MIRCEN Journal, 1987, **3**, 255–274

Growth of *Candida utils* on single- and multicomponent-sugar substrates and on waste banana pulp liquors for single-cell protein production

K. C. Aker & C. W. Robinson*

Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Received 27 April 1986; revised and accepted 12 February 1987

Introduction

Among the wide variety of agricultural wastes which are amenable to upgrading into microbial protein by relatively simple fermentation processes, cull bananas could be of particular interest to many tropical countries. The major banana-producing countries include: Brazil, India, the Philippines, Ecuador, Thailand, Indonesia, Honduras, Costa Rica and Venezuela (FAO 1978).

Some 3.7×10^7 t/a of bananas are produced world-wide (FAO 1978), and some 0.65×10^7 t/a are exported (FAO 1975), mainly by sea. Significant accumulations of cull bananas occur near harbours because quality-control measures cause rejection of damaged, ripe and over-ripe fruit. For example, in 1972 in Panama alone over 2.5×10^5 t of banana wastes occurred (LIFE 1972). Since electrical power is normally available near harbours, an aerated submerged batch fermentation process of minimum technical complexity appears to be an attractive proposition for producing protein-enriched animal feedstuffs (i.e., microbial protein) from these waste bananas. Many banana-producing and exporting countries are deficient in indigenous supplies of high-quality protein animal feeds.

Lefrançois (1970) proposed a design for a 15-t fresh-weight banana pulp per day capacity fermentation process, in which the final product would be 2500 kg dry weight/day of flour with a protein content of about 18%. Chung & Meyers (1971) cultivated *Pichia spartinae* on waste bananas, but did not develop a full-scale process flow chart. Aegerter & Dunlap (1980) tested the use of acid-producing, anaerobic bacteria mainly for their effect on prolonging the shelf-life of bananas.

The present study was undertaken as an initial step in the development of a full-scale process for the bioconversion of the fermentable sugars in waste whole banana by the nutritionally well-accepted yeast *Candida utilis*. For use in animal fodders, a low-moisture content product of at least 40 to 45% crude protein was desired. To

*To whom correspondence should be addressed.

foresee the potential fermentation pattern of the multi-carbohydrate substrate in liquors derived from ripe whole bananas, soluble-substrate fermentations were initially conducted using glucose, fructose and sucrose, alone and in mixtures of the three corresponding to the ratios expected in fully-ripened bananas.

In this paper, we report the growth kinetics, biomass yield and protein content and the specific oxygen respiration rate of *C. utilis* for the various fermentations investigated. In addition, we report and compare values of the maximum specific growth rate constant (μ_{max}) as determined by four different methods: dry weight, optical density, crude protein and dissolved oxygen history (Hill & Robinson 1974). According to Agar (1985), '. . . a technique for measuring growth rate may only be valid or suitable under a particular set of conditions. For this reason . . . the selected technique must be proven, and sometimes modified, in each case'. We considered comparison of four relatively simple growth-rate measurement techniques to be a useful complement to the basic investigation. Based on these initial studies of banana-pulp liquor fermentations, a preliminary process flowsheet and material balances are given for the case of a 20,000 kg fresh weight/day cull banana batch bioconversion process.

Materials and methods

LIST OF ABBREVIATIONS AND SYMBOLS

CP	crude protein (N × 6.25)
DO	dissolved oxygen tension, kPa
DW	dry weight, kg
FW	fresh weight, kg
OD	optical density
OUR	oxygen uptake rate, mol $O_2/m^3/h$
Q_{O_2}	microbial specific respiration rate, mg O_2/g DW/h
R	DO chart recorder reading, mV
R_O	DO chart recorder reading at oxygen saturation with respect to air, mV
S	substrate concentration, kg/m^3
t	time, h
vol/vol/min	volume per minute of sparged air per volume of fermentation broth, min^{-1}
x	dry weight biomass concentration, g/l
$Y_{x/S}$	biomass-from-substrate-utilized yield factor, $Y_{x/s} = \triangle x/\triangle S$, kg/kg
μ_{max}	maximum specific growth rate, $\mu = (1/x)\,(dx/dt)$, h^{-1}

Microorganism

Lyophilized *Candida utilis* (ATCC 9226) was maintained on agar slants at 5°C. The slants had the following complex medium composition (g/l): glucose, 20; yeast extract (Difco), 6; malt extract (Difco), 6; peptone, 10; agar, 50. The glucose was autoclave-sterilized separately from the other constituents and the two solutions combined aseptically after cooling. Periodic transfers of the slant cultures were made once every two months.

