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Aquatic Biomass as a Source of Fuels and Chemicals

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NOTICE

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AQUATIC BIOMASS AS A SOURCE OF FUELS AND CHEMICALS

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ABSTRACT

The Aquatic Species Program (ASP) addresses the development of technologies that produce and utilize plant biomass species which naturally inhabit wetlands or submerged areas. Processes being developed through this program take advantage of the rapid growth rates, high yields, and extraordinary chemical compositions inherently associated with aquatic species. Emphasis is placed on salt tolerant species for cultivation on poorly utilized, low-value lands, where conventional agriculture is not economic. Candidate species are identified from 1) microalgae - unicellular plants that are natural factories for converting sunlight into high quality oils; 2) macroalgae - large, chemically unique plants that can be easily fermented to methane gas or alcohols; and 3) emergents - plants that grow rooted in waterways and bogs, but are partially exposed above water.

Within the next five years, the conditions and resources necessary for sustained systems operations are to be defined, design parameters examined, and experimental facilities developed. Succeeding years are planned to focus on resolving major technical hurdles in systems operations, integration, and component performance.

This paper updates the technical progress in this program, describes several aspects of evolving systems concepts, and attempts to provide some perspectives based on potential economics.

INTRODUCTION

Biological organisms have had millions of years to evolve and improve the apparatus and mechanisms for transforming sunlight into chemical energy. One result of this progression is that numerous plants now exist that are capable of producing a wide variety of chemicals, albeit correspondingly coupled with a wide variety of efficiencies. At this moment in time, plant species can be found that, in aggregate, can yield virtually every organic compound utilized by industry.

This broad capability of chemicals production is no where better represented than by aquatic plants, i.e. plants that in nature occupy wetland or fully submerged habitats. Aquatic plants are noted for their high levels of productivity and photosynthetic efficiencies, as well as the extraordinary range of chemicals they produce. For example, research conducted at, or through the sponsorship of SERI has shown that microalgae are capable of producing a variety of oil compounds, including hydrocarbons and mono-, di-, and triglycerides having fatty acid moieties ranging from C_{10} to C_{38} in chain length, in saturated or unsaturated forms, cyclic, branched, or straight chain, attaching or devoid of linkages with oxygen, with these characteristics dependent upon species and/or culture conditions. Other aquatic microorganisms like the archaebacteria synthesize di- and tetra ethers of various polyols like glycerol; photosynthetic bacteria like *Rhodomonas* produce hydrogen directly while decomposing organic substrates, and acetogenic bacteria effectively degrade complex organics to simple chemicals such as acetate, formate, and propionate. Larger aquatic plants, such as seaweeds, water hyacinth, bulrushes and cattails, are excellent sources of carbohydrates and specialty derivitives such as agars, alginates, thickening agents and fibers.

Aquatic plants are credited with extraordinary productivity capabilities. Shelef and Soeder (1980) note that 55-95 tons $ha^{-1}yr^{-1}$ are within the productivity range commonly observed for microalgal species. Andrews and Pratt (1979) reported 40 dry tons $ha^{-1}yr^{-1}$ for cattails having only a 5 month growing season. Neushul, et al. (1981) recently demonstrated productivities as high as 66 dry tons $ha^{-1}yr^{-1}$ for the giant kelp *Macrocystis pyrifera*. Similarly, Ryther (1982) reported year round sustained production at 82 tons per $ha^{-1}yr^{-1}$ for

Gracillaria. These compare with 20-50 dry tons $ha^{-1}yr^{-1}$ for some of the most productive terresterial crops known, corn and sugar cane (Kresovich, et al. 1981).

A further characteristic that lends to the attractiveness of aquatic plants is their capacity to proliferate in areas and under conditions not suitable for growing conventional agriculture crops. Many species tolerate, in fact require, waters having salinities in excess of what can be used for conventional farming. This is an all too well recognized fact in the western and central United States where every milligram increase in irrigation water salinity above 700 ppm, causes the value of regional American agriculture to decrease by $472,000 annually. Halophilic aquatic plants are capable of reversing this trend by making beneficial use of saline waters. Species of microalgae exist that grow well in waters approaching salt saturation (between 4 and 5 molar salt), while simultaneously providing an output of protein, glycerol, and β-carotene (Ben-Amotz and Avron, 1980).

