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PRODUCTS AND PROCESSES OF MICROALGAE – A REVIEW

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Abstract
The utilization of biologically derived materials, generally referred as bio products, is becoming increasingly popular for a number of environmental reasons and associated health benefits. Microalgae are favored as a feedstock for the production of many bio products because they are relatively easy to cultivate, they do not require arable land and fresh water to grow, and they have faster growth rate than most other energy and energy-related crops. Microalgae have a high potential as a feedstock for bio-products, but the type, quantity and quality of the products obtainable depends on the microalgae species and the conversion process (es) employed. Different bio products (biofuels, phytochemicals, and nutritional food/feed) can be produced from microalgae via different technical routes depending on the desired end product(s).
This paper examines the bio products that can be produced from microalgae different microalgae species and their corresponding production routes. All aspects microalgae processes are reviewed – from upstream process of cultivation (and harvesting) to downstream (pretreatment/feedstock preparation, and) conversion into desired product(s) and co/by-products. The processes of biofuels production including thermochemical, chemical and biochemical routes are reviewed. The processes for the extraction of intracellular components like microoalgal oil and phytochemicals are discussed.
The review has highlighted some significant opportunities for the integration of different processes to produce multiple bio products from a single feedstock of microalgae. This will eventually have an impact on the economic feasibility of employing microalgae as a viable feedstock for the now popular bio refinery concept.

Introduction
The continuous increase in global population is causing rising global demand of essential resources like energy, nutritional foods, and other related commodities like phytochemicals. This hiking demand of resources is evident both in the developed, and the developing economies. A report published by the United Nations (UN) predicts that world population would increase to 8.3 billion by 2030 meaning additional 1.3 billion people more will need these resources.

From the environmental perspective, studies have been indicating that the recent climate change is attributed to the continued rise in atmospheric carbon dioxide concentration resulting from large scale combustion of fossil fuel. Therefore, substituting some percentage of the fossil fuels with biofuels will help in combating this environmental concern. Additionally, through the last quarter century, the proportion of the amount of known oil reserves to annual production (the R/P Ratio) has hovered between 40 and 50 [1], which indicates fast depletion of the oil reserves.

This increasing global energy consumption with static supply, coupled with geopolitical and socio-economic crisis has caused supply interruptions and led to an unprecedented rise in the price of fossil fuels. The global spot crude oil price was less than US$24/bbl in august 2001 to almost US$90/bbl today [18] – about four-fold increase in less than one and a half decade.
Adopting biological sources to replace the fossil sources for energy production will reduce the consumption of fossil fuels, and hence minimize the environmental concerns resulting from the use of fossil fuels. Doing so will also alleviate some socio-economic and geopolitical risks associated with the supply chain of the fossil fuel.

Additionally, some microalgae species contain valuable chemicals (i.e. phytochemicals) that are said to help in maintaining good health in humans. Exploiting these chemicals alongside the biofuels production could serve as a good way of improving the microalgae economy.

The objective of this paper is to show the biofuels and the different phytochemicals that can be produced together either as co-product(s) or a product and a by-product from a single feedstock of microalgae. As a way of introduction, the paper stars by shedding some lights on biofuels generally and biofuels from microalgae specifically; about phytochemicals from microalgae; and what makes microalgae a viable feedstock for the production of biofuels and other bio-products.

The second section of the paper reviews microalgae – their biology, biochemical compositions, cultivation and harvesting. The third section discusses biofuels production from microalgae. This is followed by the production of phytochemicals from microalgae.

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Then, followed by the integration of phytochemicals and biofuels productions to improve the economics of both products. Finally the concluding remarks summarized the key issues highlighted by the paper.

Biofuels and Bio-chemicals

Biofuels are biomass-derived fuels which can be in the form of gases, liquids or solids, while bio-chemicals are the corresponding chemicals produced from the similar biomass sources using the same or very similar technical routes. Bio-based fuels and chemicals contain carbon that is absorbed from atmospheric carbon dioxide during photosynthesis, when used they return that carbon as carbon dioxide to the atmosphere, making them either very near- or completely-carbon-neutral. There are considerable differences among biofuels, some might be apposite for use in the transport sector because they are liquid at a standard conditions while some might not.