Bananas

The bananas (reputedly *Musa sapientum* var. 'Gros Michel') were purchased as

required from a local supermarket. As received, the skin was normally yellow with only a few black-brown spots, corresponding to colour number 7 on the United Fruit Company colour classification scale (Poland *et al.* 1938). As is usually the case, the bananas had been shipped green from the producing country and had been artificially ripened in a carbon dioxide- and ethylene-enriched atmosphere before delivery to the retailer. The purchased bananas were stored for up to several days in the laboratory at room temperature before use; the skin appearance and the pulp texture did not change appreciably during this period.

Fermentation media

The following carbon source-limited medium used in the fermentation studies contained (g/l): carbon source, as specified ($\leqslant$ 20); KH_2PO_4, 8; $(NH_4)_2SO_4$, 8; $MgSO_4 \cdot 7H_2O$, 2; $CaCl_2$, 0.6; KCl, 0.2; $FeSO_4 \cdot 7H_2O$, 0.012; $MnSO_4 \cdot H_2O$, 0.004; $ZnSO_4 \cdot 7H_2O$, 0.008; $CoSO_4$, 0.004; biotin, 10^{-8}; calcium panthothenate, 2×10^{-5}; nicotinamide, 2×10^{-5}; riboflavin, 10^{-5}; thiamine·HCl, 2×10^{-5}; pyridoxine·HCl, 2×10^{-5}; inositol, 10^{-4}; *p*-aminobenzoic acid, 10^{-5}. The trace elements and vitamins were prepared separately as concentrated solutions. The carbohydrate source, $MgSO_4 \cdot 7H_2O$, $CaCl_2$ and KCl were autoclave-sterilized together, but separately from the other minerals and trace elements. The two separate solutions were combined aseptically after each had cooled. The solution of vitamins was filter-sterilized (0.2 μm nominal pore-size membrane; Sartorius GmbH, Göttingen, FRG) and added to the solution of the other constituents.

Glucose, fructose and sucrose were used separately as the sole carbon source for batch-growth experiments at initial concentrations of 20 g/l. Two additional experiments were done using a mixture of glucose, fructose and sucrose having initial concentrations of 7.33, 4.0 and 4.83 g/l, respectively.

The banana medium was prepared as follows: 50 g of whole banana was chopped in a Waring blender, together with 0.3 l deionized water and 0.84 g of potassium alum $[Al_2 (SO_3)_3 \cdot K_2SO_4 \cdot 24H_2O]$ used as a flocculating agent. The pH of the resulting solution/slurry was adjusted to 6.2 ± 0.1 with 10% (w/v) NaOH. Fifteen batches of the resultant pH-adjusted solution/slurry were made and placed in the 14 l stirred tank fermenter, which then was autoclave sterilized for 2 h. A 575-ml volume of carbohydrate-free fermentation medium was sterilized separately and after cooling was added aseptically to the fermenter. Vitamins were added separately as previously described. The above quantities were used to ensure that the initial sugar concentration as estimated from the reported sugar content of ripe bananas (Barnell 1941) did not exceed 20 g/l.

Inocula preparation

The inocula for the glucose, the combined pure carbohydrates and the banana liquor experiments were grown at 35°C in 0.3 l of the fermentation medium on 20 g/l glucose in shaken flasks. For the fructose and sucrose growth experiments, the carbon source was the respective sugar at 20 g/l initial concentration. Two transfers under the above conditions were made. The final inoculum was 5% (v/v) in all experiments.

