ALCOHOL PRODUCTION FROM NIPA *(NYPA FRUTICANS* WURMB.*)*: PRELIMINARY STUDIES ON ECOLOGY AND CHEMISTRY

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Abstract

Preliminary investigations were made on the ecology of nipa groves and the chemistry of the plant sap at Paombong, Bulacan. Nipa shoots tended to grow faster with a decrease in temperature and an increase in rainfall (wet season). At another station, however, there was an inversion in the response of the plants to temperature. The alcohol content of the sap showed a marked decline by about 33% of the highest values obtained as the colder and rainy season progressed. Generally, shoot elongation and alcohol production were greater under the tidal conditions in Station 1, when compared to those in the more elevated, hence, drier conditions in Station 2. The extent of utilization of nipa and its products varied widely among the sites selected for the study.

Post-fermentation analysis revealed that nipa sap contained mostly sugar and solids. Under natural conditions, the pH changed slightly within the 15-day period. However, there was a mean increase in the percentage of alcohol, particularly after the tenth day of fermentation. The tendency of the alcohol concentration to increase with the days continued regardless of the amount of inoculum *(Saccharomyces cerevisiae)* introduced into the medium.

The problems encountered in the study are discussed and the necessity for more research on nipa is emphasized as an integral aspect of nonconventional energy-based development in the country.

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Introduction

The effect of the increasing cost of petroleum-based fuel is now felt all over the world. For this reason, more research and development efforts are directed to nonconventional energy sources. The Philippines is no exception. Here, the potential of plant biomass as fuel source is being realized. This is partly attributed to the fact that the country yields natural stocks of plants and crops that can supply liquid biofuels. One of these plants is nipa (*Nypa fruticans* Wurmb.).

The nipales, or nipa groves, support a wide spectrum of renewable resource utilization. At the component level, sap from the cut inflorescence stalks of the plants is a fresh drink, a source of sugar and fermented drink (tuba), vinegar and alcohol; the fruit is used directly as food and sweetmeat while the leaves are used as house shingles, in the manufacture of hats, mats, coarse baskets and pails, brooms, tying and sewing materials, and the trunk is used as fuel and floating log. At the ecosystem level, nipa communities, together with the mangroves, function as coastline stabilizers, a retainer and builder of land, buffer against waves and storms, a reservoir in the tertiary assimilation of wastes, and in the global cycle of sulfur and nitrogen, they, too, are wildlife sanctuaries, nursery grounds for fish and shellfish and places for habitation and homestead. The extremely rapid denudation of mangrove forests at the rate of 24,285 ha per year in the last 10 years (1), brought about mainly by indiscriminate forest products extraction, fishpond development and industrial pollution, imposes a serious threat of local extinction of the species. The necessity arises to develop a simple, cost-effective and rural-oriented technology on the cultivation of nipa palms before any long-term substantial benefits could be derived from them.

Fermented nipa sap was used in the country as an alcoholic beverage even before the arrival of the Europeans in 1521. However, the production of high-grade alcohol for industrial purposes and for fuel has not yet been reported. The nipa palm has a lifespan of 50 years and yields an average of 50 liters of sap of 14% sucrose concentration per season. Recently, microorganisms which ferment palm sap to give more than 30% alcohol content have been reported. The importance of nipa in the history of our alcohol industry and the favorability for growth of the species in the country justifies detailed studies on the optimization of biomass and alcohol production from this renewable resource.

Existing knowledge on nipa are primarily scattered floristic accounts tied up with mangrove surveys. This is particularly true in Southeast Asia where mangroves grow extensively, with the nipa communities, when present, considered merely as minor ecological associates. In this

region, other taxonomically related palm species receive relatively more attention as these yield products which rank high in the priority list of extractable and useful forest resources, dictated by the country's specific needs. This is true in Malaysia, Thailand and Borneo where *Metroxylon*, *Corypha*, *Arenga* and *Caryota* are the main sources of starch and sugar. Alcohol production from palms in these parts is relatively not developed.

