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ARTICLE

Spirogyra cultured in fishpond wastewater for biomass generation

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ABSTRACT

Algae are aquatic organisms that can be found in a wide range of water bodies. Algae, a form of aquatic organism, is found in many different water types. Besides being relatively easy to maintain, algae are also numerous, making them a good choice for biomass production. The filamentous *Spirogyra* sp, a common green alga, tends to grow in freshwater. It is said that this macroalga has a wide variety of biotechnological applications. Research in this area highlights biomass's creation and builds on our understanding of the composition of macroalgae generated in fish farm wastewater. A study of *Spirogyra* in undisturbed fish farm wastewater was conducted in this study. Various algal species were evaluated for their qualities, including biomass yields and productivity, protein, fat, and carbohydrates. This investigation has confirmed that the nutrients in fish farm effluent are suitable for cultivating algal biomass. Protein, lipid, and carbohydrate levels in unaltered fish farm effluent were the highest for *Spirogyra*, with percentages of 19.03, 8.38, and 45.71%, respectively. Thus, it was the most suitable organism for various biomass-based applications and nutrient removal.

1. Introduction

Aquatic biomass is being investigated as a feedstock to supplement terrestrial biomass in the biochemical synthesis of food and fuels, with algae being a prospective algal feedstock (Ramaraj et al., 2013, 2014a,b). Macroalgae are photosynthetic multicellular organisms. They have a fascinating evolutionary history and can be found all over the world. They're usually low in lipids and high in carbs, which can be employed for a variety of metabolic processes. Algae already have a commercial demand,

either as a food source or as a source of polysaccharide and hydrocolloid extraction feedstock. However, when contrasted to the scale of cultivation required for macroalgae to be considered a significant contributor to biomass, it is insignificant (Ramaraj et al., 2015a,b). New production in places not currently used for micro/macroalgae growing would be required to prevent competing pressures on existing resources.

Macroalgae refer to a diverse collection of photosynthetic eukaryotic marine organisms. Macroalgal species are multicellular and have plant-like characteristics. In most marine situations, a

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blade or lamina, a stipe, and a holdfast are used to secure the entire construction to hard substrates. In terms of the general features of these structures, the macroalgae groupings differ substantially. Macroalgae are divided into three groups based on the composition of photosynthetic pigments: Phaeophyta (brown algae), Rhodophyta (red algae), and Chlorophyta (green algae). Green macroalgae are evolutionarily and biochemically connected to higher plants (Tipnee et al., 2015; Nithin et al., 2020; Saengsawang et al., 2020). It exhibits a wide range of life cycles, including yearly and eternal life cycles, sexual and asexual reproduction methods, and generation alternation. Also, it can be found on every continent.

Nomenclature and abbreviation

| | |
|-----|----------------------------|
| BCG | Bio-Circular-Green Economy |
| COD | Chemical oxygen demand |
| TSC | Total suspended cells |
| TSS | Total suspended solids |
| SD | Standard deviation |

They require nearshore waters with a sufficient substrate for attachment and are prevalent in freshwater resources and coastal settings. Macroalgae are commercially viable as a food source and a source of biochemicals. Human food products, which are mostly related to the Asian market, account for between 83 and 90% of the total value of macroalgae. The remaining value is made up of chemical compounds produced by macroalgae. A high concentration of commercially valuable structural polysaccharides can be found in the varied species (Garcia-Vaquero & Hayes, 2016). Other agricultural applications include fertilizers, soil conditioners, and animal feed. However, macroalgae as a feedstock for biocircular economic models are not being used commercially.

