High-value chemicals from marine diatoms: a biorefinery approach

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Abstract. Nowadays, we are going a step forward into the new era for the sustainable production of industrial commodity products such as energy, fine-chemicals, active compounds and materials from renewable biomass. Marine diatoms offer great potential as an untapped living factory for the generation of valuable commodity chemicals. As a photosynthetic microorganism, diatoms contain pigments, which have a high market value in the cosmetic, pharmaceutical and food colorant industries. Their unique metabolism to utilize the soluble silica in seawater for their porous silica cell wall (frustule) opens an opportunity for the nanoporous material industry. Diatom’s lipids consist fatty acids, which could be catalytically upgraded into high-quality fuels like fatty acid alkyl esters (biodiesel) or hydrocarbons (green diesel). In the analysis reported here, we present the potential of biorefinery pathways of valuable components in marine diatoms. Understanding the biochemistry of them and the application of their valuable chemicals are discussed to gain insights for the opportunities and the key barriers in the development of marine diatoms-based biorefinery.

1. Introduction
In recent years, microalgae have attracted much attention as a potential alternative renewable feedstock for the sustainable production of carbon-based fuels. It is particularly triggered by its fast growth rate and high lipid content. Owing to their high photosynthetic efficiency (~12.6%) [1], the maximum specific growth rate (μmax) of microalgae is estimated to be able to reach 10 folds than the terrestrial plants under the optimum condition [2, 3]. Accordingly, the biomass production of microalgae requires lower amount of land area and time compared with the terrestrial plants to get an equal number of biomass. Furthermore, microalgae utilize atmospheric carbon dioxide, water and sunlight to accumulate lipids through photosynthetic process, especially, in form of an ester of glycerol and fatty acids (triglyceride). It was reported that the lipids content of microalgae is commonly observed in the range of 8-50% d.w., with a possibility to up to 55-80% d.w. for some species under appropriate condition [4-6]. Lipids isolated from microalgae were reported to have an energy content of 35,800 kJ kg⁻¹ or nearly 80% of the average energy contained in crude petroleum
Hence, microalgae are expected to have promising productivity for alternative biofuel (biodiesel) feedstock, even compared with the oleaginous crops [8, 9].

The cultivation of microalgae also does not meet the land availability issues. The land has multiple functions to support our lives; e.g. for the production of food, feed, fiber, materials; human living place; nature conservation; climate protection [10, 11]; and thus, its utilization for the development of dedicated energy terrestrial plants should be limited. In contrast, microalgae could be grown in the photo-bioreactor or in open ponds, which do not require large scale of land use for their culture system. Even, the cultivation of microalgae could be conducted in non-arable land.

In spite of their eminences, the commercialization of microalgae for biofuels is still far from realized. Many demonstration projects reported that producing algal fuels solely is uneconomically viable [1], particularly after the decline of petroleum prices in August 2014. For instance, the techno-economic analysis of algal oil production from a freshwater microalgae Chlorella vulgaris, with 25 wt.% of lipid content and 0.5 g L\(^{-1}\) of growth culture density in raceway ponds model, exhibited that the cost is estimated to reach $4.10 L\(^{-1}\) [12]. It is significantly higher than the price of biodiesel derived from vegetable oils ($0.89 L\(^{-1}\) in the US market in 2017) [3]. The US Department of Energy has identified and found four main challenges for the commercialization of microalgae’s biofuels including culture stability, scalable system designs, nutrient sources and water sustainability [1]. These contribute to 95% of the total capital investment [12] and to 25-87% of the total production cost [1, 12, 13], especially for open pond raceway systems.

To improve the economic viability, Togarcheti et al. investigated the study on the co-production of biodiesel and biogas from microalgae’s lipid and the spent biomass resulted from lipid extraction, respectively [13]. However, this integration process did not give a significant effect on the total cost of the process; e.g. the overall energy process is only reduced by 2%. It is related to the low economic value of biofuel itself despite its high market volume [14]. In this respect, another financial incentive is required to assure the profitable of the utilization of microalgae for energy production.

The co-production of biofuels and other valuable products with high economic value, viz. biorefinery, could be a potential strategy to improve the return of investment (ROI) and the sustainability of the commercialization of microalgae process. It is considered since microalgae could synthesize various kinds of biochemical compounds that are potentially used for the production of high-value products in the foods, cosmetics, pharmaceuticals and nutraceuticals industries. In fact, the revenue per unit of microalgae biomass for biochemicals production is much higher than that for biofuels (US$2.60 and US$0.39 kg\(^{-1}\), respectively) [15]. This has also made some industries to focus their market products in biochemicals production instead of biofuels. For instance, Saphire Energy Inc. (USA) and Aurora Biofuels Inc. (USA) have expanded their market products from biofuels investments into Omega-3s and EPA, respectively [1].

