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Mass Production of the Blue-green Alga *Spirulina*: An Overview*

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ABSTRACT

Progress has been made in the past decade in developing appropriate technology for microalgal mass cultivation. This review details basic requirements required in order to achieve high productivity and low cost of production. There is a need for a wide variety of algal species and strains that will favorably respond to the varying environmental conditions existing outdoors. Another essential requirement is for better bioreactors, either by improving existing open raceway types or developing tubular closed systems. The latter solution seems more promising. These developments must overcome the main limitation confronting the industry today which is the overall low areal yields which fall too short of the theoretical maximum and which are associated with scaling up microalgal culture to commercial size.

Key words: Algal mass culture, blue-green algae, *Spirulina*, algal ponds, productivity.

1 INTRODUCTION

The past few years has seen considerable activity relating to production of microalgae for commercial purposes. From a modest beginning with *Chlorella* tablets in Japan in the late fifties, new endeavors have emerged as specialized industries the world over, aiming to produce health food,

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food additives, animal feed, biofertilizers, and an assortment of natural products.[1-3] So far, three algal species have attracted major commercial interest — *Chlorella*, *Spirulina* and *Dunaliella*. *Spirulina* is of commercial importance due to its overall nutritional qualities: high protein content (60% to 70% of dry weight), low fat, high vitamin content (particularly B_{12}) as well as its content of the essential fatty acid gamma-linoleic acid.[4] The pigments of *Spirulina* also make this algae useful in aquaculture, particularly as feed for tropical fish.[5-7]

The history of *Spirulina* as a staple in the human diet is unique. There is evidence from the annals of the Spanish conquest of Mexico, early in the sixteenth century, that the Aztecs harvested from Lake Texcoco mats of algal biomass reminiscent of *Spirulina*, from which they made dry bricks which were eaten as cheese would be eaten today in the Western World. Likewise, for many generations dried *Spirulina* has been used as a food by the Kanembu tribe, which lives along the shores of Lake Chad in Central Africa.[8]

Today, *Spirulina* is commercially cultivated in several countries, with a total annual production of a few hundred tonnes. The main production sites are summarized in Table 1. A few other sites have ceased production, owing to high cost of production. *Spirulina* products in the form of pills and spray-dried powder are produced in Mexico, Taiwan, USA, Thailand, Japan and Israel for the health food market. Small amounts of *Spirulina* are extracted for the production of phycocyanin, known commercially as 'lima blue', used as a blue colorant for food and cosmetics. In addition, some *Spirulina* products are fortified with extracts of various herbs as well as some vitamins and minerals and sold as a relief for premenstrual syndrome.[7] Another mode of production based on local inputs and simple techniques in rural, developing regions, yields human food as a supplement for protein-deficient diets. Indeed, the concept of mass cultivation of *Spirulina* is being pursued by people of two different social categories. One is the affluent industrialized society, where people (particularly vegetarians) are looking for natural foods and health-food additives for their diet.[9] The other is represented by Third World societies in search of a rich source of protein that can be produced under local conditions using local resources, i.e. marginal land and saline water not suitable for conventional agriculture as well as animal and human wastes. In addition the possibility of using *Spirulina* as a source of animal feed, particularly for fish, has attracted attention.[10-12]

Much progress has been made in the last 20 years in developing the biotechnology for algal mass culture. Improvements have been mainly in the management of outdoor cultures based on a better understanding of the biology of dense cultures grown on a large-scale as well as of the technological aspects, such as pond design, mixing systems, and modes

TABLE 1
Spirulina Production Plants

Company	*Location*	*Type and size of ponds (ha)*		*Annual Production (tonnes dry wt)*
		Native	*Lined*	
Sosa Texcoco	Lake Texcoco, Mexico	12		300
Earthrise Farms	California, USA		5	90
Siam Algae	Bangkok, Thailand	1·8	2	100
Blue Continent Chlorella	Taiwan (a few locations)		4–15	up to 300
Nippon — Spirulina	Japan		1·5	30
Cyanotech	Hawaii		2	40

of harvesting.[13,14] High figures for algal biomass production under outdoor conditions i.e. up to 50–60 g m^{-2} day^{-1} have been reported,[15] but such reports are based on outputs from small ponds (*c.* 10 to 100 m^2), which were operated continuously for only a few weeks and from which the biomass was removed by dilution. As far as we know, such high values have never been achieved in any large-scale commercial set-up. In large scale ventures, annual production does not exceed 30 tonne ha^{-1} $year^{-1}$ representing an average of less than 10 g m^{-2} day^{-1} i.e. a net photosynthetic efficiency of somewhat over 1%, significantly less than the theoretical limit.[16]