Several studies reported that the productivity, growth, and biochemical composition of *Spirogyra*, a representative of major freshwater macroalgal species, as a first step in identifying target freshwater macroalgal species for biomass applications. Our next chose a primary target species and evaluated its capacity to compete with other species across a range of stocking densities (Tipnee et al., 2015). Our overall goal was to identify freshwater macroalgae that could be grown on a big scale in industrial wastewater streams to supply biomass for various end-product applications. This is accomplished by concentrating on filamentous species of freshwater macroalgae belonging to the genus *Spirogyra*. These genera were chosen because they have a wide geographic distribution, represent the macroalgae found in various freshwater habitats, grow rapidly, and become pest species when nitrogen levels are high. A novel and rapidly evolving industrial ecology concept are to culture macroalgae in wastewater as a bioremediation technique, with the biomass then used as a feedstock for algal-based end products such as food, feed, and bioenergy (Ramaraj & Dussadee, 2015; Tsai et al., 2015, 2016, 2017). Due to their capacity to thrive in various wastewaters and the suitability of the resulting biomass for a variety of uses,

freshwater macroalgae represent a very creative possibility across numerous industries.

Aquaculture is one of the fastest expanding sectors of global food production. However, it has been demonstrated that the rapid growth of fish farms and present fish farming practices have a harmful influence on the ecosystem (Whangchai et al., 2018). Uncontrolled fish farming causes organic matter and nutrient loading, largely from uneaten feed and feces, impairs nearby ecosystems' water and sediment quality. Furthermore, the formation of harmful algal blooms is aided by continuous nutrient enrichment. Furthermore, high nutrient levels deplete oxygen and impair sediment quality. Thus, nutrient enrichment induced by higher fish meals and fish waste impacts water quality and sediment conditions. On the other hand, wastewater from fish farms (i.e., effluent) can be used to produce macroalgae without an artificial medium. Thus, our purpose is to highlight and analyze recent research on the culture of freshwater macroalgae in fish farm effluent and their biotechnological capabilities and present an overview of the products produced by freshwater macroalgae *Spirogyra*. Finally, we demonstrate how freshwater macroalgae can be integrated into an industrial ecology system and Bio-Circular-Green Economic (BCG) model.

2. Materials and Methods

2.1. Study species and site

Spirogyra is a genus of green algae with unbranched filaments. This genus is found worldwide and is a prevalent component of natural ecosystems, where it grows adhering to the substrate or as free-floating mats. *Spirogyra* has been discovered as a strong and competitively dominant genus. *Spirogyra* sp. stock cultures were obtained from the Faculty of Fisheries Technology and Aquatic Resources at Maejo University in Thailand. Tilapia are cultured in vast outdoor ponds that are supplied with clean bore water continuously. To minimize suspended particles, pond effluents are passively treated utilizing reed beds and settlement ponds. The settlement pond's effluent water was pumped out and screened using a sand filter in this experiment. This water was then utilized to recirculate water in tanks. *Spirogyra* was kept in tumbling culture in these tanks with the use of a central aeration ring. A 750 μ m mesh screen was utilized to prevent *Spirogyra* filaments from exiting the tank with the incoming water, allowing for water exchanges without sacrificing biomass.

2.2. Water quality analysis

Temperature, pH, dissolved oxygen, and turbidity were measured in situ using a multimeter (TOA DKK WQC- 22A Model, Japan). In addition, alkalinity, total nitrogen, total phosphorus, and total suspended solids were determined in the laboratory by standard methods (APHA 2005).

2.3. Biomass and chemical composition analysis

Total suspended solids (TSS) were used to determine the algal biomass using Whatman GF/C filter paper. Since we hypothesized that algal biomass was in the proportion of total suspended cells (TSC), we used the gravimetric dry weight method to estimate algal biomass directly. Additionally, TSS is another method for directly determining algal biomass by weight, and the measurement is practical and efficient for bioengineering purposes. The procedures for measuring protein, carbohydrate, and lipids were adapted from Tipnee et al. (2015). The approach for determining antioxidant activity was adapted from Bhuyar et al. (2020).

2.4. Algal growth system and medium preparations

Lab-scale ponding system unit design and operating conditions were shown in Figure 1, consist of the operational and working parameters including detention time (10 days), reactor design (200 L), water level and water volume (150 L), feeding, feed filter size, mixing speed (by sponge bubbling) and light intensity (natural light), operation period 30 days. The daily feed of natural freshwater was collected from the effluent of tilapia pond at the Faculty of Fisheries Technology and Aquatic Resources, Maejo University. The collected water was filtered through by 0.45 μm filter paper and used as the medium.