Marine microalgae have great potential for the sustainable production of industrial commodity products, including fuels, bulk/high-value chemicals, and materials. Particularly, special attention should be directed to diatoms because they are the major group of microalgae in oceans. The biomass productivity of diatoms was reported in the range of 5-25 g m\(^{-2}\) day\(^{-1}\), which is in line with the type of green microalgae 10-25 g m\(^{-2}\) day\(^{-1}\) [16]. Furthermore, about 60% of the potential microalgae for commercial lipid production was reported from diatoms [17]. In fact, diatom’s lipid has been confirmed as the major source for petroleum and some of their lipid’s components; i.e. steranes, 24-norcholestanes, and highly branched isoprenoids; are used as the biomarkers to determine the petroleum reservoir in earth [17]. Since they could be grown in seawater, the cultivation of diatoms will not meet with freshwater issues as well.
The selection of the biorefinery strategy depends on the product portfolio and the chemical substances in biomass. In general, it maximally exploits the chemical components of biomass to generate multiple valuable products. As another type of microalgae, diatoms contain great variety of chemical compounds, however, their utilizations are mainly used in the limited aspect. As an example, many types of research have been conducted to generate lipids for fatty acid methyl esters (biodiesel) \cite{18,19}. Current reviews have also reported the potential of diatoms for nanomaterial development \cite{20,21}. In this research review, we highlight the potential of valuable chemicals that could be derived from diatoms. The insights on the chemical substances of diatoms are discussed in order to explore the market's opportunity of them besides for biofuels. Afterward, we propose a possible integrated process for the production of valuable chemicals from diatoms. It is expected that this review could give an insight as another strategy to improve the economic viability of the utilization of diatoms.

2. The biology of diatoms

2.1. A general overview of diatoms

The “Diatoms”, which originally refers to the genus of *Diatoma* De Candolle 1805, is a general term to describe the phytoplankton species of the class of Bacillariophyceae \cite{22}. It is a unicellular organism, which is characterized by their “frustule” (cell wall) that is made from silica. The frustules of diatoms consist of two different main parts, epitheca and hypotheca \cite{22,23}. Approximately, there are more than 100,000 species of diatoms with 200 genera \cite{23}. Taxonomically, diatoms are divided into two order, which are: (1) Biddulphiales, or commonly known as centric diatoms, which have distinct characteristic of radial symmetrical valve or frustules (Fig. 1A,B) ; and (2) Bacillariales, or pennate diatoms, which have elongated frustule with a bilateral symmetry form (Fig. 1C,D) \cite{22-24}. Fig. 1 shows some diatoms species from these two groups.

![Figure 1. Microscopic images of some diatoms: Coscinodiscus sp. (A), Lauderia sp. (B), Navicula sp. (C) and Pseudo-nitzschia sp. (D)](image-url)
The population and the growth rate of diatoms are affected by their environmental living habitats, such as light, salinity, temperature, pH and nutrient level. In particular, diatom’s diversity is generally regulated by two major forces; *i.e.* (1) light availability, which regulates the photosynthetic activities, and (2) water mass movement; which distributes the nutrients into the surface layer [24]. As a photosynthetic organism, diatoms require light for their photosynthesis process. As such, the changes in the light’s availability; either due to increasing water turbidity, sinking, or changes in season; could affect their growth rate. In this case, diatoms need to remain in the photic layer of water and avoid fast sinking rate towards the aphotic layer. Due to the lack of the specific organelle, diatoms maintain their buoyancy by several strategies, such as: (1) developing special structures like spines, setae, or filaments; (2) forming colony, either in chain or filamentous; (3) manipulating the cell’s ionic regulation; (4) oil or lipid production and storage; (5) produce low-density metabolic by-products or secondary metabolites; or (6) forming mucilage [24, 25].

Diatom’s primary productivity is generally limited by the C:N:P ratio of the Redfield Ratio (106:16:1) [26]. In general, marine diatoms have a C:N ratio close to the Redfield ratio of 6.6. However, the N:P ratio in the diatom community is generally lower than the Redfield ratio (N:P) of 16 [26]. At high Si:P and Si:N ratios, diatoms could undergo rapid and uncontrollable reproduction rates (bloom). Furthermore, iron (Fe) and Silica (Si) are the important nutrients that regulate diatoms community by limiting their metabolic processes, such as photosynthesis, respiration, and cell wall synthesis [26].

Herein, the addition of Fe favors the large size of diatoms [27].

