

FINAL REPORT

THE FEASIBILITY OF ETHANOL PRODUCTION FROM

THE CATTAIL TYPHA LATIFOLIA



Submitted to:

California Department of Food and Agriculture

June 1984

By

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Submitted to
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TABLE OF CONTENTS

BACKGROUND	PAGE 1
DESCRIPTION OF THE PHYSICAL AND EXPERIMENTAL DESIGN	PAGE 3
Project Location	PAGE 3
Energy Farm Layout and Harvest Procedures	PAGE 6
MATERIALS AND METHODS	PAGE 6
Harvest Procedures and Peak Biomass Determination	PAGE 6
Sugar and Starch Analysis	PAGE 8
Methane Production Studies	PAGE 8
RESULTS	PAGE 9
Peak Biomass Production and Harvest Strategies	PAGE 9
Free Sugar Content	PAGE 12
Starch Content	PAGE 14
Methane Production	PAGE 14
ALCOHOL AND METHANE PRODUCTION POTENTIAL OF TYPHA LATIFOLIA-	PAGE 15
ENVIRONMENTAL EFFECTS ASSOCIATED WITH TYPHA BIOMASS PRODUCTION	PAGE 16
MARKETABILITY AND COST EFFECTIVENESS OF TYPHA SP. BIOMASS PRODUCTION	PAGE 19
Market	PAGE 19
Cost Effectiveness	PAGE 20
CONCLUSIONS AND RECOMMENDATIONS	PAGE 21
BIBLIOGRAPHY AND REFERENCES CITED	PAGE 23
APPENDIX 1 - FREE SUGAR ANALYSIS METHOD	PAGE 25
APPENDIX 2 - STARCH ANALYSIS METHOD	PAGE 30

BACKGROUND

Freshwater marshes are typically formed by advanced eutrophication of lakes, ponds, river deltas and shallow low spots with poor drainage in areas of moderate to heavy rainfall. Because of the continual inflow of mineral and detritus-laden water from streams and other sources, marshes are seldom nutrient limited and are very highly productive. Since a large percentage of the food web is occupied by microbes with short life cycles, nutrient cycling is rapid. One acre of productive wetland fixes up to ten tons of nutrients per year, several times more than an average wheat field. These marshes are important ecologically in that they provide resting, feeding and breeding habitat for many species of animals. Through natural and man-made plant succession there is today only 475,000 acres of the original 910,000 acres of freshwater marsh present in California.

In addition to the expansive areas of wetland normally considered marshes, productive wetland ecosystems exist in such restricted but accessible areas as drainage ditches, waste treatment lagoons and ponds, canals and along the sides of rivers and streams. The plants often do so well that a great deal of energy is expended in removing them periodically to clear the ponds and canals. The plants are removed either chemically using herbicides or mechanically, then transported to land fills. In other areas where insects have become a problem because of the aquatic plant habitat, the plants are actually burned off at a high energy cost. When these aquatic plants infect man-made or man-maintained areas they are termed nuisance aquatic weeds.

One of the reasons that these aquatic plants are troublesome is because of their fast growth. This fast growth in turn creates a great deal of biomass. The biomass produced by wetland plants are a good source of fermentable and digestible products.

Freshwater marshes in California are characterized by several prevalent species of aquatic plants. These include emergents such as cattails, Typha sp.; Bulrushes, Scirpus sp.; various floating plants such as Lemna sp., Rorippa sp., Hydrocotyl sp., and submergents Potamogeton sp. and others. Typha sp. appears particularly hardy, for once it has gained a foothold in an area it will generally outcompete other species (Gearheart et al, 1983). For this reason Typha sp. is found in many diverse aquatic habitats. The fast and hardy growth exhibited by Typha sp. in addition to its relatively high fermentable carbohydrate and relatively low crude fiber makes Typha sp. appear ideal as an energy crop particularly for alcohol production. Therefore, it was proposed that Typha latifolia could be used for ethanol production with the intent of determining economic feasibility.

A proposal was submitted to the California Energy Commission (CEC) in response to RFP number 500-80-503 (Hull, Wilbur and Klingenspor, 1981). Under this \$46,153 proposal, 10 acres of artificial wetland would be planted, harvested and converted to ethanol and methane. The objective was to determine, at a demonstration scale, if Typha sp. could be used as an energy crop to be utilized to lessen the City of Arcata's dependence on petroleum. Unfortunately, the CEC Energy Farm Program was deleted from the commissions 1981-82 budget.

Before funding was deleted however, Arcata's proposal had been selected as a finalist and had been rated high by the commission staff. The proposal was rated so highly because "it proposed to produce high yielding marsh plants on relatively marginal areas in California with the dual benefit of marsh enhancement and energy production." The commission also concluded that "your project represented a unique opportunity to obtain data to determine the feasibility of producing marsh plants for conversion to fossil fuel substitutes."

These comments and recommendations were forwarded to the California Department of Food and Agriculture (CDFA) in the summer of 1981. At that time CDFA had a \$500,000 Biomass Farming Loan Program. This program was set up for loans and not grants although the possibility of a small grant would be considered. Thus, in the fall of 1981 a \$3,325 grant proposal was submitted to CDFA through the Humboldt State University Foundation (Wilbur et al, 1981). The proposal was accepted and funded in early 1982 and work began soon after. Because the project was funded at a level less than 10 percent of the original CEC proposal, many tasks and the scale of the project had to be greatly reduced. This report describes the results of the "Typhahol Project" (ethanol produced from Typha sp.) which was structured around the following objectives.

- 1) To determine the time of year that Typha sp. has its greatest biomass productivity,
- 2) to determine the time of year that Typha sp. has its greatest sugar and starch content and
- 3) to determine the feasibility of Typha sp. as an energy crop.

DESCRIPTION OF THE PHYSICAL AND EXPERIMENTAL DESIGN

Project Location

The Typhahol Project was located at the north end of Humboldt Bay in Arcata, California. Arcata is the second largest city in Humboldt County, with a population of 14,000. The largest city, Eureka, which is also the County seat, is located seven miles south on Humboldt Bay. The majority of Humboldt County's 110,000 people live within 25 miles of the Arcata-Eureka area. As shown in Figure 1, the Typhahol Project had very good access, including U.S. Highway 101, Highway 255, and Southern Pacific Railroad (now inactive). The City of Arcata operates its corporation yard and waste treatment facility within one-fourth of a mile from the Typhahol Project site therefore transportation of materials and equipment was minimal.

The climate in Arcata is wet and mild, averaging 57 inches of rainfall per year. The rain falls mainly from November through May. Summers are fairly rain-free but foggy. The mean annual temperature is 52 F. Seasonal daytime temperature variation is slight, ranging from a high of about 70 F to a low of about 30 F. It should be noted that with this type of weather pattern very few agricultural crops can be grown in this area. The few that do grow here, however enjoy a very long growing season.

The marsh used for the Typhahol Project was one of 12 artificial wetlands (Figure 2) created to test the effects of secondarily-treated wastewater on a freshwater marsh and also to examine the effect that these wetlands have on wastewater effluent quality. The marshes were constructed, maintained and monitored by the City of Arcata with funds from an EPA grant (Gearheart et al, 1983.)