Large regional expanses, such as the American Southwestern Deserts, which have few population or industrial centers, little potable water, and hot arid climates, are ideal for aquatic plant production since land is readily available, inexpensive, and comparatively unproductive. Further, water limitations do not exist provided saline water can be used, such as in microalgal production. Figure 1 shows the major regions within the continental U.S.A. where significant reservoirs of saline ground water have been located. Figure 2 reduces and restricts this area to the arid Southwest, where average daily insolation equals or exceeds 450 cal cm^{-2} day^{-1}. Under this condition, the horizontal land area overlying saline reservoirs approaches 36 million hectares for which there is little competing present use, but which might be suitable for halophilic aquatic biomass production.

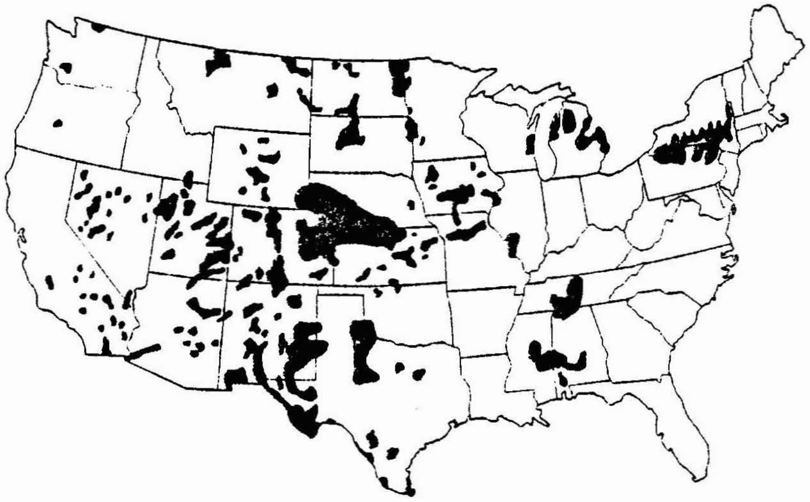

Fig. 1. Shallow saline ground water reservoirs (salinity ≃3,000 ppm) based on USGS report HA-199, 1965.

Thus, aquatic biomass appear to have significant potential for impacting and contributing to the fuels and chemicals markets on the basis of chemical composition, productivity, compatibility with available resources and lack of competition for water, land, and sunlight. With these advantageous, it is reasonable to question why industry has not responded to this technical challenge. The answer is based upon technical, logistic, and economic questions of significant proportion, sufficient to place development risks well beyond the capacity of the private sector to accept. It is towards providing these answers as well as practical solutions to technical hurdles that the SERI/Department of Energy Aquatic Species Program is directed.

The Aquatic Species Program

The Aquatic Species Program is guided by one overriding technical objective—to develop technologies that utilize aquatic plants for producing petroleum replacement products. The realization of this objective ultimately requires that biomass energy development be based upon the creation of systems, the external boundaries of which lie outside both biomass production and the processing of products in volumes responsive to market demands. Once conceptual systems are described, the R&D needs can be more specifically defined and evaluated with respect to their importance in achieving technical success, given the usual constraints of time and budget.

Figure 3 shows a conceptual system that might be considered applicable to aquatic plants in general, but which is specifically directed to the microalgae. Each major system component—production, harvesting, processing and conversion—is shown along with interdependencies in an overall scheme for converting sunlight into fuels, petrochemicals, feeds and specialties. Various R&D opportunities are identified which may lead to reductions in capital investment requirements and/or operating costs while concurrently improving efficiency.

Fig. 2. Locations of saline ground water reservoirs are illustrated for the American southwest. 36 million hectares of land overlays these subterranean deposits, and potentially could be deployed for production of halophilic plants.