One unique trait of several diatom species is the ability to produce toxins under specific nutrient limitation conditions, such as domoic acid, a potent neurotoxin produced by some *Pseudo-nitzschia* species, which produced and accumulated in the cells under Si depletion condition [28]. Concern about *Pseudo-nitzschia* blooms is increasing worldwide particularly since the domoic acid is the cause of amnesic shellfish poisoning (ASP) in human and poisoning cases in marine animals [28, 29]. Therefore, blooms of toxic diatoms could pose immediate threats to both marine lives and human communities in the coastal areas. As a note, even if the diatoms that bloom in the ecosystem did not produce toxin, it still causes damages to ecosystem by forming high density of biomass, leading to oxygen depletion and increased turbidity and shading by those flocculated biomasses [30].

2.2. Reproduction of diatoms

Diatoms are capable of reproducing both vegetatively, by binary fission (mitosis), or sexually, by meiosis which produces a zygote that will end up as an auxospore [22-24]. In binary fission process, the parent cell frustule will divide and form new individuals from the parent’s epitheca and hypotheca. However, vegetative reproduction in diatoms produces a side effect of size reductions of its offspring, which will reduce the average cell size of the diatom population in the ecosystem. At one point, the cell size will be too small and need to be restored via sexual reproduction, which produced auxospore [22-24]. The centric (Centrales) and pennate (Pennales) diatoms differ in their sexual reproduction strategy, in which the formerly produced flagellates oogamy and the later produced isogamy without undergoing flagellated stage [22]. As a note, sexual reproduction of diatoms sometimes could be triggered by changes in environmental conditions, particularly changes in salinity, light, nutrient, or temperature. Those factors could induce a transformation from asexual into sexual reproduction in the vegetative cells of the diatoms [23].

The formation of resting spores, those generally formed under unsuitable environmental conditions, is one important survival strategy of diatoms, in which the spores will later germinate when the water condition has improved or restored. Interestingly, the formation of resting spore in planktonic diatoms is more commonly observed in centric diatoms and rarely in pennate diatoms [22]. Based on its position in relation to the parent cell, resting spore could be divided into three types: (1) exogenous resting spores, in which the matured spore is completely separated from the parent cell; (2)
semiendogenous resting spore, with one of the parent cell theca enclosing the spore’s hypovalve; and (3) endogenous resting spore, which located inside the parent cell’s frustule [22]. The ability to produce resting spores under unfavorable conditions is a key factor that enables some diatoms species to undergo jump dispersal via ballast water tanks of the ships. It was reported that resting spores of *Chaetoceros* and *Odontella* species sampled from the sediments in the ballast water tank could still produce viable diatom cultures, even after months of voyages in a total dark and low-temperature environment (at 4°C) [31].

2.3. Role of diatoms

Aside from its role as the “silica pump” in the ecosystem [25, 32], diatoms also play a major role in the cycles of Carbon (C), phosphorus (P), nitrogen (N), and iron (Fe) [26]. In fact, diatoms play a great role in the ecosystem by contributing up to 45% of the total primary production in the ocean [26, 32]. Diatoms are also considered as the most important key player in the carbon cycle of the ocean via CO₂ air-to-sea exchange and by transferring nutrients and organic carbon (C) into deeper water layers [25, 26, 32]. Diatoms also act as an important link in the energy transfer cycle within the food chain, which supports the life of other marine organisms at the higher trophic levels [33]. For example, higher growth rate and healthier fish larvae were observed in an ecosystem with a food chain that is primarily supported by diatoms, in which, the fish larvae are feeding on copepods with high fecundity that consume the diatoms [33]. In that case, diatoms support the most productive fisheries area in coastal waters, while diatoms provide food (organic matter) to the deep-water organisms by sinking from the surface to deeper water in the open ocean [34].

3. Biochemicals in diatoms

3.1. Pigments

As common photosynthetic organisms, diatoms have pigments in their cells. Their pigments typically consist of two groups, chlorophylls and carotenoids. Chlorophylls are responsible for light energy absorbing, converting it to “chemical energy” in form of chemical compounds rich in energy such as carbohydrates or lipids. Meanwhile, the later pigment has role in photoprotection of cell’s photosystem from the oxidative damages. In particular, carotenoids have ability to absorb light and quench excess energy in photosynthetic metabolism. They protect the photosynthetic components in the cell from photo-oxidation and stabilize the integrity of lipid membrane from peroxidation [35]. Examples of these carotenoids are astaxanthin, canthaxanthin, and echinenone [36]. Some carotenoids such as fucoxanthin also participate in light-harvesting together with chlorophyll a (Chl a) and chlorophyll c (Chl c) [37]. Other carotenoids, that also involved in the photosynthetic components, are α-β-carotene, lutein, violaxanthin, zeaxanthin, and neoxanthin.