The wetland used for the Typhahol Project was designated "Cell 10" and was chosen for this study because plant surveys indicated that Typha latifolia was the dominant plant species and was fairly evenly distributed throughout the marsh. Cell 10 was 200 feet long, 20 feet wide and 12 inches deep and was continually irrigated with wastewater at a rate of 4 gallons per minute. Cell 10 was completed in August, 1980 and has never been harvested although the vegetation was burned off at water level once in April, 1982.

Energy Farm Layout and Harvest Schedule

Cell 10 was divided into eight, 25 foot long, 20 foot wide plots (Figure 3). Each plot was given a designation of Plot 1-8 with Plot 1 at the head end of the marsh. Cell 10 was to continue having its water quality monitored to determine the effects of harvesting and resultant open water areas, thus it was decided to leave Plot 1 intact and harvest alternate plots beginning with Plot 2.

To meet the objectives of the Typhahol Project, several specific tasks were outlined. These included:

- 1) Literature search for appropriate methods of sugar and starch analysis.
- 2) Harvest different plots of the marsh at three times during the Typha sp. growing season and once during the dormant season.
- 3) Determination of sugar and starch concentrations from the four sample periods.
- 4) Actual alcohol production from Typha sp. feedstock.

MATERIALS AND METHODS

Harvest Procedures and Peak Biomass Determination

Although all of our harvests were done by hand our objective was to choose a specific harvest technique that could be applied to larger scale harvest and mechanization. We postulated that two techniques were possible for large scale Typha sp. harvest. These were:

Technique 1) Pulling up the entire plant much as a mechanical harvester does when removing corn stalks. The biomass recovered in this manner was termed "as pulled up" or "APU". This method would leave some roots and rhizomes intact on the bottom of the harvested Plot.

Technique 2) Mowing the leaves at water level (above water), skimming them off then raking the bottom to remove all roots and rhizomes (below water).

The three harvests completed during the growing season were June, 15; August 6 and September 22, 1982. The dormant season harvest was completed February 1, 1983.

There appeared to be advantages to both techniques thus at each harvest a portion of the plot was harvested by Technique 1 and at the September and February harvests by Technique 1 and 2. To assure samples of equal area a one-square meter quadrangle (Quads) was constructed and used during each of the harvests. Quads sampled by Technique 1 (APU) had all Typha sp. pulled up that was above the water surface. To determine the amount of biomass left

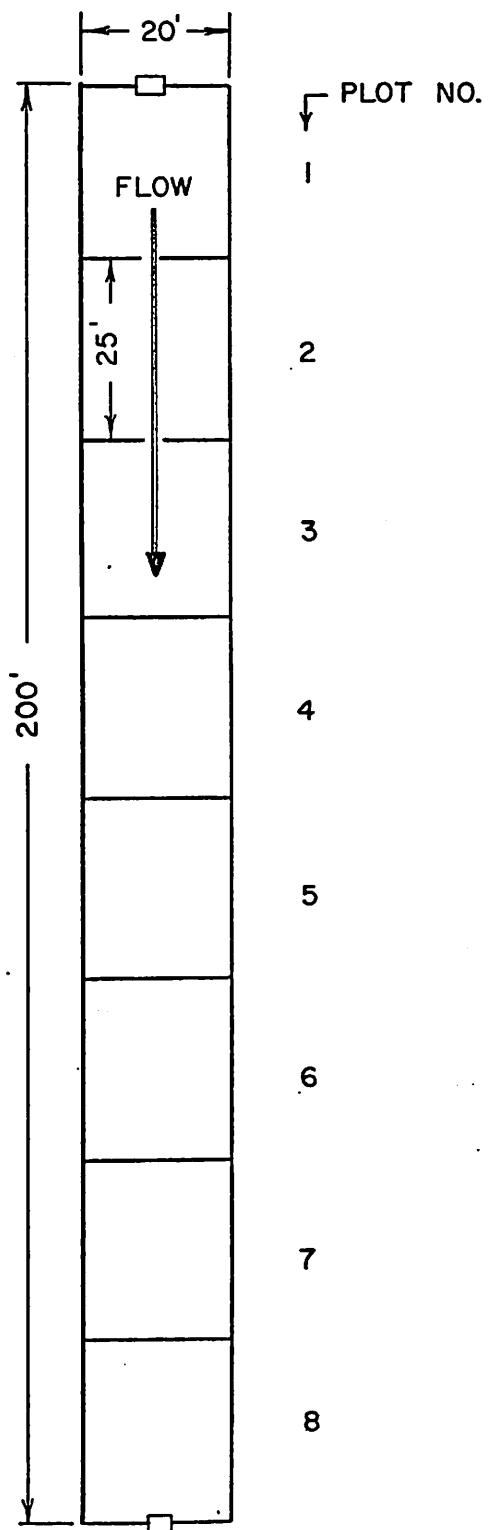


Figure 3: Typhahol Project site, Cell 10.

below water by this technique, all Typha sp. remaining underwater was also removed. Quads sampled by Technique 2 had all leaves cut at the water surface with hedge trimmers then all Typha sp. biomass remaining below water was removed. All quad samples were weighed wet then dried at 50-70 C for approximately seven days to remove all moisture. The dry weight was then recorded for each sample and used as the basis of comparison between quads and harvests.

Sugar and Starch Analysis

Samples of Typha sp. from each harvest were frozen to allow time to perfect our sugar and starch analysis techniques. The sugar analysis technique used for this project is the Somorgyi (1945) alkaline copper test (Appendix 1).

Three portions of Typha sp. were tested for free-sugar. These were leaves, stalks and rhizomes. Each of these portions were tested for each harvest in order to determine the time when free-sugar is at a maximum.

Kausch, et al (1981) indicated that Typha sp. rhizomes contain 27-45 percent starch by dry weight. Roots were found to contain 8-23 percent starch. There are several methods for quantitatively determining starch concentrations in plant tissue, few of which would give results applicable to alcohol production. The method we chose consisted of first measuring the free-sugar in the rhizomes, then enzymatically converting the starch to sugar, measuring free sugar again and finally, subtracting initial free sugar from converted free-sugar. The remainder would be the amount of starch that was (and could be) converted to useable sugar - and alcohol. Commercial enzymes from BIOCON and marketed under the names Hitempase and Gasolase were used in starch conversion tests.

Methane Production Studies

Anearobic digestion of Typha sp. to methane was studied because the production of methane can easily be interfaced with ethanol production to maximize energy yield and minimize external energy requirements.

Normally, ethanol production from biomass is limited in feasibility due to the high energy cost of distillation to fuel-grade alcohol. In order for distillation to be cost and energy efficient, a cheap abundant source of heat which is less suitable than the ethanol for the envisioned use (e.g. automotive fuel) must be used. Traditionally, wood has been suggested. This may be fine for very small scale production but the commercial use of wood heat to distill ethanol has many difficulties. It is difficult and expensive to transport, dirty to burn and not well suited to the precision required to efficiently distill alcohol. Solar heat is also considered feasible in some areas. In this case the high initial capital investment as well as the availability only during daylight hours on clear days severely limits its usefulness as a heat source for commercial-scale alcohol distillation in the Pacific Northwest.