The future of this industry is greatly dependent on developments that should result in increased yields with a corresponding decrease in cost of production. To achieve this end, the limiting factors of growth and of net output in large-scale mass cultures must be better understood. Nutrient limitation as well as inhibitory pH may be readily managed in mass cultures, and thus should not present difficulties in pond management. Large-scale production systems of photoautotrophs are thus practically dependent only on environmental factors, which in turn may be modified through the technological features of the reactor.

The following is a short summary of some basic achievements in mass cultivation of *Spirulina* and the major problems still outstanding. The latter must be solved before a viable industry of algal mass production with global annual outputs of many tens of thousands of tonnes can become a reality.

2 THE PRESENT STATE OF THE ART

The main technological and biological achievements to date have been as follows:

2.1 Maintenance of monoalgal cultures

Contamination by different algae may represent a very severe problem for microalgal cultures grown in outdoor reactors. In a previous paper,[17] we described conditions which prevent contamination of outdoor *Spirulina* cultures by *Chlorella*. Since then, we have successfully tested the proposed measures in large-scale commercial ponds. In most cases, the steps which proved effective in preventing contamination by *Chlorella* were: maintaining a high bicarbonate concentration (e.g. 0·2 M); taking precautions to maintain the dissolved organic load in the culture medium as low as possible; and increasing winter temperature by heating. Development of grazers in the culture, mainly the amoeba type, presents another problem. Vonshak *et al.*[17] noticed amoeba grazing on *Chlorella*, and amoeba grazers of *Spirulina* were also observed in some commercial ponds which were improperly maintained. Addition of ammonia (2 mM) arrested the development of these grazers. Similar observations have been reported by Lincoln *et al.*,[18] who showed that the population of grazers was significantly reduced when ammonia was used as the main nitrogen source. Furthermore, when a population of *Chlorella* cells growing in mass cultures of *Spirulina* increased in number (above 10^6 ml^{-1}), repeated treatment with 1 mM ammonia was found to prevent further proliferation of *Chlorella* (Vonshak & Boussiba, unpublished results).

In conclusion, contamination of outdoor *Spirulina* cultures grown outdoors can be controlled. Experience indicates that as a rule contaminating organisms do not present a serious difficulty as long as good growth is maintained in a monoalgal culture. It is worth noting that no cyanophages attacking *Spirulina* have been observed so far.

2.2 Design and mixing of the open-channel raceway

Almost all commercial reactors for production of *Spirulina* are based on shallow raceways in which algal cultures are mixed in a turbulent flow sustained by a paddle wheel. One exception is Sosa Texcoco (Mexico) where a natural lake and certain facilities of a production plant for sodium bicarbonate were adapted for producing *Spirulina*. At other production sites for algal mass cultivation in the USA, Israel, India, Thailand, and Taiwan, two types of open raceway ponds are used: the first, the most capital intensive, lined by concrete (Thailand, USA); the second is a shallow earthen tunnel lined with PVC or some other durable plastic. The cost and durability of the lining influences significantly the capital costs and thus the economic feasibility. Benemann *et al.*[19]

estimated that any durable liner will add up to $0·5 to the cost of production of each kilogram of algal biomass produced, pointing out the need for cheaper lining such as low-cost clay sealing. Such lining has yet to be tested for its durability under turbulent flow and periodical cleaning of the pond.

The size of commercial ponds varies from 0·1 ha to 0·5 ha. Stirring is accomplished in all ponds with paddle wheels, the design of which varies significantly from large wheels (diameter up to 2 m) with low revolution speed (i.e. 10 rpm) to small wheels (diameter of 0·7 m) revolving two to three times faster. Culture depth is usually maintained at 15–18 cm. In most places this depth is limited by technical requirements, such as ground levelling and the depth of immersion of the paddle wheel.