The production of alcohol from nipa has long been of commercial importance and is unique to the Philippines. Gibbs (2) wrote an excellent review of commercially important palms in the country including nipa, coconut, buri, and sugar palms, as well as some propagation techniques of nipa which open themselves for modification and improvement using present technologies. Brown (3) gave taxonomic and forestry accounts on nipa, emphasizing the possibilities for commercial exploitation of nipales, and more recently, a review of the uses of the palm has been made (4). No reliable data exist, however, to explain the conspicuous gap in our knowledge to understand the biology of nipa and its potential as a stable and dependable source of alcohol from the first quarter of the century onwards.

Among the locally growing palm species, nipa has long been considered as the most promising source of industrial alcohol. As the sap is collected, the inversion of sucrose is completed and fermentation is well under way. Sometimes acetic acid fermentation also progresses to a considerable extent. A large proportion of fermentable sugars is also lost in the handling of the sap which contains approximately 15% sucrose concentration.

The distilleries receive the tuba in its partially fermented condition usually contaminated by many forms of undesirable microorganisms. It has been reported (2) that there is a great loss of the substance in the distilleries through failure to obtain the expected sucrose content from the sap. Nevertheless, nipa is still considered a very low cost source of alcohol.

Very little work has been reported concerning the nipa sap, except as raw material in the manufacture of tuba. The microflora of the fermenting sap has been studied (5) and microbiological investigations have been made (6, 7) to preserve palm wine in general. Use of chemical preservatives for palm wine has also been explored (8). Recently, an ethanol-producing bacterium, *Zymomonas congolensis*, which produces as much as 30% w/w ethanol from sucrose, was reported (9). In the Philippines, there is no reported work using seed cultures of screened microorganisms to increase the alcohol yield of nipa sap.

Materials and Methods

Ecology

Ground inventorial surveys were conducted at some selected nipales in the country for actual hectarage and density and general structure of the nipa community. An intensive study area (ISA), at which transect-stations were made, was selected for in-depth preliminary management studies. Selection of the site was based on plant abundance, industrial (alcohol) history, accessibility, and general physiographic and ecological contrasts.

The management studies concentrated on the monthly monitoring of some operationally significant environmental factors which influence the biomass production and alcohol yield as well as the development of the plants. These factors include: leaf/stand density, water chemistry (salinity, pH, and dissolved oxygen), chemical and physical analysis of the sediments, and temperature and rainfall. The socio-economic aspects that are directly or indirectly associated with the nipa industry at the sites were obtained from locally available data and from interviews of persons concerned.

Chemistry

Proximate analysis of nipa sap and its residue was done using general assay procedures. Alcohol content was determined both by the specific gravity method and by refractive index. pH was determined in a Beckmann model SS-2 pH meter. Total solids were analysed and the method for water analysis prescribed by the U.S. Environmental Protection Agency (1979) was used. Phosphate was determined by the dry ash method for sample preparation followed by the EPA colorimetric method using a Perkin-Elmer UV-VIS Model 551 spectrophotometer. Sugar was determined as glucose by the DNS method (10). The residue after fermentation was used for fat analysis and ether extraction was done on a Raffatec extractor Model 3000-301. The residue was also used for crude protein analysis using a Buchi N_2 analyzer.

Natural anaerobic fermentation was done at room temperature inside 2-liter flasks with a CO_2 inlet and sample collecting tube. pH and alcohol concentrations were monitored with time. Anaerobic fermentation, with varying amounts of pure cultures of *Saccharomyces cerevisiae* (UPCC 2110) was done inside similar flasks as above. Into each flask was added 1 liter of sterilized nipa sap. Varying amounts of the inoculum were added to the cooled sap. The initial pH was determined and the temperature maintained at 25°C. Alcohol and glucose concentrations

were likewise determined. The effect of adding the additive from mangrove bark, "tangal", was also studied.