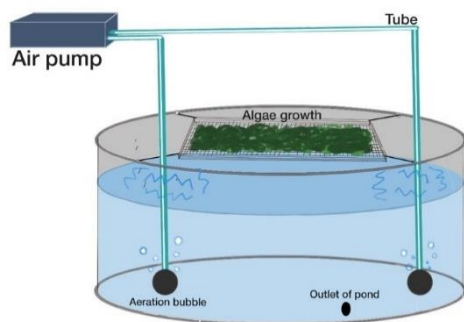


Fig. 1. Open pond algae growing system

2.5 Data analysis

All results are presented as means \pm standard deviation (SD) of three replicates. One-way ANOVA performed statistical analysis and comparison of group data. Relationships were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Water quality and algal medium

Water samples were collected from cement ponds at Maejo University's Faculty of Fisheries Technology and Aquatic Resources (tilapia fishpond effluent). Table 1 summarizes the physicochemical parameters of fishpond wastewater. Temperature and other physical variables have an effect on the numerous compounds dissolved in water. Gains in water quality refer to changes in water features that result in increased production,

whereas degradations in aquaculture systems refer to changes that result in output loss. This is a key concept in aquaculture, as densely planted aquatic crops frequently affect water quality. These changes would not constitute water quality degradation unless they harmed the target organism's production, safety, or value. Certain species require a better grade of water than others. For example, the traits that enhance tilapia production may work against rainbow trout. It is usual for aquaculture species to be selected for their tolerance of poor water quality. As a result, it is required to conduct water quality analyses concerning planted species. This study used an unsupplemented tilapia fishpond effluent medium prepared according to the method described by Ramaraj et al. (2017).

Table 1.

Physicochemical characteristics of fishpond effluent

| Parameters | Value |
|-------------------------|-------------------|
| Temperature | 30.1 \pm 1.01 |
| pH | 7.17 \pm 0.21 |
| Dissolved oxygen (mg/L) | 4.58 \pm 0.14 |
| Turbidity (NTU) | 137 \pm 2.53 |
| Alkalinity (mg/L) | 17.24 \pm 3.33 |
| COD (mg/L) | 48.56 \pm 2.17 |
| TP (mg/L) | 1.78 \pm 0.02 |
| TN (mg/L) | 11.53 \pm 0.07 |
| TS (mg/L) | 332.47 \pm 4.33 |
| TDS (mg/L) | 54.25 \pm 3.62 |

3.2 Algal biomass compositions

Water samples (tilapia fishpond effluent) were obtained from cement ponds at Maejo University's Faculty of Fisheries Technology and Aquatic Resources for physicochemical analysis. Table 1 summarizes the physicochemical properties of fishpond wastewater. Temperature and other physical qualities of water affect the many compounds dissolved in it. Gains in water quality refer to changes in water features that result in enhanced output, while degradations in aquaculture systems refer to changes that result in lower output. This is critical in aquaculture, as growing aquatic crops at large densities frequently degrade water quality. These changes would not be deemed degradation of water quality unless they harmed the target organism's production, safety, or value. Certain species benefit from superior water quality. For example, the characteristics that enhance tilapia productivity may be detrimental to rainbow trout. It is common to select aquaculture species that can tolerate poor water quality. Thus, water quality must be compared to farmed species. This study used an unsupplemented tilapia fishpond effluent medium prepared according to Ramaraj et al. (2017).

Freshwater macroalgae are found worldwide in both natural and manufactured streams and wastewater treatment systems. *Oedogonium*, *Cladophora*, and *Spirogyra* have been isolated from municipal and aquaculture wastewaters and naturally occurring bodies of water, irrigation canals, and wetland areas (Ge et al.