Although the paddle wheel is the most common stirring device in today's *Spirulina* commercial plants, other mixing devices have been tested. One difficulty with paddle stirring rests with the nature of the flow, which is usually not sufficiently turbulent to produce an optimal light pattern for single cell algae.[20,21] Laws *et al.*[15] introduced into the raceway arrays of foils, similar in design to airplane wings, which increased mixing owing to the vortices created and resulted in a more than two-fold increase in photosynthetic efficiency. Recently, Valderrama *et al.*,[22] following an idea originally suggested by Baloni *et al.*,[23] described a novel device for mixing of shallow algal ponds, consisting of a board which fills the pond's cross-section, except for a slit above the bottom of the pond. The board is moved back and forth, creating a turbulent backwhirl as the culture is forced through the slit. The authors claimed to have achieved 'outstanding' results in growth of *Spirulina*. This method of inducing turbulence in shallow raceways has not yet been scaled up, and a comprehensive evaluation of the system has yet to be carried out. Cultivating *Spirulina* in tubular reactors in which the flow is induced by a suitable pump seems another promising means of cultivation which has yet to be tested on a large scale. Initial work by Tomaseli and co-workers[24] points out the potential of such a system.

2.3 Harvesting and processing of *Spirulina* biomass

In all commercial production processes similar filtration devices are used for harvesting. These are basically of two types, i.e. inclining or vibrating screens. Inclined screens are 380–500 mesh with a filtration area of 2–4 m^2 per unit and are capable of harvesting 10–18 m^3 of *Spirulina* culture per hour. Efficiencies of biomass removal are high (up to 95%) and two consecutive units are used for harvesting up to 20 $m^3\ h^{-1}$ from which a slurry (8–10% of dry weight) is produced. Vibrating

screens can be arranged in double or triple decks of screens up to 72 in diameter. Vibrating screens filter the same volume per unit time as the inclining screens, but require only one third of the area. Their harvesting efficiencies are often very high. At one commercial site a combination of an inclining filter and a vibrating screen is used. Two main problems exist with the systems described above. In the process of pumping the algal culture to be filtered, the filaments of *Spirulina* may become physically damaged, and repeated harvesting leads increasingly to enrichment of the culture with unicellular microalgae or short filaments of *Spirulina*, which pass through the screen readily.

Our experience in large-scale production of *Spirulina* indicates that the vibrating screen may not be the optimum device for harvesting *Spirulina*. Its major drawback is that it effects a progressive enrichment of species with small and narrow filaments as well as of unicellular species. Thus the inclined, stationary screen seems a somewhat better solution for removing *Spirulina* biomass from the medium. The slurry (8–10% of dry weight) obtained after filtration is further concentrated by vacuum filtration, using vacuum tables or vacuum belts, depending on the production capacity. This step is also used for washing excess salts (which amount to 20–30% of dry weight) from the biomass. The washed cake is frequently homogenized before being dried. Drying is accomplished by spray- or drum-drying. The end product should have an ash content of about 7%. For good preservation under storage, moisture should not exceed 3–4%.

2.4 Production of *Spirulina* by simple technology and locally available inputs

The production of *Spirulina* may be greatly simplified, avoiding the high technology systems described so far. Such a mode of production results in relatively low outputs, which are compensated for by the much reduced cost of production and the fact that the production is based fully on local resources.

Becker and Venkataraman[25] and Seshadri and Thomas[11] pioneered work in this field formulating *Spirulina* growth media based on low-cost nutrients obtainable from rural wastes, such as bone meal, urine, or the effluent from biogas digesters. Indeed, *Spirulina* culture lends itself readily to simple technology: cultivation may be carried out in unlined ditches in which the flow is low (e.g. 10 cm s^{-1}), stirring being provided by a simple device driven by wind energy or man-powered; harvesting may be readily performed using some suitable cloth, and the biomass is dehydrated in the sun. The quality of the *Spirulina* product obtained in