In the preliminary identification of sap microflora, 2 methods were used: the broth and the plate methods. In the first, a differential medium was prepared which is composed of the following: malt extract (0.15 g), yeast extract (0.15 g), glucose (1 g), peptone (0.25 g), actidione (20 mg), and distilled water (20 ml). The pH of the broth was adjusted to 4 using dilute HCl or H_2SO_4. The medium was dispensed into screw-capped 25 ml test tubes and autoclaved for 15 minutes at 15 psi. One ml of nipa sap was added to each test tube which was provided with an inverted Durham tube. These were inoculated at 25°C for 2 to 6 days.

In the plate method, culture media for *Zymomonas* and *Serratia marcesens* were prepared. The *Zymomonas* medium consisted of: bacto-peptone (10 g), yeast extract (10 g), glucose (20 g), agar (15 g), actidione (20 mg), and distilled water (1 l). The medium for *S. marcesens* consisted of 25 g of nutrient agar per liter of distilled water. The latter medium was placed at the bottom of several petri dishes while the former, on their covers. The covered petri dishes were sealed with paraffin and these were inverted and incubated at 25°C for 3 to 4 days. Whitish colonies in the *Zymomonas* media were harvested and inoculated in another series of petri dishes under the same condition. The microbial growths were identified in slides by gram staining and microscopic examination.

A piece of cotton moistened with a few drops of pyrogallol was attached to the outer cover of 6 sterilized petri dishes with aluminum foil and masking tape. Approximately 10 ml of *Zymomonas* media was placed in each dish together with 1 ml of nipa sap diluted to 10^6. The cover was placed upside down over the petri dish and the whole dish wrapped in ordinary paper. This was incubated at 25°C for 3 days.

Several different colonies were observed. Each was successively inoculated into nutrient agar, *Zymomonas* culture media and yeast media. Identification was done by gram staining and microscopic examination.

In the dehydration process, alcohol vapors from solutions heated in 300 ml flasks were led through a short glass tube (18 mm x 115 mm) containing the activated dehydrating agents. The vapor was then led through a vertical condenser and into a receiver. In another set-up, dehydrating agents were placed in a vertical condenser heated with circulating water the temperature of which was maintained at 90°C. The distillates were then assayed for alcohol concentration.

Results and Discussion

Ecology

Figs. 1 and 2 show that in Station 1, the shoots of nipa tended to grow faster with a decrease in temperature and an increase in rainfall. This response is well marked from May to September which coincides with the pronounced wet season recorded for the region. In Station 2, however, the shoots showed a suppression in growth rate with a decrease in temperature but with an increase in rainfall, although these trends are not as clear as those the plants in Station 1 showed. It thus appears that physiological processes resulting in the maximum elongation of the shoots of nipa in Station 1 favor the annual temperature means and maximum wetness. In addition, ripening of the fruits generally occurred during the first half of the wet period and this developmental process might have utilized much of the metabolites accumulated from the previous season, leaving only a minimum amount for growth. As the fruits ripen, however, growth of the leaves commences, but only to be suppressed as the sap content of the plants reaches a maximum. This is indicated by the decrease in the growth rate of the plants from late October to December which coincides with the *tuba season* (i.e., the tapping of the sap), the latter period lasting for about 6 months onto the dry season. The dry period starts from November till April at the later half of which flowering commences. The inversion of the response of nipa at Station 2 suggests a possible adaptation of the plants to grow better during the drier and warmer months of the year. This agrees with the observation that Station 2 is located at an elevated portion of the mangrove area above Station 1, relatively more frequently uninundated by tides.

The alcohol (ethanol) content of nipa sap from both stations showed a marked decline of about 33% of their highest values (which was recorded in June) as the colder and rainy season progressed. The significant depression in alcohol production in August appears to be a generalized response of the plants to excessive rainfall and low temperature conditions. Generally, shoot elongation and alcohol production were greater under the tidal conditions in Station 1, when compared to those in the more elevated, hence, drier conditions in Station 2.