2018; Obey et al., 2019). The presence of freshwater macroalgae *Spirogyra* sp. in wastewater streams, as well as their competitive dominance of high yield biomass productive over other species (Table 2), demonstrates their inherent capacity to thrive in tilapia fish pond effluent wastewater and thus their potential as an algal biomass producer and biochemical compositions of protein, carbohydrate and fat were 19.025 ± 1.25 , 45.713 and 8.375%, respectively.

3.3 Biocircular economy and industrial ecology systems of *Spirogyra*

Freshwater algae offer a once-in-a-generation chance to alter a variety of industries by utilizing wastewater streams to generate algal biomass that can be turned into lucrative bioproducts. Figure 2 depicts a conceptual model of the integrated freshwater

macroalgae *Spirogyra* and a bio-circular-green economy (BCG) model. Freshwater macroalgae can be grown on-site in nutrient-rich wastewaters in agricultural systems, improving water quality and accumulating N, P, and other minerals and trace elements in their biomass. This biomass can subsequently be used as a high-quality feed ingredient or slow-release fertilizer, and the cleaned water from macroalgal cultures can be reused in animal production or industrial processes. In urban waste treatment systems, freshwater macroalgae can be cultivated on-site in nutrient-rich wastewaters, reducing the quantity of dissolved nitrogen and phosphorus in the wastewater. The biomass produced can then be converted into biocrude, biogas, bio-oil, or ethanol, and the treated water from macroalgal cultures can be recycled or safely released into the environment. Additionally, the biomass might be converted to charcoal and used as a soil improver. Freshwater macroalgae can be grown on-site in waste effluents in industrial systems, eliminating toxins and purifying water.

Table 2.

Comparison of wastewater based freshwater macroalgae culture system and biomass productivity.

| Species | Wastewater source | Reactor type | Volume (L) | Light source | Biomass productivity ($\text{g.m}^{-2}\text{d}^{-1}$) | Reference |
|---|--|-------------------|------------|-----------------------------|---|-----------------------------|
| <i>Oedogonium</i> sp. | Primary (PW), secondary (SW), dissolved air flotation unit (DAF) | Cylindrical tanks | 20 | Outdoor/ Natural | PW: 12.7-13.8 SW: 6.3 DAF: 9.2 | Neveux et al. 2016 |
| Mixed Species (<i>Oedogonium</i> , <i>Spirogyra</i> , <i>Ulothrix</i> , <i>Vaucheria</i>) | Pre-chlorination effluent | Shallow trays | 5 | LED | 3.37 | Yun et al. 2014 |
| <i>Microspora willeana</i> , <i>Ulothrix ozonata</i> , <i>Rhizoclonium hieroglyphicum</i> , <i>Oedogonium</i> | Swine Manure | Tray type | 205 | 2 x 400 W Metal Halide 23:1 | 7.1-9.4 | Kebede-Westhead et al. 2006 |
| <i>Oedogonium</i> sp. | Aquaculture system | Cylindrical Tanks | 853 | Outdoor/ Natural | 3.8-23.8 | Cole et al. 2014 |
| <i>Ulothrix</i> , <i>Spirogyra</i> , <i>Microspora</i> | Agricultural drainage | Raceways | 1200 | Outdoor/ Natural | 5 | Kangas and Mulbry, 2014 |
| <i>Spirogyra</i> sp. | Fish farm effluent | Cement pond | 150 | Outdoor/ Natural | 12.52-16.72 | This study |