this fashion would not be as high as attainable in 'clean cultures', but the product could very well serve as animal feed. In one study,[26] *Tilapia mossambica* was cultivated in artificial ponds with relatively high stocking density and fed with solar-dried *Spirulina*, cultivated and processed using low-cost technology, added to ground-nut cake. The mean food conversion ratio was lower than that observed in control fish fed with the usual fish ration. The yield of *Tilapia* fed on *Spirulina* mixed with ground-nut cake was higher by 41% compared to that of fish fed on ground-nut cake alone. Bai[27] has recently summarized information available concerning production of *Spirulina* biomass in a village in India. According to Chung *et al.*,[28] *Spirulina* grows well in diluted fermented swine manure, provided the concentration of ammonia nitrogen is adjusted to 100 mg liter^{-1} and some nutrients are added. *Spirulina* may also be grown on sea water enriched with urea, after excess Ca^{2+} and Mg^{2+} have been precipitated.[29] In recent reports[30] the use of sea water for cultivating *Spirulina* without any pretreatment of the water was described. Bicarbonate concentration used was about one-tenth lower than usual, the pH was maintained at 9·0 and the medium was not recycled. Yields of 10 g m^{-2} day^{-1} were obtained, suggesting the possible use of sea water as a cheap medium for mass cultivation of *Spirulina*.

3 BIOLOGICAL PROBLEMS AND LIMITATIONS

The major biological limitations for production of *Spirulina* in mass culture must be understood in order to obtain maximum outputs. These limitations may serve as criteria for selecting outstanding strains of *Spirulina*. The main points are discussed below:

3.1 Maintenance of steady-state production in outdoor cultures

The open-channel raceway has its merits in that it is relatively simple to operate and maintain. Yields of about 30 tonnes ha^{-1} year^{-1} may be obtained by this method (results from Siam Algae, personal communication). It is becoming clear, however, that high net photosynthetic efficiency, i.e. approaching the theoretical maximum, may not be achieved in open-channel raceways. One difficulty lies in maintenance of the optimum temperature in the culture throughout the diurnal cycle and the year round. Indeed, even when the maximum day temperature is at optimum, the morning temperature could be some 10°C or 15°C below the optimum for species such as *Spirulina*, preventing the full exploita-

tion of 4 to 6 hours of morning radiation in the summer. During the winter, *Spirulina* cannot be grown in open ponds, except in the tropics. In the winter, when the ponds are covered with transparent plastic sheeting or glass, the cultures survive, but production is greatly reduced, mainly because the temperature is still too far from the optimum throughout all or most of the day and because irradiance is reduced under the cover.[20,21]

To achieve sustained production of *Spirulina* biomass, it is therefore very important to maintain the temperature as close to the optimum as possible for growth during the entire day-light period and reduce it quickly with the onset of darkness. Effective utilization of high intensity solar irradiance (prevalent in arid zones) is the most basic and challenging issue in the production of photoautotrophic biomass in general and in *Spirulina* in particular. In a continuously mixed dense culture, the cells are illuminated intermittently, thus a most important parameter affecting productivity is the light regime to which the cells are exposed. The light regime is governed by the intensity of solar irradiance and the duration of each single light exposure of the cells entering the photic zone in the reactor and its frequency, as well as the duration of their exposure to light intensity below the compensation point upon leaving the photic zone.[13,20,21] Clear interrelationships exist between photon flux density, cell concentration, extent of mixing, and the output rate.[20,32] Our results indicate that increasing the degree of turbulence or decreasing the culture depth shifts the optimum cell concentration to a higher value. This is a great practical advantage, because the higher the density of a culture, the higher the efficiency of harvesting. In most of the commercial ponds, a good deal of the production potential is lost because the cultures are maintained at much too low a turbulence and at much too high a cell density (Vonshak & Richmond, unpublished data).

In summary, to the best of our knowledge, record yield of 35 tonnes ha^{-1} $year^{-1}$ of *Spirulina* from a commercial size pond for a full year's operation were achieved in the Siam Algae site near Bangkok. Even if a significant improvement is obtained in the future, we do not believe that the potential productivity of *Spirulina*, i.e. 90 tonnes ha^{-1} $year^{-1}$, may be achieved in open raceways.

3.2 Controlling photoinhibition

The upper layer of an outdoor algal culture is exposed, even in densely populated cultures, to a very high solar irradiance, 3 to 4 times the saturation point.[19] In most of the algal species studied, photosynthesis is saturated at 33% of the intensity of full solar radiation, and in most cases some photoinhibition may be observed with light intensity at 60 to 70%

of full sunlight. In many algal species prolonged exposure to high light intensity may cause photooxidative death.[33] We have been able to isolate *Spirulina* strains, with high light saturation values, which are more resistant to high light intensities. When tested outdoors some of these strains yielded higher production rates as compared to the more sensitive ones.[34,35] A detailed quantitative assessment of the damaging effects of photooxidation is not yet available.