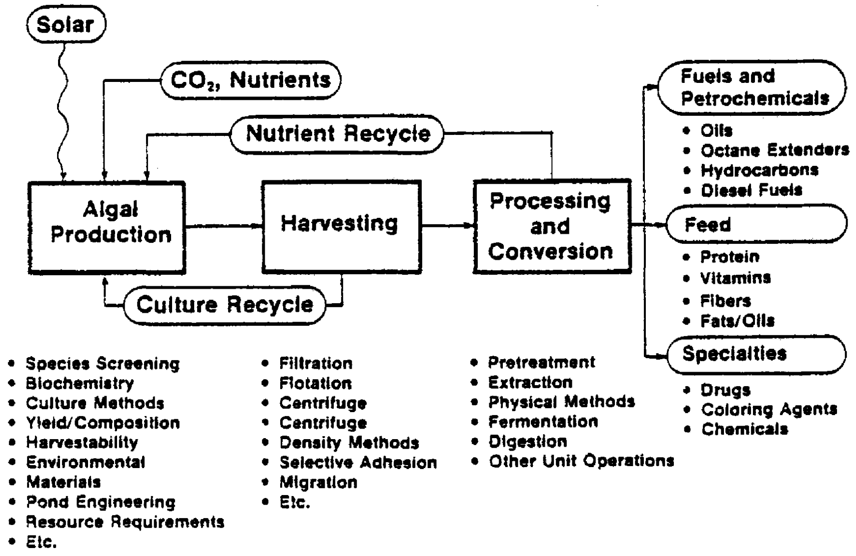

Fig. 3. A typical aquatic biomass energy system is illustrated, but emphasis is placed on microalgae.

This concept was modified recently with the objective to identify that set of R&D opportunities with the greatest combined potential for success and technology improvement for the research dollar. This is shown as Fig. 4. In this case, provisions for economies of scale, market size, supply, and demand conditions were included to allow the system to respond to its environment, rather than be independent of real world constraints. Specific cost data were gathered and employed when available; otherwise cost estimates were prepared utilizing a standard list of assumptions. These data were then varied over a range considered representative of the potential for technology improvement, both from a pessimistic and an optimistic view. This analysis indicated that improvements in biomass yield and reductions in capital investment costs were the two most important general parameters to address for the purpose of expanding the ability of microalgal products to compete on the opened market.

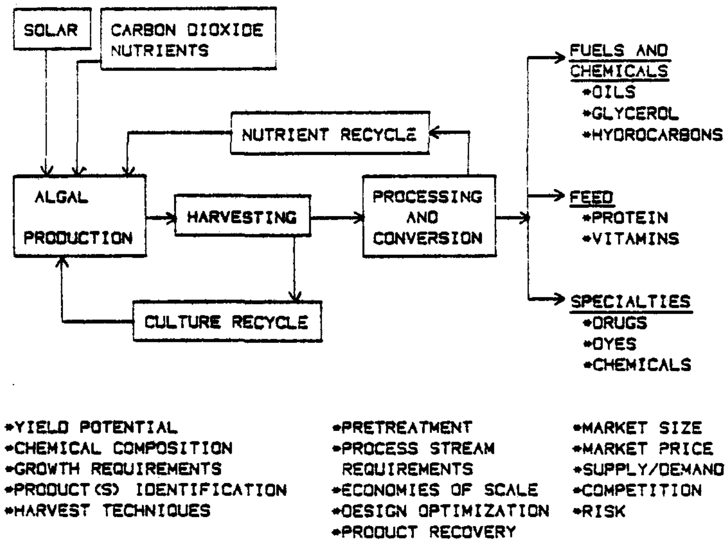

Fig. 4. Overview of microalgae production systems as seen from an economic perspective. This places the system in a real world context.

This analysis was extended to establish performance goals for research seeking to improve yield and capital investment. Historical and theoretical data were assembled to bound achievable yield and capital requirements. These limits were 23-168 dry tons ha^{-1}yr^{-1} (corresponding to easily realizable production utilizing present technology and optimistically attainable; respectively) and \$5.30 - \$32.20 m^{-2} capital investment. Constraints due to operating costs, processing costs, and return on equity requirements, indicated that yields approaching 85 dry tons ha^{-1}yr^{-1} were required simply to breakeven in a \$500 per ton market. Since \$500 per ton^{-1} is near the value for large volume chemical markets, and 85 dry ton ha^{-1}yr^{-1} yields is near the upper limit of state of the art productivity, yields must be improved before microalgal products can enter the chemicals market.

Even maximal yield and minimal capital investment do not permit entry into the fuels market. The reason is simply that operating and processing cost requirements are higher than the present market price of fuels in the United States. This observation leads to at least two possible conclusions, that microalgal systems may never impact the fuels market, or that multiple products of differing value must be derived from microalgal systems so that the average revenue obtained per dry ton of algae approaches \$500.