Natural gas would of course be the fuel of choice were energy and cost efficiency not important. Natural gas is composed mostly of methane and ethane. An entirely acceptable substitute for natural gas is methane. For a commercial scale venture by the City of Arcata, methane generated in the

cities anaerobic sludge digestors could fire the still. Under design operating conditions, the cities anaerobic digestors generate much more methane than they use and the surplus is currently vented to the atmosphere. It is envisioned that the methane yield could be substantially increased further by the addition of Typha sp. biomass not suitable for ethanol production or perhaps even the spent stillage from the alcohol distillation to the digestors. This would be accomplished by increasing the carbon/nitrogen ratio to one which is more stoichiometrically correct for methane production. The capacity for extra biomass input already exists in the digestors and will be enhanced by the treatment plant upgrade scheduled for the summer of 1985.

Bench-scale testing on the suitability of Typha sp. as a digester supplement was completed by using small batch digestors and various combinations of Typha sp. and primary solids. The five combinations ranged from 100 percent Typha sp. to 100 percent primary solids and the tests ran for 510 hours or to the point where gas production slowed considerably. The resultant gas production was then recorded and compared with control of 100 percent primary solids.

RESULTS

Peak Biomass Production and Harvest Strategies

Table 1 shows harvest and dry weight biomass of Typha sp. per square meter and in kilograms per hectare. APU dry weights ranged from a mean of 4,945 grams/meter² in February to a high of 16,330 grams/meter² in August. Remaining below water biomass ranged from 2,540 grams/meter² in August to 4,221 grams/meter² in February. Dry weight of above water biomass harvested by Technique 2 ranged from 1,050 grams/meter² in February to 2,246 grams/meter² in September. Dry weight of below water biomass harvested by Technique 2 ranged from an average 3,362 grams/meter² in September to 8,586 grams/meter² in February.

The ability to predict dry weights from wet weights measured in the field may be a convenient tool for monitoring productivity. Table 2 shows the mean percent water content of the various harvest portions for the two harvest techniques. Our data shows that the average water content of Typha latifolia varies from 82 percent in the above water portions to 90.5 percent in the below water portions. The data in Table 2 and Table 1 can be combined to show that, for prediction purposes, APU dry weight equals 1.5 to 4.9 times the above water weight and below water dry weight equals 1.6 to 1.9 times the APU remainder weight.

Converting the data in Table 1 to a graphical form shown in Figure 4 shows that the peak Typha sp. standing crop occurred in August. Literature productivity values are based on dry weights of above water and below water samples (Technique 2). Morton, 1975 states that Typha sp. below water dry weight ranges from 6,739 to 65,144 kilograms/hectare. Jervis, 1969 noted that at peak standing crop Typha sp. above water dry weight was 15,700 kilograms/hectare. Our September data shows that our biomass production is 39 percent greater for above water and 17-91 percent greater for below water biomass. We attribute most of this increase in biomass to the nutrient laden wastewater which was used to irrigate the Typha sp. in this project.

Table 1: Mean wet and dry weights for Typha latifolia from four harvest dates.

HARVEST	TECHNIQUE	PLOT NO.	\bar{X} grams/m ² (wet)	\bar{X} grams/m ² (dry)	\bar{X} kg/ha (dry)
June	1 (APU)	2	43,047	5,625	56,416
August	1 (APU)	4	71,125	16,330	163,296
	1 (Remainder)	4	24,041	2,540	25,402
September	1 (APU)	6	46,177	4,710	47,100
	1 (Remainder)	6	32,387	2,955	29,548
	2 (Above water)	6	17,985	2,246	29,548
	2 (Below water)	6	53,842	3,362	78,247
February	1 (APU)	8	30,663	4,945	49,452
	1 (Remainder)	8	34,508	4,221	42,213
	2 (Above water)	8	4,445	1,050	10,500
	2 (Below water)	8	67,042	8,586	85,859

Table 2: Mean percentage water in various harvested portions of Typha latifolia.

HARVEST	TECHNIQUE	PORTION	\bar{X} % WATER
1	APU		86.9
1	Remainder		89.4
2	Above Water		82.0
2	Below Water		90.5