![Figure 2. Molecular structure of Chl a and Chl c in diatoms](image-url)
Generally, diatoms have two kinds of chlorophylls, i.e. Chl \(a\) and Chl \(c\) \([37]\). As other photosynthetic organisms, Chl \(a\) plays the main function on the photochemical energy conversion during photosynthetic process. Meanwhile, Chl \(c\) has similar function to Chl \(b\) in higher plants and green algae. The most abundant among of Chl \(c\) types in diatoms are Chl \(c_1\) and Chl \(c_2\). Figure 2 shows the chemical structure of Chl \(a\) and Chl \(b\).

Due to the extended \(\pi\)-delocalization at the edge of their cyclic tetrapyrrole (porphyrin) skeleton, chlorophylls have major absorption bands in the visible light; i.e. “red” (Q-) band and “blue” (Soret or B-) band \([38]\). These characteristics are commonly used for the practical identification of chlorophylls by the UV-Vis spectroscopy measurement. However, the absorption values are mainly depended on their organic extraction solvent. In the acetone solution, the “red” and “blue” bands of Chl \(a\) are generally located at 661-663 and 430-431 nm, respectively (Fig. 3). Meanwhile, Chl \(b\) has typical maximum absorbance at 644-647 and 453-457 nm, respectively. In this context, the typical ratio of maximum absorbance \((A_{\text{Soret}}/A_{Q})\) of Chl \(a\) and Chl \(b\) are reported in the range of 1.27 and 3.89, respectively \([39]\). Typically, the “red” absorption maximum of Chl \(a\) shifts from 660 to 650 nm and the blue absorption maximum shifts from 428 to 432 nm with increasing the polarity of the solvent \([40]\). Similarly, Chl \(b\) shifts from 642 to 652 nm and 452 to 469 nm, respectively.

![Figure 3](image_url). UV-Vis absorption spectrum of acetone extract of marine diatom *Navicula* sp., *Thalassiosira* sp. and astaxanthin (A) compared with a green marine microalga *Chlorella* sp. and some pigments standard (B). *Navicula* sp. and *Thalassiosira* sp. are obtained from microalgal culture collection of Mariculture Laboratory, Research Center for Oceanography-LIPI. Figure 3B is adapted from reference \([39]\) with permission.
Carotenoids are lipophilic compounds that usually have a yellow, orange or red color. These compounds show intense UV absorption in the range of 400-500 nm [37], in which, their maximum absorbance is generally observed in the region of 470 nm [40]. Basically, they are built based on isoprene units (terpenoid) and divided based on the presence of oxygen in their structure. Carotenoids with the presence of oxygen (hydroxyl or keto-) are classified as xanthophylls groups such as astaxanthin, antheraxanthin, fucoxanthin, diadinoxanthin, diatoxanthin, zeaxanthin, and violaxanthin. Meanwhile, carotenoids with only hydrocarbon structure are grouped as carotenes such as α-carotene, β-carotene, and lycopene. As such, xanthophylls are relatively hydrophilic due to the presence of oxygenated functional groups at the end of their rings. Fig. 4 shows some molecular structures of carotenoids that are found in marine microalgae.

![Molecular structures of carotenoids](image)

**Figure 4.** Some common carotenoids in marine microalgae

| Table 1. Fucoxanthin content in some species of macroalgae and diatoms [41, 42] |
|-----------------------------------------------|
|                             | Macroalgae (mg g⁻¹ d.w.) | Diatoms (mg g⁻¹ d.w.) |
| Cystoseira hakodatensis       | 1.3                        | 8.6-16.5               |
| Sargassum horneri             | 2.4                        | 6.0-18.2               |
| Sargassum muticum             | 0.6                        | 26.6                   |
| Undaria pinnatifida           | 2.3                        | 2.2                    |
| Alaria crassifolia            | 0.3                        | 18.5                   |
| Nitzschia sp.                 |                            | 4.9                    |

Typically, diatoms contain seven kinds of carotenoids, i.e. β-carotene, antheraxanthin, fucoxanthin, diadinoxanthin, diatoxanthin, zeaxanthin and violaxanthin [37]. β-carotene has an orange-yellowish color which is used for food or nutritional supplement because it is a precursor of vitamin A (retinol) [43]. Among them, fucoxanthin is the most attractive diatom’s pigment (Table 1). It is a component of fucoxanthin-chlorophyll protein (FCP) complexes in diatom’s cell, which has function on performing light-harvesting during photosynthesis [37]. The fucoxanthin content in diatoms is reported in the range of 2.2-18.2 mg g⁻¹ d.w. [44], which is 10-20 folds higher than the present commercial fucoxanthin sources in the brown seaweeds of Laminaria japonica, Eisenia bicylis, Undaria pinnatifida, Hijkia fusiformis, Sargassum sp. and Fucus sp. (c.a. <0.1-2.4 mg g⁻¹ d.w.) [36, 41, 45-47]. The two main species of diatoms for the fucoxanthin production are Phaeodactylum tricornutum and Isochrysis galbana. However, the pigment compositions, as well as the content, are greatly depended on the environmental conditions and the species of diatoms.