Table 1: Classification of biofuels

Biofuel(s) Classification	Examples of the biofuels and their sources
Conventional biofuels	
First generation	Biodiesel from vegetable oils
	Bioethanol from sugarcane and corn
	Biodiesel from non-edible oils like jatropha oil
Second generation	Biodiesel and bioethanol from woody biomass
Advance or third generation biofuels	Biodiesel and other biofuels from microalgae

Biofuels are commonly classified into first, second and third generation biofuels, or in some literatures 'conventional' and 'advance biofuels', depending on the type of feedstock used or maturity of the technology employed for their production. Some examples of these are presented in Table 1.

The first generation biofuels have the downside of causing a competition between food and biofuel applications. Subsequently, second generation biofuels which are based on non-edible sources (like Jatropha and other non-edible oil crops, grasses, and woody energy crops) were explored. However, the second generation biofuels also have some drawbacks, because they require arable lands and freshwater for their cultivation, thus also leading to some sort of resource competition with edible agro-materials. Hence, advanced biofuels like microalgae based biofuels are being considered as the most feasible option to tackle both the problems associated with fossil fuels and those impeding the large scale productions of the conventional biofuels [3]

Phytochemicals from microalgae

Phytochemicals (plant chemicals) are plant compounds especially those that may affect human health by their preventive or protective properties. They are non-nutritive and nonessential to human beings, but are demonstrated to protect against diseases

There are many varieties of phytochemicals in plants generally, and in different microalgae species. The most commonly known phytochemicals obtained from microalgae include beta carotene, lutein, and other carotenoids, vitamin E, vitamin B, etc etc (Table 2).

The phytochemicals are extracted from microalgae mostly using different solvent extraction methods. The most commonly used methods of phytochemicals extraction from microalgae are discussed hereunder.

Microalgae as a source of bio-products

One of the yardsticks of a sustainable biofuel is being competitive to fossil based fuels in terms of cost and performance efficiency. Microalgae have been promoted as one of the more promising third generation biofuels [22] due to their many advantages over other potential sources of biofuels. These include:

- Microalgae are easy to cultivate, can grow with little or even no attention, using water unsuitable for human consumption and easy to obtain nutrients [4].
- They can utilize salt water (from seas and oceans) and wastewater streams, thereby reducing freshwater demand and serving as pollution control agent
- Microalgae grow very rapidly, commonly doubling their body mass within 24 hours [10], the doubling time are usually as short as 3.5 hours during the exponential growth [5]
- They have higher biomass and oil productivities per unit ground area than all other oleaginous (oil) crops [11].

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Table 2: Phytochemicals and other bio-chemicals from different microalgae species

Phytochemical	Microalgae strain	Application	Ref
Arachidonic acid	*Phorphyridium cruentum*	Infant formula, nutritional supplement	
Astaxanthin	*Haematococcus pluvialis Chlorella vulgaris,*	Antioxidant, anti-inflammatory, anti-cancerous, immune system enhancer, anti-depressant, treating carpal tunnel syndrome, food supplement and colorant, animal feed additive, cosmeceutical applications in protection against skin aging,	20, 21
Beta-carotene	*Chlorella vulgaris, Dunaliella salina, Spirulina platensis*	Antioxidant, Anti-inflammatory, anti-depressant, food supplement, feed surrogates	
Carbohydrate extract	Chlorella	Immune system booster, anti-flu	20
EPA (Eicosa Pentaenoic acid)	*Chlorella vulgaris, Haematococcus pluvialis,*	Anti-inflammatory, anti-depressant, nutritional supplement, aquaculture	22
Chlorophyll	*Chlorella vulgaris,*	Antioxidant, anti-cancerous, constipation reliever, food colorant	
Glycerol	*Dunaliella salina*	Food additive, humectant, lubricant and laxative	21
Lutein	*Chlorella vulgaris*	Nutritional supplement especially for patients with degenerative human diseases, like AMD (age-related macular degeneration) or cataract, and also for skin health	23
Phycoerythrin	*Haematococcus pluvialis*	natural colorants in food, cosmetics and pharmaceuticals	21
Phycocyanin	*Spirulina platensis*	natural colorants in food, cosmetics and pharmaceuticals	21
Crude Polysaccharides	*Chlorella vulgaris* and *Phorphyridium cruentum*	Antioxidant, Anti-inflammatory, antiviral	
Sulphated polysaccharide	*Phorphyridium cruentum*	Antioxidant, Anti-inflammatory, antiviral	20, 21,
GLA (Gamma Linolenic Acid)	*Spirulina platensis*	Infant formula, nutritional supplement Health foods, cosmetics	
Vitamin B12	*Spirulina*	Helps immune system	

