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A Review on Production of Biodiesel from Microalgae: A Biofuel for the Future that Degrades Naturally

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ABSTRACT:

Microalgae have emerged as a promising renewable feedstock for biodiesel as the quest for alternatives to fossil fuels continues. The photosynthetic single-celled microorganism is called a Microalgae. By utilising CO_2, sunlight, sugar, N_2, P, and K as nutrients, self-rejuvenation and rapid growth enable the production of lipids, proteins, and carbohydrates in enormous quantities over a short period of time. Recent technological developments have increased the efficiency of the growing, harvesting, pre-treatment, lipid extraction, and transesterification processes, bringing microalgae biodiesel closer to being commercially viable. Although microalgae are the best alternative, production costs and biofuel yield remain difficult to achieve. The algal biodiesel/green diesel has the potential to develop into a substitute supply of biodiesel to meet the rising global energy demand, it can be said. The benefits of algae-based biofuels for economic development are numerous. Many socioeconomic benefits that contribute to an outcome that can be maintained by the general public can be obtained by developing an algae cultivation-based biofuel sector.

KEYWORDS: Microalgae, transesterification, lipid production, algal biodiesel.

1. INTRODUCTION:

Non-renewable fossil fuel reservoirs will be depleted < 50 years due to their higher demand and over exploitation. The urgent need for alternative fuel sources has been justified by the rapidly growing global population, ongoing energy crises, the quick depletion of non-renewable energy sources, the explosive growth in vehicle use, the pollution risks from fuel emissions, and the related health diseases [1]. Over the ensuing two decades, it is anticipated that the global energy consumption would continue to increase [2]. Coal, petroleum-based products, and natural gas are the main sources of fossil fuels that today meet the majority of the world's energy needs. When it became clear that there is a very finite supply of fossil fuels and that burning them causes a number of additional environmental issues, including global warming, the demand for these alternative fuels increased [3]. Many greenhouse gas emissions are produced when conventional fuels like petroleum are used. Using biofuels is the best approach to cut emissions [4]. India consumes almost five times more diesel fuel than gasoline, whereas, almost all other countries in the world use more gasoline than diesel fuel. India needs 200 billion gallons of biodiesel annually at the current rate of consumption without use of petroleum fuel. The Bioenergy Road Map Vision 2020" to develop new technologies and policies for biofuel production in India. Any fuel that is produced using plant, algae, or animal waste biomass is referred to as a biofuel. Biofuels are regarded as the most environmentally friendly natural energy source [5]. Due to the fact that many microalgal strains have the capacity to accumulate lipids and that they grow their biomass at a faster rate and produce more photosynthetic energy than their terrestrial counterparts, microalgae have also become recognised as a potential feedstock for the production of biofuels [6]. Producing this much biodiesel, require microalgae to be grown over an area of 13 million acres (5.4 million ha) i.e. only 2% of the India geographical area. Marine Microalgae are good option for biodiesel production in India because we have coastline of 7,517 km. Marine microalgae have greater lipid productivity, easy to mass production and produce lipids with high purity [7]**.** Biodiesel is a sustainable fuel that can replace petroleum-based diesel and lowers CO_2 emissions [8]. The design of the various subsystems, such as algae growing, harvesting, and lipid extraction, determines the initial investment cost of the current algal biofuel production system. The majority of biofuel companies have decided to construct large algae growth facilities near the coast in order to use seawater as the primary water source and lower the cost of water usage [9].