Pigments provide the highest revenue from the microalgal biomass (US$39.2 kg⁻¹) and their market volume is estimated to be over US$1 billion [15]. Mainly, carotenoids are the attractive pigments in the pharmaceuticals and cosmetics industries because of their potential bioactivities (Table 2). The global carotenoid market is about US$1.24 billion in 2016 and it is estimated to achieve US$1.53-3.8
billion by 2021 [6, 36]. The market interest of carotenoid pigments is mainly β-carotene, astaxanthin, zeaxanthin, lutein, lycopene, and canthaxanthin. Currently, fucoxanthin also has a growing market because it has potential benefits for anti-obesity functional food, anti-cancer, and anti-inflammatory. Chlorophylls also possess potential market and have been used as a control of geriatric patients, dietary supplements, antimutagenic and anticarcinogenic agent [43]. Although some synthetic pigments are already available, the market demand for natural pigments is still increasing due to their unique properties which cannot be found in the artificial one as well as the consumer’s vision for healthy natural sources.

| Carotenoid | Potential bioactivity |
|------------|-----------------------|
| Astaxanthin | Anti-oxidant, anti-inflammatory, anti-cancer, cardio protective, neuro protective, anti-diabetic |
| Lutein     | Prevent cataract and age-related macular degeneration, anti-oxidant, anti-cancer, prevent cardiovascular diseases, protecting agent against macular degeneration of the eye |
| β-carotene | Prevent night blindness, anti-oxidant, prevent liver fibrosis, the cardioprotective effect |
| Lycopene   | Anti-cancer, prevent cardiovascular diseases, radiation protection, anti-oxidant |
| Fucoxanthin| Anti-obesity, anti-oxidant, anti-diabetic, anti-angiogenic, anti-metastatic |
| Cantaxanthin | Creates tan color, anti-oxidant |

Diatoms have great potential advantages for pigment production, especially fucoxanthin. Besides their high pigment content, diatoms have higher biomass productivity than macroalgae. Their growth cells could be double per day [41], implying the high pigment productivity derived from diatoms. Moreover, their cultivation does not depend on the season since the environmental condition of the diatoms culture can be well adjusted and optimized regularly, either in open pond systems or closed photobioreactors. On the contrary, macroalgae are often seasonally dependent and their cultivation usually takes >3 months to reach harvestable size (approximately 3% biomass increase per day).
3.2. Lipids and their derivatives

The major component of diatom’s lipids is triglycerides of fatty acids with the chain length of C14:0, C16:0, C16:1, C18:0 and C18:1 (Fig. 5). In normal conditions, diatoms contain 15-25% d.w. of fatty acids during exponential growth [17]. Under optimal conditions, it could reach in the range of 25-45% d.w. of biomass. Mainly, it is obtained in the condition of limited nutrients, especially low amount of nitrogen. Typically, the nitrogen deficiency suppresses the productivity of microalgae biomass since it is required in the biosynthesis of amino acids as well as protein for the growth of the cells. In fact, amino acids are decreased to 1/20 amount in nitrogen-deficient condition during in the nitrogen assimilation and the N-transporting metabolisms [48]. In contrast, molecular species of triglycerides; e.g. diacylglycerols, palmitic acid, stearic acid, oleic acid; are increase significantly [48], leading the accumulation of lipids of triglycerides in their biomass for their energy storage.

Enhancing lipid accumulation in diatoms can also occur through the silicon starvation. Levitan et al. mentioned that this approach could increase the lipids content of diatoms from 28% d.w. into 68% d.w., with an average of 45±10% d.w. [17]. Through this strategy, high lipid productivity could be achieved without suffering the growth productivity of diatoms. It is because silica has no significant effect on the physiological growth rates of diatoms compared with that in the nitrogen starvation.

