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ABSTRACT—The yeast, *Candida utilis,* was cultivated on hydrochloric acid hydroysate of northern Minnesota peats. Carbon limitation, nutrient limitation and inhibition due to salt concentration are discussed. An efficiency of 76 percent (grams dry yeast/grams reducing substances) was obtained.

Among the major products obtainable from peat are peat carbohydrates. These carbohydrates can be used to cultivate yeast for protein (in livestock feed) or alcohol (Fuchsman, 1978). In Russia the production of peat hydrolysate for the culturing of yeast involves a sulphuric acid hydrolysis procedure (Fushsman, 1978). Evdokimova, et al. (1974) estimate the yield of reducing compounds from peat at 50 percent and yeast biomass at 60 to 70 percent of the reducing compounds used. The purpose of this study was to assess the suitability of northern Minnesota peat as a source of carbohydrates for the cultivation of yeast. Specifically we were interested in the effect of a nutrient solution on the growth rate of yeast, a comparison of the growth rates of yeast grown on peat hydrolysate with those grown on yeast-mold broth (Y–M broth), and the feasibility of a hydrochloric acid hydrolysis procedure.

Hydrolysis method and peat source

Peat samples were obtained from Wilderness Farms (Wild Bog and Sod Field), and Brookston Bog located in northeast Minnesota (Fuschsman et al. 1979). Bitumens were removed, using the procedure of Fuchsman, et al. (1979). Three grams of peat were refluxed in 450 mls. of 5 percent HCI for five hours. This procedure will hydrolyse the hemicelluloses, but not any cellulose present (Fuchsman, C.S., personal communication). The samples were then filtered while hot and neutralized using 7M. NaOH. After cooling they were refiltered to remove any precipitate. Five ml. aliquots of hydrolysate were transferred to four Spectronic 20 cuvettes. An equal number of cuvettes contained 4 mls. of hydrolysate and 1 ml. of micronutrient solution (Table 1). All tubes were autoclaved and inoculated with five drops of *Candia utilis* culture (American Type Culture Collection No. 22023) which was in log phase growth. Samples were incubated at room temperature (23 C) and the optical density taken daily. Tubes were agitated and read at 720 nm in a Bausch and Lomb Spectronic 20 against an uninoculated cuvette. The relationship between optical density and cell numbers was determined by growing *Candida utilis* on different concentrations of Y–M broth (Difco 0711-02). Cell numbers were counted using a Levy Hemocytometer.

Specific conductivity of each solution was measured with a Beckman RC-16B2 conductivity bridge, to determine the relationship between specific conductivity and the concentration of Na C1 added to yeast-mold broth. Excess salts were removed from the hydrolysate using a dialysis apparatus consisting of a beaker covered with dialysis tubing inverted in distilled water. The distilled water was changed twice daily for two days. Biomass was determined by filtering the cuvette samples at their growth peak and reweighing the dried filter. The percentage hemicellulose and reducing substances in the Brookston Bog were determined using the stances in the Brookston Peat Bog were determined using the method cited by Fuchsman et al. (1979).

Results and discussion of limiting factors

Optical density of the cultures was related to cell number by the regression line $Y=74.92x10^6X - 3.72x10^6$, where X is the optical density at 720 nm and Y is the number of yeast cells per ml., $r^2=0.958$. Nutrient containing samples from the Brookston Bog and Sod Field peats produced the highest peak optical densities (Figures 2 and 3). However, the Wild Bog peat samples produced higher optical densities without the addition of nutrients.

When yeast were grown on Y–M broth with NaC1 added, up to 5 percent NaC1 acted only to slow the rate of growth:

Table 1 – after Miller et al. (1978)

Final concentration of micronutrients as salts and elemental concentration (ug l^{-1}) in distilled or de-ionized water.

Compound	Concentration (ug l^{-1})	Element	Concentration (ug l^{-1})
H_3BO_3	185.520	B	32.460
$MnCl_2 \cdot 4H_2O$	415.610	Mn	115.374
$ZnCl_2$	3.271	Zn	1.570
$CoCl_2 \cdot 6H_2O$	1.428	Co	0.354
$CuCl_2 \cdot 2H_2O$	0.012	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.260	Mo	2.878
$FeCl_3 \cdot 6H_2O$	160.000	Fe	33.051
$Na_2EDTA \cdot 2H_2O$	300.000	-	-

Final concentration of macronutrients as salts and elemental concentration (mg l^{-1}) of distilled or de-ionized water.