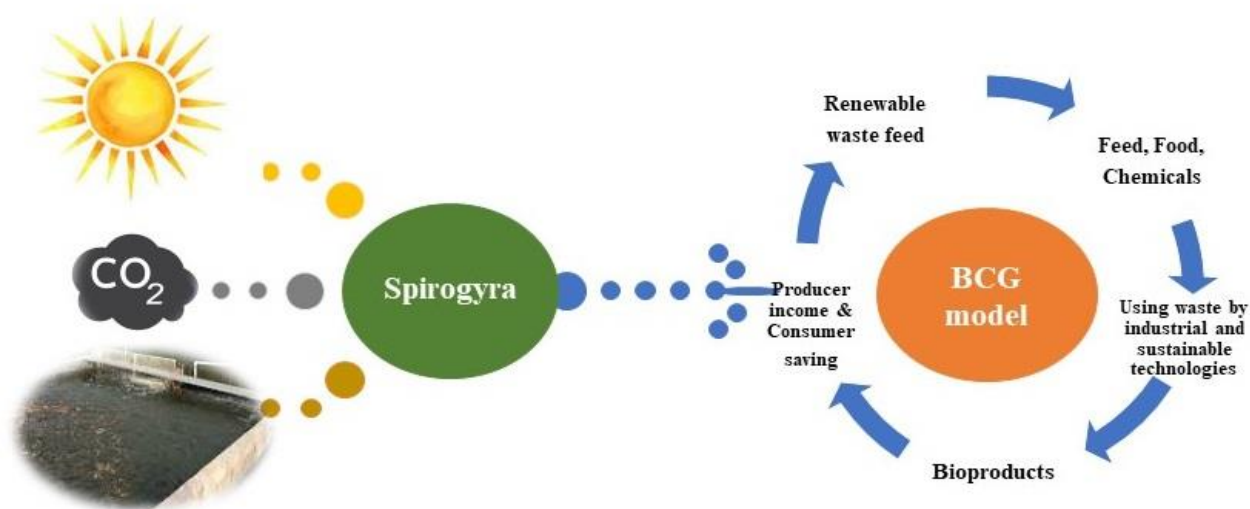


Fig. 2. Conceptual model of integrated freshwater macroalgae *Spirogyra* and Bio-Circular-Green Economy (BCG) model.

According to Dourou et al. (2018), fish farm effluents can be utilized as culture media for marine microalgae, the cell mass of which is a good source of bioactive chemicals for fish. Various fish farm effluents were evaluated as algal culture media in the current experiment. Alternatively, the biomass produced can be turned into a variety of bioenergy resources, including biocrude. Finally, macroalgal culture-cleaned water can be recycled or safely returned to the environment. The numerous commercial applications of microalgal mass in a variety of sectors, including the food and pharmaceutical industries, cosmetology, agriculture, and aquaculture, where it is used as animal feed or live feed for zooplankton and other aquatic species, has sparked considerable interest in macroalgal production in recent years. The term 'bioeconomy' refers to sectors of the economy that utilize renewable biological resources from land and sea, such as forests, animals, and microorganisms such as algae, as well as biological processes and principles, in order to create sustainable food, components, and energy (Seghetta et al., 2018). As a result, a 'circular bioeconomy' is a closed system that produces no waste, which is the global goal for assuring a long-term future. The bioeconomy has the ability to convert algae to fuel, recycle plastic, repurpose food waste, and turn industrial waste into valuable biobased commodities.

Spirogyra are known for their rapid growth, which enables them to convert sunlight, carbon dioxide, and nutrients into organic matter (biomass) and hence produce a vast number of sustainable bioproducts. It reduces our reliance on petrochemicals by sequestering CO₂ and fostering the development of carbon-neutral enterprises. *Spirogyra* are generally easy to culture: They don't need sophisticated growth media formulas or carefully controlled ambient conditions; all they need is light, water, and basic nutrients. As a result, its productivity per square meter of land is far higher than that of all crop plants. Algae have an exceptional production efficiency, as their conversion of solar energy to chemical energy via CO₂ fixation is tenfold that of terrestrial plants. To achieve economic and environmental

sustainability, algae are cultivated on non-arable land, in seawater, or even in wastewater, lowering freshwater usage and removing competition from food production, which is environmentally sustainable (Ramaraj et al., 2016a,b). Aquatic biorefining is another function that advanced biorefineries can perform that can contribute to a circular bioeconomy. The development of an algae-based circular bioeconomy will result in the creation of clean, green, and sustainable industries and new supply networks for manufacturing, clean energy, and ecological services.