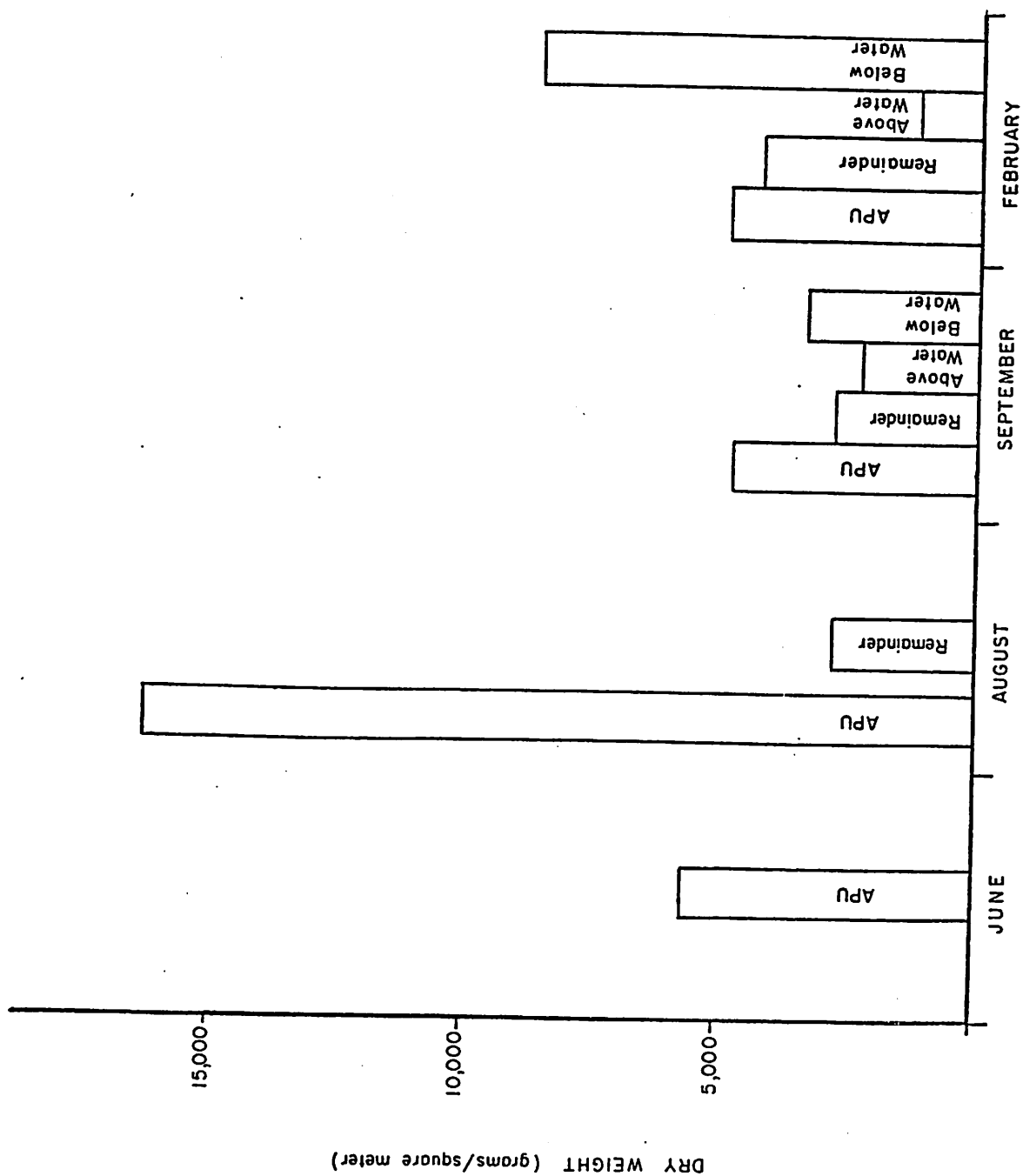


Figure 4: Dry weight biomass of various harvest portions of *Typha latifolia*.

Free Sugar Content

Table 3 shows the percent of sugar in Typha sp. leaves for each harvest. Percent leaf sugar ranged from 0.02 percent in February to 0.2 percent of wet weight in August. September leaf harvest of 205,032 kilograms/hectare wet weight at 0.1 percent sugar equals 205 kilograms of sugar per hectare. Comparing Table 3 with Figure 4 shows that maximum leaf sugar correlates with peak biomass standing crop. No sucrose was found in any of the leaf samples.

Free sugar was present in the cattail stalk samples from all four seasonal samples and ranged from 0.007 to 0.29 percent of the wet weight (Table 3). Peak sugar concentration was found in the September samples while the lowest stalk sugar concentrations were found during the June harvest. Average stalk and leaf sugar values were almost identical for August samples.

Table 4 shows the percentage of total sugar recovered from stalk samples that was glucose and sucrose. Sucrose ranged from 0 percent in June to 37.9 percent of the total sugar present in the September harvest samples.

Free sugar was also found in all root/rhizome samples. Peak sugar concentrations were found to be 0.28 percent of the wet weight and occurred in September. Lowest values were found in August samples at concentrations of 0.07 percent of the total wet weight.

Sucrose ranged from 0 to 94 percent of the total sugar recovered in the root/rhizome samples. Table 4 shows that 94 percent of the sugar recovered in June samples was sucrose while no sucrose was present in August samples. September and February samples showed that greater than 50 percent of the total sugar present in the roots/rhizomes was sucrose.

Table 3: Percent free sugar in Typha leaves from four harvest periods.

HARVEST	% FREE SUGAR		
	LEAVES	STALKS	ROOTS/RHIZOMES
June	0.04	0.007	0.21
August	0.20	0.17	0.07
September	0.10	0.29	0.28
February	0.02	0.11	0.25

Table 4: Percentage of the total free sugar present in sucrose and glucose by plant portion.

	% SUCROSE	% GLUCOSE
LEAVES		
June	0	100
August	0	100
September	0	100
February	0	100
STALKS		
June	0	100
August	17.6	82.4
September	37.9	62.1
February	30.0	70.0
ROOTS/RHIZOMES		
June	94.0	6.0
August	0	100
September	50.2	49.8
February	79.1	20.9

Starch Content

Using the enzyme technique we were only able to recover 25 percent of a starch standard and only 1-2 percent wet weight in winter Typha sp. rhizomes - much lower than the literature values. Another method was selected to determine starch content of the Typha sp. rhizomes. The Anthrone method, described in Appendix 2, appeared to be acceptable however funding limitations made the use of this method impossible. Steve Schaffer, CA Department of Food and Agriculture offered the services of his lab to determine the starch content of the rhizomes. Typha sp. samples were sent to his lab however an inappropriate method was used and the results obtained were questionable. The remaining Typha sp. samples were also sent to the Food and Agriculture lab along with a copy of the Anthrone method. The Department of Food and Agriculture also has budget limitations thus as of this writing the samples remain in their possession awaiting funding to complete the analysis.

Methane Production

Table 5 represents methane gas production by weight and volume for five batch digesters used in this study to assess methane production potential of Typha sp. It also represents the percent difference with the control digester which was composed of 100 percent primary solids.

Table 5: Results of batch digester methane study.

Digester	Volume		Weight		Gas Production		Percent Increase	
	Percent Typha	Percent Sludge	Total (lb.)	Ash (lb.)	Volume (cu.ft./L)	Ash Weight (cu.ft./lb.)	Volume	Ash Weight
1	100	0	4.50	0.98	0.136	0.323	42.9	261.0
2	75	25	4.43	1.24	0.121	0.263	49.2	194.0
3	50	50	5.17	2.13	0.102	0.129	7.5	44.3
4	25	75	5.75	2.76	0.073	0.069	-32.8	-22.7
Control	0	100	6.03	3.33	0.126	0.089	-	-

ALCOHOL AND METHANE PRODUCTION POTENTIAL OF TYPHA LATIFOLIA

The amount of alcohol produced from a batch of mash depends on the concentration of sugar available to the yeast for conversion. The theoretical maximum yield of ethanol from glucose or sucrose is 51 percent by weight. The remaining 49 percent is theoretically composed of carbon dioxide. In practice, however, yeast converts sugars to a variety of compounds, not just ethanol and carbon dioxide. Therefore actual alcohol yield is always less than 51 percent of the weight of the glucose. A realistic yield is 40-45 percent in a well run batch fermentation which uses a good strain of yeast (Bradley and Drew, 1980). Forty-five percent yield was used for calculating the ethanol yields of Typha sp. based on the data presented in Table 3.

Harvest Technique 1 involved pulling up and processing the entire plant. Based on maximum biomass and maximum free sugar content, September would be the month to harvest Typha sp. by this technique in the Humboldt Bay area. Alcohol yields by this method would be approximately 68 gallons per acre or approximately that of rice (Table 5).

Harvest Technique 2 would allow the mowing of the leaves for processing then the removal of the stalks and rhizomes (below water biomass). Based on the data in Table 3, leaf free sugar content is at its peak in August, as is peak biomass production. If the leaves were mowed and processed in August, ethanol yields of 27 gallons per acre could be realized. This is roughly equivalent to the yields of sorghum cane (Table 5).

Table 3 shows that the stalks and roots/rhizomes have the greatest sugar content during September. If the below water biomass was harvested and processed for sugar only during September, yields of ethanol would be approximately 96 gallons per acre. At that yield, Typha sp. ethanol production approximates that of yams and raisins (Table 5). Combining leaf (above water) and below water yields, Technique 2 would yield 123 gallons of ethanol per acre per harvest season.