2. BIODIESEL:

Alternative diesel fuel known as biodiesel is produced from sustainable biological sources like vegetable oils and animal fats. It has low emission profiles, is harmless and biodegradable, and is therefore good for the environment. Because it is derived from renewable resources and has positive environmental effects, biodiesel has recently gained in popularity. According to their sources, biofuels are typically divided into three generations. First generation biofuels, such as those produced from biomass made up of sugar, starch, and vegetable fats and oils, are those that are acquired straight from the food source [10]. The second generation of biofuels are those that are created from plant biomass, which is primarily made up of lignocellulosic materials, as this creates the majority of the abundant and cost-effective non-food chemicals that are available from plants [11,12]. Previous studies have outlined the major capabilities of green algae inferred biomass for the production of a better kind of third-age biofuels, which have been sought after as practicable and practical alternatives to non-renewable energy sources [13]. Microalgae can develop more quickly than terrestrial crops because they do not need to manufacture structural materials like cellulose for leaves, stalks, or roots and because they can be produced floating in a nutrient-rich media. Also, compared to conventional crops, they may convert a substantially larger percentage of their biomass to oil, for example, 60% versus 2-3% for soybeans [14]. The biggest barrier to the product's commercialisation, however, is the price of biodiesel. The key choices to be taken into consideration to reduce the price of biodiesel include the use of spent cooking oils as raw materials, adaptation of the continuous transesterification process, and recovery of high-grade glycerol from the by-product (glycerol) of biodiesel [15]. Direct usage and blending, microemulsions, thermal cracking (pyrolysis), and transesterification are the four main processes used to create biodiesel. Vegetable oils and animal fats are trans-esterified, which is the technique that is most frequently utilised [16]. The molar ratio of glycerides to alcohol, catalysts, reaction temperature, reaction duration, free fatty acids, and water all affect the transesterification reaction. Biodiesel will continue to be crucial for enhancing the sustainability of our transportation system in the future decades. A liquid fuel with a high energy content is biodiesel. This makes it perfect for applications requiring a lot of power, like those in ships, aircraft, and busy roads.

3. ALGAE:

The unique characteristics of algae include their ability to adapt to saline water, their ability to absorb CO_2 during growth, and the fact that they require less space for growth than other food crops. They also have a high lipid content. There is a wide variety of species available worldwide, and many of them contain an oil content of about 80% of their dry weight [17]. Algae are mostly categorised based on their physical characteristics. The chief among these are pigment constitution of the cell, chemical nature of stored food materials, kind, number, point of insertion and relative length of the flagella on the motile cell, chemical composition of cell wall and presence or absence of a definitely organised nucleus in the cell or any other significant details of cell structure [18]. Macroalgae and microalgae are the two basic classifications of algae. Microalgae are minute and unicellular, whereas macroalgae are the macroscopic, multicellular variety of algae. Macroalgae typically include 80–90% water in addition to 50% carbohydrates, 1% lipids, and 7–38% minerals in their dry weight. Microalgae are seen as a potential alternative source for the generation of biodiesel because of their high lipid content [19]. Microalgae are tiny, single-celled organisms that predominantly use photosynthetic processes. Despite being single-celled, some of them have the ability to group together to create colonies, such as filaments or spheres of the same species. The pigment used in photosynthetic processes enables them to photosynthesize [20]. The four main forms of metabolism found in microalgae are photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic [21]. In photoautotrophic microalgal metabolisms, light (as a source of energy) catalyses the photosynthesis reaction that turns inorganic carbon (CO2) and water into biomass [22]. Organic carbon is required by heterotrophic microalgae as a source of energy and carbon. In terms of producing biodiesel, they are more promising than photoautotrophic species. Their manufacture is carried out in closed bioreactors. Even while photoautotrophic microalgae have large levels of lipids, they often have lower biomass production than heterotrophic microalgae in photobioreactors and open ponds. Because some microalgae species can grow in both light and darkness and use both inorganic and organic carbon sources, they also exhibit a mixotrophic metabolism [23]. Microalgae with photoheterotrophic metabolism require light for both a source of energy and an organic carbon supply. The carbon molecules found in microalgae can be used to make biofuels, medicines, cosmetics, and dietary supplements [24]. They can also be used to clean wastewater and reduce atmospheric CO2. Researchers from several nations have compiled substantial collections of microalgae over the past few decades. The wide range of diverse microalgae that can be chosen for usage in a wide range of applications, including value-added products for pharmaceutical uses, food crops for human consumption, and as an energy source. Many bioproducts, such as polysaccharides, lipids, pigments, proteins, vitamins, bioactive substances, and antioxidants are produced by microalgae [25]. A new direction in biorefinery has been spurred by interest in microalgae as a sustainable and renewable feedstock for the manufacture of biofuels [26].

