mono-species algal cultivation, suggesting that diverse assemblages of algal species can be constructed that result not only in high algal biomass productivity, but also reduced invincibility by undesired algal strains, and reduced susceptibility to algal diseases [54].

Aquatic Species Program (ASP) Discussion

From 1978 to 1996, the U.S. Department of Energy's Office (USDOE) of Fuels Development funded a program known as the Aquatic Species Program (ASP), to develop renewable transportation fuels from algae oil. Much of the program's research focused attention on the elusive lipid trigger. This "trigger" refers to the observation that, under environmental stress, many microalgae appeared to flip a switch to turn on production of triacylglycerols (TAGs) [49]. Nutrient deficiency was the major factor studied. Lipids are a generic name for TAGs, the primary storage form of natural oils. Biodiesel is commonly produced from oils or fats using transesterification and is a liquid similar in composition to fossil/mineral diesel. Its chemical name is fatty acid methyl (or ethyl) ester (FAME). TAGs consist of three long chains of FAMES attached to a glycerol backbone. Oils are mixed with sodium hydroxide and methanol (or ethanol) and the chemical reaction produces biodiesel (FAME) and about 10% (w/w) glycerol as the main by-product.

Nutrient deficiency, typically nitrogen or silicon deficiency is well known to enhance the lipid content of algae [55]. Lipid content has been reported under both nutrient replete and deficient growth conditions [56, 57]. Nutrient levels have been reduced by differing amounts across studies reported. For the purposes of this review, the following definitions are used:

- Nutrient replete: Stoichiometrically balanced nutrient conditions were assumed where no evidence of nutrient reduction or depletion in the medium was provided; and
- Nutrient deficient: Specified nutrient was completely removed from the culture medium or reduced below stoichiometric requirements for growth, either by changing the medium, or maintaining a batch culture until nutrient levels in the culture medium have been shown to be severely depleted.

The ASP program only investigated the autotrophic growth mode of algae. Autotrophic growth provides several advantages. For example:

- Microalgae can harvest the radiant energy from the sun into valuable products at the expense of inexpensive natural resources (e.g. CO_2 and H_20) [58], which contributes to global CO_2 reduction; and
- Microalgae can bloom at places where salty water, excessive sun exposure, and lack of vital nutrients inhibit other crops to grow [59, 60].

It has been argued that among microalgae species that colonize the photic earth zones, many organisms suitable for outdoor mass culture and biofuel production might be found. Consequently, it has been suggested that there is no apparent need to genetically modify microalgae so as to achieve the requirement for stable mass cultures with relatively high oil contents and lipid productivity [61]. Instead it may be prudent to limit projections to what can be achieved with natural strains. Notably, USDOE ASP had collected over 3,000 strains of oil-producing organisms, which after screening, isolation and characterization efforts, the collection was narrowed down to 300 species, mostly green algae and diatoms. Sixty % of these were diatoms, which were chosen based on criteria such as high growth rates and lipid yields, tolerance of harsh environmental conditions, and performance in large-scale cultures [49].

Algae Production Unit Model

Microalgae are a product of simple biochemical components: carbon dioxide, water, and nutrients generally in the form of inorganic salts. The growth medium must provide the inorganic elements that constitute the algal cell. Essential elements include carbon (C), nitrogen (N), phosphorus (P), and in the case of diatoms, silicon (S). It can be inferred that algae production requires large amounts of water, carbon and nutrients. When nutrients are not limited, the Redfield molar element ratio C:N:P for most phytoplankton is 106:16:1. Diatoms need, in addition to other nutrients, dissolved silicic acid, $Si(OH)_4$ [62, 63], which is taken up in low concentrations by specific silicic acid transport proteins, and at high concentrations by diffusion across the plasma membrane [64]. The Redfield-Brzezinski molar element ratio C:Si:N:P in most diatoms is 106:15:16:1 [65]. Nutrients such as phosphorus must be supplied in significant excess because the phosphates added complex with metal ions, therefore, not all the added P is bioavailable.