The real potential for producing ethanol from cattails lies in the conversion and utilization of the starch contained in the rhizomes. We have been unable to adequately convert the rhizome starch to sugar as of this date. Kausch et al (1981) data though, provides a conservative range of starch values which can be used to predict ethanol yields from converted rhizome starch. Kausch's data shows that cattail rhizomes contain 27-45 percent starch by dry weight. Using these figures and the dry weight below water biomass values for the September harvest the result is that cattail rhizomes could be converted to 556-920 gallons of ethanol per acre. Starch data from Morton (1975) suggests that 823-1,230 gallons of ethanol per acre are possible.

From the bench scale study it appears that the addition of Typha sp. biomass to the City of Arcata's digester may be a feasible disposal alternative for the excess biomass of low alcohol potential e.g. Typha sp. leaves and stalks. The methane production per pound of wet weight would enhance current gas production by as much as 49 percent. Although the figures for a 75:25 ratio of Typha sp. to sewage solids appears to be an attractive mix, it is impractical with regard to the current capacity of the digesters.

ENVIRONMENTAL EFFECTS ASSOCIATED WITH TYPHA BIOMASS PRODUCTION

The major environmental impacts arising in conventional production of biomass from an energy farm are erosion and the use of scarce resources such as water and land. In addition, the use of herbicides, insecticides and fertilizer can also have detrimental environmental effects.

A wetland energy farm has several unique characteristics which make the impacts from the above mentioned items minimal. To illustrate this point, each potential impact will be considered separately.

Erosion

The rate of erosion is dependent on soil type, slope, hydraulic loading and ground cover. In a wetland energy farm, the soil is generally a mixed loam with a clay base. The slope is negligible, and the ground cover would be a mixed marsh community, dominated by emergent vegetation. As a result, the marsh acts as a zone of deposition rather than erosion.

Water Usage

In addition to runoff, another source of water for a wetland energy farm is treated municipal wastewater. Studies such as Gearheart et al (1983) indicate that wetlands can be quite effective in treating wastewater in addition to increasing the productivity of the aquatic plants. The total amount of water lost to evaporation and transpiration depends on the climate. In the Humboldt Bay area, only about 10-20 percent is lost to evaporation and transpiration, which amounts to 5-7 inches per year. Evaporation is suppressed compared to evaporation pan studies and transpiration is increased over results of terrestrial plant transpiration studies.

Land use

Presently, California has essentially two general types of wetlands; those that are or could be valuable representatives of a diminishing habitat and those of less or little habitat value such as former wetland reclaimed for pastureland or drainage ditches that are cleared periodically. A wetland energy farm would only be feasible if established in the latter. This would protect those wetlands of high or potential value to wildlife and instead make use of lands too degraded, impacted or already in marginal agricultural production. Such agricultural land around Humboldt Bay is only slightly productive as pastureland because of the low elevation, poor drainage and past history as wetlands. These pastures can remain too wet for cattle to utilize them for months at a time. Therefore, in general a wetland energy farm utilizes areas of little habitat or economic value. If the City of Arcata continues research and eventually scales up an ethanol production facility, aquatic biomass removed from productive wetland areas during routine maintenance and ultimately the use of marginal pastureland specifically for Typha sp. production would be the sources of feedstock.

Herbicides

None are used, therefore no impact.

Fertilizer

While fertilization can increase production of Typha sp. biomass, because of the nature of the wetland habitat it is not necessary. Fertilizing a wetland energy farm with treated domestic wastewater results in both an increase in biomass as well as improved water quality. The City of Arcata will begin discharging treated wastewater into 36 acres of artificial wetland in the fall of 1985. Increases in biomass from the addition of wastewater should be similar to that seen at the Typhahol Project site.

Insecticides

One of the major drawbacks to a wetland biomass farm is the creation of habitat for both pest and nuisance species. The Department of Public Health and local Mosquito Abatement Districts spend large sums of money to control mosquitos and other diptera found in wetland areas.

There are three broad catagories of control practices: source elimination, chemical control and biological control. Harvesting the dense stands of emergent vegetation improves the effectiveness of all three methods. In many cases, the removal of vegetation during the peak emergence times enables natural predators to keep the mosquito population in check. It can be concluded that by properly harvesting aquatic macrophytes, the dependence on insecticides for mosquito control can actually be reduced or eliminated.

A wetland energy farm may only be feasible in certain areas of the State and not in others because of the insect problem. For example, in the Humboldt Bay area, wetlands composed of dense stands of Typha sp. and fertilized with wastewater show no more potential to produce mosquitos than any other local wetland. In the Sacramento or San Joaquin Valleys, where there are already great problems with mosquitos because of rice farming and the warm temperatures, a wetland energy farm composed of Typha sp. would undoubtedly require intense mosquito management.

Outline of Outstanding Features

- A. High potential net energy yield from a large amount of otherwise unused land.
 - 1) Doesn't use land which could be used to grow crops.
- B. No requirement for high quality water.
 - 1) Agricultural runoff (Typha sp. fairly salt tolerant)
 - 2) Municipal sewage
 - 3) Urban runoff
 - 4) Industrial waste - cannery waste
- C. Very low capital requirement for maintenance
 - 1) No herbicides
 - 2) Little or no fertilizer, depending on water source
- D. May be coupled with control of nuisance "aquatic weeds" and mosquitos
- E. May be managed to enhance wildlife habitat by prudent management and selective harvesting
- F. Adapability of aquatic biomass to a variety of uses
- G. Useful byproducts in addition to fuel may include animal feed, fertilizer

MARKETABILITY AND COST EFFECTIVENESS OF TYPHA SP. BIOMASS PRODUCTION

Market

One large market for automotive fuel-grade alcohol produced by the City of Arcata has been tentatively established. The Arcata Police Department has indicated an interest in converting some or all of the patrol cars to burn ethanol. The current gasoline consumption of the Police Department is approximately 30,000 gallons/year. This results in a demand for about 31,500 gallons of ethanol/year since average mileage is usually decreased somewhat. The city mechanic has attended a seminar on conversion of gasoline and diesel powered vehicles to ethanol use. Furthermore, it is envisioned that if successful in the police department, other city vehicles would be converted in proportion to the supply of ethanol.

Conversion of city vehicles to ethanol is practical because they all fuel at the same location in the corporation yard where the alcohol production facility would be located. Most city vehicles never leave the immediate vicinity so ethanol fuel availability would never be a problem.

In addition, a major industry in Humboldt County is dairy farming. Spent stillage were it not all used as digester supplement could be sold as a high protein feed supplement for cattle (Table 6 and 7). A number of local dairy farmers have expressed tentative interest in purchasing the stillage.

Table 6: Nutrient analysis¹ of Typha latifolia roots/rhizomes from August and September samples (in percent of dry weight).

COMPONENT	AUGUST	SEPTEMBER
Protein	1.45	1.30
Fat	0.168	0.102
Fiber	1.71	1.41
Ash	4.08	3.94
P205	0.19	0.16
K20	0.06	0.12

¹Analysis courtesy of CA Department of Food and Agriculture.

Table 7: Nutrient Analysis of entire Typha latifolia plants compared with alfalfa hay.

