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Microalgae based production of single-cell protein

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Microalgae express high protein levels and can be produced in contained cultivation systems with low water requirements and complete fertilizer use. The production potential is 22–44 tons of protein per hectare per year although the current production scale is small. Techno economic analyses have shown good potential for scale-up and cost reduction. Large-scale production of microalgae in the post-fossil era will rely on the capture of carbon dioxide from the air, or sugars from crops. Microalgal amino acid composition matches well with requirements for food and feed, which, in combination with novel biomass pre-treatment steps, will guarantee high-quality microalgal protein. For a broadening of the microalgae species available as single-cell protein, novel food approval is required.

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Microalgae as a source protein

Microalgal biomass production has been long considered a promising way to close the predicted 'protein gap'. Microalgal protein content and amino acid profile depend strongly on the species and also production conditions [1,2•,3,4]. Reported crude protein content of microalgal biomass varies between 30 and 80 mass percent: for example, *Chlorella vulgaris*, 51–58%; *Arthrospira (Spirulina) platensis* 60–71%; *Tetraselmis chui* 31–46%; *Nannochloropsis oceanica* 35–44%; *Dunaliella salina*, 50–80%; *Galdieria sulphuraria*, 62% [1,2•,5–8]. As such, microalgal protein content generally is higher than that of dried skimmed milk (36%), soy flour (37%), chicken (24%), fish (24%), and peanuts (26%). When it comes to nutritional value, the amino acid profile of several microalgal species matches the reference profile of a well-balanced protein, as defined by WHO/FAO [7–9]. Specifically, *Arthrospira* (*Spirulina*), *Dunaliella*, and *Galdieria* stand out [2•,3,7], where *Galdieria* grown under phototrophic conditions expresses a relatively high proportion of essential sulfur amino acids.

Despite the high protein content and the favorable amino acid composition direct use of microalgal biomass as single-cell protein (SCP) is limited by its digestibility [2•,9,10]. Digestibility of cyanobacteria such as *Arthrospira* (*Spirulina*) appears to be higher than that of green algae such as *Chlorella* which possess a rigid cellulosic cell wall [2•]. For a similar reason, *Dunaliella* species are suggested to be advantageous as a protein feedstock because they completely lack a cell wall [8]. Clearly, the accessibility of the high crude protein content of microalgal biomass by both humans and animals has to be studied in more detail to tackle the problem of suboptimal digestibility. For this reason, there is an ongoing search for efficient biomass pre-treatment steps to pave the path for the application of microalgal biomass as a source of proteins for food and feed [11–13]. Moreover, it is envisioned that biomass pretreatment can be followed by specific mild extraction processes resulting in several more or less purified biomass components. Among others, protein extracts can be generated with techno-functional properties (e.g. foaming, emulsification, gelation), which can match those of established protein sources such as soy, egg, and whey proteins [14,15].

When microbial biomass is used for human consumption, or feeding long-living animals, high nucleic acid content could lead to gout and kidney stones. The total nucleic acid content of microalgal SCP is generally lower than that of yeast and bacteria, which proliferate faster [6]. Values lower than 2% were reported for *Chlorella* and *Arthrospira*, which would allow for direct use of the biomass [2•]. Other studies, however, present 5–6% for similar algal species [16] suggesting this value should be closely monitored. In case nucleic acid levels are too high they should be removed in a dedicated treatment step which already is in place for other sources of SCP [6]. Also high chlorophyll content could reduce product quality because chlorophylls affect color and taste (bitterness). For this reason, these green pigments are preferably removed before human consumption, and mild treatment steps have been developed for this [14,17]. In addition, the chlorophyll content of crude microalgal biomass can be reduced by adapting cultivation conditions or selecting 'pale' low chlorophyll strains [18].