3.3 Decreasing dark respiration

Outdoor mass cultures are not illuminated during the night. Preliminary measurements of the dark respiration losses in outdoor cultures of *Spirulina* have revealed that up to 35% of the total biomass produced during the day may be lost through respiration at night.[36,37] Clearly, rapid cooling of the culture after sunset would be advantageous, providing an important incentive to develop reactors of as low areal volumes as possible. Algal strains with a low dark-respiration rate or a high ratio of light-dependent O_2 evolution to O_2 uptake in the dark have an advantage for mass cultivation.

3.4 Decreasing oxygen tension in the culture

Maintaining adequate turbulence is a technically demanding task in large commercial ponds. One purpose for inducing turbulence, in addition to effecting a proper light-regime, is to remove oxygen evolved during photosynthesis. In relatively small ponds, where high flow rates can be maintained, the oxygen concentration can be kept at levels no higher than 200% of saturation. In large ponds, where the water flow is relatively low (10 to 20 cm s^{-1}), the O_2 concentration may reach as high as 500% of saturation when high photosynthetic rates exist. High concentrations of O_2 inhibit photosynthesis and growth and may sometimes lead to a total loss of the culture, but not all species respond in a similar manner to elevated oxygen tension. The ill effects of high O_2 tension in the pond have not yet been quantitatively evaluated. Nevertheless, it seems safe to assume that strains tolerant to high oxygen concentrations may be isolated from strains existing in nature in addition to induced mutants.

3.5 Diurnal fluctuation in temperature

Many arid zones are suitable for algal mass cultures, since they offer high temperature, high light intensity and large expanses of land. As a rule, however, diurnal fluctuations in temperature of more than 20°C are

common. During the morning, when irradiance is high enough to support intense photosynthesis and high growth rates, water temperature in large ponds is still significantly below air temperature and could be much below the optimum for growth. Only at midday in summer does the rise in temperature approach the optimal for *Spirulina* growth. Strains with a wide optimum temperature range for growth would be advantageous in outdoor cultivation and closed systems must be preferred for *Spirulina* in many regions. The temperature of the culture at night is yet another very important factor in determining the net output rate of biomass. In itself, the effect of temperature on cell respiration is, of course, very well documented, but the magnitude of loss in biomass due to temperature-induced dark respiration outdoors is not sufficiently understood, recent evidence indicating it has been greatly underestimated.[36, 37]

3.6 Sensitivity to high osmoticum

Evaporation rates of 1 to 2 cm day^{-1} are quite prevalent in large open-channel raceways in arid zones. In 15- to 20-cm deep cultures operated in a continuous mode by recycling the culture medium, this evaporation leads to a continuous increase in the salt concentration of the medium. In the course of two months the salinity may become twice the original concentration, effecting an osmotic stress. Although we have demonstrated that *Spirulina* can be grown under elevated NaCl concentrations without a significant reduction in photosynthetic oxygen evolution,[38] a reduction in the output rate can be expected owing to elevated activity of dark respiration. Thus an algal strain well adapted to increasing osmoticum without a significant increase in its respiratory activity will be advantageous for mass cultivation.

4 ECONOMICS AND FUTURE PROSPECTS

Economic evaluation

Many attempts have been made to establish the cost of production of algal biomass produced on large scale.[19, 39] Rather than present another economic calculation for the production of *Spirulina*, we elaborate on the economic basis of commercial production plants and point out the reasons for the large discrepancy between the actual cost of production and the cost predicted according to some of the early calculations.