- Their biomass productivity has been estimated to be more than 50% that of one of the fastest growing terrestrials plants (i.e. switch grass) [7, 5]
- They have a high lipid content of up to 70%, but most commonly between 20 - 50% of their body weight (dry cell basis). Higher productivities of 80% [8] to 90% have been reported in some species under certain conditions, although contents reaching 75% and above are associated with low productivities [3].
- Microalgae can be harvested batch-wise nearly all year round, providing a reliable and continuous supply; and have a very short harvesting cycle (from planting to harvesting) of 1 – 10 days [12]
- Microalgae have great photosynthetic efficiencies leading to reduced fertilizer and nutrients inputs, thus results in lesser waste generation [12].

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- Microalgae can couple CO_2 neutral fuel production with CO_2 sequestration because they can take up to ten times the CO_2 consumed by other plants for equivalent growth, and their cultivation is not directly linked to human consumption [4]
- Depending on the microalgae species other compounds with valuable applications (like omega 3 fatty acid) may also be extracted to improve the economics of the biofuel(s) production

Microalgae – biology and physico-chemical properties

Microalgae are multicellular and unicellular photosynthetic organisms, which comprised of a diverse mix of organisms with different characteristic [2, 3]. They are plant-like organisms but do not have roots or stem, and are predicted to be amongst one of the oldest form of life on earth [3]. Microalgae are mainly classified into two categories prokaryotic (cyanobacteria) and eukaryotic (Figure 1). Each of these categories is estimated to have thousands of species totaling to more than 50,000 species extant [4].

