THE DEVELOPMENT OF AN UNCONVENTIONAL FOOD REGENERATION PROCESS: QUANTIFYING THE NUTRITIONAL COMPONENTS OF A MODEL METHYLOTROPHIC YEAST

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INTRODUCTION

Closing the food loop for extended space missions is a function of the economics of transportation versus regeneration. The determinants of that function are 1) the weight/volume requirements and 2) the suitability or palatability of the food produced. The latter determinant is so compelling as to limit the number of choices for food regeneration to largely conventional plant agronomy. However, the need for system redundancy, protein supplements and production efficiency argues for the inclusion of unconventional food regeneration schemes. (1,2) This use of unconventional food production becomes especially tenable given the highly developed state of food processing which can render almost any basic food commodity palatable. In addition, if the weight/volume factors can be shown to be advantageous for unconventional food regeneration, then these routes will be useful from the economic standpoint.

Work in our laboratory at JPL has focused on a hybrid

chemical/biological approach to unconventional food regeneration. Carbon dioxide and water, the major wastes of human metabolism would be converted to methanol by one of several physico-chemical processes available (thermal, photocatalytic, etc.). Methanol is then used to supply carbon and energy for the culture of microorganisms which in turn produce biologically useful basic food stuffs for human nutrition. Our work has focused on increasing the carbohydrate levels of a candidate methylotrophic yeast to more nearly coincide with human nutritional requirements. Yeasts were chosen due to their high carbohydrate levels compared to bacteria and their present familiarity in the human diet. The initial candidate yeast studied was a thermotolerant strain of Hansenula polymorpha, DL-1. This paper describes the quantitative results that permit an evaluation of the overall efficiency in hybrid chemical/biological food production schemes. A preliminary evaluation of the overall efficiency of such schemes is also discussed.

DETERMINING THE NUTRITIONAL PROFILE OF H. POLYMORPHA

Table I gives a summation of data from an earlier report and shows the carbohydrate profile of the methylotroph and the common yeast, Saccharomyces. (3). This data also provides the necessary information required to evaluate the conversion efficiencies of the proposed approaches. Obtaining the analytical data required the adaptation of available procedures and the development of new approaches. The methods involved

the use of oxido-reduction analyses, enzymatic analyses, and difference calculations. The total CH_2O, protein, and glycogen analyses were obtained by direct analysis of whole cell preparations. The glycogen assays were most difficult to obtain and required significant efforts to correlate qualitative and quantitative data. (3-5) The table shows mass balance calculations. These were made to confirm the validity of the results. Some cellular components needed to be estimated such as nucleic acid, unhydrolyzed polysaccharides, lipids and ash. Reasonable estimates of these fractions were made using published data and other experimental data. Strains of Saccharomyces were also analyzed to provide comparative data. An examination of the data in Table I reveals that the data are accurate based on mass balance calculations. An additional confirmation of this data comes from earlier qualitative data which correlates closely with the data in Table I and the independent analysis of protein and carbohydrate values given by Johnson Space Center's Biomedical Laboratories (references 4, 5 and Table I).

An initial premise was made to treat this microbiological food regeneration scheme as a primary food source. Since the edible carbohydrate level was naturally low in aerobically grown yeasts (see Table I wild type H. polymorpha and Saccharomyces), strains of H. polymorpha were selected which had enhanced levels of the intracellular storage carbohydrate,

TABLE I

Composition Profile of H. polymorpha and Saccharomyces

	FRACTION OF DRY WEIGHT (%)							
	H. polymorpha					Saccharomyces		
ANALYSIS FOR	DL1	#13	#122	#84	#84[a]	S. cerevisiae	S. uvarum	S. diastaticus
TOTAL CH_2O	69.8*	69.6	65.5	70.8	75.2	42.2	27.5	33.2
Glycogen	6.5	14.0	0.0	20.0	-	2.9	-	-
Mannan	39.9	32.9	42.5	26.0	-	26.8	-	-
Trehalose & Acid Soluble ω-Glucan	23.4	22.7	23.0	24.8	-	12.5	-	-
PROTEIN	17.0*	24.4	20.9	12.5	13.7	48	58	47
CH_2O + Protein	86.8	94.0	86.4	83.3	88.9	90.2	85.5	80.2
Other Cellular Components (Estimated)	10	10	10	10	10	8	8	8
MASS BALANCE TOTAL	96.8	104	96.4	93.3	98.9	98.2	93.5	88.2

[a] Data from Johnson Space Center Biomedical Laboratory. This analysis from JSC Biomedical Laboratory also provided an actual value for fat and fiber of 1.2% and 0.8%, respectively.

glycogen. Table I shows that strains were selected that had two to three times as much glycogen as the parent strain (#13 and #84).