Under natural growth conditions phototrophic algae absorb sunlight and assimilate CO_2 from the air and nutrients from the aquatic habitats. The two systems that have been deployed most often in algal production are based on open pond and closed photo bioreactor technologies. A raceway pond is made of a closed loop recirculation channel that is typically about 20 – 50 cm deep. Mixing and circulation are produced by a paddlewheel. Flow is guided around bends by baffles placed in the flow channel. During daylight, the culture is fed continuously in front of the paddlewheel where the flow begins. Broth is harvested behind the paddlewheel, on completion of the circulation loop. The paddlewheel operates all the time to prevent sedimentation. Raceway ponds for mass culture of microalgae have been used since the 1950s. Extensive experience exists in the operation and engineering of raceways. Raceways are perceived to be less expensive than photobioreactors because they cost less to build and operate. Although raceways are low-cost, they have a low biomass productivity compared with photo bioreactors [59].

Unlike open raceways, photo bioreactors permit essentially single-species culture of microalgae for prolonged durations. Photobioreactors have been successfully used for producing large quantities of microalga biomass [66, 67, 58]. A tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass. This tubular array, or the solar collector, is where the sunlight is captured. The solar collector tubes are generally 0.1 m or less in diameter. Tube diameter is limited because light that is necessary for ensuring a high biomass productivity of the photobioreactor does not penetrate too deeply in the dense culture broth. Microalga broth is continually circulated from a reservoir, through the array, and back to the reservoir.

Reference [68] states that a key challenge in algal cultivation efforts is to achieve a high biomass energy content, high areal productivity and a high efficiency of solar energy capture. These authors suggest that one solution to solving some of the major challenges of microalga biomass production is to leverage the affordability of open raceway ponds and the stability and productivity of photobioreactors by employing a hybrid photobioreactor-raceway system. They present results from an innovative two-stage turbidostat culture system that supported sustained exponential growth at different rates and lipid accumulation in the halophilic green alga *Dunaliella sp*. They suggest that continuous biomass transfer from a stage 1 photobioreactor to a stage 2 raceway ponds should reduce the competitive ability of contaminating algae in the open pond system, and conclude that their system should be compatible with incorporating multiple stages for biological contaminant control [54].

Whether it be open raceways or photo bioreactors or some combination thereof, the recovery and drying of the microalga biomass from the algal broth is necessary for extracting the oil used in the Biodiesel transesterfication process. Biomass is recovered from the broth by various means followed by drying, with the cost drying being a significant portion of the overall recovery. Biomass recovery from photobioreactor cultured broth costs a fraction of the recovery cost for broth produced in raceways. This is because the typical biomass concentration produced in photobioreactors is nearly 30 times the biomass concentration generally obtained in raceways. Thus, in comparison with raceway broth, a much smaller volume of the photobioreactor broth needs to be processed to obtain a given quantity of biomass. Alternative biofuel strategies are also being pursued to convert biomass constituents beyond lipids to bio-oils (i.e., whole biomass conversion), such as Hydrothermal Liquefaction (HTL). Using HTL, wet biomass can be processed at medium temperatures (280-370 °C) and high pressures (10-25 MPa) into a liquid biocrude, so biomass feedstock drying is no longer needed [69, 70]. The biocrude produced, with an energy value close to fossil petroleum [71], is not directly suited as transportation fuel, but it is expected to be a suitable renewable feedstock for co-refining in existing fossil-based refineries. Additional research in this area in underway.