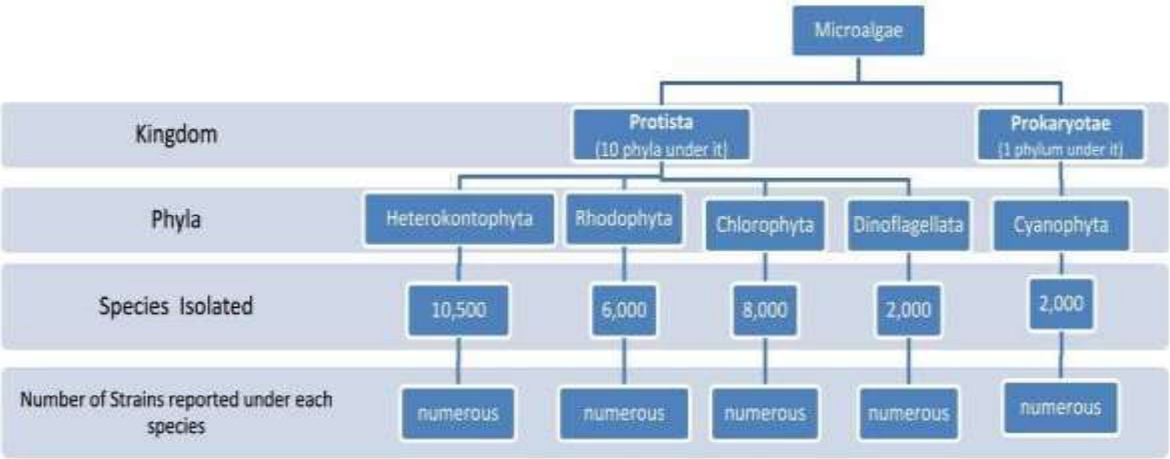

Figure 1: Microalgae classification

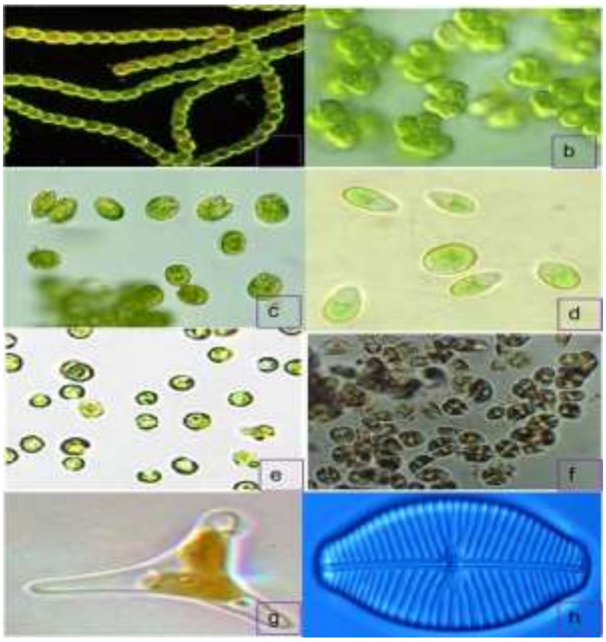

Figure 2: Different microalgae species
a) Cyanobacteria (blue green algae) b) Cholorococcum sp. c) Tetraselmis sp.
d) Dunaliella sp. e) Nannochloropsis sp.; f) Amphora sp. g) Phaeodactylum tricornutum h) Navicula sp.

Each species consists of numerous strains, and each strain has specific physico-chemical properties like size and shape (Figure 2), growth requirement and biochemical compositions.

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The two defining characteristics for algae as a biofuel are its growth rate (generally given in grams/m2/day) and lipid content (generally given as a weight percent of total biomass) [23] both quantities vary greatly within the literature as they depend on variables including algae type and weather conditions, among many others [23].

Biochemical compositions of microalgae

The main biochemical components in microalgae are lipids (or oil), carbohydrates, and proteins. Each of these three is present in varying proportions depending on the microalgae species, and the conditions under which it is grown. Other component are present depending on the species of microalgae (section 1.2).

Lipids are hydrocarbons essentially insoluble in water, used for the storage of energy. Microalgae contain lipids in the form of oil, both as membrane component and storage products.

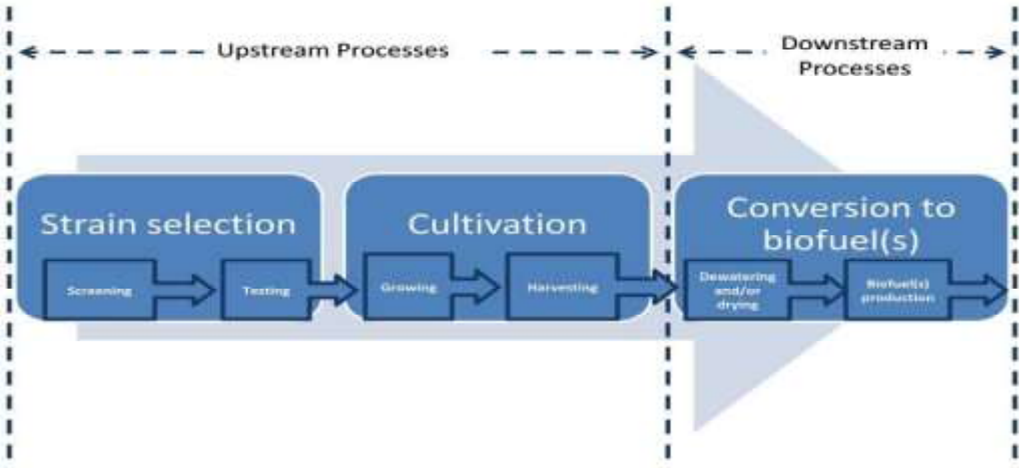

Figure 4: Whole chain of microalgae to bio-products production processes

The lipids help control the flow of materials into and out of the cell wall, and they are responsible for the fluid nature of some microalgal cells which allow materials to be transferred through the cells. The mechanism of lipids production is not fully understood but is said to vary with environmental condition. For example, different culture conditions (temperature, pH, light intensity, etc.), genetic factors, and varying the nutrients (like sulphur and nitrogen) affects the microalgal lipids production [13, 14].