Compound	Concentration (mg l^{-1})	Element	Concentration (mg l^{-1})
$NaNo_3$	25.500	N	4.200
$MgCl2 \cdot 6H_2O$	12.164	Mg	2.904
$CaCl_2 \cdot 2H_2O$	4.410	Ca	1.202
$MgSO_4 \cdot 7H_2O$	14.700	S	1.911
K_2HPO_4	1.044	P	0.186
$NaHCO_3$	15.000	Na	11.001
		K	0.469
		C	2.143

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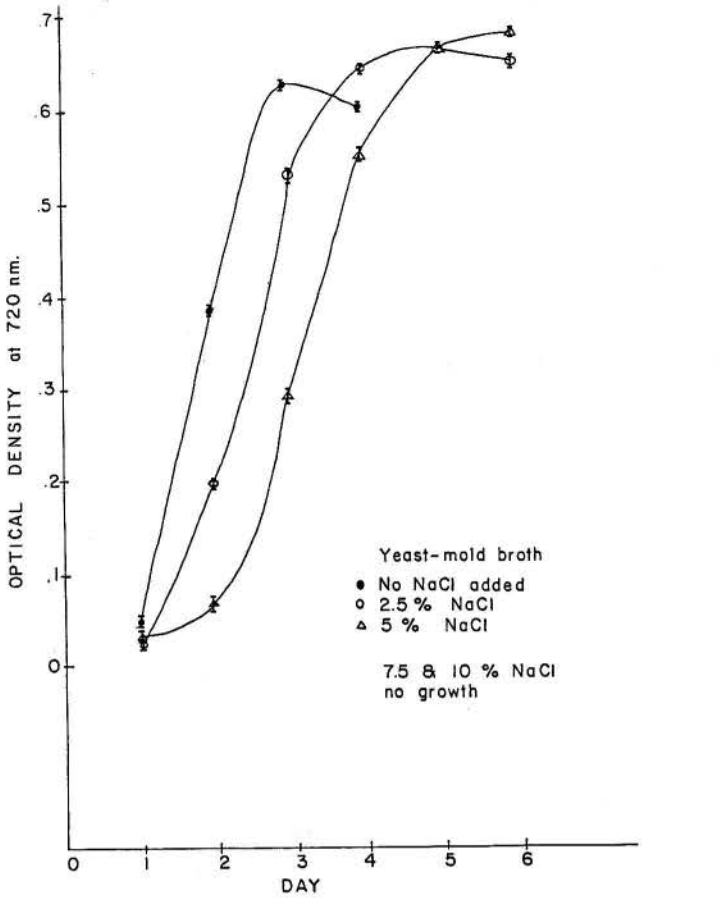

Figure 1. Effect of different salt concentrations on the growth rate of Candida utilis.

