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Extracellular Polysaccharides of Algae: Effects on Life-Support Systems

Abstract. *The amount of extracellular polysaccharide produced by eight species of green and blue-green algae ranges from 174 milligrams per liter to 557 milligrams per liter. Most of the polymers are composed of four monosaccharides: a hexose, a pentose, a methyl pentose, and uronic acid. The production of excessive amounts of these photosynthetic end products will undoubtedly influence the effective recycling time of growth media in life-support systems.*

In "life-support systems" where media will be recycled for the continuous growth of algae, algal end products will undoubtedly influence the efficiency of the recycling process. Few microorganisms have been shown to be capable of breaking down their respective polysaccharides for carbon and energy (*1*). Therefore, in an efficient medium recycling process, not only will it be necessary to replace depleted minerals but some provision will have to be made to remove the accumulated extracellular by-products. Accordingly, the selection of an alga to be used in a life-support system will depend not only on its efficiency in utilizing CO_2 and producing O_2 but also on the quantity and nature of its extracellular by-products under the cultural conditions employed.

Since the early 1950's, studies have been carried out to explore the possible use of algae as a source of food for overpopulated regions of the world (*2*). In more recent years, the use of algae in life-support systems designed for long space flights has been studied (*3*). These efforts were initially designed to study mass culture, efficiency of oxygen production, suitable substrates, and so forth. One of the basic problems now concerns the production of extracellular end products of algal metabolism and their influence on the growth-medium recycling processes which would be used in life-support systems.

At least three classes of organic compounds are known to be liberated by some species of freshwater algae: organic acids (*4, 5*), nitrogenous material such as polypeptides and free amino acids (*6*), and carbohydrate polymers (*5, 7, 8*). Lewin (*7*) has presented quantitative studies on the production of extracellular polysaccharides by 18 species of green algae isolated from soil samples. The yields of the extracellular polymer ranged from 3 to 113 milligrams per liter.

In this report we describe the production and composition of extracellular polysaccharides by eight species of mucoid green and blue-green algae used for studies on life-support systems. Quantitative data are also presented for capsular and water-soluble intracellular polysaccharide production.

Table 1. Yields of polysaccharide and dry weight of cells from eight species of freshwater algae. The cultures were bacteria-free, unialgal cultures isolated from samples of fresh water and oxidation-pond water.

Algal culture	Incubation temperature (°C)	EP* (mg/liter)	CP (mg/liter)	IP (mg/liter)	TP (mg/liter)	Cells (mg/liter)
Anabaena flos-aquae	40	557	13	126	696	1379
Nostoc sp.	40	415	15	23	453	1315
Palmella mucosa	25	271	36	196	503	2133
Chlorella vulgaris	25	235	34	75	344	3203
C. ellipsoidea	25	234	15	26	275	1959
Chlamydomonas sp.	25	224	19	62	305	1391
Oocystis sp.	25	197	22	48	267	2206
Chlorella sp.	25	174	15	26	215	1959

*EP, extracellular polysaccharide; CP, capsular polysaccharide; IP, water-soluble intracellular polysaccharide; TP, total polysaccharide.

Table 2. Increase in dry weight of cells and production of extracellular polysaccharide by *Anabaena flos-aquae*.

Time (days)	Extracellular polysaccharide (mg/liter, as glucose)	Cells (mg/liter)
2	25	268
4	44	323
6	50	1128
8	77	1203
10	138	1748
12	205	2068

Bacteria-free, unialgal cultures were isolated from samples of fresh water and oxidation-pond water gathered in the vicinity of this laboratory. The algae were cultured for 12 days in modified Knop's mineral medium (*9*), *p*H 7.0, contained in sterilized 2-liter Pyrex columns (48 mm in diameter). The cultures were maintained either at 25°C or 40°C in the presence of 13.4 kilolux of continuous light intensity supplied from a bank of white, 40-watt fluorescent lamps, and were aerated with a mixture containing 5 percent of CO_2 in air.

At 2-day intervals, samples were removed for the determination of polysaccharide in the cell-free medium and for determination of the dry weight. An appropriate amount of the cell-free medium was treated with 2 volumes of absolute ethyl alcohol, mixed, and centrifuged; the precipitate dissolved in 1 ml of distilled water, and the polysaccharide was determined as the glucose equivalent by the anthrone procedure (*10*). Dry weight was determined by drying overnight at 100°C.