Carbohydrates are organic compounds which perform numerous roles in living organisms. In microalgae, carbohydrates serve as energy storage and structural components, either in the form of complex carbohydrate like starch or as simple carbohydrates like sugars.

Proteins are the other essential part of the microalgae and their composition varies depending on the microalgae strain.

Microalgae strain selection

The whole chain of biofuels and other bio-products production from microalgae starts with the selection of the microalgal strain, through its cultivation and final conversion to biofuel(s) and/or phytochemical(s) (Figure 4). Each of these three steps has specific technical requirements and associated challenges.

The pioneer research on microalgae strain selection and cultivation is that of the Aquatic Species Programme (ASP) at the National Renewable Energy Laboratory (NREL), Golden, Colorado, under the U.S Department of Energy (DOE) from 1978 and until it ended in 1996. The ASP objectives was to initiate the use of plants for the production of transport fuels, with a specific focus on algae. The work initially started on using algae to produce hydrogen, and later in the 1980s the emphasis was switched to biodiesel production and selecting specific naturally occurring strains that are most suited for biodiesel production. The selected strains were grown using advance biotechnological techniques to improve and optimizing their properties, and demonstrate the processes required for cultivating mass quantities of algal for biodiesel production [9].

Large Scale Production of Microalgae

Microalgae cultivation

Different microalgae species and/or strains require different conditions to grow. Some microalgae can grow autographically, meaning they perform photosynthesis by naturally absorbing sunlight, CO2 and inorganic salts (especially nitrates, phosphates and

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potassium salts) to grow. Autotrophic cultivation can be either be outdoors in open ponds, or in closed systems called photo bioreactors (PBRs). Other species/strains can grow heterotrophically, without sunlight, relying on external substrates (like simple sugars) as their sources of energy, mostly in stirred tanks or fermenters. Some species of microalgae are capable of growing both autotrophically and hetetrophically, a system known as mixotrophic.

The open systems are in the form of open tanks or ponds (or race ways) where either one or different strains of microalgae can grow together without strict control of the environmental conditions and growth environment. The open ponds can be mechanically mixed or unmixed; they can be circular or rectangular; covered or uncovered; and the bottom can be lined or unlined.

PBRs are installations used for cultivation of microorganism which require isolation from their natural environment to ensure better control and avoid contamination from unwanted microorganisms, as well as improve mass transfer and temperature conditions. PBRs are associated with higher growth rates and biomass concentration, which makes harvesting a little easier. The key disadvantages of PBRs systems include very high capital and operating costs compared to the open systems, and being more energy intensive.

The current cost of PBRs makes them too expensive for the cultivation of microalgae for biofuels production alone, making them only fit for the production of microalgae for high value commodities (like phytochemicals). Only unlined (dirt bottom) open ponds are said to be economically feasible for biofuels production and the need for enriched CO2 restricts the sighting of these ponds near CO2 sources like power plants, ammonia plants, CO2 reservoirs, etc.
Commercial production of microalgae is commonly done in an outdoor cultivation systems like open, race way type or circular ponds, mixed by paddlewheel [16, 17]. But research emphasise is given to the improvement of the technical design of enclose PBRs for a more efficient microalgae cultivation system.

There are also hybrid systems utilizing the advantages of each of the two systems. A hybrid two-stage cultivation method combines distinct growth stages of PBRs and open ponds. The first stage was in a PBR where controllable conditions can be utilized, and then transferred to open ponds for the second stage of the cultivation.

The concept of using microalgae evolves during the twentieth century when it is used as a source of human nutrition. The initial small-scale industrial cultivation started in Japan, and United States, and then followed by other countries. The first microalgae cultivated at an industrial scale is Chlorella (green microalgae).

Presently the most commonly commercially cultivated microalgae are Dunaliella, Spirulina, and Chlorella – all three being cultivated for human food, animal feed and for the production of valuable chemicals. Another commercially cultivated microalgae is Haematococcus, generally produced for the production of carotenoids (specifically astaxanthin) [15]. Other products being produced from microalgae include ω-polyunsaturated fatty acids and β-carotene. These products are produced and sold as enhanced value for human food, animal feed, and for applications in the pharmaceutical and cosmetics industries.