The quantitative result of this analysis was the preliminary base from which an initial set of performance goals could be drawn. Yields approaching 135 tons ha^{-1}yr^{-1} and obtained from facilities having capital costs less than \$10 m^{-2} are targets for R & D to strive for if microalgae are to meet the program objective.

This attempt to obtain preliminary assessments of microalgae production indicates that low capital costs and high yields of algae may be required to penetrate the bulk fuels and chemical market. Other process improvements may help to reduce the need for the low capital cost and high yield. While it is expected that data refinements will help identify other improvement requirements and provide more realistic cost estimates, a reasonable basis has been provided for specifying objectives for this program area. These are:

- Develop microalgal energy systems that yield multiple products.
- Reduce the costs associated with maintaining high productivity.
- Define the design parameters required to sustain productivity at the 135 dry tons per hectare level.

These broad objectives are sought through carefully coordinated and focused R & D in the following four areas:

1) Selection of species to provide maximum yields of biomass of desired compositions. Species are selected according to data comparing growth rates, light requirements (both intensity and wavelength responses), temperature tolerance, nutrient demands, salinity and pH limitations, as well as chemical composition, which may be controlled through the manipulation of the chemical and physical culture environment.
2) Definition of cultivation and resource requirements. This entails defining the contributions of production system design parameters to productivity, as well as identifying and matching the physical and chemical resources required for sustained operations.
3) Development of processing technology. This involves harvesting, fractionation, and conversion of biomass into fuels and high-energy chemicals.
4) Systems integrations. This requires the definition and joining of components required for fully functional, practical systems.

Program Status

Research conducted at, or through the sponsorship of SERI/DOE has concentrated on these four main areas. When this research began, the state of the art focused on producing meso or thermophilic green and bluegreen microalgae with a primary view on world protein production. While aspects of productivity and protein content typical of vegetative and expontentially growing microalgae provided inputs to this effort, concerns were not openly expressed regarding high nucleic acid concentrations and incomplete amino acid profiles of widely cultivated species like Chlorella and Scenedesmus. Spirulina, however, could be regarded as an exception, having a documented protein value approaching egg-yolk (Durand-Chastel, 1980).

Regardless of intended end-use , no fully autotrophic outdoor production system had achieved the productivity levels estimated to be required for entry into the markets to be addressed by the SERI/DOE Aquatics Species Program (see Table 1). Clearly, advances had to be made in cultivation technology; the list of suitable species needed to be expanded, and techniques had to be identified for maintaining desired chemical composition. Considerable advances can now be reported in all areas.

TABLE 1 Yield Performance Standards for Microalgae Production in 1979

Location	Species	Productivity Metric Tons $Ha^{-1}Yr^{-1}$	Reference
South Africa	Chlorella sp.	68.5	Toerien and Grobbelaar, 1980
Taiwan	Chlorella ellipsoidea Chlorella pyrenoidosa	109.6 (mixotrophic)	Kawaguchi, 1980
India	Scenedesmus acutus	73-91	Becker and Venkataraman, 1980
Mexico	Spirulina maxima	36.5	Durand-Chastel and Clement, 1975 Durand-Chastel, 1980
Israel	Spirulina platensis	60-80	Richmond, et al. 1980
Thailand	Scenedesmus acutus	55	Sinchumpasak, 1980

1) Species Selection

Microalgal species are collected, cultured, and screened for oil production using a SERI developed rapid screening technique. Three new oil producing species have been identified, two Chlorella strains and a Scenedesmus, the latter containing hydrogenase in addition. The goal for FY 83 is to develop a resource pool of 80 species. Experiments have been initiated to explore the biosynthetic regulation and control mechanisms affecting lipid production in macroalgae.