Microalgae cultivation and protein yield potential

Microalgae are photoautotrophic microbes and the most obvious cultivation strategy is based on sunlight energy

Figure 1

Production of *Galdieria* in a tubular photobioreactor at AlgaePARC (Wageningen University and Research). *Galdieria* is rich in protein (60% w/w) when cultivated under phototrophic conditions.

and carbon dioxide (CO_2). The production of single-cell protein for food and feed applications requires controlled cultivation conditions and so-called good manufacturing and agricultural practices (GMP and GAP) to guarantee product safety. This is often neglected and it is frequently suggested in the scientific literature that microalgae can be used to supply both feedstuffs and treat waste streams rich in inorganic nutrients. This will only be possible for waste streams of high quality and consistency typically found in the food and feed producing and processing industry [19]. In the case of large-scale production of microalgae as single-cell protein, a large part of the production will most likely rely on the application of inorganic NP fertilizers identical to those applied in today's agriculture and horticulture (e.g. ammonium and nitrate salts, ammonium-nitrate, urea, phosphoric acid, and different phosphate salts). In order to guarantee GMP also the use of contained production systems (called photobioreactors) is required [20]. Because of this containment fertilizers can be used at 100% efficiency, which is in strong contrast to today's agriculture where only half of the fertilizers are used by the crop [21] and the remainder drains away to surface and ground water causing pollution and eutrophication.

Mostly used production systems are closed tubular photobioreactors and so-called raceway ponds [20,22]. Raceway ponds can be placed in greenhouses to offer better containment and product control. Tubular photobioreactor units (Figure 1) can be constructed at scales of approximately 1000 m^2, and further scale-up is carried out by the multiplication of units [23,24]. Sunlight is the key driver of microalgal growth, and the light supply rate directly dictates the productivity of the photobioreactor or raceway pond (Table 1). Year-round average light supply rates vary between 20 mol PAR photons m^{-2} d^{-1} in North-Western Europe up to 40 mol PAR photons m^{-2} d^{-1} on the Arabian Peninsula (simulated in Meteonorm 7.1; Meteotest AG, Bern, Switzerland) (Table 1). These values can be converted into biomass productivity based on the biomass yield on PAR photons (i.e. the photosynthetic efficiency, PE). Under outdoor conditions, this efficiency is reported to vary between 0.2 and 1.0 g dry matter per mol of PAR photons, depending on system design, cultivation conditions, and algal species [25,26,27••]. Taking an average value of 0.6 g mol^{-1} (equivalent to PE of 2.75% on sunlight) we arrive at a biomass production ranging between 12 and 24 g m^{-2} d^{-1} for a wide range of geographical locations, which corresponds to 44 and 88 tons of dry biomass per hectare per year. Assuming a protein content of 50% w/w we can produce 22–44 tons of single-cell protein per hectare. This simple calculation exemplifies the big promise of microalgal single-cell protein as compared to soybean with a yield of approximately 1.2 ton protein per hectare [28] (USDA, 2021; Charts and maps, Field Crops, Soybeans Yield by Year, US; https://www.nass.usda.gov/, Accessed 26-10-2021).

Cost reduction of microalgae cultivation

Regardless of the high potential protein yield of algae cultures large-scale application is currently limited by costs. Investments in materials and equipment are substantial, as well as the power required to mix the cultures, supply carbon dioxide (CO_2), and remove photosynthetically produced oxygen (O_2). An analysis of Ruiz *et al.* from 2016 [29] revealed a production cost price in the range of 5–9 € kg^{-1} dry matter algal biomass when adopting tubular photobioreactors and use of non-renewable CO_2 from the industry (see Table 2 for overview cost factors). Again assuming 50% of protein, this number translates into 10–18 € kg^{-1} dry protein mass, but it must be addressed that processing of the concentrated biomass is not included in this cost price. A simple breaking of the microalgae will add around 0.5 € kg^{-1} biomass, while extraction and purification of soluble protein leads to an additional cost of 4.5–13 € kg^{-1} protein [30]. Clearly, a further reduction in cost price is necessary to be competitive with other potential sources of single-cell protein (SCP) such as yeast or fungal cultures grown on cellulose-containing residues [31], with an estimated protein cost ranging from 5–9 € kg^{-1}. Whole biomass from hydrogen-oxidizing or methane-oxidizing bacteria is currently estimated to be as expensive, or more expensive, than algal biomass [32•] (Table 2). The cost price of algal biomass can be reduced by increasing the photosynthetic efficiency. An efficiency of 1.2 g mol^{-1} (5.5% PE, Table 1) is possible at a laboratory scale by the