3.4 Smart idea of the antioxidant properties of *Spirogyra* biomass

The concept direction of *Spirogyra* biomass on the antioxidant process was illustrated in Figure 3. By creating a diverse array of natural and sustainable components and products such as proteins, pigments, fatty acids, and bioplastics, freshwater macroalgae contribute significantly to an environmentally friendly society. In addition, natural antioxidants found in plants are critical in the fight against stress-induced oxidative damage caused by the generation of reactive oxygen species within the cellular milieu and many illnesses and aging processes (Bhuyar et al., 2019). As a result, such chemicals can improve general health and well-being and be used medicinally, notably to treat a wide variety of human illnesses and afflictions. Thus, numerous natural plant forms such as micro- and macroalgae, lichens, micromycetes, and plants have developed into the primary bioresources for natural antioxidants that effectively prevent and treat a variety of ailments. Algae are a primary focus of our search for natural antioxidants since they have been shown to have considerable antioxidant activity and free radical scavenging characteristics.



Fig. 3. concept direction of *Spirogyra* biomass on the antioxidant role.

Additionally, *Spirogyra* is a common component in South-East Asian cuisine and has been shown to provide health benefits due to its high protein, mineral, vitamin, lipid, and carbohydrate content. As a result, the food industry may commercially employ it to produce healthy foods (Tipnee et al. 2015). Certain *Spirogyra* species have lately gained interest for their antioxidant activities both in vivo and in vitro (Vogel and Bergmann 2018). Algae are a source of alternative nutrients that contain a high concentration of functional dietary components. While numerous research has demonstrated the positive effects of macroalgae, this study focuses on the antioxidant activity of *Spirogyra*, a freshwater macroalga, in animal models.

Spirogyra hot water extraction showed antioxidant effects (lab data not shown in this paper). Our earlier research established the antioxidant activity of a warm water extract of algal biomass in vitro. However, because the hot water extract of biomass includes a higher phenolic content than the warm water extract, this study examined the in vivo antioxidant activity of the hot water extract of biomass. The biomass levels employed in the analysis were not harmful to laboratory animals, ranging from human daily ingestion to fourfold overdose. Antioxidant enzymes are critical for the cell's defense against oxidative stress. For example, catalase is an enzyme that reduces hydrogen peroxide to water and molecular oxygen, thereby preventing extremely hazardous hydroxyl radicals. These findings indicate that *Spirogyra* may be exploited to produce novel medications and as a source of natural antioxidants and antibacterial agents (Vogel and Bergmann 2018).

3.5 Smart idea of the *Spirogyra* biomass for future food

Food is essential for human survival, and its proper balance is essential for human health and happiness. The food supply has undergone a remarkable transformation as society and technology have grown, and customers now seek food that meets their health and nutritional needs. Protein is an essential part of the human diet since it provides all of the necessary nitrogen. Humans cannot synthesize a subset of amino acids; thus, they must be received

through food (Ge et al. 2018). Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are all essential amino acids. Arginine, cysteine, glutamine, glycine, proline, and tyrosine are among the other amino acids that are required on a case-by-case basis.

The concept direction of *Spirogyra* biomass for food products outlines shown in Figure 4. Following hydrolysis, algal starch, cellulose, or other accumulating carbohydrates can be used to make ethanol. The generation of bioethanol from algae does not compete with terrestrial or aquatic food production, and both marine and freshwater algae can be employed. They also do not have to compete for food with other people. Algal cells are rich in carbohydrates, including starches and sugars, which can be fermented to make bioethanol (Sulfahriet et al., 2016). While measuring the overall protein content in living cells is extremely useful, isolating the desired phenotype using a cell sorter would be wonderful. A suitable production technique with a high protein content will need to be developed, as will the ideal strain. Appropriate nitrogen source and concentration and other micronutrients will be critical in developing suitable media for high protein production. Additionally, producing algae in autotrophic or heterotrophic systems can substantially alter cell composition. Thus each strain must be grown in the optimal environment. Finally, a favorable amino acid profile is critical in which all essential amino acids are present in appropriate amounts. If the desired amino acid is not present in the selected strain, it may be added via a protein or peptide that is extremely rich in the desired amino acid.