Table 1 gives the approximate hectarage of nipales and their prevalent resource use and degree of management practices at 7 selected sites in the country. It should be noted that the extent of utiliziation of nipa varies widely among the areas and is a function of whichever extracted product (mostly tuba, vinegar, shingles) commands the higher price in the market. An interesting case is that in Iwahig Penal Colony in Pala-

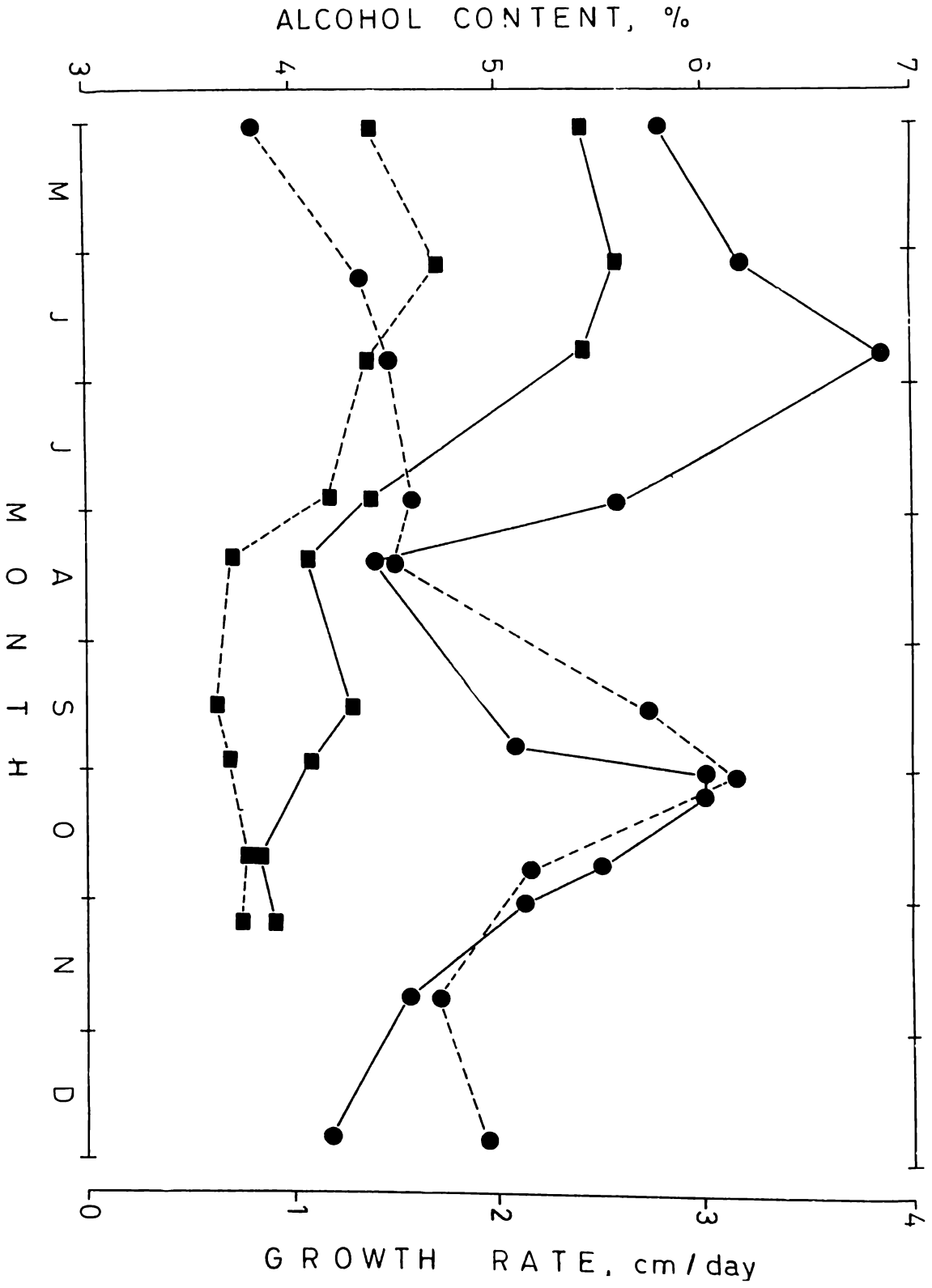

Fig. 1. Growth rate and alcohol production of nipa plants plotted against the months of collection. (Solid line, alcohol production; broken line, growth rate; circle, Station I; square, Station 2).