Table 1

Characteristic numbers on light and photosynthetic growth. PAR stands for photosynthetic activity radiation and represents that part of the light spectrum (400–700 nm) that can be used in oxygenic photosynthesis. PAR is expressed as a photon flux

Characteristic number	Value	unit	Reference
PAR content sunlight	1.98	$mol_{ph}\,MJ^{-1}$	ASTMG173
PAR intensity Wageningen – 52° N, temperate maritime climate			
Yearly average	20.0	$mol_{ph}\,m^{-2}\,d^{-1}$	Simulated[a]
21 March – 21 Sep	30.8	$mol_{ph}\,m^{-2}\,d^{-1}$	Simulated[a]
PAR intensity Dubai – 25° N, hot desert climate			
Yearly average	39.6	$mol_{ph}\,m^{-2}\,d^{-1}$	Simulated[a]
21 March – 21 Sep	47.4	$mol_{ph}\,m^{-2}\,d^{-1}$	Simulated[a]
Yield of biomass on PAR			
Theoretical maximal[b]	1.68	$g\,mol_{ph}^{-1}$	[53]
Laboratory maximal[c]	1.2 – 1.3	$g\,mol_{ph}^{-1}$	[36]
Outdoors	0.2 – 1.0	$g\,mol_{ph}^{-1}$	[25,26,27••]
Enthalpy of combustion biomass	0.53	$MJ\,kg^{-1}$	[54]
Mass of 1 carbon mole biomass	22−27	$g\,mol_{C}^{-1}$	[54]
LED PAR yield on electricity	3.4	$mol_{ph}\,MJ^{-1}$	Signify 2021
Maximal LED PAR yield (550 nm)	4.6[d]	$mol_{ph}\,MJ^{-1}$	Plank's Law

[a] Meteonorm 7.1; Meteotest AG, Bern, Switzerland.
[b] Excluding photosaturation and cellular maintenance requirements; assuming photon requirement of 10 for photosynthetic CO_2 reduction to triose and 0.7 biomass carbon yield (molar) on photosynthetic triose.
[c] Obtained under simulated outdoor conditions of vertical reactor orientation; solely light limitation.
[d] Hypothetical yield assuming 100% conversion efficiency electrical energy to light energy. Because of constraints imposed by the second law of thermodynamics this yield cannot be reached.

Table 2

Overview discussion cost factors algae production for single-cell protein

Process		Costs/€ kg^{-1}		Reference
		Biomass	Protein	
Yeast or fungal biomass on cellulose-containing residues			5–9	[31]
Bacterial biomass on hydrogen or methane		5–24	10–48[a]	[32•]
Algal biomass on sunlight and carbon dioxide[b,c]		5–9	10–18[a]	[29]
Extra costs:	Cell breakage[c]	0.5	1[a]	[30]
	Soluble protein extraction-purification[c]		4.5–13	[30]
	Artificial light instead of sunlight	9	18[a]	
Improvements:	Doubling PE by reactor design, or strain selection	Reduction production costs is inversely proportional to PE increase		[29]
	Direct air capture (DAC) CO_2	+0.18	+0.36[a]	[42–44]
		Process becomes carbon neutral and circular		
	Selection strain with higher temperature tolerance – crop rotation	7.5% reduction production costs		[29]
		Minimal fresh water use		
		25% reduction production costs		[29,51]
	Mixotrophic cultivation on sucrose	4 fold lower land requirement, and 50 fold lower water requirement, compared to soy protein		

[a] Assuming 50% protein.
[b] Tubular photobioreactor and non-renewable CO_2.
[c] Includes cell harvesting by microfiltration and centrifugation (0.2–0.3 € kg^{-1} biomass).

selection of optimal strains [33••,34] and improved reactor design and control [35•,36]. Moreover, considering microalgal strains have been selected that thrive well at different temperatures, ranging from 10 to 45°C [37,38], costs for temperature control can be omitted by selecting the right strain for the right location and adopting algal crop rotation over different seasons.