Under subcontract with SERI, the University of California, San Diego has been characterizing marine phytoplankton responses during period of nitrogen sufficiency and nitrogen starvation (Thomas, 1982). Using dense thin cultures, various algal species have been screened for their lipid, protein, and carbohydrate content. Both nitrogen deficiency and light intensity effects have been assessed. The highest yield (21.5 gm dry weight m^{-2} day^{-1}) and efficiency (12.2%) was obtained with Phaeodactylum at a light intensity equaling 39% of maximum sunlight at La Jolla, California and N sufficiency. Lipid and protein yields were 5.62 and 13.0 gm dry weight $m^{-2}day^{-1}$, respectively. Dry weight, lipid, and protein yields translate to 68.3, 17.7, and 40.9 metric tons $ha^{-1}yr$. Comparative higher plant yields average 7.3 gm dry weight $m^{-2}day^{-1}$. Increased light intensity or cell density did not increase yields or efficiencies. Nitrogen limitation increases Phaeodactylum lipid content from 20% to 30% of the dry weight, but lipid yield is reduced (to 2.39 gm m^{-2} day^{-1}) because of low overall dry weight yield. No other species (Dunaliella, Monallantus, Tetraselmis, Isochrysis or Botryococcus) gave as high yields (dry weight, lipid or protein) or efficiency as Phaeodactylum. Nitrogen deficiency did not increase lipid content in any of these other species and, in Dunaliella and Tetraselmis,carbohydrate, rather than lipid, levels were greatly increased by deficiency. Table 2 provides comparative data on the yields and efficiencies for these species.

2) Culture and Resource Requirements

Under subcontract with the University of Hawaii, a 50 m^2 outdoor (Laws, 1982) experimental algal production outdoor raceway (APR) has been designed, constructed, and tested. Nutrient, temperature and lighting requirements were established through laboratory experiments, using Phaeodactylum tricornutum as test species. The APR has been operated, achieving cell densities up to 42 million per millimeter, at specific growth rates of 0.35 to 0.45 per day. Chemical composition is 25% lipid, 55% protein, and 20% carbohydrate. Photosynthetic efficiencies of approximately 15% PAR have been obtained. Five $8m^2$ raceway units have been designed and are currently being constructed. These units will be used to study the effects of variations of design and performance parameters. Established values will be incorporated into the $50m^2$ system which will be operated continuously for at least six months to test sustained yield performance.

TABLE 2 Yields and Photosynthetic Efficiencies of Several Microalgae at Moderate Light Intensities and Nutrient Sufficiency

Alga	Yield (gm dry wt. m^{-2} day^{-1})	PAR* Efficiency (%)	Lipid yield (gm m^{-2} day^{-1})	Protein yield (gm m^{-2} day^{-1})
Phaeodactylum				
(Batch culture)	21.7	12.2	5.62	13.0
(Continuous culture)	16.2	6.2	3.22	9.5
Dunaliella				
(Batch culture)	8.7	3.5	—	—
(Continuous culture)	12.0	3.8	2.59	8.4
Monallatus				
(Batch culture)	7.1	4.0	1.42	2.8
Tetraselmis				
(Batch & cont. cult.)	1.3	7.6	4.48	—
Isochrysis				
(Batch culture)	6.7	2.8	1.77	—
Botryococcus (Batch culture)	4.0	1.7	1.20	—

*PAR = Photosynthetically active radiation, or light energy between wavelengths of 400 and 700 mm.

Performance projections based on current data were presented by Laws (1982) as follows:

- The optimum efficiency of solar energy conversion in the system will probably be about 10%.
- The optimum obtainable yield will probably be about 40-50 g dry weight m^{-2} day^{-1}.
- The optimum lipid and protein production rates from P. tricornutum will probably be about 8-10 g m^{-2} day^{-1} and 16-20 g m^{-2} day^{-1} respectively.
- The energy yield from the system could be as much as 250-300 kcal m^{-2} day^{-1} if the entire product were combusted. The energy yield from the lipid would be about 70-90 kcal m^{-2} day^{-1}, equivalent to 170-220 bbl $ha^{-1}yr^{-1}$ of crude oil.

3) Processing Technology

A subcontract is in force with the Georgia Institute of Technology to perform chemical analyses of microalgae, conduct studies of the effects of cultivation conditions on chemical composition, and identify and evaluate process techniques for lipid extraction and recovery. Tornabene (1982) recently provided a detailed analysis of the halophilic microalga Neochlorisis oleoabundans cultivated in mineral medium deficient in nitrogen. The yield of oily lipids was 35-45% of cell dry weight. Triglycerides comprised 80% of the total lipids. Aliphatic hydrocarbons, sterols, pigments, glycolipids and phospholipids comprised the remaining lipid fraction. Saturated, monunsaturated and diunsaturated octadecanoic acid represented approximately one-half of the total fatty acids. Detailed characteristics are provided in Tables 3 and 4.