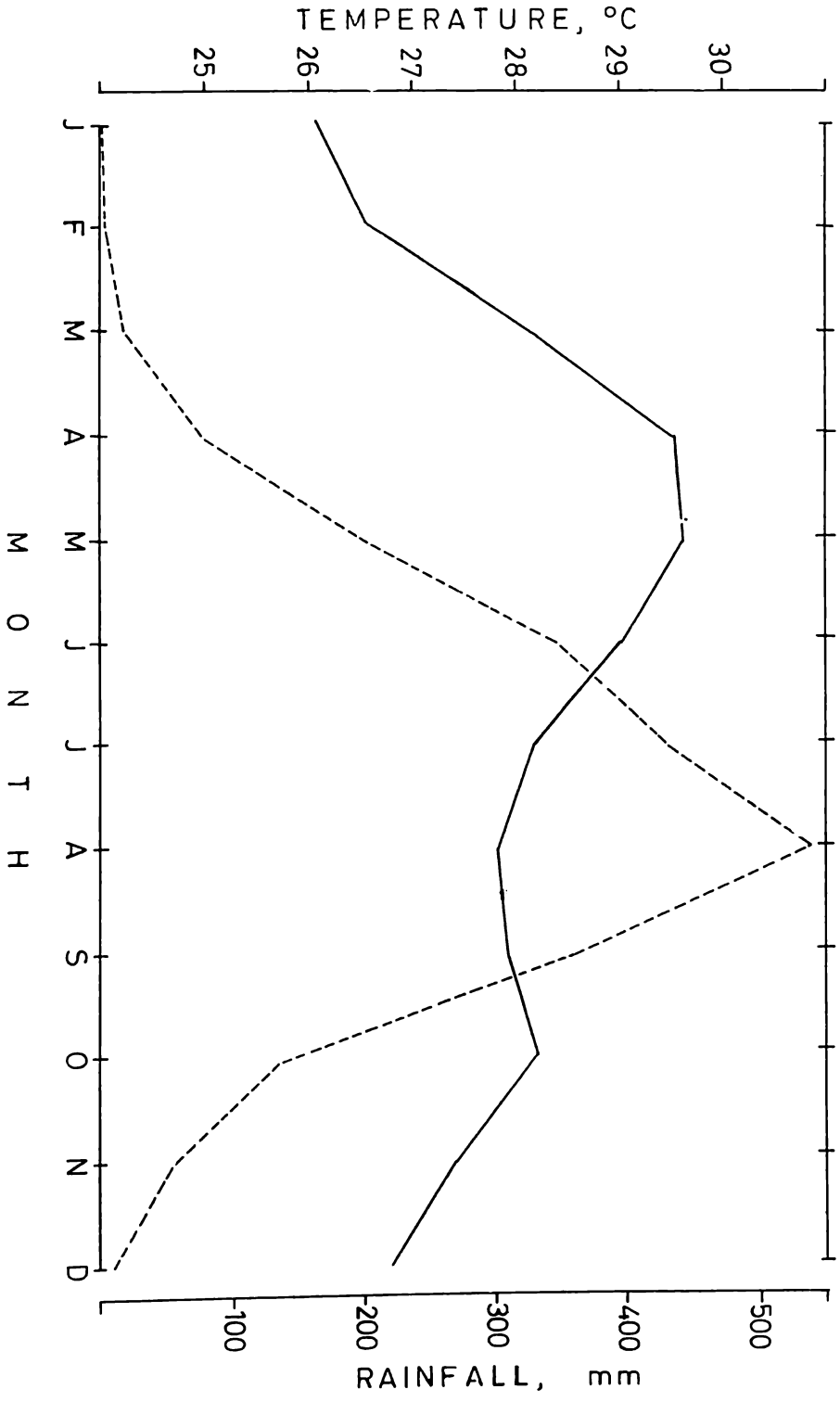

Fig. 2. Temperature (solid line) and rainfall (broken line) recorded for the region of Bulacan for the year 1981.