In the context of cost reduction the two most important substrates for photoautotrophic production of algal

biomass must be discussed, light and CO_2. Light-emitting diodes (LEDs) are being adopted rapidly in horticulture replacing traditional high-pressure sodium lamps. LED efficiency reached 3.4 mol photons per MJ of electrical power (Table 1) with a 36 000 hour lifetime (Signify, Philips GreenPower LED toplighting compact, https://www.lighting.philips.nl/, Accessed 26-10-2021). Installation costs dropped down to 0.3 € per unit photon flux (μmol/s) (JH de Vree, LGem BV, The Netherlands, 2021). Combined with an assumed electricity cost of 0.11 € KWh^{-1} and photosynthetic efficiency of 1.2 g mol^{-1} this leads to a cost price addition of 9.4 € kg^{-1} biomass (Table 2) for the use of artificial light only (80% of which is electricity cost) and an additional electrical power requirement of 245 MJ kg^{-1}, which is one order of magnitude larger than the energy content of algal biomass (Table 1). Even considering further improvements of LED efficiency direct use of the sunlight is the most attractive for large-scale production of microalgal SCP.

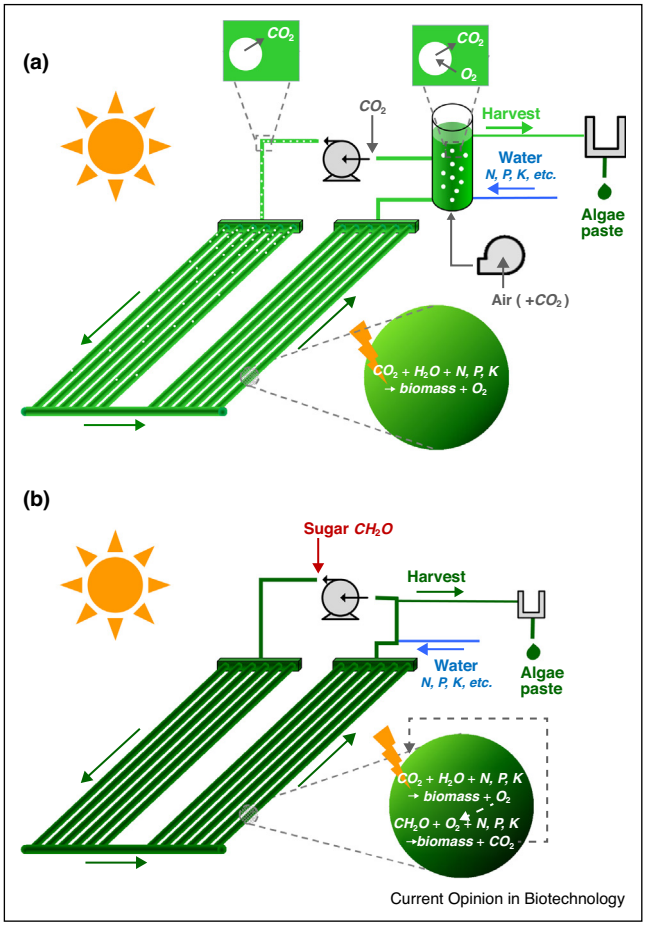
Carbon dioxide supply is the other substrate that poses fundamental challenges. Dissolved CO_2 levels must be maintained high for productive microalgal cultures, and at the same time, the relative CO_2 loss via the off-gas has to be minimized. This can only be achieved by employing CO_2-rich gas streams and an optimized design of the gas-liquid transfer in microalgal cultivation systems [39]. In a future scenario, a concentrated and renewable form of CO_2 can only be obtained by direct air capture (DAC) of CO_2 from the atmosphere [40••,41]. This challenging technology is under development and is essential for enhancing the photosynthetic growth of algae and plants in contained environments such as photobioreactors and greenhouses. Current projections suggest a CO_2 cost price of around 100 € ton^{-1} when employing DAC [42–44]. This translates into a CO_2-related cost of algal biomass production of 0.18 € kg^{-1} dry matter (Table 2) and an additional energy requirement of 5–10 MJ kg^{-1} (mostly heat required to liberate absorbed CO_2).

Another aspect that is often reported to result in high costs of production of microalgal biomass is related to harvesting the cells. Because of the small cell size (2–10 μm) and the diluted nature of the microalgae mass cultures (0.25–5 g/L), harvesting of the cells, and subsequent dewatering, are energy-intensive and capital-intensive processing steps. A list of available technologies includes centrifugation, chemical flocculation, filtration, sedimentation, and auto-flocculation. The analysis of Ruiz *et al.* [29] has shown that a combination of filtration and centrifugation is attractive at a large scale and will add 0.2–0.3 € kg^{-1} of dry matter (Table 2).