Table 1. Lipid content of different types of microalgae

MICROALGAL SPECIES	LIPID CONTENT (%)
Chlorella vulgaris	20 - 32%
Chlorella sorokiniana	51%
Chlorella protothecoides	48.7%
Scenedesmus sp.	16 - 40%
Dunaliella salina	45-55%
Dunaliella tertiolecta	22 - 42%
Botryococcus braunii.	24%
Nannochloropsis oculata	37- 60%
Chamydomonas reinhardtii	65.85%
Spirulina platensis	4 - 16%
Spirogyra sp.	0.69 - 4.24%
Chlorococum humicola	13%
Thalassiosira pseudonana	38.1%
Tetraselmis saecica	5 - 58%
Isochrysis *sp.*	8.5 – 23%

Arthrospira platensis	30.23%
Porphyridium cruentum	18.4%
Phaeodactylum tricornutum	24.39%
Haematococcus pluvialis	34.85%
Euglena gracilis	50%
Oocystis sp.	13.9%

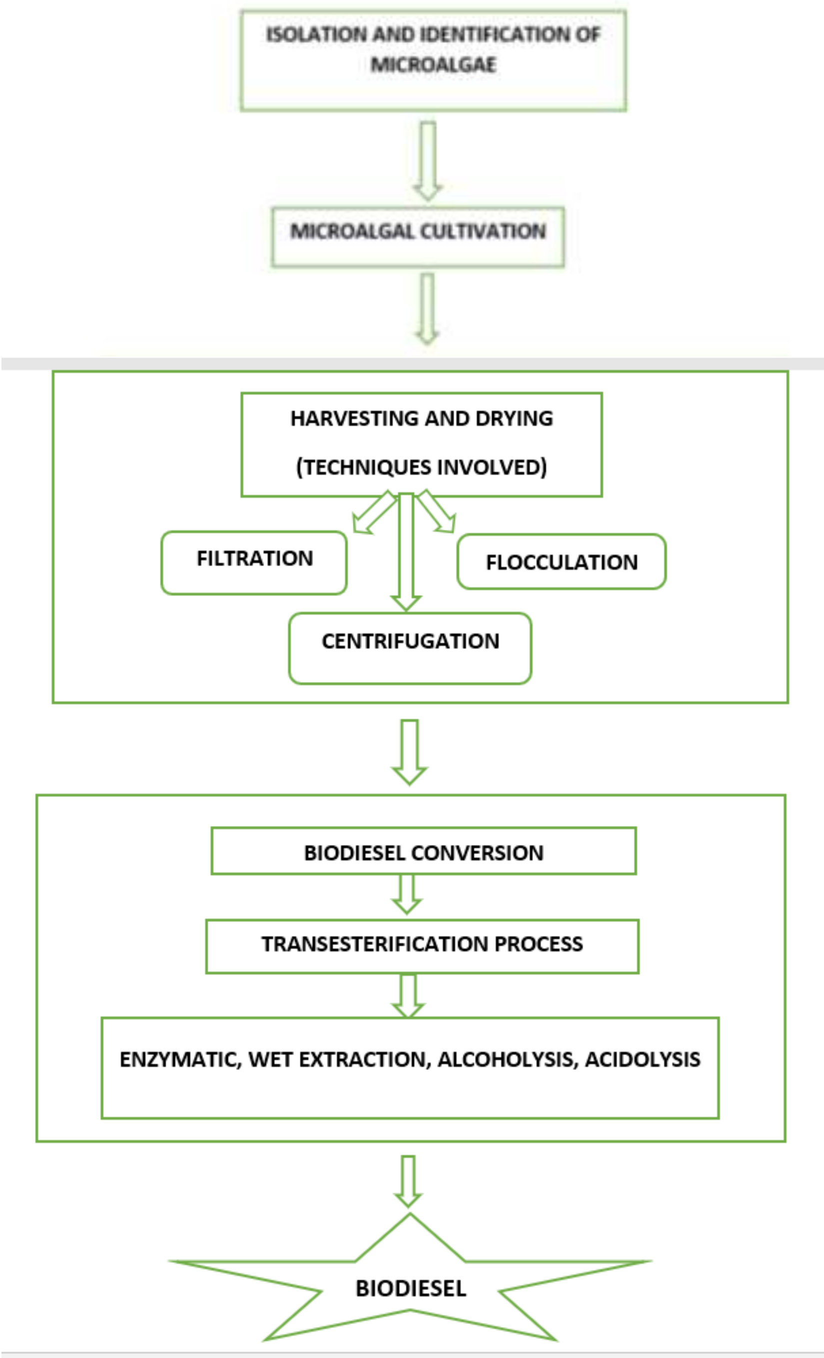
4. BIODIESEL FROM MICROALGAE:

Midway through the 19th century, the first biodiesel plant in the world advocated using algae as a source of both food and energy. During the Second World War, large-scale *Chlorella* algae farming was started in Japan, England, and Israel. The idea of employing these algae to produce energy was diverted to the production of food staples due to the abundance of fossil fuels [27]. Recently, there has been a lot of interest in the idea of using algae to produce alternative fuel. Algal biomass is mostly composed of lipids/natural oils, proteins, and carbohydrates. Microalgae are the only focus of the algae-to-biodiesel industry since the majority of the natural oil produced by them is tri-glycerol, the ideal type of oil for creating biodiesel. Because of their quick biomass output and relatively high oil content, microalgae have long been considered potential promising sources for biofuel production. Algal mass culture can be carried out on non-arable areas utilising non-potable saline water and waste water since microalgae grow far more fast than terrestrial crops do [28]. Several microalgae have oil production rates that are far higher than those of the best oil crops. The generalised approach for producing biodiesel from vegetable oil can be utilised to convert the lipids in algae. According to reports, microalgae have a greater potential to produce more energy per hectare of land than conventional crops do. The ability to create microalgae-derived biodiesel year-round is one of its main advantages. Because it can be produced on undeveloped terrain, microalgae cultivation has no requirement for pesticide use and has a higher oil output than any other crop. It also has the capacity to grow quickly with oil levels that range from 20 to 50% of the dry weight of biomass. An environmentally beneficial and renewable energy source, biodiesel is created by trans-esterifying plant or animal fats with short-chain alcohols. In contrast to conventional feedstocks like rapeseed and soybean, microalgae have a high oil content and may grow quickly on non-agricultural land or in brackish water. Algae has garnered the most interest among the several feedstocks for biodiesel production because of its quick growth rate, lack of competition with the availability of food and feed, and capacity to collect CO_2 [29]. Mono-alkyl esters are the main component of the biofuel known as biodiesel. Via the process of transesterification, these esters are produced from organic oils, algae, plants, or animals [30]. However, biobutanol and bioethanol are made from the sugars in algae [31].

5. SOURCES OF MICROALGAE:

A kind of plants known as algae are typically found in water. Microalgae are single-celled eukaryotic organisms found in freshwater or the ocean. They are a class of autotrophic microbes that thrive in soil, freshwater, and marine habitats and synthesize organic compounds by photosynthesis. Another element influencing the growth and development of microalgae is the salinity of the solution. Microalgae, particularly those that live in freshwater, have highly specific salinity requirements. Microalgae can generally be classified based on their tolerance to salinity. Algae, like other plants, use the pigment chlorophyll to convert sunlight into nourishment. All types of waterways, including freshwater, brackish water, and saltwater, include algae. By consuming extremely basic nutrients, microalgae may thrive in a range of environments, including fresh water, sea water, and treated industrial waste water [32]. Algae are prevalent in bodies of water, frequent in terrestrial settings, and can even be found in unexpected places like on snow and ice. While most seaweeds are found in shallow marine waters, less than 100 metres, some have been found as deep as 360 metres. The amount of light, its wavelength, and the photoperiod that the cells are exposed to during cultivation all affect how well microalgae develop [33]. The production of biologically active compounds, biomass growth, and photosynthesis in microalgae are all directly impacted by nutrient scarcity. The amount of light, its wavelength, and the photoperiod that the cells are exposed to during cultivation all affect how well microalgae develop. A direct correlation exists between light intensity and the photochemical stage of photosynthesis. Depending on the situation, the light source may be artificial or natural (sunlight), with the latter being the most practical and affordable [34]. Microalgae among all the feedstock, has been suggested to be more viable and a rich source of lipids for biodiesel production because it does not compete with food production, farmland, or fresh water in any way. Microalgae grows on saltwater, sludge, contaminated, or wastewater on non-arable or marginal lands [35]. Microalgae have the potential to grow on marginal ground with water that is unsuitable for irrigation, which is a significant advantage over plants [36].

