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INCREASE OF LIPID YIELDS FROM SOME ALGAE BY ACID EXTRACTION

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Key Word Index—Algae; lipids; extraction; yields; acid extraction.

Abstract—The amounts of total lipids extracted from some but not all the algae examined were increased significantly by adding HCl to the usual chloroform–methanol extraction mixture. The yield of phospholipid fraction relative to the glycolipids and neutral lipids increased significantly with acid extraction. Acid extraction also increased the yield of phosphatidyl serine, fatty acids, chlorophyll (or its derivatives) and several unknown compounds.

INTRODUCTION

Addition of acetic or formic acid to cells prior to chloroform extraction increases the lipid yield [1]. Refluxing dried *Ochromonas* cells with HCl followed by extraction with ethanol also increases the yield of fatty acids [2, 3] and mild acid hydrolysis of yeast increases the yield of sterols [4]. During recent studies involving the extraction of lipids from 42 samples of 19 species of lyophilized algae we found that most of them yielded significantly larger amounts of lipids when extracted with chloroform–methanol to which HCl had been added.

RESULTS AND DISCUSSION

Table 1 indicates that many algae yielded significantly more total lipid if extracted with acid solvent rather than solvent alone. Tables 2 and 3 compare results of several extraction procedures on three lipid fractions of *Scenedesmus* sp. from Dortmund. In our *Scenedesmus* samples, total lipid yields obtained by using acidified solvent for the whole 8 hr of the extraction were lower (27%) than those obtained by 4 hr solvent extraction followed by 4 hr acidified extraction (34%). In a 24 hr extraction at room temperature the increase in lipid yields by acidified solvents was considerably higher than those obtained by Soxhlet extraction.

The observed increases in total lipid yields are mainly due to the release of additional polar lipids by the acidified extraction procedure. This release is further enhanced if acidified extraction follows solvent extraction. When a 4 hr solvent extraction was followed by a 4 hr acidified solvent extraction and the extracts were analysed separately, the second (acidified) extract was 2–6 times higher in its content of polar lipids than the previous one, with a concomitant decrease in the neutral lipid content.

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Table 1. Increased extraction of algal lipids by acid extraction

	Total lipids recovered (as % dry wt)			
	$CHCl_3$–MeOH*	$CHCl_3$–MeOH–HCl†	Total	% Increase due to acid extraction
Botryococcus braunii	44.4	8.6	53.0	19
Chlorococcum oleofaciens	18.3	3.3	21.6	18
Chlorosarcinopsis negevensis	17.0	26.0	43.0	153
Chlorella-Euglena‡	21.9	0.7	22.6	3
Cylindrotheca fusiformis	21.8	6.3	28.1	29
Fragillaria construens	3.3	12.7	16.0	385
Micractinium‡	14.0	3.4	17.4	24
Oocystis‡	11.3	8.6	19.9	76
Phaeodactylum tricornutum	11.6	8.3	19.9	72
Stichococcus bacillaris	26.5	5.4	31.9	20
Unidentified, pennate, marine diatom	18.2	10.6	28.8	58

* $CHCl_3$–MeOH, 2/1, v/v for 4 hr followed by:
† $CHCl_3$–MeOH + 2 drops 11 M HCl/100 ml solvent, for additional 4 hr.
‡ High rate sewage oxidation pond algae.

Table 2. Effect of extraction treatment on yield of total lipid and lipid classes from *Scenedesmus* sp.

Treatment	Total lipid (% cell dry wt) recovered	% of total lipid as: Neutral lipid fraction	Glycolipid fraction	Phospholipid fraction
8 hr Soxhlet without acid	24 ± 0	58 ± 1	32 ± 2	10 ± 1
8 hr Soxhlet; 4 hr without acid + 4 hr with acid	34 ± 3	43 ± 2*	41 ± 5	17 ± 7
8 hr Soxhlet with acid	27 ± 6	36 ± 5*	25 ± 2	40 + 6
24 hr without acid 25°	18	33	40	27
24 hr with acid 25°	29	23	22	55

* No free fatty acids or Me esters of fatty acids were noted in the neutral lipid fraction on TLC in petrol–Et_2O–HOAc, (90:10:1).

Table 3. Comparison of 4 hr Soxhlet extractions of *Scendesmus* sp.

Extraction procedure	Sample and average No.	Wt(g)	Lipid yield (g)	% of cell dry wt	% of total lipids: Neutral	Glyco-lipids	Phospho-lipids
4 hr no acid	1	1.42	0.310	21.8	46.5	34.2	19.4
4 hr acid following 4 hr without acid	2	1.42	0.126	8.8	7.6	12.7	79.7
4 hr acid	3	1.73	0.436	25.2	46.7	31.1	22.2

* Mean value for two samples.

In *Botryococcus braunii* samples, which were high in neutral lipids (73 % of total lipids) after 4 hr of extraction, no further increase in lipids could be detected by an additional 4 hr of acid extraction. The glycolipids fraction did not show any consistent trend. Similar increases in phospholipids were noted by Allen *et al.* [1].

TLC of equal volumes of the phospholipids from the *Scenedesmus* samples in Table 3 showed little difference between the phospholipids of samples 1 and 3 but 2 showed no phosphatidyl choline or phosphatidyl ethanolamine. Detailed TLC and spectroscopy of 2 indicated that this acid-extracted sample contained phosphatidyl serine, fatty acids, and chlorophyll (or its derivatives) as well as several unknown spots. No evidence for glycolipids (diphenylamine spray [8]) or choline-containing lipids (Dragendorff spray [8]) were found. Some of the spots giving positive ninhydrin and lipid reactions may be similar to the proteolipids isolated from brain tissue extracted with $CHCl_3$–MeOH–11 M HCl (200:100:1) [5].

EXPERIMENTAL

Marine and fresh water algae were grown in mineral media [6, 7] *in vitro* to the stationary phase of growth or large samples were obtained from algae grown in high rate sewage oxidation ponds in Israel or in large scale culture in the Federal Republic of Germany. *In vitro* algal samples were lyophilized. Sewage oxidation pond algal samples were drum-dried. All dried algal samples were stored in a freezer. Algae were extracted by refluxing for 4 or 8 hr in a Soxhlet extractor with $CHCl_3$–MeOH (2:1) alone or with 2–3 drops of 11 M HCl added to each 100 ml of extraction fluid or incubated in extraction fluid for 24 hr at room temp. with or without acid. Attempts to neutralize the acidity after extraction led to the production of large quantities of salt which interferred with further work and neutralization was discontinued.

Lipid extracts were reduced in vol. under N_2 and red. pres., redissolved in a known vol of $CHCl_3$ (usually 5, 10 or 25 ml) and aliquots taken for gravimetric determination by drying samples to constant wt at 55°. The lipid samples (with and without HCl) were fractionated by CC on silicic acid to give neutral, glycolipid and phospholipid fractions by the procedure of ref. [8]. Phospholipids were examined by TLC [8, 9] on Si gel G. Lipids were characterized by TLC with the following reagents [8]: Rhodamine G for lipids, I_2 vapor for unsaturated lipids, UV (360 nm) for chlorophyll and its derivatives, ninhydrin for free amino groups, phospray for phospholipids, diphenylamine for glycolipids and Dragendorffs for choline-containing lipids.

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