Mixotrophic cultivation of microalgae for protein

An alternative approach to photoautotrophic production of microalgal biomass and SCP is so-called mixotrophic growth [45]. In this growth mode microalgae are cultivated on organic substrates with the aid of sunlight energy. Several microalgal species can take up and metabolize certain organic molecules, most commonly hexoses and acetic acid [46]. These algae (e.g. *Chlorella*, *Galdieria*) can therefore combine chemoorganotrophic growth with photoautotrophic growth. The CO_2 released from the organic substrate can be almost completely reused by the photoautotrophic metabolism within the same cells. The oxygen is completely recycled [47•,48]. As such, production costs of microalgal biomass can be reduced, by saving on the energy and equipment required for gas–liquid transfer in traditional microalgae cultivation systems (Figure 2). Moreover, both biomass concentration and reactor productivity can be doubled at the expense of supplying an organic carbon source. The biomass concentration is important for the harvesting and processing of the suspended microalgal cells. Doubling of the biomass

Figure 2



Simplified representation of tubular photobioreactors operated either in photoautrophic mode **(a)**, or mixotrophic mode **(b)**. Darker green of mixotrophic mode represents higher biomass concentration which can be maintained, and for the same reason, the harvest unit is shown to be smaller.

Table 3

Current global production of photosynthetic microalgae [52]

Species	Tons of dry weight per year
Spirulina	18 000
Chlorella	9500
Dunaliella	1700
Aphanizomenon flos-aquae	500
Haematococcus	300
Nannochloropsis	150
Euglena	50
Total	30 206

concentration will half the volume of water to be separated from the biomass, which on its own is estimated to lead to a 25% cost reduction [29].

Although microalgae are often positioned as an alternative to traditional crops, the cultivation of sugar crops and microalgae can support each other yielding more protein at lower land and water requirement than traditional protein crops. Sugar beet is extremely productive yielding 14 tons of sucrose per hectare per year in North-Western Europe [49] with considerable potential for further improvement [50••]. Employing mixotrophic cultivation this sucrose can be converted into high-quality microalgal single-cell protein at a very high efficiency of 90% [47•]. Combining both the area and water requirements of mixotrophic algae cultivation with those of sugar beet cultivation microalgal single cell protein can outperform soy protein on land and water requirement although current production costs are still estimated to be higher [51]. It is estimated that mixotrophic cultivation of *Chlorella* on sugar beet sucrose requires a land area of 0.23 ha for the yearly production of 1 ton of protein. This compares favorably to 0.80 ha required for 1 ton of soy protein. Moreover, it is estimated that the freshwater requirement can be reduced 50-fold in comparison to soy [51].

Market developments on the use of microalgal biomass

During the last 70 years, microalgae have several times attracted attention as promising candidates for industrial exploitation. In comparison with plants, microalgae have rapid growth and high potential for nutritional uses either as whole-cell biomass or as a biorefinery feedstock. Markets for *dry* microalgal biomass (mostly *Spirulina* and *Chlorella*) and carotenoids (ß-carotene and astaxanthin) for human health food and aquaculture feeds developed in the 1990's. It was a breakthrough to be able to separate microalgae production and the use of the product. In the 'peak oil' days (2005–2010), where crude oil prices rose above $100 per barrel, automotive fuels were the main driver behind a surge in interest for microalgae production. But similar to the objective of 'Staple food for the world population' after the Second World War, also the objective of 'Biofuel after peak oil' lost industrial relevance. Most microalgal production companies have shifted towards food supplements and other niche products. For 3–4 years, many microalgal R&D projects and industries have started operating within the rapidly developing markets for meat replacement and soy protein replacement.