At the end of the growth period, the cells were removed by centrifugation. The cell-free supernatants were concentrated to one-tenth volume with a rotary evaporator at 60°C, deionized with weak ion-exchange resins, and the extracellular polysaccharides were precipitated with two volumes of absolute ethyl alcohol. The stringy precipitates were collected in tared alundum crucibles and weighed after drying. The ash content of all extracellular polysaccharides, determined by combustion at 600°C, was subtracted to give yields of organic matter. The harvested cells were killed with 2 ml of a 2:1:1 mixture (by volume) of chlorobenzene,

dichloroethane, and chlorobutane, the capsular polysaccharides were extracted with distilled water at 10°C for 24 hours, precipitated with 2 volumes of absolute ethyl alcohol, and weighed. Intracellular polysaccharides (water-soluble) were extracted by subjecting the cell suspensions to sonic vibrations (frequency = 10 kcy/sec) for 15 minutes; the soluble protein and soluble pigments were removed with cold 10 percent trichloroacetic acid, and the polymer was precipitated with 2 volumes of absolute ethyl alcohol. For the determination of monosaccharide constituents of the extracellular polysaccharides, 50-mg samples were hydrolyzed with 4 ml of 1*N* H_2SO_4 in sealed ampules at 100°C for 6 hours. Barium carbonate was added to neutralize the H_2SO_4 to congo red and the $BaSO_4$ was removed by centrifugation. The hydrolyzates were then concentrated to 0.5 ml for chromatographic analysis. Portions of the hydrolyzates and standards were placed in spots on Whatman No. 1 filter paper and developed with a mixture of phenol and water (100:39 by weight), and a mixture of butanol, acetic acid, and water (2:1:1, by volume). Sugar spots were located and differentiated on the chromatograms with the aniline-oxalate mixture described by Horrocks and Manning (*11*). The tentative identification of the monomeric constituents of the polysaccharide was made by comparison of the unknowns to standard reference sugars.

The yields of polysaccharide in the various fractions and cells are given in Table 1. These strains produced much more extracellular polymer than those reported by Lewin (*7*). The range of extracellular polysaccharide production was from 174 mg per liter to 557 mg per liter. The yield of extracellular polymer was greatest (557 mg/liter), with the high-temperature blue-green alga, *Anabaena flos-aquae* (*12*). Another high-temperature blue-green alga, *Nostoc* sp., produced the next highest amount of extracellular polymer (415 mg/liter). Among the lower-temperature (25°C) green algae, the mucoid *Palmella mucosa* produced the most extracellular polymer (271 mg/liter). Yields with the other room temperature green algae (25°C) were slightly lower. The yield of capsular polymer did not deviate widely among the eight cultures tested, that is, 13 to 36 mg per liter. The yield of intracellular polymer was highest with *Palmella mucosa* (197 mg/liter), and next highest with *Anabaena flos-aquae* (126 mg/liter). The yields with all other cultures were somewhat lower (23 to 75 mg/liter). Synthesis of extracellular polysaccharide occurs more extensively than the accumulation of capsular or intracellular polysaccharides. Very little capsular polymer was expected to be produced because of the vigorous aeration of the cultures during growth.

Table 3. Monosaccharides detected in the hydrolyzates of the extracellular polysaccharides investigated. G, glucose; Ga, galactose; A, arabinose; X, xylose; R, ribose; Gl, glucuronic acid; F, fucose; Rh, rhamnose.

Algal culture	G	GA	A	X	R	Gl	F	Rh
Chlamydomonas sp.	X*			X			X	
Nostoc sp.	X		X			X	X	
Chlorella ellipsoidea	X		X			X	X	
C. vulgaris	X			X		X		X
Palmella mucosa	X		X			X	X	
Oocystis sp.		X	X			X	X	
Chlorella sp.	X		X			X		X
Anabaena flos-aquae	X			X	X	X		

* X indicates the presence of a particular tentatively identified monosaccharide.

With all of the cultures, the production of the extracellular polymer was dependent upon the age of the culture, the most significant increase being during the late log phase of the growth cycle. These results are in contrast to those reported for some species of marine algae in which the extracellular carbohydrate accumulated only after the cultures reached the stationary phase of the growth cycle (*13*). Polysaccharide production continues slightly after the cells reach the stationary phase of the growth cycle. The data for *Anabaena flos-aquae* are given in Table 2.

The large values for extracellular polysaccharide may have been the result of the vigorous aeration afforded in column culture. Conversely, Guillard and Wangersky (*13*), and Lewin (*7*) obtained much smaller yields from gently aerated flask cultures.

The monosaccharide components of the extracellular polysaccharides were hexose, uronic acid, pentose, and methyl pentose (Table 3). None of the polymers contained more than four components. The most common were glucose, arabinose, fucose, and glucuronic acid. Organisms which did not show this general pattern were *Chlamydomonas* sp, *Chlorella vulgaris*, and *Anabaena flos-aquae*. In the extracellular polysaccharides of the two green algae, (*Chlamydomonas* sp and *Chlorella vulgaris*), no uronic acid moiety was found. In the extracellular polysaccharide of the blue-green alga, *Anabaena flos-aquae*, no methyl pentose was found, and the polymer contained two pentose components, ribose and xylose. Hydrolyzates of capsular and water-soluble intracellular polysaccharides from *Palmella mucosa* and *Anabaena flos-aquae* yielded the same constituents as found in the extracellular polysaccharide of the respective alga.

The results of the extracellular polysaccharide screening tests designed to measure the amount of soluble extracellular carbohydrate polymers produced by certain algae used in studies of life-support systems show that these metabolic end products accumulate in the growth medium. The accumulation of these end products will undoubtedly decrease the effective use of growth media.

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