Directions of Future Research and Development

The long-range goal of the Aquatic Species Program is to identify and to research innovative biomass energy systems that have significant potential for meeting continued national requirements for fuels and chemicals. Recognizing the high risks associated with the development of such options, the program has elected to pursue a dynamic strategy that can respond to projected fluctuations in product supplies, market demands, national needs, and new technical developments. Close associations and coordinated activities are established and maintained with industry, other research organizations, and governmental agencies to obtain the information needed to guide research and to encourage timely transfer of research results.

Analyses performed to date have indicated substantial advantages to biomass energy systems conceived to operate in underutilized areas, such as arid lands, marginal lands, and wetlands, to utilize saline or otherwise nonpotable waters, and to have beneficial impacts on environmental resources. These factors eliminate or minimize land-use conflicts, competition for water, and difficulties in environmental degradation that could adversely influence the long-term operations of the systems. The program area is carefully focused to incorporate these factors, and make major improvements in the production and conversion of aquatic biomass into useful fuels and chemicals.

Major decisions will need to be made as requirements are better defined for technical developments and sustained systems operations. In order for aquatic species, particularly microalgae, to produce oils, certain physical and chemical requirements must be met, including land, water, salts, nutrients, carbon dioxide, and sunlight. While it appears this can be achieved using arid lands that overlay shallow saline groundwater reservoirs, field studies must be conducted to verify this potential. Algal species, capable of growing and producing oils from these water supplies, need to be identified, characterized for product potentials, and selected. Appropriate systems must be technically feasible for algal mass culture. Each of these research areas is essential to the success of the program and will constitute major determining factors over the next five years.

TABLE 3 Characteristics of Neutral Lipid Components of N. Oleoabundans (From Tornabene, 1982)

Av. R_f Values	Eluates From Silic Acid Column					Probable Identification of Compounds
	Hexane	Benzene	$CHCl_3$	Acetone	MeOH	
0.88	0.7	—	—	—	—	Hydrocarbons
0.79	—	1.2	—	—	—	Steryl esters
0.69	—	0.2	0.1	—	—	Methy esters
0.59	—	—	73.5	7.1	—	Triglycerides
0.50	—	0.2	0.1	0.3	0.1	Pigment
0.48	—	0.3	0.1	0.4	0.1	Pigment
0.47	—	0.2	0.1	0.4	0.1	Pigment
0.46	—	0.6	—	—	—	Pigment
0.46	—	—	0.7	—	—	1,3 diglyceride
0.42	—	0.5	—	—	—	Pigment
0.42	—	—	0.1	—	—	1,2 diglyceride
0.39	—	0.7	0.5	—	—	Free fatty acids and free sterols
0.39	—	—	—	1.1	—	Pigment
0.30	—	—	—	1.1	—	Pigment
0.25	—	—	—	0.1	—	Pigment
0.18	—	—	0.1	—	—	Monoglyceride
0.00	—	—	—	1.3	8.0	Polar lipids

Data obtained from TLC developed with neutral lipid solvent A, R_f values for authentic lipids were found to be eicosane, 0.88; cholesteryl oleate, 0.79; methyl stearate, 0.69; Tripalmitin, 0.5; 1,3-dipalmitin, 0.45; 1,2 dipalmitin, 0.41; myristic acid 0.39; monopalmitin, 0.17. Values, expressed as percentage of the total lipids were determined with a recording Zeineh soft laser scanning densitometer.

TABLE 4 Fatty Acid Methyl Esters of Triglycerides of N. oleoabundans (from Tornabene, 1982)

Peak #	Fatty Acid Identity	Molec. Wt.	Area %	Position of Double Band
1	14:1	240	0.4	
2	14:0	242	1.6	
3	iso-15:0	256	1.0	
4	15:0	256	0.4	
5	16:2	266	2.5	
6	16:1	268	3.5	7
7	16:0	270	15.0	
8	iso-17:0	284	8.4	
9	C17:1	282	1.0	
10	17:0	284	3.3	
11	18:2	294	7.4	
12	18:1	296	36.0	9
13	18:0	298	11.0	
14	iso-19:0	312	0.5	
15	19:1	310	0.1	
16	19:0	312	0.3	
17	20:1	324	2.5	[illegible]
18	20:0	326	2.1	

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