Table 1. APPROXIMATE HECTARAGE, DOMINANT RESOURCE USE AND EXTENT OF MANAGEMENT PRACTICES OF NIPALES AT THE 7 STUDY SITES

	Alabat (Quezon)	Hamtik and San Jose	Sorsogon NW	Bay N	Hamtik Iwahig (Palawan)	Paombong (Bulacan)	Pinamukan (Aklan)
hectarage	100	45	19	17	57	105	351[1]
resource use:							
shingles	xxx	xx	xx	xx	xx	xx	xx
tuba	x x		x		x	xxx	x
alcohol			xx			x	
fishpond	x	xxx	x		xx	xx	xx
others	x	x	x	x	xx	x	x
management	xxx	xx	x	xx	xx	xxx	xx

[1] Includes mangrove areas

xxx, dominant or intensive; xx, less dominant or moderate; x, trace or nil

wan, where alcohol and vinegar manufacture is completely prohibited. The "inmates", however, exercise division of labor in what could be a most systematic and successful venture to boost the nipa-based industry. The greatest setback against this nipa industry in all 7 sites, however, arise largely from the indiscriminate cutting of the trees. This denudation adversely affects the growth of the plants which in turn reduces the source of "barok" and "tangal" which are utilized to improve the alcohol quality of local whiskey. Marinduque and Palawan are reportedly the best source of the additives, obviously because of the extensive mangrove areas and nipales which still exist in these places.

It appears that relative acidity (pH 5.2), low phosphorus (13.3 ppm), high sodium (17.4 ppm) and potassium (1.7 ppm) content of the sediments where the nipa plants thrive favor optimum alcohol production (Table 2). Calcium and magnesium, on the other hand, appear to be not as important as the other elements in exerting differences in alcohol productivity in nipa.

Table 2. CHEMICAL AND PHYSICAL ANALYSIS OF SEDIMENTS COLLECTED IN THE 3 SHORE PORTIONS AT STATION 1. RESULTS ARE ON OVEN-DRY BASIS.

Portion of Shore	Chemical Analysis (pH)	Elements (ppm) P	Ca	Mg	Na	K	Mechanical Analysis (%) Sand	Silt	Clay
Landward	6.4	18.4	19.4	7	12.5	1.1	12.4	47	40.6
Middle	5.2	13.3	22.8	8.6	17.4	1.1	0.4	58	41.6
Frontal	5	21.8	21.2	11.3	16	1.4	12.4	52	35.6

Similarly, low percentage of sand (0.4) and high percentage of silt (58) favor growth and alcohol production in the plants (Table 2). Interestingly, these conditions characterize Station 1 which lies along the river mouths of tidal rivers in the low wetlands, subject to overflow of brackish water as the tides rise each day. Station 2, on the other hand, approximates the conditions at the landward portion of the transect where growth and alcohol production were relatively lower than those recorded in Station 1.

Chemistry

Proximate analysis reveals that after fermentation, nipa sap contains mostly sugar and total solids, with phosphate and ash comprising a small

percentage (Table 3). Fat and crude protein were conspicuously intraceable although these were the only components detected in the residue.

Table 3. PROXIMATE ANALYSIS OF NIPA PALM SAP AND ITS RESIDUE AFTER FERMENTATION

	Sap	Residue
Total solids	4.08%[1]	—
Phosphate	2.71 ppm	—
Sugar	11.61%[1]	—
Ash	0.79%	—
Fat	—	0.27%
Crude protein	—	32.76%

[1] 8 hours after collection

Under natural conditions of fermentation, there was only a slight change of pH of the sap within the 15 day period (Table 4). However, there was a mean increase in the percentage of alcohol, particularly after the tenth day of fermentation.