Plant	Crude Protien	Crude Fiber	Nitrogen Free Extract	Ash
alfalfa hay	16.91%	31.60%	40.55%	8.84%
<u>Typha latifolia</u>	3.2-12%	20-30%	50-60%	2.3-10%

Cost Effectiveness

The City currently pays \$1.05/gallon for unleaded gasoline, and has indicated a willingness to pay the same price for fuel-grade ethanol. In addition, the price for ethanol production is not expected to fluctuate as fast as gasoline prices, resulting in ever-increasing cost effectiveness and budgetary stability.

Since this project was experimental in nature, cost effectiveness cannot be determined simply by net income after expenses. Several expenses such as site preparation, planting, and construction of fermentation and distillation equipment are one-time expenses and their cost must be amortized over the expected lifetime of the project. David Blume of the American Home Grown Fuel Company has shown that fuel-grade ethanol can be produced for as little as \$0.60/gal when inexpensive heat is used and the by-product stillage is sold at market value. This value does not include labor, profit or overhead, but we feel that the margin is more than adequate. Since our biomass will initially simply be had for the harvesting, we expect to be able to match this price. This estimate is of course tentative, since the actual production of ethanol from Typha sp. has not yet been demonstrated on a commercial scale.

CONCLUSIONS AND RECOMMENDATIONS

This study has shown that cattails can be used as an energy crop which is especially important because they are normally treated as a nuisance and a wasted resource. When productivity is increased through wastewater addition, as with Arcata's system, not only is the resultant water quality improved but the cattails growth and biomass is increased 17-91 percent over that of cattails inhabiting a typical freshwater marsh.

In the Humboldt Bay area, cattails have typically been dredged from drainage channels, ponds etc. and trucked to landfills. This study has shown that at worst if cattails were completely harvested in September and processed for ethanol without any starch conversion, at least 123 gallons of ethanol per acre could be produced. With more work it is estimated that 556 to 1230 gallons of ethanol per acre could be produced if the rhizome starch were either chemically or enzymatically converted to sugar before fermentation.

This study has also given background data and ranges of the water content, harvesting technique, free sugar content of the leaves, stalks and rhizomes and productivity over four different harvest times, namely June, August, September and February. In general, it can be concluded that the average water content of cattails in the Humboldt Bay area is approximately 86 percent of the wet weight. Peak biomass was recorded in the August samples at 16,330 grams/meter² dry weight. Sucrose was the dominant sugar in the rhizomes while the leaves contained glucose exclusively. Approximately 25 percent of the cattail biomass occurred above water and the remaining 75 percent occurred below water.

Bench scale anaerobic digestions demonstrated that excess biomass of low alcohol potential, e.g. Typha sp. leaves and stalks could enhance methane production when added to primary sewage solids. When Arcata achieves a commercial scale of ethanol production it is envisioned that the leaves and stalks would be harvested, added with primary sewage solids in the City's anaerobic digester and the excess methane would be used to fire the ethanol distillation apparatus. Typha sp. rhizomes would then be used as the feedstock for ethanol production. Using different parts of the cattail for different stages of the alcohol production process allows for the minimum of production expenses and hence, lower cost per gallon of ethanol produced.

The City of Arcata, in its long commitment to sound environmental planning, energy and resource recycling, and self sufficiency, as a policy has a continued interest in utilizing excess biomass produced at its wetland wastewater treatment complex. When continued research is completed and the results are judged favorable and economically feasible, Arcata will consider dedicating land specifically for biomass production which would then be converted into fuel grade ethanol. The ethanol would then be used to power City vehicles thereby lessening Arcata's dependence on petroleum.

It is recommended that two areas of research be completed before the City of Arcata utilizes cattails as an energy source. This is not to say that Arcata should not consider utilizing another proven energy crop in the interim to begin the move to self sufficiency. A crop such as fodder beets could be irrigated with treated wastewater and grown for ethanol production. When the processes of efficient starch conversion and materials handling have been

researched and perfected the City could switch to cattails and undoubtedly increase the yield of alcohol. With the capability of potentially producing more alcohol per acre than sugar cane, the cattail may then make the switch from nuisance weed to high yield energy crop.

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Somogyi Micro Copper Method

Probably most used for general research work is the alkaline copper method developed over many years by Shaffer, Hartmann, and Somogyi (81-86). Copper reagents are more highly specific for sugars than ferricyanide or hypiodite (4, 85, 87, 88) and, therefore, are widely preferred for analyses of biological material. The main objection, back-oxidation by air, was reduced considerably when Somogyi added sodium sulfate to the combined alkaline copper reagent to repress entry of air (84, 85). However, air-oxidation is still the most important factor to be controlled, and variable autoreduction of alkaline cupric tartrate upon heating is a persistent, minor drawback (86, 89, 90). Nevertheless, the method has been widely and successfully applied on both milligram and microgram scales, titrimetrically and colorimetrically. Its accuracy over a wide range of sugar concentrations, ease and rapidity of operation, and proved reliability place it above other micro oxidation methods. In conjunction with chromatographic separations of unsubstituted reducing sugars, it is recommended in both the titrimetric (10, 12, 28, 80, 86, 88, 90) and colorimetric (4, 91-93) modifications.

Many different compositions have been proposed for the alkaline copper reagent, some for use with the iodimetric titration and some for use with the Nelson arsenomolybdate colorimetric reagent. To keep a clear distinction between the differently composed reagents, titrimetric and colorimetric procedures are presented separately.

Titrimetric Method

Several different alkaline copper reagents have been recommended for use in conjunction with the iodimetric determination of reduced copper. The principal modifications can be designated by the years in which they were introduced as the 1926, 1933, 1937, 1945, and 1952 reagents. The 1926 and 1933 reagents are still recommended for certain analyses (1, 11), but, in view of the shortcomings of the older reagents that have been discussed by Shaffer and Somogyi (83-86), there is little justification for their continued use, with the exception of the 1945 reagent.

Somogyi's 1945 phosphate-buffered reagent does possess an advantage over the 1952 carbonate-buffered reagent, in that amyloses are held in solution in the 1945 reagent but are precipitated from the 1952 reagent (94).

* From: *Methods in Carbohydrate Chemistry Volume I - Preparation of Sugars.* 1962.

The 1945 reagent apparently is better suited to determining relative molecular weights of oligo- and polysaccharides (95, 96). Sugar equivalents of the 1945 reagent will vary from operator to operator and from time to time (86, 89, 90, 97); therefore, sugar standards should be run frequently and preferably with each group of analyses. On the other hand, sugar equivalents of the 1952 reagent remain unchanged for several years (86). The following procedure applies to both 1945 and 1952 reagents.

Precision of the method is ± 0.01 mg. for D-glucose and maltose, or about $\pm 2\%$, averaged throughout the range 0.3 to 3.0 mg. of glucose. A variation that will determine 5 γ of sugar in plant tissue with a precision of $\pm 5\%$ has been reported (90). Most consistent results are obtained when the copper reagent is de-aerated and when mixing and heating operations are carried out under an inert atmosphere (89). Preheating of the alkaline copper reagent (without iodate) is recommended for greater precision in the microgram range (90).

Apparatus

Test tubes.—25 mm. diameter by 200 mm.

Test tube rack.—To hold tubes separately upright in a boiling water bath so that all tubes may be inserted and removed as a unit; see illustration in ref. 98.