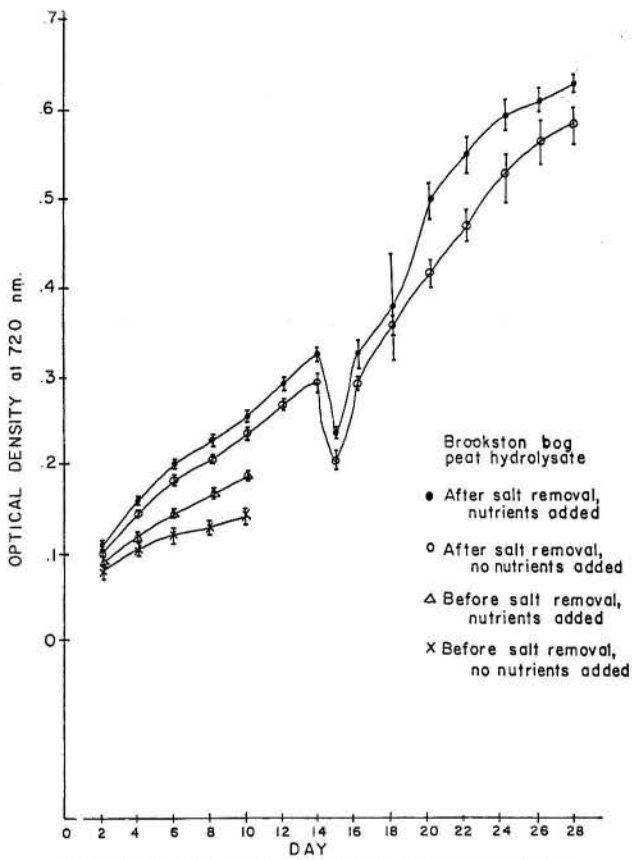

Figure 2. Effects of salt removal and nutrient addition on the growth rate of Candida utilis.

it did not decrease the peak optical densities (Figure 1). Salt concentration of 7.5 percent and 10 percent completely inhibited growth. Conductivity measurements indicate that the concentration of salts in the hydrolysates were equivalent to a 6 to 7 percent salt solution and may have been limiting in our initial experiments. Dialysis reduced the conductivity in all hydrolysate samples from 68,000-72,000 to 2,620-2,850 umhos/cm which represents a 96.1 percent reduction in salt (Table 2). The reduction in salt produced a marked increase in the growth rate in all three peat hydrolysates (Figures 2, 3 and 4).

In order to determine if the yeast growth was carbon limited, 1 ml. of 5 percent dextrose solution was added to each of the Brookston Bog samples on day 15. There was an initial decrease in the optical density due to dilution, however optical density increased rapidly after that, indicating that the yeast were carbon limited (Figure 2). Distilled water was added to the Sod Field samples on the same day to determine if the resultant growth after dextrose addition was simply a result of the dilution of inhibiting substances. No appreciable change in the growth rate occurred after this treatment (Figure 3).

The addition of a micro nutrient solution had a small but significant effect on the growth rates of the Brookston Bog samples. However in the Wild Bog samples it decreased the growth rate of the yeast. In the case where there is a sufficient carbon source, nutrients may be a limiting factor. However when no extra carbon source was added to our samples they were not nutrient limited. Thus the two most prominent limiting factors appeared to be salt concentration and carbon availability. The samples not treated with dextrose solution did achieve an efficiency of 76 percent (grams dry yeast/grams reducing substances). This compares favorably with Soviet estimates of 60 to 70 percent (Evodkimova, et al. 1976). However any error in the determination of reducing substances, or in obtaining dry weight of yeast may have given deceptively high values.

Growth rates on the different peats did not appear to differ to any great extent (Figures 2, 3 and 4). However

Table 2

Media	Peak Optical Density at 720 nm	Specific Conductivity unhos-cm	Yield gm dry yeast/ gm dry peat
Y-M broth Full strength	.743		
Y-M broth Half strength	.572		
Y-M broth 1/4 strength	.452		
Y-M broth 1/8 strength	.365		
Wild Bog	.157	69,000	
Sod Field	.078	68,000	
Brookston Bog	.206	72,000	
Wild Bog after dialysis	.41	2,620	.057
Sod Field after dialysis	.37	2,670	.034
Brookston Bog after dialysis	.59	2,850	.085
Y-M broth 2.5% NaCl	.66	35,000	
Y-M broth 5.0% NaCl	.68	60,000	
Y-M broth 7.5% NaCl	.10	80,000	
Y-M broth 10.0% NaCl.	0	96,000	

yeast grown on peat hydrolysate achieved a peak optical density of only 55 percent of that grown on Y–M broth. Furthermore this value was attained after 24 days whereas yeast grown on Y–M broth achieved a peak optical density after only 3 days.

While the hydrochloric acid hydrolysis is fairly efficient, it produces excessive concentrations of salt. In order for this procedure to be feasible salt concentrations in the hydrolysate need to be reduced and the available carbon increased. There is reason to believe (Sherry Nelson, graduate assistant in Environmental Studies, Bemidji State University, personal communication) that one or two extra post-hydrolysis rinses of the hydrolysis residue may increase the yield of reducing substances by as much as 20 to 30 percent. However, what the subsequent increase of yeast produced would be is unknown. Further work needs to be done on the optimization of the hydrolysate as a carbon source, on peat from different bogs and on peat from different strata within the bogs.