There was a general tendency for the alcohol content of the sap collected from the 2 stations to increase with the number of days it was allowed to ferment (Tables 5 and 6). This response was exhibited regardless of the amount of inoculum (*Saccharomyces cerevisiae*) introduced into the medium. Glucose content of the sap, however, showed a pronounced decrease as the days progressed.

Among the 6 chosen dehydrating agents, cornstarch and Avicel exhibited the greatest promise since they effected a substantial increase in the concentration of the final solution (Table 7). Likewise, recovery using these drying agents were highest. Cornstalk gave the lowest alcohol concentration as well as the lowest recovery.

Nipa sap is a low cost source of alcohol. However, there are 2 major problems, each one involving great complexities in optimizing the alcohol yield.

The first problem is fermentation. Assuming that the quality of the sap that reaches the laboratory can be improved, still the alcohol con-

Table 4. ANAEROBIC FERMENTATION OF NIPA PALM SAP

	Sample I		Sample II		Sample III		Sample IV		Sample V	
Days	pH	% ETOH	pH	% ETOH	pH	% ETOH	pH	% ETOH	pH	% ETOH
1	3.40	6.50	3.35	5.25	3.15	4.82	3.20	6.20	3.15	5.83
2	4.05		3.55		3.86		3.72		3.55	
3	3.85		3.72		3.95		4.25		4.30	
4	3.75	8.08	3.72	6.72	3.65	8.23	3.82	7.78	3.72	7.85
5	3.72		3.72		3.65		3.65		3.69	
6	3.68		3.50		3.50		3.50		3.65	
7	3.75	8.35	3.55	8.58	3.60	8.96	3.65	8.95	3.85	8.58
8	3.70		3.40		3.50		3.55		3.40	
9	3.58		3.35		3.40		3.50		3.40	
10	3.60	9.04	3.40	7.89	3.40	9.20	3.50	8.50	3.38	8.35
11	3.65		3.40		3.40		3.60		3.45	
12	3.60		3.40		3.40		3.60		3.45	
13	3.58	8.20	3.40	7.21	3.35	8.66	3.55	7.97	3.45	8.27
14	3.60		3.33		3.37		3.55		3.40	
15	3.58		3.40		3.38		3.58		3.40	

Table 5. EFFECT OF VARYING AMOUNTS OF *SACCHAROMYCES CEREVISIAE* (2.5×10^2/ml) ON NIPA SAP FERMENTATION

Amount of Inoculum	Sample I				Sample II			
	10 ml		20 ml		30 ml		50 ml	
Fermentation time (hrs)	Alc (%)	Glu (mg/ml)	Alc (%)	Glu (mg/ml)	Alc (%)	Glu (mg/ml)	Alc (%)	Glu (mg/ml)
Start	5.64	36.41	5.64	36.41	5.85	15.75	5.85	15.75
12					5.20	10.17	5.78	9.92
24	6.00	19.60	5.05	15.28	5.56	9.24	4.63	9.33
36	6.15	16.00	6.60	12.00	5.71	7.37	5.42	7.99
48	6.30	11.27	5.93	8.11	5.42	5.61	5.27	5.59
60	6.23	7.37	6.45	5.54				
72	6.08	5.05	5.85	3.82				
96	5.85	3.18	6.98	2.43				
108	6.30	2.15	6.08	1.54				
120					6.53	3.61	6.23	3.61
144					6.45	0.61	5.56	0.56
156	7.05	0.74	6.60	0.58				