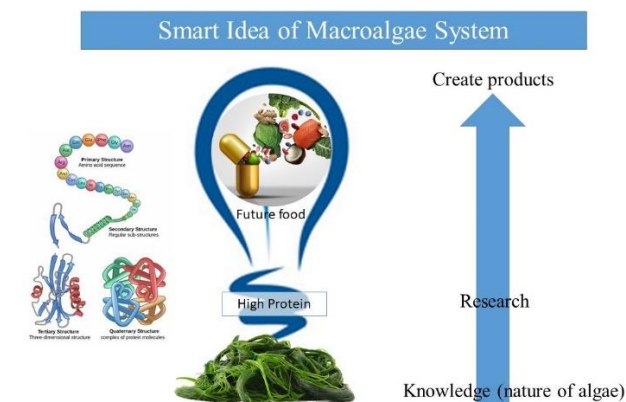


Fig. 4. The concept direction of *Spirogyra* biomass for food products.

To express the high protein phenotype, a method for manufacturing high protein will need to be devised. Making the best media choices to promote protein production, including choosing the best nitrogen sources and concentrations, will be crucial. In addition, the cell composition must be investigated, and the best growth system for each strain must be selected. To be more specific, an amino acid profile that contains a suitable proportion of essential amino acids is required. This can be performed by combining concentrated concentrations of specific

amino acids in a protein or peptide. As a result, *Spirogyra*, which is high in protein, may be used in future cuisine.

3.6 Smart idea of the *Spirogyra* biomass for biodiesel production

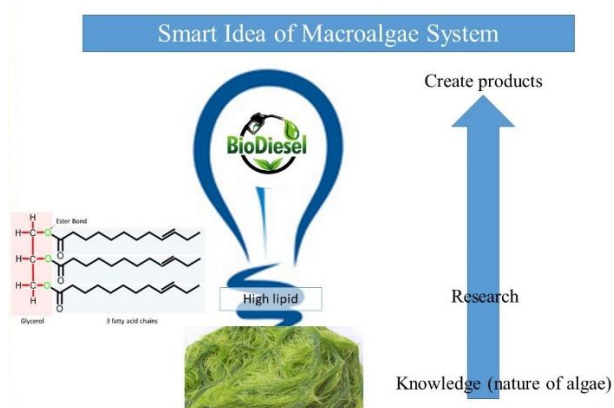


Fig. 5. The concept direction of *Spirogyra* biomass on the role biodiesel.

Pollution is generated at every stage of the fossil fuel production process. Solar, wind, and geothermal energy all have their own generating and supply restrictions. Biofuels can reduce pollution while also generating electricity. Algae oil is a viable energy source since it consumes less water and energy than other crops while producing more oil. Transesterification of plant seed or algal oil is the most extensively used process for creating biodiesel at both the micro and macro levels. The transesterification process increases the viscosity of crude oil. The molar ratio and kind of transesterification procedure are key parameters in the entire transesterification process, as are the type and number of catalysts, the reaction method, the type of alcohol, and the temperature, duration and character of the alcoholic beverages. Enzymatic transesterification is utilized to produce both biodiesel and glycerol at high purity. It eliminates the need for soap production and is also environmentally friendly.

The concept direction of *Spirogyra* biomass on the role biodiesel approach was presented in Figure 5. Algae can be used to preserve pollution and oil by utilizing nutrients and carbon dioxide from wastewater to grow. The *Spirogyra* sp. material contained a significant oil reserve that might be exploited to make biodiesel. Therefore, *Spirogyra* has been identified as a valuable source of oil that may be used to synthesis biodiesel. *Spirogyra* oil is appropriate for biodiesel synthesis due to its acid, saponification, peroxide, density, viscosity, and iodine.