Test tube closures.—Glass bulbs or hat-shaped hemispherical thimbles with flanged rim.

Stirring rods.—Plunger type for titration. Glass rod (5–6 mm.) with 20 mm. diameter ring at bottom, with plane of ring standing at right angle to the stem and with upper stem bent outward for a handle.

Reagents

Alkaline copper reagent (1945).—Rochelle salt (40 g.), disodium hydrogen phosphate dodecahydrate (71 g.) (or 53 g. of the heptahydrate), and *N* sodium hydroxide (100 ml.) are dissolved in 500 ml. of water. Eighty ml. of a solution containing 8.0 g. of cupric sulfate pentahydrate is stirred in, followed by a solution of 0.892 g. of potassium iodate in about 100 ml. of water.¹ Finally, 180 g. of anhydrous sodium sulfate is dissolved; the solution is diluted to 1 liter and allowed to stand 3 days so that impurities settle out.

¹ To determine widely variable amounts of sugar and to avoid excessive blank titers with small amounts of sugar, iodate may be omitted from the copper reagent and added in closely regulated amounts to each tube just before the titration. For example, to determine less than 1 mg. of D-glucose, 5.00 ml. of 0.01 *N* potassium iodate (0.3567 g./liter) and 0.5 ml. of 2.5% potassium iodide are added without mixing after the oxidation and before acidification (97). To determine 5 γ of D-glucose with an accuracy of $\pm 5\%$, iodate is omitted and the alkaline copper reagent is heated 20 min. at 100°, cooled, and filtered just before use (90).

The clear supernatant solution is siphoned through a fritted glass filter. The pH is about 9.5, and the solution is stable at least 1 year.

Alkaline copper reagent (1952).—Rochelle salt (30 g.), anhydrous sodium carbonate (30 g.), and *N* sodium hydroxide solution (40 ml.) are dissolved in 200 ml. of hot water. A solution of 8.0 g. of cupric sulfate pentahydrate in 80 ml. of water is stirred in, then the solution is boiled to expel air. A solution of 180 g. of anhydrous sodium sulfate in 500 ml. of water also is boiled, then cooled, and mixed with the copper solution. Eight g. of potassium iodide is dissolved in water and added to the combined reagent. To determine 0.015 to 0.5 mg. of glucose or the equivalent, 5 ml. of *N* potassium iodate is added; to determine between 0.5 and 1.5 mg. of glucose, 12 ml. is added, and to determine up to 3.0 mg. of glucose, 25 ml. of *N* potassium iodate is added, before diluting to 1 liter with boiled water.

Potassium iodide, 2.5 %.—(For use with the 1945 reagent) A solution of 2.5 g. of reagent grade potassium iodide in 100 ml. of water is made alkaline with sodium carbonate or sodium hydroxide. This solution should remain colorless when acidified and treated with a drop of starch indicator.

Sodium thiosulfate.—Slightly stronger than 0.1*N*, accurately standardized. Withdraw 50 ml. by pipet, add to 5 ml. of *N* sodium hydroxide in a 1-liter volumetric flask and dilute to the mark with boiled water to make 0.005*N* sodium thiosulfate as needed.

Starch indicator.—1 % soluble starch is added to boiling water.

Phenol red indicator.—Phenol red, 100 mg., is dissolved in 28 ml. of 0.0*N* sodium hydroxide and diluted to 250 ml. with water.

Procedure

If the sample is acid or extremely alkaline, it should be neutralized with phenolphthalein.² The sample is put in a 25 × 200-mm. test tube, and the volume is brought to 5 ml. with water. Five ml. of the alkaline copper reagent is pipetted in and mixed thoroughly, preferably with a stream of pure nitrogen. The tube is closed with a glass bulb and then placed in the rack. Duplicate blanks are prepared with 5 ml. of water and 5 ml. of alkaline copper reagent; usually appropriate standard sugar solutions are also prepared. The rack of tubes is immersed in a vigorously boiling water bath.

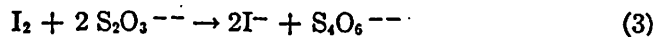
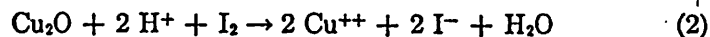
² Sample size is adjusted to the capacity of the reagent as determined by the amount of iodate present. The 1945 copper reagent, containing 0.892 g. of potassium iodate per liter, will allow accurate determination of a maximum of 3.0 mg. of D-glucose (or 5.2 mg. of maltose hydrate) and a minimum of 0.03 mg. of D-glucose by the procedure. Chlorides and nitrates (82), citrates and oxalates (83, 99), ferric, calcium and magnesium salts (100), and suspended precipitates (84) in the sample may alter the normal reducing values. Chloroform and phenol will decompose and probably interfere with the titration. If alcohol is present, an equal volume should be used in the blank.

to about 5 cm. difference in level inside and outside the tubes. With the 1945 reagent, 10 min. is allowed to determine glucose or fructose, 20 min. for arabinose, galactose, or maltose, and 30 min. for mannose, lactose, or polysaccharides. With the 1952 reagent, 15 min. is allowed for glucose or fructose and appropriately longer times for the slower reacting sugars. The rack of tubes is placed about 3 min. in a cold water bath to cool to 25–30°. The tubes should not be agitated during heating or cooling periods.

With the 1945 reagent, 2 ml. of 2.5 % potassium iodide is added to each tube *without mixing*; the 1952 reagent already contains the iodide. From a fast-flowing buret, 1.5 ml. of 2*N* sulfuric acid is run into each tube with shaking so that the liberated iodine will oxidize all reduced copper. After 5 min., the tubes are re-shaken. The excess of liberated iodine not reduced by cuprous ions is then titrated with 0.005*N* thiosulfate using plunger-type stirring rods. When the solution is light yellow, two drops of starch indicator and two drops of phenol red indicator solution are added, and the titration is continued until disappearance of the starch-iodine blue.

Calculations

Equations for the iodimetric reactions are:



The blanks will require about 25 ml. of 0.005*N* thiosulfate when the copper reagent contains 0.892 g. of potassium iodate or 25 ml. of *N* potassium iodate solution per liter. Titer of the sample subtracted from titer of the blank is equivalent to copper reduced by the sugar. From titrations of standard sugar solutions at three or more concentrations in the expected range, a straight-line relationship is obtained upon plotting "mg. of sugar" against "net ml. of 0.005*N* thiosulfate." By using the slope of this line as the factor, mg. of sugar per ml. of 0.005*N* thiosulfate, unknown sugar contents can be calculated.

APPENDIX 2
STARCH ANALYSIS METHOD

WHOLE STARCHES AND MODIFIED STARCHES

[9] Quantitative Determination of Starch in Plant Tissues

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Introduction

Most higher plants contain starch. The amount of this polysaccharide found in any tissue at any specific time is determined by the physiological activity of that tissue. The starch content of various plants and of the different tissues of the same plant may vary to a great extent. The amount in leaf tissue is subject to diurnal variation and may be as low as a fraction of one per cent. Starch accumulates and becomes especially abundant in seeds, bulbs, and tubers. Some seeds or grains contain as much as 70% starch. The tubers and roots of pithy stems of certain palms may contain 25-30% starch.