Sample I — sap from Station 2
Sample II — sap from Station 1

Table 6. EFFECT OF VARYING AMOUNTS OF *SACCHAROMYCES CEREVISIAE* (2.5×10^7/ml) ON NIPA SAP FERMENTATION

Amount of Inoculum	10 ml		30 ml		60 ml	
Fermentation time (hrs)	Alc (%)	Glu (mg/ml)	Alc (%)	Glu (mg/ml)	Alc (%)	Glu (mg/ml)
Start	4.35	36.41	4.35	36.41	4.35	36.41
12	4.49	35.39		34.83	4.63	25.96
24	5.42	13.31	5.49	14.52	5.64	9.40
36	6.23	9.13	5.42	9.67	5.34	9.27
48	6.83	4.65	5.42	4.66	5.64	4.32
60	6.08	2.51	6.08	2.59	6.38	2.29
72	6.53	2.26	6.23	2.64	6.14	2.26
96	5.64	0.58	6.30	0.63	5.78	0.44

Sap sample from Station 1 only

Table 7. EFFECT OF DEHYDRATING AGENTS ON ALCOHOL SOLUTIONS OF VARYING CONCENTRATIONS

Initial Alcohol Conc. (%)	Drying Agent	Final Alcohol Conc. (%) [1]	Increase in Alcohol Conc. (%)	Recovery (%)
48.67	Cornstarch	61.13	12.46	70
48.67	NaOH	53.97	5.30	68
48.67	Avicel	63.44	14.77	63
49.33	Rice hull	62.33	13.00	39.2
49.33	Cornstalk	65.50	16.17	32
49.33	Coir dust	72.25	22.92	45.6
23.71	Cornstarch	59.56	35.85	73
23.71	Avicel	45.11	21.40	70
23.86	Rice hull	34.04	10.18	34
23.86	Cornstalk	44.17	20.31	36
23.86	Coir dust	46.84	22.98	39.2
15.50	Avicel	43.20	27.70	69.2
10.00	Avicel	35.44	25.44	67
10.00	Rice hull	17.26	7.26	32
10.00	Cornstalk	14.96	4.96	19.2
10.00	Coir dust	25.26	15.26	16

[1] Average of 3-4 trials

tent is very low. Natural fermentation fails to give more than 10% alcohol. The use of pure yeast cultures does not seem to improve the yield. Neither did the addition of the mangrove bark, "tangal" which is used by the local fermentors to increase alcohol yield.

This study focuses on the need for more efficient fermenting organisms. It is certain that *Zymomonas* is a natural component of the sap microflora. It is reportedly responsible for the alcohol content and the frothing because of CO_2 formation. All fermentation equations reported in the literature show that *Zymomonas* strains produce more than 1.5 mole alcohol per mole of glucose. They grow and ferment the sap very fast. For these reasons, they seem to be promising agents for industrial alcohol production from sugary juices.

Until recently few *Zymomonas* strains were available. Exact and more extensive information on some isolates of the bacterium remains scarce. However, enough features reported permit the identification of *Zymomonas*. For this reason, attempts were made to detect, isolate and identify the microorganisms in nipa palm sap. Preliminary experiments using recommended detection medium, while yielding bacterial isolates, are inconclusive. Some basic features of *Zymomonas* are not known. Therefore, the process of isolation is presently being continued and refined. It is hoped that pure isolated cultures can be used in succeeding fermentation runs.

The second problem is the dehydration of the alcohol obtained. While distillation still remains the standard procedure for alcohol rectification, another technique very recently reported was tried to bring down the cost of the energy-intensive process. This involves the removal of water by using cellulosic materials.

CaO was reported to be capable of drying ethanol 40 years ago. Other dehydrating agents which are relatively unknown have been tried in the present study. Although the water absorbing properties of starch and cellulose are known, preferential absorption of water in the presence of alcohol was unexpected. Initial runs indicate these capabilities of cellulosic materials. However, recovery is low, a problem which may be solved through modification of the present set-ups. The principle of drying alcohol demonstrated by CaO may, therefore, be extended to other cheap and readily available dehydrating agents. Since processes for cheap and energy-efficient alcohol production are needed, it seems appropriate that research along this line be renewed.

We would like to acknowledge the financial support from the Energy Research and Development Center of the Philippine National Oil Company.

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