Lipids of diatoms have similar potency to be converted into multiple valuable chemicals like that of plant oils. The most common approaches are producing fatty acids alkyl esters (FAAEs) via transesterification reaction, either by using acid or base catalysts. Since diatoms are a single-cell microorganism, the reaction could be performed by using the raw diatom’s biomass directly without conducting the extraction of their lipids at first [49]. This process offers advantages such as minimizing the use of chemical reagents as well as the toxic wastes, faster and more efficient compared with the preparation of FAAEs from terrestrial plants. Importantly, the profiles of the

![Figure 5. Lipids profile of some marine diatoms (adapted from reference [19])](image-url)
obtained fatty acids are not significantly different compared with the conventional method; i.e. two steps by lipids extraction and transesterification of lipids.

\[
\begin{align*}
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3 \\
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3 \\
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3
\end{align*}
\]

+12\text{H}_2 \rightarrow \text{C}_9\text{H}_6 + 6\text{H}_2\text{O} + 3\text{CH}_3(\text{CH}_2)_{10}\text{CH}_3 \quad \Delta G = -303.0 \text{kJ mol}^{-1}

(A)

\[
\begin{align*}
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3 \\
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3 \\
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3
\end{align*}
\]

+3\text{H}_2 \rightarrow \text{C}_9\text{H}_6 + 3\text{CO}_2 + 3\text{CH}_3(\text{CH}_2)_{10}\text{CH}_3 \quad \Delta G = -145.5 \text{kJ mol}^{-1}

\text{decarboxylation}

(B)

\[
\begin{align*}
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3 \\
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3 \\
\text{H}_2\text{C} & \text{-O-} \text{C}_3\text{H}_2(\text{CH}_2)_{10}\text{CH}_3
\end{align*}
\]

+6\text{H}_2 \rightarrow \text{C}_9\text{H}_6 + 3\text{CO} + 3\text{H}_2\text{O} + 3\text{CH}_3(\text{CH}_2)_{10}\text{CH}_3 \quad \Delta G = -99 \text{kJ mol}^{-1}

\text{decarbonylation}

\text{Scheme 1}. Chemo-catalytic synthesis of hydrocarbons from tristearin as a model of triglyceride via hydrodeoxygenation (A) and decarboxylation as well as decarbonylation (B) [50]

Despite their mature technology, FAAEs have low oxidation stability and cold flow properties. Moreover, they have limited kinematic viscosity which causes some issues on the atomization pump, carbon deposition and ring sticking in the combustion engine. As such, the use of FAAEs as a liquid fuel always needs to be blended with conventional diesel. Currently, much attention has been focused on the chemo-catalytic hydrogenolysis reaction of triglycerides with some active metals as a catalyst. This reaction generates the deoxygenated products (hydrocarbons) which have physicochemical properties similar to that of petroleum diesel [51]. This reaction could be conducted via hydrodeoxygenation, decarboxylation, and decarbonylation to remove oxygen as water, CO\textsubscript{2} and CO as a by-product, respectively (Scheme 1).

As an oleaginous resource, diatoms have potential for the production of green diesel (biohydrocarbons) via catalytic deoxygenation reaction of their lipids. HDO is directed to remove the oxygen in the structure of triglycerides in the form of H\textsubscript{2}O (hydrodeoxygenation), CO\textsubscript{2} (hydrodecarboxylation) or CO (hydrodecarbonylation), leading to the formation of a hydrocarbon that is chemically similar to petroleum diesel called as green diesel (Scheme 1). Due to this property, green diesel has higher quality than fatty acid alkyl esters and can be blended with fossil-based diesel in any proportion without modifying the engines. These reactions are generally conducted by reacting triglycerides with pressurized hydrogen gas in the presence of active metal catalysts such as Pt, Cu, Ni and Mo on the solid support catalyst. Hydrodeoxygenation has been applied in a number of commercial processes such as Neste Oil’s NExBTL™ and UOP/Eni’sEcofining™ [50].
Scheme 2. Biorefinery of glycerol to some valuable industrial commodity chemicals (adapted from [52]. Copyright 2018, Royal Society of Chemistry)

Glycerol is another valuable product that could be derived from diatom’s lipids. It is obtained as a co-product of the transesterification reaction. This polyhydroxy compound is one of the top building block chemicals in biorefinery [53, 54] because of its versatility to be converted into multiple industrial commodity products (Scheme 2). Its market demand is predicted to expand with an annual average rate of 7% during 2007-2021; with 6 million tonnes in 2025 [55]. For instance, glycerol could be catalytically oxidized into dihydroxyacetone by Fe-containing of MFI zeolite with 88% selectivity at 350°C for 1.5 h [56], which could be further converted into lactic acid [14, 52]. Glycerol is also able to be dehydrated into acrolein; i.e. a precursor of acrylic acid; with an acid catalyst at hot compressed water [57].