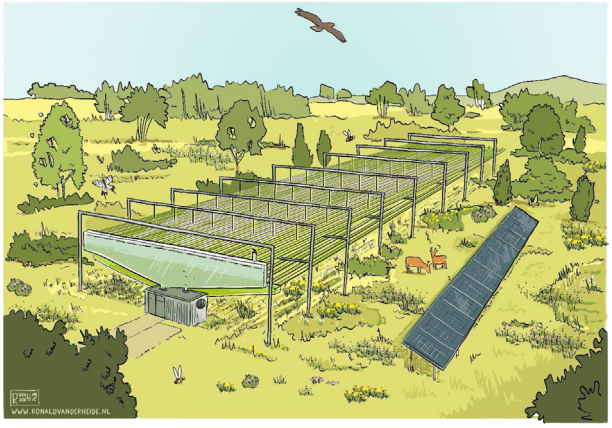
The scientific and industrial community working on microalgal applications increased tremendously in the last years all over the world, bringing along technological developments some of which are discussed here. Over the past 10 years, the market has approximately tripled, nevertheless, it remains small, considering the large public interest in microalgae. Currently, the global market of autotrophic microalgae is about 30 000 tons of biomass, distributed over only 7 major species (Table 3) [52]. These are the species that are available on the market. In addition, microalgae are used as live diets in aquatic hatcheries, for example for bivalve rearing, but these algae are mostly produced on-site.

Table 4

Microalgae Species and microalgae-based ingredients with Novel Food rights

Species for novel food	Appl. date	Status	Final decision
Chlorella vulgaris		Consumed prior 1997	Approved 1997
Chlorella pyrenoidosa		Consumed prior 1997	Approved 1997
Chlorella luteoviris		Consumed prior 1997	Approved 1997
Arthrospira platensis		Consumed prior 1997	Approved 1997
Odontella aurita	2002		Approved 2002
Tetraselmis chui	2011		Approved 2014
Nannochloropsis gaditana	2011		Pending
Euglena gracilis	2018	Positive report of EFSA_March_2020	
Additives/supplements			
Ulkenia sp. oil	2004		Approved 2009
Dunaliella salina oil (additive and supplement – E 160a (iv) or food orange5)	1977		Approved 1997
Astaxanthin-Rich Oleoresin from *Haematococcus pluvialis*	2014	Positive report of EFSA_Dez_2019	Approved 2019
EPA-rich oil derived from the microalgae *Phaeodactylum tricornutum*	2016	Negative report of EFSA_June_2019	
Euglena gracilis food supplement		Positive report of EFSA_March_2020	
Phycocyanin from *Arthrospira platensis* (food colorant – additive)			Approved 2013

Figure 3



Artist impression of intensive, contained, and zero-waste cultivation of microalgae within a natural ecosystem (by Studio Ronald van der Heide, The Netherlands).

In order to place microalgae-based SCP in the European Food market, a strict set of rules must be followed, that is, the European food law, to guarantee the highest standards on food safety. The process for submission and approval as Novel Food is time-consuming (ca. 4 years) and expensive (300 k€–400 k€ per ingredient), which makes it unaffordable to most SMEs. This is a very big barrier to the market uptake of algae ingredients in the food market. As a consequence, there is presently a very limited number of microalgae strains and ingredients approved as novel food. Up-to-date only 6 microalgae species and 4 microalga-based ingredients are considered novel food (Table 4). Most new food applications of microalgae are based on Spirulina (*Arthrospira) platensis* and *Chlorella* as these algae do not require novel food approval due to the long history of consumption of those species for food. Recently *T. chuii* has been approved as novel food and *Nannochloropsis* is pending.

Outlook

For a broadening of the microalgal species base suitable as SCP, production companies should join forces and cooperate for the expensive and lengthy novel food approval. In order to meet the protein market demands, multiple producers together need to supply larger quantities, similar to in agricultural crop production. Robust and cost-effective production methods are essential for microalgae production companies to accept the risks and costs of developing new microalgal products based on new species. For this, next-generation technologies must be implemented for a targeted reduction of process costs in the post-fossil era, such as strain improvement, light dilution, direct air capture of carbon dioxide, or mixotrophic cultivation. Special attention should be given to developing microalgae cultivation processes focusing on zero-waste and 100% fertilizer use (Figure 3), and high product quality (e.g. maximal digestibility, palatable, safe). Increasing market penetration for microalgae-based products as sustainable alternatives to the currently available options in the market will be crucial for the success of microalgae as a sustainable protein source

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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