EVALUATING CONVERSION EFFICIENCIES

The conversion efficiency of methanol to edibles can be determined directly from the data obtained in Table I. Table II shows the data from Table I redisplayed by substate source into the edible fractions, protein and carbohydrate. The carbohydrate fraction is the sum of glycogen and trehalose fractions (trehalose is estimated by a difference procedure described in reference 3). The data is shown for H. polymorpha and three of its mutant strains (one strain was a glycogen deficient strain used as in internal control, #122) and three strains of the common yeast Saccharomyces. The Saccharomyces strains were not grown on limited nitrogen as were the methylotrophs and the lower carbohydrate levels are indicative of this nutritional difference. Total edibles for the methylotrophic strains approaches the glucose grown strains only in the two high glycogen mutants. The ratio of protein to carbohydrate is roughly reversed for the Saccharomyces strains.

These data can be converted into a carbon efficiency if the levels of methanol consumed can be correlated with total

TABLE II

Edible Fractions of Hansenula Polymorpha

and Saccharomyces Cerevisiae

		Fraction of dry weight (as %)		
Substs	YEAST SPECIES	EDIBLES		TOTAL EDIBLES
		PROTEIN	CARBOHYDRATE	
	H. polymorpha			
	Wild type	17.0	27.9	44.9
Me	#13 (High glg)	24.4	34.7	59.1
	#84 (High glg)	12.5	44.8	57.3
	#122 (Low glg)	20.9	21.0	41.9
	S. cerevisiae*	48.0	13.4	61.4
	S. uvarum	55.0	20.4	75.4
Glu	S. diastaticus	47.0	21.2	68.2

* Approved by FDA for human consumption

cell mass produced. This is easily done using gas chromatographic analysis of growth media for methanol and using gravimetric determinations of the cell mass as a function of time. Typical conversion to cell mass has been reported at 35% for autotrophs (6). In other experiments in our laboratories with the methanol grown strain, Hansenula capsulata, conversion of methanol to cell mass was measured at 30-35% in early stationary phase (data not shown). Thus a 30-35% conversion of methanol to cell mass was considered reasonable for H. polymorpha. Using the data from Table II and this estimated conversion efficiency, the overall yield for edible food regeneration in this methanol grown system can be calculated to be 16-21%. For the glucose grown Saccharomyces strains, the values are about 5% higher at 21-26%.

Based on this data it is possible to estimate a total overall conversion efficiency of a hybrid chemical/biological regeneration process if the conversion of CO_2 to methanol can be estimated. A reasonable estimate can be made by considering the individual known and estimated conversion efficiencies involved in the splitting of water to H_2 and O_2, the reduction of CO_2 to CO, and the reduction of CO to methanol by H_2. Using photocatalytic and photoelectrocatalytic processes, an estimate of 32% conversion from solar energy to methanol can be made with reasonable confidence (V. Miskowski and S. DiStefano, personal communication). Combining this with the biological

efficiencies estimated above, an overall conversion efficiency for this candidate hybrid chemical/biological system was calculated to be 5.1-6.7% (using 35% as the biological value).

SIGNIFICANCE

A preliminary evaluation for a candidate foor regeneration system has been accomplished. The system involving CO_2 reduction to methanol, followed by yeast growth on the methanol to produce protein, edible carbohydrate and lipids appears to be a worthy candidate for further development. We have been able through genetic modifications and specific cultural conditions to increase the content of digestible carbohydrates in a methylotrophic yeast to better meet human dietary requirements.

The energy efficiency calculations also suggest that the system is at least on a par with conventional agriculture and may have some significant advantages over such food regeneration. While many factors must be considered in final selection of food regenerating systems, the hybrid system described herein merits consideration for further development. If not this specific system, then at least the "class" of systems employing chemical reduction of CO_2 and water to simple organics, and the utilization of microorganisms growing on these reduction products to produce human food nutrients, should be considered in any overall food regeneration system in CELSS.

FUTURE EFFORTS

Efforts are underway to subject another methylotroph and an FDA-GRAS approved yeast strain (Candida boidinii and Candida utilis, respectively) to the same types of analyses to further validate the approach and to provide a larger body of data on possible candidates for unconventional food regeneration schemes.

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