3.3. Silica
Diatoms have a unique capability to utilize the soluble silicic acid in sea waters and metabolize it to build their silicate cell-wall called frustules. The biosynthesis process of these silicified materials is complex and involves multiple silicon transport mechanisms. Herein, the dissolved silicic acid is uptake into the cell and intracellularly transported by silicic acid transporters [16]. The formed internal soluble silicon pool is then transported into silica deposition vesicle, which subsequently condensates the soluble silicic acid into the insoluble amorphous silica of the cell-walls catalyzed by specific proteins (silaffins) and polyanionic peptides (silacidins) [58, 59]. The frustules are the major component of diatoms besides lipids. Their content could reach up to 50-60% d.w. of diatom’s biomass [16, 60]. This material has the typical X-ray diffraction of the amorphous silica; i.e. a broad signal at 2θ of 15-35° with the maximum intensity at 2θ of 22-23°. Quantitative elemental analysis and FTIR spectroscopy measurement confirmed that these materials are made from silica in the form of $[\text{Si}_n\text{O}_{2n+2}\text{H}_{n/2}]$ (c.a. 89-98%) with several surface defects such as silanol (Si-OH) and Si-H groups [61, 62]. Nevertheless, the frustules of diatoms also contain some organic residual resulted from cell-wall proteins (c.a. 2%) such as silaffins, silacidins and pleuralins [63, 64]. This organic residual could be removed by calcination at ≥550 °C, although, the condition should be controlled to hinder their structure deformation.
Figure 6. Electronic microscope images of the frustules diatom of Coscinodiscus sp. (A), Pseudo-nitzschia pungens (B) and the high magnification of TEM image of the nanopores of Pseudo-nitzschia pungens (C)

Interestingly, our observations found that diatoms create these silicified materials with porous structures (Fig. 6). In particular, transmission electron microscopic measurement clearly shows the precise hierarchical porous structures in the frustules of diatoms. The shapes and the physical characteristics of the frustules are varied depending on the species. For example, Coscinodiscus sp. is a dish-like diatom with diameter of 60-200 µm with the inner pore size of 900-950 nm [65, 66]. Pseudostaurosira trainorii has a similar shape with diameter and pore size of 4-5 µm and 150-200 nm, respectively [61]. Talassiosira sp. and Skeletenome sp. are two centric diatoms with 10 µm in size and 64-156 m² g⁻¹ of surface area [67, 68].

The hard silica cell-walls are benefiting to provide mechanical protection of their cells from zooplankton as their predators, high-pressure conditions and collision by solid particles in seawater. The silica cell-wall also protects the membrane cells from the penetration of viruses and bacteria. Meanwhile, their porous architectures have multiple advantages for diatoms such as; providing the fluid dynamic structure for easy movement in water environment, enhancing the optical scattering for optimized light as well as energy harvesting, and optimizing molecular sieving for nutrient uptake [21, 69].

Owing to these characteristics, the hierarchical porous structure of the diatom’s frustules has potential applications for the nano-biotechnology industries. The silanol group on the frustule’s surfaces could be functionalized with some reactive groups to become coupling points for immobilization of biomolecules, which have applications for the drug delivery systems and biosensor. The unique optical property of the frustules has been used to detect NO₂ pollution via photoluminescence [69] and photoelectric devices [21]. Commonly, the frustules of diatoms are used as a support to make nano metal composites for energy storage (supercapacitors, H₂ storage) [21, 70], catalysts [68] and adsorbents [59, 71].

To date, silica-based porous materials are obtained by chemo-synthetic processes which are usually time-as well as energy-consuming. Moreover, the process involves toxic precursors and generates a large number of wastes. In consideration with that, diatoms are a promising resource for the sustainable production of high-purity of silica. Particularly, it could be a potential alternative natural source for the manufacture of hierarchical porous silica materials. The use of diatoms offers minimum impact on environment as well as health issues.

4. Biorefinery approaches
It is clearly seen that diatoms contain multiple chemicals components that have many potential applications in industries (Table 3). Besides lipids, pigments and silica are the diatom’s components that should be maximally used because both of them and their derivative products have high economic value as well as market demand. As such, maximizing the use of those components and the
manufacture of their derivative products (Scheme 3) should be considered rather than the utilization of single component of them solely: e.g. lipids for biofuel production. It is because this could offer potential revenue and is expected to improve the economic viability of the process [54]. In fact, fuels derived from petroleum are one of products that are resulted in the petroleum refinery industries, besides chemicals and materials.