The main difficulty in attempting to evaluate the economics of existing commercial production plants stems from lack of information owing to commercial secrecy. We estimate that in none of the intensive production sites (Thailand, USA, Japan, Mexico) is the cost of production, not including capital cost, lower than US$10 per kg of spray-dried *Spirulina* powder. Some of those estimates are summarized in Table 2. Manpower represents a major component of the cost amounting to 20–30% of the running cost. This reflects one of the main difficulties in attempting to cut down costs. Even in the biggest production sites in the USA, consisting of ten 0·5-ha ponds, the total production area is too small to realize significant reduction of manpower per unit product. According to some published analyses, the minimal economic plant size is no less than 10 to 100 ha, consisting of 1 to 10-ha ponds. Such large production sites have yet to be constructed and tested, and hence the question concerning the effect of size of production on the cost of unit product remains open. Another open question is the effect of increased supply at reduced cost on the consumer's demand.

In addition to labor, a major component in the cost of production is the cost of nutrients, particularly carbon, varying from 15% to 25% of the total operation costs. The main reason for this high cost is the relatively low efficiency in the conversion of the nutrients to algal mass. When nitrogen is supplied in the form of liquid ammonia a significant part of it may be lost to the atmosphere as ammonia gas due to the high pH (*c.* 10·0) used for the cultivation of *Spirulina*. This problem is avoided when NO_3^- is used as the nitrogen source, but nitrate is significantly more expensive than ammonia. Loss of nitrate through denitrification may take place particularly in large ponds with low mixing rates, in which anaerobic pockets may occur in still corners. We calculated a loss of up to 50% of the nitrate in one commercial plant. The carbon requirement

TABLE 2
Cost of Production of *Spirulina platensis* Biomass[a]

Site	*Cost (US$)*	*Comments*
Siam Algae	10	for spray-dried, food quality product
Earthrise	13	based on annual production of 60 tonnes
Sosa Texcoco	5–8	sun-dried or drum-dried
Israel	15–20	based on small production sites 0·1–1 ha

[a]Cost estimates not including investment and capital.

is usually supplied as CO_2 following an initial supply of 0·2 M sodium bicarbonate in the commonly used Zarrouk's growth medium.[40] The highest efficiency of CO_2 conversion into biomass obtained in commercial reactors of *Spirulina* is in the range of 80%. This figure is high and can be obtained in relatively small operation units (0·1–0·2 ha), in which an exact monitoring and control systems exist. In most of the production sites, where one carbonation system is used for large ponds (0·3–0·5 ha), lower efficiencies are obtained. When growing *Spirulina* at a pH level higher than 9·6, some of the carbon requirements are met by atmospheric CO_2.[41] The overall low efficiency of nutrient utilization currently obtained also stems from a lack of know-how concerning the use of recycled medium for long periods of time. Depending on the production site and local experience, the medium must be changed completely 3 to 6 times a year in order to sustain production and to avoid deterioration of the culture. At least in one case, reuse of this 'low quality' medium for so called 'feed grade biomass' is practised. Clearly, more information concerning recycling of the culture medium may result in less frequent changes of expensive nutrient medium being required, cutting nutrient cost significantly. The cost of energy represents in most cases some 15% of the operational cost, reflecting the requirement of power for mixing, pumping, and drying. A breakthrough in economic utilization of solar and/or wind energy would have to take place for this cost to decline significantly. There is no doubt that, in order to make *Spirulina* biomass a widely used commodity, the cost of production must be reduced to the range of 2–3 US$ per kg of dry matter. This figure will be attained only if much higher production rates, at least three times higher than those obtained in the present commercial plants, are achieved.

The economic analysis of low-technology endeavors, based on local resources, is far more complicated, as no exact figures for estimating the cost benefit of locally produced proteins are available. The cost of man-power may become irrelevant when considering social factors, such as added jobs, improvement in the standard of living and prevention of migration of small farmers to urban centers.

The future of algal biotechnology rests, to a large extent, on development of suitable reactors.[42] Closed systems have several advantages over open raceways. In closed systems, cultures are better protected from contaminants and thus the maintenance of monoalgal cultures should be easier.[43,44] Loss of water and the consequent increase in salinization of the medium is prevented. This mode of production opens the possibility of using sea water with low bicarbonate concentrations, thus saving on the cost of water and medium. Areal volumes may be kept much smaller,

due to much higher cell densities, reducing the cost of harvesting. Finally, an optimum temperature may be established and maintained more readily in closed systems, ensuring higher output rates. The latter is an essential aspect in the production of a micro-organism such as *Spirulina*, with a growth temperature of 37°C. Presently, however, no information exists concerning productivity of *Spirulina* in closed systems of commercial size.

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