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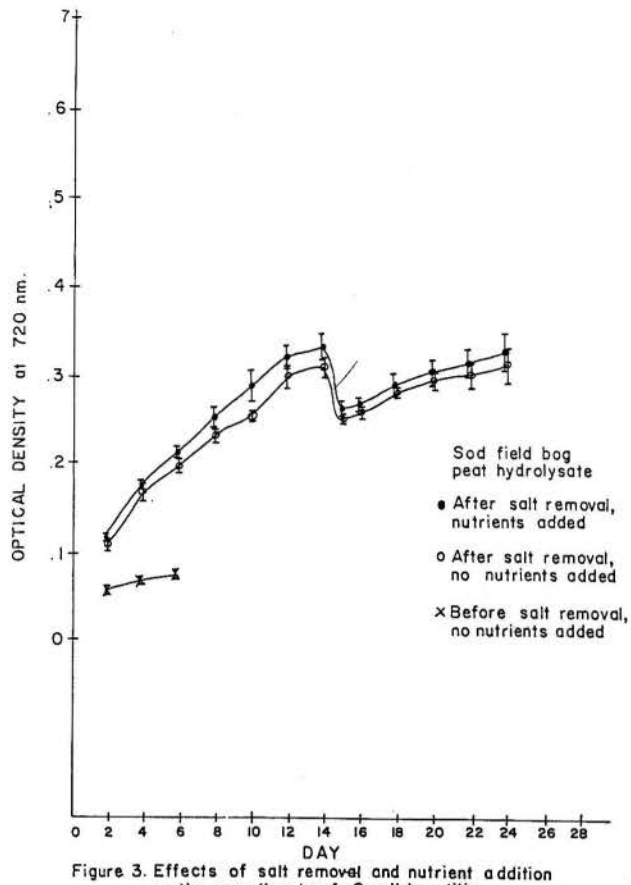

Figure 3. Effects of salt removal and nutrient addition on the growth rate of Candida utilis.

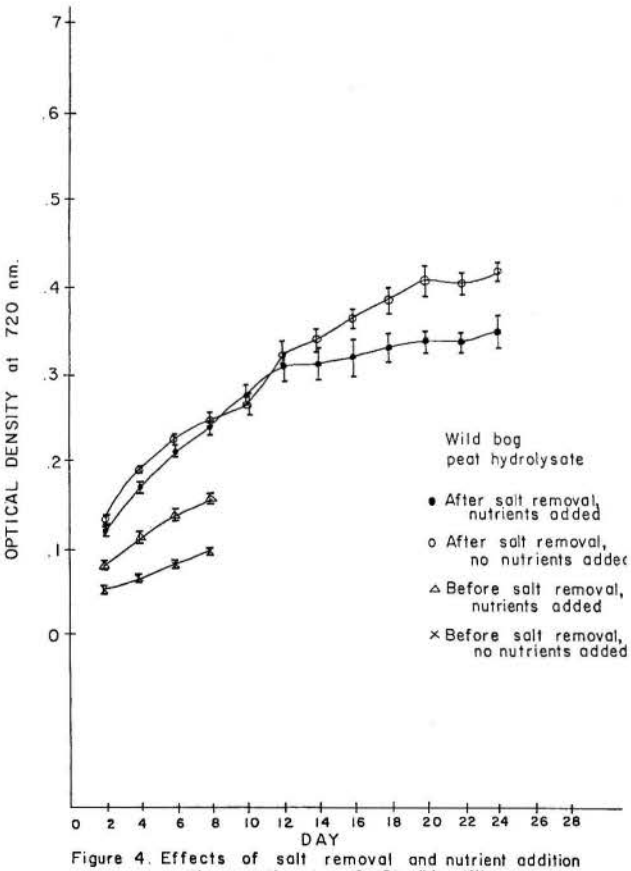

Figure 4. Effects of salt removal and nutrient addition on the growth rate of Candida utilis.