Table 3. Some species of diatoms and their potential markets in biotechnological industries

| Diatom species               | Potential chemicals                              | Potential markets and applications                                                                 | References |
|------------------------------|--------------------------------------------------|-----------------------------------------------------------------------------------------------------|------------|
| Chaetoceros gracilis         | High lipid content (30-70%)                       | Aquaculture larval feed; lipid-based fuel production                                               | [72]       |
| Chaetoceros calcitans        | Fucoxanthin (5.2 mg g\(^{-1}\))                  | Fucoxanthin production                                                                           | [47]       |
| Thalassiosira sp.            | Lipids (24-39%)                                   | Aquaculture larva feed                                                                           | [18, 20, 73] |
| Phaeodactylum tricornutum    | EPA (2.2-3.9 %-d.w.)                              | Functional foods (for coronary heart disease treatment, hyper-triglyceridemia, blood cholesterol level reduction, preventing the risk of arteriosclerosis and inflammation); lipid producers | [20, 74]   |
| Nitzschia sp.                | EPA (1.9-4.7 %-%-d.w.); arachidonic acid (0.6-4.7 %-%-d.w.) | Food supplment                                                                                   | [20]       |
| Coscinodiscus sp.            | Nanoporous silica                                | Biosensors                                                                                        | [20]       |
| Skeletonema costatus         | Bioactive compound                                | Antiphagogenic secondary metabolite                                                                | [75]       |
| Cyclotella sp.               | Lipids (42%)                                      | Lipid-based biofuels                                                                              | [18, 73]   |

As previously discussed, pigments have high economic value among other chemical components in diatoms. In consideration of their stability, hence, the extraction of pigments should be strictly considered at first to minimize the loss of these valuable components. Pigments extraction could be done by using organic solvents such as acetone, alcohols (methanol, ethanol) and N,N-dimethylformamide (DMF) [37]. The efficiency of the extraction is varied depending on the characteristics of the pigments targeted, the duration time, the cell concentration and the pre-treatment of the biomass. In case of diatoms, most attentions are commonly focused to extract carotenoids, especially, fucoxanthin.
Scheme 3. Biorefinery concept of diatoms for producing lipids, pigment, and silica. T and t are the abbreviations of temperature and time, respectively.

Methanol and acetone are two organic solvents that are widely applied for extracting photosynthetic pigments from microalgae. Although methanol could give higher efficiency than acetone, the use of methanol could promote the degradation of chlorophylls [37]. Meanwhile, acetone was found to inhibit the activity of chlorophyllase; i.e. an enzyme that is responsible for chlorophylls degradation [37, 76]. In acetone, Chl a is reported to be stable at ≤ 1 h extraction while fucoxanthin has good stability even extracted for 2 h [76]. Because of the similar polarity index of acetone and methanol as well, hence, the former solvent is more preferably used for pigment extraction, especially in the extraction of fucoxanthin [77]. Furthermore, acetone has lower boiling point than methanol, which gives advantages for its separation process. In addition, this solvent extraction is reported to have no effect on the silica’s cell integrity of marine diatom Cylindrotheca closterium [76]. It is important in order to obtain the unique silica of the frustules of diatoms.

Acetone is not effective to extract lipids from microalgae [78, 79]. Moreover, the frustules of diatoms are not soluble in acetone. As such, the acetone extract containing pigments could be easily separated from the remaining biomass. Meanwhile, the lipids could be extracted by non-polar organic solvents such as n-hexane or a mixture of chloroform-methanol. The obtained lipids could be catalytically transformed to generate valuable chemicals such as FAAs with co-formation of glycerol, hydrocarbons or fatty acids via transesterification, hydrogenolysis, and hydrolysis, respectively.

After extracting the organic components, the remaining materials of diatoms could be utilized for silica-based materials development. The hierarchical porous structure of the frustules of diatoms offers potential applications for nanobiotechnological industries such as catalyst, adsorbents, biosensors, etc.
5. Conclusions and outlooks

Diatoms are a class of marine microalgae that are promising for the sustainable production of industrial commodity products. Almost all of their biomass components could be utilized for profitable use. Lipids of triglycerides are the main interest of diatoms, which could generate fatty acid alkyl esters and glycerol for fuels and bulk chemicals, respectively. Diatom’s lipids are also potentially used to be converted into biohydrocarbon (high-quality fuels) via catalytic hydrodeoxygenation. As a photosynthetic microorganism, diatoms contain pigments that have high economic value as well as market demand in the food, cosmetic and nutraceuticals industries. Particularly, diatoms are a potential source of carotenoids because of the high productivity of these pigments in diatoms compared with other resources, even with green microalgae as well as macroalgae. Meanwhile, the frustules of diatoms, which are made of silica with hierarchical nanopore size, exhibit potential applications in the field of nanobiotechnological silica-based industries.

The main bottleneck on the development of diatoms-based biofuels is related to its low ROI due to the biochemical component in diatoms is expected to increase the revenue of the process, leading the biofuels production to be more competitive. It is considered because of the potential economic value of those biochemical-based products derived from diatoms, especially in the foods, nutraceuticals, pharmaceuticals and nano-biomaterials industries besides biofuels.

Author Contributions
A Bayu led the writing and organizing the content of the manuscript. A Rachman contributed to the biology of diatom’s section. D R Noerdjito, M Y Putra and W B Widayatno supported the editing of the contents.

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