FIG. 1. STEPS AND TECHNIQUES FOR THE PRODUCTION OF BIODIESEL FROM MICROALGAE



6. ISOLATION AND IDENTIFICATION OF MICROALGAE:

From ponds, lakes, and rivers, water samples with a visible microalgal population were taken. Whether marine or freshwater microalgae, cultures from collections, or native wild species are most suitable for large-scale production should be the determining factor in microalgae isolation [37]. Standard plating techniques were employed to divide algal populations from the field water samples in order to isolate single microalgal species. The colonies were isolated using several medium recipes. These diluted samples were placed in sterilised plastic petri dishes containing agar media. Throughout the course of 14 days, algae were allowed to grow. On the basis of morphological analysis of the colonies on an agar nutrient medium, microalgal cultures were divided. A microscopic slide is used to hold the isolated material. Slide observation is best done at 40X or 100X magnification. To effectively observe species using a compound microscope, a high power more than 100X is required. The identification of algae can be done by drawing or taking a picture. Following microscopic analysis, the shape of the individual cells served as the basis for the identification of these isolates to the genus level [38].

7. CULTIVATION OF MICROALGAE:

The fastest-growing species is microalgae. The growing microalgae need light, water, fertilisers, CO_2, and a temperature range of 20° to 30°C in their growing environment. Microalgae absorb CO_2 and use photosynthesis to turn it into fuel. They are capable of capturing CO_2 from the atmosphere, heavy industry waste gases, and soluble carbonates, three different sources. By altering the growing conditions, numerous efforts have been made to increase the productivity and lipid content of particular microalgae species [39]. To maintain high algal yields, microalgae cultivation needs a steady supply of numerous inorganic nutrients, including nitrogen (N), phosphorous (P), and potassium (K). Open and closed microalgae growing systems are the two main types. The most often used of these systems are lawn scrubbers and open ponds with lanes such as Race-way Ponds. Closed systems, also known as photobioreactors, offer a greater variety of shapes and configurations, with tubular, Bubble Column, Air-lift, and Flat Panel being the most popular [40]. Moreover, wastewater from palm oil milling and home sewage can be used to cultivate microalgae, which can help with the bioremediation of wastewater [41,42].

8. HARVESTING PROCESS:

In order to get the lipid profile, the farmed algae must be dewatered. Instead of appearing as a liquid that flows freely, the dewatered algae resemble a solid-liquid transition zone [43]. All harvesting methods generally attempt to remove as much culture media from the microalgae biomass as possible to make room for subsequent downstream processing, like the extraction of bioactive chemicals. Filtration, centrifugation, flocculation, and flotation are just a few of the harvesting techniques that have been employed to gather biomass [44]. In Filtration a permeable material is used, so that the algae biomass may be held in place and the liquid can still pass through. A pressure difference across the filter is necessary for this procedure, and it can be generated by vacuum, pressure, or gravity. According to the size of the pores, membrane filters can be categorised as having macro filtration (pores larger than 10 10 m), micro-filtration (pores between 0.1 and 10 m), ultrafiltration (pores between 0.02-0.20 m), and reverse osmosis (pores smaller than 0.001 m) [45]. Filtration techniques include: magnetic, deep-bed sand, cross-flow, pressure, and vacuum [46]. As the membrane's pore size is expanded, less pressure is needed to push the fluid through it. Microalgae cells in the suspension can be concentrated by filtration processes by up to 5–18%, and the cost of operation varies from $10 to $20 per gallon. Using filtration techniques, the harvesting efficiency ranges from 20% to 90% [47]. centrifugation, has a high efficiency that is almost 100% but has the potential to use more energy than it produces. The disc-stack centrifuge, solid-bowl centrifuge, hydro-cyclone, tubular centrifuge, and multi-chamber centrifuge are the most often used centrifugal equipment that has been proven to be useful for harvesting algal biomass. A physical dewatering technique called centrifugation relies on the creation of a centrifugal force that acts radially to speed up the movement and separation of particles based on the disparity in densities between the particle and the medium in which it is contained. The more-dense particle in this scenario will migrate outside, while the less dense particle will move inside. By using the right rotating rates, centrifugation is dependable for separating highly diluted liquids [48]. One of the least energy-intensive methods for collecting microalgae biomass is coagulation-flocculation. The biomass of the microalgae was efficiently harvested using ferric chloride (72-96 mg/L). A biomass Paste with a solid content of between 10 and 30 percent can be produced in the second stage, depending on the needs of the downstream processing, after the microalgae biomass has been dewatered in the first step to a slurry with a solid content of between 1 and 2 percent. Particularly for low-value products, flocculation of microalgae cells may be a viable preliminary choice for extracting microalgae biomass [49]. Using the self-floating and relatively low density of microalgae, flotation is a more efficient and cost-effective method of harvesting them [50]. Typically, negatively charged bubbles with a big size and hydrophobic (HPO) surface are successful at flotation. In order to reduce the energy input, several efforts have been made to produce smaller bubbles for collecting algae cells as opposed to enlarging the size of the algal particles [51,52]. Moreover, there is a disconnect between flotation efficiency and the degree of hydrophobicity and algal floc size, which are crucial elements in flotation success. The most popular and efficient way to create algal flocs is to mix chemicals, such as aluminium salts, into the algal suspensions to increase the collision activity of algal cells with bubbles. Because to their higher surface area, flocs facilitate the removal of algae by increasing bubble generation at the surface, bubble entrapment, and bubble entrainment [53]. According to the research, a single litre of cultivated media contains just 0.1% dry matter. Drying algae is accomplished with the use of flocculation and membrane filtration [54].