Although starch can be solubilized in boiling water, its complete extraction from plant tissue is difficult to achieve because of its high molecular weight and colloidal properties. Perchloric acid was found to be a most efficient solvent for starch.

A number of methods of starch determination are reported in the literature (1-4). The procedure described here is partly based on that developed by Pucher and co-workers (1), which proved to give satisfactory results for the analysis of starch in tissues of various plants.

The method consists of extraction of the soluble sugars from the dried plant tissue with 80% ethanol. The sugar-free residue is then treated with perchloric acid solution; the extracted starch is precipitated with iodine, and the starch-iodine complex is decomposed with alkali. The liberated starch is then determined colorimetrically with anthrone reagent.

Procedure

Reagents

80% Ethanol.—1680 ml. of 95% ethanol are diluted with water to make 2 liters.

Perchloric Acid, 52%.—270 ml. of 72% perchloric acid are diluted with 100 ml. of water. The solution should be stored in glass-stoppered containers.

* From: *Methods in Carbohydrate Chemistry Volume IV - Starches.*
1962.

Iodine-Potassium Iodide.—7.5 g. of iodine and 7.5 g. of potassium iodide are ground with 150 ml. of water; the resulting solution is diluted to 250 ml. and filtered if necessary through a Whatman No. 3 paper with suction.

Ethanolic Sodium Chloride.—350 ml. of ethanol, 80 ml. of water, and 50 ml. of 20% aqueous sodium chloride solution are diluted to 500 ml. with water.

20% Aqueous Sodium Chloride Solution.

0.25N Ethanolic Sodium Hydroxide.—350 ml. of ethanol, 100 ml. of water, and 25 ml. of 5N sodium hydroxide are diluted to 500 ml. with water.

Anthrone Reagent (0.1% in 72% v/v sulfuric acid).—The reagent is prepared by dissolving 1 g. of purified anthrone¹ in 1 liter of cooled sulfuric acid solution containing 760 ml. of conc. sulfuric acid, sp. gr. 1.84; it is filtered, if necessary, through fritted glass. The solution is stable at 4° for several days but must be discarded when it turns green. (Note: Because the anthrone solution reacts with lint and other contaminants, it is essential to wash with cleaning solution all glassware which may come in contact with this reagent.)

0.01% D-Glucose Solution.—This solution is prepared daily by diluting a stock 1% solution of pure D-glucose. The stock solution is prepared in 0.1% sodium benzoate solution or is frozen.

Method

It is important that the plant tissue for starch determination be dried rapidly in a vacuum oven at 90–95°. Slow drying at a low temperature may allow some hydrolysis of the starch by amylase present in plants.² A ventilated oven provided with a fan to circulate air over the tissue at 79–80° may also be used for that purpose. The dry material is ground to pass a 50–80-mesh screen. Depending on the starch content of the tissue, a 50–250-mg. sample is placed in a 50-ml. centrifuge tube; a few drops of 80% ethanol are introduced to wet the ground material, so as to prevent clumping; 5 ml. of water are added, and the mixture is thoroughly stirred. The soluble sugars are then extracted as follows: the tube is im-

¹ Anthrone of sufficient purity can be obtained commercially from a number of chemical companies, or it can be prepared according to Meyer (5).

² In the case of fresh leafy material and tissues containing small quantities of starch and having a high water content, the fresh material may be first weighed and then dropped in boiling 95% ethanol to inactivate the enzymes. The soluble sugars are extracted by repeated treatment with hot 80% ethanol or by continuous extraction in a Soxhlet apparatus. The insoluble residue is then dried and weighed. In order to calculate the percentage of starch on a dry weight basis, the ethanolic extracts should be combined and concentrated to dryness. The residue is weighed and added to the weight of the ethanol-extracted residue.

mersed in a hot water bath; 25 ml. of hot 80% ethanol is added, and the mixture is stirred several minutes and centrifuged. The ethanolic solution is decanted and discarded. The extraction is repeated three more times in order to completely remove the soluble sugars.

The sugar-free residue is then heated 15 min. with 5 ml. of water in a boiling water bath in order to gelatinize the starch. The suspension is cooled to room temperature, and 6.5 ml. of 52% perchloric acid is added while stirring. The tube is kept cool by immersion in a water bath at about 25°. Stirring is continued about 5 min. and thereafter occasionally during 15 min.; 20 ml. of water is then added, and the mixture is centrifuged. The aqueous starch solution is decanted into a 50-ml. volumetric flask, and the extraction with perchloric acid is repeated without preliminary heating. The tube and rod are washed with water. The combined extracts and washes are diluted to the mark and centrifuged, and the supernatant is decanted through glass wool.

A 10-ml. aliquot of the starch extract is transferred to a 50-ml. conical centrifuge tube; 5 ml. of 20% sodium chloride and 2 ml. of iodine-potassium iodide reagent are added, and the solution is mixed. (If the concentration of starch is excessive, it may be necessary to add more iodine-potassium iodide reagent.) After standing at least 20 min., the tube is centrifuged, and the supernatant liquid is removed with extreme care to avoid loss of precipitate. The precipitated starch-iodine complex is then suspended in 5 ml. of ethanolic sodium chloride wash solution and centrifuged. The supernatant fluid is discarded, and the washing process is repeated at least one more time.

To the packed precipitate, 2 ml. of 0.25*N* ethanolic sodium hydroxide is added, and the tube is gently shaken and tapped until all the blue color is discharged. A stirring rod must not be used, as the gummy precipitate would stick to it, and ample time for decomposition of the complex must be allowed. The liberated starch is then centrifuged and washed several times with 5-ml. portions of ethanolic sodium chloride as before.

About 5 ml. of hot water is added to the precipitated starch in the tube, and the mixture is stirred with a glass rod until the starch has dissolved. The solution is quantitatively transferred to a 50-ml. volumetric flask; the tube and rod are thoroughly washed with warm water, and the wash is added to the contents of the flask, which are then diluted to volume and thoroughly mixed. The concentration of starch is determined by the procedure of Fairbairn (6) as follows.

Aliquots of the starch solution expected to contain 20–200 μg . of starch are pipetted into acid-washed test tubes and diluted with water to 2.0 ml. At the same time, a series of tubes containing 0–200 μg . of D-glucose in 2 ml. of solution are prepared. The tubes are immersed in cold water; 10

ml. of anthrone reagent is added to each from a pipet or buret, and the reactants are well mixed in the tube. (The anthrone may precipitate at this point, but this will not affect the determinations.) The tubes are placed in a boiling water bath and heated 15 min. (until maximum color develops). They are then rapidly cooled by immersion in cold water, and the absorbance is measured at 620 m μ . In calculating the amount of starch from the standard curve of D-glucose, the D-glucose reading must be multiplied by 0.90.

Alternatively, the starch may be hydrolyzed with acid (1), and the reducing value of the hydrolyzate determined as D-glucose by any of the standard procedures (7, 8, Vol. I [115]).

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