Photoautotrophic vs. Heterotrophic Production

Algae are sunlight-driven cell factories that can convert carbon dioxide into a wide variety of biofuels, foods, feeds, and high-value biomolecules [72]. Microalgae/SCOs hold promise as an aquafeed source because of its potential for converting solar energy more efficiently and with less negative environmental impact than the alternatives, especially terrestrial biofuel crops such as corn and canola/soybean. Reference [73] presents photosynthetic efficiency (PE) as the fraction of light energy fixed as chemical energy during phototrophic growth [74]. Reference [75] calculated a theoretical maximum PE of 13% for a green-type plant in bright sunlight. This estimated value is the theoretical ‘‘upper limit’’ of PE, as it does not account for other factors that could decrease efficiency and conversion (e.g. poor light absorption, photosaturation, and photorespiration) and significantly reduce PE. Because of such impacting factors, most terrestrial plants attain PE levels far below the theoretical estimates, with global averages typically between 1% and 2% [75]. However, because of their simple structure, microalgae can achieve substantially higher PE values compared to terrestrial plants. For example, [77] and [78] recorded PE values of 15% and 21.6% for the microalga *Phaeodactylum tricornutum*, respectively. Other findings include 20% PE for *Chlorella* [79], and 19% PE for *Tetraselmis suecica* [80]. Although highly species and class specific, overall the evidence suggests that microalgae could be a very efficient biomass resource for biofuel and aqua feed production [42].

The concept of autotrophic growth for microalgae for production is technically feasible; however, in practice it is difficult to reach a high biomass density since light penetration is inversely proportional to the cell concentration [81, 82]. Additionally, mutual shading of cells can cause light insufficiency, which leads to low algal lipid productivity resulting from low biomass productivity [83]). Furthermore, low biomass concentration also increases the biomass harvesting cost [73, 84]. To develop cost-effective algal oil production, microalgae can be cultured in heterotrophic conditions where organic carbons, such as sugars and organic acids, serve as carbon sources. This mode of culture eliminates the requirement for light and therefore, offers the possibility of greatly increased cell density and productivity [85]. Some microalgae can grow rapidly heterotrophically [86, 87, 88, 46, 89]. Heterotrophic algal cultivation has been reported to provide not only a high algal biomass productivity, but high cellular oil content as well [90, 89, 91]. In the case of *Chlorella protothecoides*, heterotrophic growth on corn powder hydrolysate results in a 3.4 times higher biomass yield than that from autotrophic growth while the lipid content is increased 4.2 times.

However, even though the biomass and lipid productivities are significantly higher compared with those from autotrophic growth, the cost of the organic carbon sources (usually in the form of glucose or acetate) is high when compared against all other added nutrients. To overcome this high carbon cost, a cheap resource must be found. Crude glycerol, which is derived as a by-product from biodiesel production, is capable of providing such a supply [92].

Diatoms

Much work has been reported on green algae but the use of diatoms has received limited attention in comparison. Initial interest by the author in the use of diatoms was peaked during periods of down time during extended offshore COMPS buoy service cruises in the Gulf of Mexico [92] when natural slicks appearing on the water surface were frequently encountered. Follow-on literature searches revealed that these slicks were usually confined largely to regions of near-shore upwelling or shallow water wind-induced Langmuir circulation, where organic production is high [93]. Laboratory investigations of sampled “natural slick” water revealed contaminate films of organic oil probably derived from diatoms; certain species of which contain droplets of intercellular lipids (oil) in their cell to assist in flotation and/or as an emergency food supply. And even earlier in the literature, [94] proposed the mass cultivation of diatoms to produce urgently needed fat in World War II.

Diatoms are unicellular microalgae of the heterokont class characterized by silica-based cell walls and high productivity. Diatoms are among the most productive and environmentally flexible eukaryotic microalgae on the planet. They are estimated to be responsible for 20% of global carbon fixation and have become the dominant primary producers in the ocean [95, 96]. In addition, to being highly abundant, they are also highly diverse, with estimates of over 100,000 species [97], with the majority of species between 10 and 50 µm in length. Diatoms are excellent lipid accumulators with a substantial portion of the cells’ volume is occupied by lipid droplets that accumulated rapidly, especially under Si limitation [62].

A literature survey by Griffiths and Harrison of 55 microalga species from various classes showed that diatoms, as a class, performed above average at 27.4% (19% better than average) for total lipid content under nutrient replete conditions and 36.1% (13% better than average) under N-deficient conditions. Diatoms also accumulate lipid as a result of Si limitation, and the average lipid content for diatoms limited for Si relative to N deficient conditions was 41%, which was 28% higher compared with all species and 13% higher when compared within the diatoms. On silicon deprivation, the average lipid content increased from 24% to 41% dried weight (DW). Species grown under Si deficiency have lipid content between 30% and 50% DW. A positive increase the diatoms. The translation of an increase in lipid content into an increase in lipid productivity is dependent on the degree of growth retardation caused by the nutrient deficiency [55].