Microalgae harvesting, dewatering, and thickening
Harvesting (i.e. the separation of microalgae biomass from water, the growth medium) is a key technical challenge in commercial cultivation of microalgae, and consequently in the microalgae biofuels and other biochemical production.

Harvesting process, which accounts for 20 – 30% of the total microalgae production cost, is a challenge because of the nature of microalgae cultivation. Additionally, microalgae are microscopic in size (e.g. *Chlorella sp.* < 5 µm in diameter, [16] and *Spirulina* sp. is 20–100 µm long (Brennan and Owende, 2010); have low concentration (a few hundred mg/L) in the growth medium; and low standing biomass ranging from 50 g/m^2 to 5000 g/m^2 depending upon the cultivation method and/or time of cultivation [16].

There are many harvesting methods employed for microalgae processes, generally based on the conventional solid-liquid separation techniques used in wastewater treatment. Most of these conventional processes are used microalgae harvesting with little or no modification, and with varying – but generally not significant – degrees of success. Harvesting is generally achieved via two stages:

1. Bulk (or primary) harvesting — separating the biomass from the bulk suspension to increase the microalgal biomass concentration from <3 g/L to 10-20 g/L.
2. Thickening (or secondary harvesting) — concentrating the slurry after primary harvesting to higher concentration of 100-200 g/L. The secondary harvesting is generally a more energy intensive step than bulk harvesting.

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Choice of harvesting technique under each stage is dependent on characteristics of microalgae, like size, shape, density, as well as the value of the target products.

Downstream processes for the production of biofuels and bio-chemicals from microalgae

There are several methods of converting microalgae feedstock to liquid and gaseous fuels, these processes are similar to those of converting other biomass to fuels, namely chemical, thermochemical, and biochemical conversion methods (Table 2) [16].
The choice of biofuel to be produce and the method to be used for the biofuel production depends on the microalgal strain selected and its biochemical composition. For example, high lipid content is particularly important for biodiesel production via Tran's esterification method, while carbohydrate content determines the yield when bioethanol is the target product. Furthermore, not all the lipids fractions contained in microalgae are good enough for conversion to biodiesel, thus it is important to not only note the lipid content but also their composition when selection a microalgae strain for biodiesel production [10] via Trans esterification. The protein content is not an issue for most microalgal derived biofuels. But for some, especially those produced by thermochemical processes (like liquefaction), the protein decomposes and yields nitrogenous compounds which are problematic in further processing and results in NOx formation when the biofuel undergoes combustion.

High protein microalgae are mostly grown for the production of nutritional supplements for human consumption.

Table 2: Technical routes for the production of biofuels and phytochemicals from microalgae

Processing Route		**bioproduct**
Thermochemical Conversion	Gasification	Syngas
	Pyrolysis	Bio-oil, Bio-char, bio-gas
	Liquefaction	Bio-oil
	Combustion	Electricity
Biochemical Conversion	Anaerobic Digestion	Biogas
	Fermentation	Ethanol
	Photobiological hydrogen production	Hydrogen
Chemical conversion	Oil extraction and transesterification	Biodiesel
	Oil extraction and hydrogenation	Light and Middle distillates (Renewable petrol, kerosene or diesel)
Phytochemicals extraction	Solvent, supercritical or pressurized liquid extractions	PUFAs, carotenoids, and proteins

Ultimately, it is the biochemical content of a microalgae that determines the best biofuels that can be produces from the microalgae, and the potential biochemical that can be produced. Cell rapture to obtain the intracellular components like lipids and then extract them to chemically or biochemically convert them to biofuels. The biomass residue can then be converted to biogas using anaerobic digester. The same process stages from cultivation to extraction apply when producing high value products like carotenoids

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Concluding remarks

Although production cost (associated with microalgae cultivation and conversion to biofuels) is still an issue with microalgae biofuels, the trend of research holds optimism that in the near future the economics will be highly improved [20], and microalgae will serve as the best alternative source to fossil based fuels.

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