9. CONVERSION OF MICROALGAL BIOMASS TO LIPIDS:

One mole of a complex ester triglyceride reacts with three to four moles of alcohol to form simple esters through a process known as transesterification (Biodiesel). The transesterification process is frequently catalysed by a number of acids, including sulphonic acid and sulphuric acid, as well as bases like NaOH, KOH, sodium methoxide, sodium ethoxide, and K_2CO_3 [55]. Because base catalysed processes are less corrosive than acid catalysed processes, they are preferred in industrial settings. In contrast to the base catalyst, which removes a proton from the alcohol to produce a stronger nucleophile, the acid catalyst adds H+ to the carbonyl group to produce a stronger electrophile [56]. By employing methanol and ethanol, respectively, methyl and ethyl esters (biodiesel) are produced. Transesterification is driven using a heterogeneous catalysis pathway to get beyond the issues that homogeneous catalysts present [57]. Zeolites with silica alumina frameworks (MOR, HY, HZSM-5, H), mesoporous materials (MCM-41, SBA-15, MCM-48), metal oxides (ZrO_2, WO_3, CaO, ZnO, $SrCO_3$), and heteropoly acids ($H_3PW_{12}O_{40}$) have all been used for more than ten years. Composition of about 0.30 g NaOH in 2ml of methanol. FAMEs were prepared by transmethylation of FA from oil extracts of each strain & kept for 16 h until the biodiesel and sediment layers formed.

TRIGLYCERIDE + METHANOL $\xrightarrow{\text{CATALYST}}$ **METHYL ESTER + GLYCERINE**

CATALYST **(BIODIESEL)**

10. TEST FOR BIODIESEL FUEL PROPERTIES:

One of the most effective sources of energy from microalgae that may be used in internal combustion engines is algal biodiesel/green diesel. It is necessary to understand and evaluate the green diesel's fuel qualities for use in IC engines after conversion to biodiesel. More research has been done on the extraction of microalgae oil, the conversion of algae to biodiesel, and the characterisation of the biodiesel for engine performance testing and emission studies. The biodiesel (B100) displayed higher heating values of 41 MJ/kg, 0.864 kg/L density, and 5.2x10-4 Pa s viscosity at 40 °C. According to the literature, different species of microalgae have varied heating values for biodiesel [58]. Various property tests were carried out such as Viscosity, Api Gravity determination, Determination of acid value, Hydrogen carbon ratio, Flash point, Determination of Saponification value, Calorific value and Aniline point.

11. CONCLUSION:

According to the literature, microalgae biodiesel/green diesel is an alternative to petroleum-based diesel for internal combustion engines that is both affordable and environmentally beneficial. In terms of net energy balance and impact reduction due to greenhouse gas emissions, microalgae production is the most effective. Another viable/achievable option is the large-scale cultivation of microalgae for the manufacture of biodiesel. It might also function admirably as a natural CO_2 harvesting system.

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