A wide variety of TAG composition has been identified in diatoms, with fatty acid (FA) side-chains ranging from 14:0/16:0/16:1 to 16:1/20:5/22:6 [98]. The data suggest that diatoms are at least on par with, if not superior to, other classes of algae in terms of their ability to accumulate lipids. Diatom FAs are among the most highly enriched in C14 chain lengths compared with other classes of algae [99] especially chlorophytes, which appear to lack this particular length [98, 100, 101]. C18 lengths are poorly represented in diatoms, but can be a predominant form in chlorophytes, especially polyunsaturated forms [99, 100]. In general, shorter chain lengths are desirable to improve cold-flow properties of biofuels [102] and saturated FAs are more desirable because they increase the ignition quality (cetane number) of the fuel [103]. Diatoms are unusually high in EPA which is not prevalent in chlorophytes [99, 101, 104].

Diatoms have characteristics, both conceptual and proven, which make them amenable to large-scale biofuel and aquafeed cultivation. Why then are diatoms so underrepresented in production systems? According to Hildebrand, two possible explanations come to mind:

- The long history of the study of chlorophytes because of their intimate relationship with terrestrial plants (especially related to photosynthesis) and our food supply; and
- Another reason is related to the fact that diatoms dominate marine environments, whereas chlorophytes and plants dominate terrestrial environments. The ocean is a difficult system to study, and there are fewer research institutions with a marine focus than those dedicated to terrestrial environments [62].

VII. CONCLUSION - THE PATH FORWARD

The intent of this paper is to highlight a pair of significant issues facing the world today and present these issues in terms of their coupled water-food-energy nexus relationship with possible solutions. The often foretold fish and energy scarcity issue confronting us as we move into the middle 21st century and beyond is a daunting issue. Sufficient details were provided to describe the world fisheries status at hand and support a proposed solution to ensure a viable and environmentally sustainable aquaculture industry, while maintaining human nutritional health and protein requirements.

Fish derive the LC-PUFA omega-3 oils from the food they consume which is ultimately derived from lower trophic level primary producers like microalgae. Microalgae have been suggested as very good candidates for biofuel and aquafeed production because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops. Under natural growth conditions phototrophic algae absorb CO_2 from the air and nutrients from the aquatic habitats. Therefore, as far as possible, artificial production should attempt to replicate and enhance these optimum natural growth conditions. This is the concept behind IAS with microalgae promoted as the lower trophic food source.

A key consideration is the choice of algal strain. Algae are simple aquatic organisms, but there are an estimated 300,000 species, whose diversity is much greater than that of terrestrial plants [24]. The USDOE ASP collected over 3,000 strains of oil-producing organisms, which after screening, isolation and characterization efforts, the collection was narrowed down to 300 species, mostly green algae and diatoms. The literature is replete with many comprehensive and detailed process analyses and studies, the result of each often used to promote microalgae production for various purposes. However, the vast majority of these promote the use of green algae. The time has come to change gears and consider the use of diatoms. Diatoms like *Chaetoceros* would be a very good candidate for both aqua feed and biofuel production in an IAS system [105].

Other microalgae species will need to be considered as well as to whether photoautotrophic or heterotrophic conditions will be used. Along with a whole host of considerations such as:

- What inorganic nutrient supplies will be required?
- What operating equipment and conditions will be required? Hybrid photobioreactor/raceway design or something else?
- What compatibility issues with the other higher level trophic species feeding and growing with in the IAS system will be encountered?

These considerations and others could be addressed through a focused, interdisciplinary systems-wide approach towards exploring water-energy-food nexus synergies between the aquaculture and biofuels sectors. This would begin with an active collaboration between engineers, biologists and chemists towards R&D innovations in microalgae production, harvesting, and processing technologies. Where co-products would provide the push to develop and solidify the technology while simultaneously providing markets for the jointly developed main products.

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