3.7 Smart idea of the *Spirogyra* biomass for bioethanol and biogas production

Figure 6 demonstrates the concept direction of *Spirogyra* biomass for bioethanol and biogas production. Algae are attracting considerable interest as a sustainable biomass source for the

production of bioethanol, a "third-generation biofuel." Following hydrolysis, algal starch, cellulose, or other accumulating carbohydrates can be used to produce ethanol. The production of bioethanol from algae does not conflict with terrestrial or aquatic food; both marine and freshwater algae can be utilized. They also don't have to compete for food with other people. Algal cells are rich in carbohydrates such as starches and sugars, which can be fermented to make bioethanol. Algae have the advantage of being lignin-free and containing little hemicellulose, which results in increased hydrolysis efficiency and fermentation yields, cutting the cost of bioethanol production. Algal can absorb CO₂ from the environment and power plants, and when combined with appropriate technology, algae bioethanols can result in considerable reductions in GHG emissions compared to fossil and other biobased fuels (Ramaraj et al., 2006a,b).

Algae grow rapidly and flourish in various aquatic environments, including freshwater, seawater, and municipal wastewater. They have high photosynthetic efficiency; aquatic biomass, on average, has a photosynthetic efficiency of 6–8%, which is much higher than that of terrestrial biomass (1.8–2.2%). Algal cells are extremely productive and have a short harvesting cycle (1–10 days) compared to other feedstocks (harvest once or twice a year), providing sufficient supplies to meet ethanol manufacturing demands.

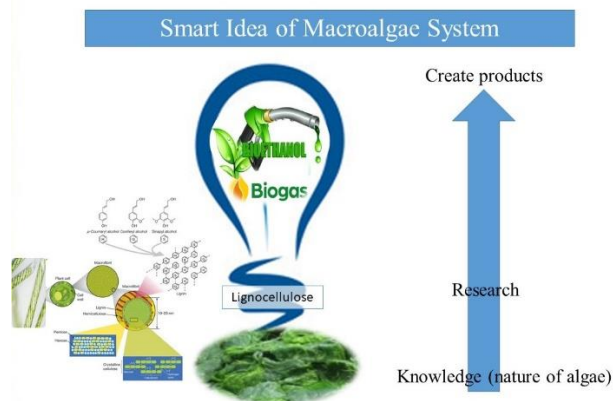


Fig. 6. The concept direction of *Spirogyra* biomass for bioethanol and biogas production

Due to the macroalgae *Spirogyra* sp.'s productivity and capacity to collect significant amounts of sugar, this biomass is also attractive as a substrate for biogas production. Because biofuel production from this macroalga is still in its infancy, the production of biogas from *Spirogyra* sp. biomass may be of interest in this research field. Anaerobic processing involves analysing the biomass for fat, protein, and carbohydrate composition (Ramaraj et al., 2015). Variations in the proportions of these elements result in varying amounts of biogas and methane concentrations. The biogas production process is accelerated when the substrate contains more carbs because the rate of carbohydrate degradation is faster, and the methane concentration can reach 50–60%. However, when more fat or protein is added, biogas production is reduced and methane concentrations are increased. It might range from 75% to 90%. Algae include important fatty

acids, carbohydrates, and protein. Thus, by measuring the chemical composition, one may ascertain the capacity for producing biogas and methane. In this case, based on the methane concentration in biogas, it is possible to determine that the amount of protein produced during *Spirogyra* processing was the highest, owing to the high methane concentration.

4. Conclusions

The raw materials for lignocellulosic biofuels and bioproducts are inexpensive, renewable, and plentiful; energy production is expensive due to the high recalcitrance of lignocellulosic raw materials. Given the fundamental hurdles to modern society's long-term progress posed by the energy crisis and pollution, macroalgal biomass as a raw material for bioproduct production is a sustainable and environmentally beneficial source. Furthermore, freshwater macroalgae, characterized by the genus *Spirogyra*, offer numerous prospects for integration into agricultural, municipal, and industrial systems for wastewater treatment and biomass production under a variety of cultivation circumstances. Finally, every method for creating new things from trash enhances sustainability and decreases environmental impact. Thus, integrating large-scale growth of freshwater macroalgae into existing production systems will greatly help both industry and the environment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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