Fermentation

All fermentations were conducted in a 14 l Magnaferm Model MF214 fermenter (New Brunswick Scientific Co., Inc., New Brunswick, NJ, USA) operated with a liquid

volume of 6 l. The dissolved oxygen (DO) tension was monitored by a Model M1016–0208 sterilizable DO electrode (NBS Co., Inc., NJ, USA) and continuously recorded by a strip-chart recorder (Model XKR, Sargent-Welch Scientific Co., IL, USA). The pH was controlled at 4.5 ± 0.05 using a sterilizable Ingold pH electrode (Model 465–25, Ingold Electrode, Inc., MA, USA) with an automatic pH-40 controller (Model M1055–7000, NBS Co., Inc., NJ, USA). Alkali (5M NaOH) or acid 2.5M H_2SO_4) was added by a coupled peristaltic pump module (Model M1055–7501, NBS Co., Inc., NJ, USA). Unless noted, polypropylene glycol P400 was used as an antifoam, being added as required by fermenter automatic control. Aeration was at the rate of 1.67 vol/vol/min; the rotational speed of the fermenter's six-blade turbine was maintained at 10 rev/sec. The temperature was controlled at 35 ± 0.5°C for all runs.

Analytical methods

Reducing and total sugars. Fermentation samples were stored at 5°C after adding a few granules of $HgCl_2$ to arrest yeast growth and that of any inadvertent contamination. Storage for up to three weeks at 5°C did not affect the subsequent analyses. Prior to analysis, samples were centrifuged (34,000 *g*; 20 min); 10 ml of supernatant liquid was membrane filtered (0.2 μm mean pore diameter), and the filtrate analysed for sugar(s). The DNS test for reducing sugars (Miller 1959) was used to detect either glucose or fructose, whereas the total carbohydrate method of Dubois *et al.* (1956) was used for the determination of sucrose when it was sole carbohydrate present.

HPLC sugar analysis. Glucose, fructose and sucrose were individually quantified by HPLC analyses for those fermentation experiments which involved mixtures of these three carbohydrates as the carbon source (either mixtures of the pure sugars in specified ratios or banana liquors derived from whole bananas). Each sample was centrifuged for 20 min at 34,000 *g* and 7 ml of the supernatant liquid was transferred to a 50 ml test tube containing 7 g of analytical-grade mixed ion-exchange resin (AG 501-X8, Biorad Labs, Richmond, CA, USA). The mixture was shaken for 10 min and then a liquid sample was withdrawn into a syringe fitted with a Seitz filter (0.2 μm mean pore size). The resulting clear and deionized solution was stored frozen for up to 20 days before HPLC analysis. The latter was performed using a column containing Aminex HPX-85 carbohydrate analysis packing (Biorad Labs, Richmond, CA, USA). Distilled, degassed and deionized water was used as the mobile phase. Eluted peaks were detected by a differential refractometer (Model R401) connected through a differential refractometer electronics unit (Model P400) to a programmable data module (Model 730) (all Waters Associates, Milford, MA, USA). The column and refractometer temperatures were maintained at 85 and 45°C, respectively. The instrument was calibrated using standard solutions of mixed sugars of known mass composition.

Biomass concentration. The optical density (OD) was determined at 620 nm (Turner Model 330 spectrophotometer, Palo Alto, CA, USA) on samples which had been diluted 40-fold. Deionized water was used in the reference cell. Dry weight (DW) concentration (x) was determined by vacuum filtration of a known volume of sample (usually 10 ml) using 0.45-μm mean pore size cellulose acetate filters (Sartorius GmbH, Göttingen, FRG) of predetermined tare weight, followed by air drying in an oven at 80°C for 24 h.

Crude protein. The crude protein (CP) content of the solids (mostly yeast cells with some residual banana insolubles) in the fermenter samples was determined using a Kjel-Foss Automatic 16210 Analyzer (A/S N. Foss Electric, Copenhagen, DK). Samples were filtered through reinforced cellulose acetate filters, washed, and then the filter cake and the filter together were analysed for total nitrogen (CP = 6.25 × N). Three fresh filters were analysed separately, and their average CP content was subtracted from that of the filter cake plus filter determinations.

Maximum specific growth rate (μ_{max}). Least-squares regression analysis of plots of the logarithm of OD, x and CP versus time yielded values of μ_{max} for each of these measurement methods. In addition, the maximum specific growth rate was calculated from the recorded DO versus time data according to the method of Hill & Robinson (1974). In that case, a plot of $\ln[R_O - R)/R_O]$ versus time gives a straight line during the exponential growth phase, the slope of which is the maximum specific growth rate when the oxygen mass-transfer parameters and the microbial specific respiration rate are constant. Here R_O is the DO chart recorder reading at $t = 0$ (i.e. of the cell-free medium immediately before inoculation, corresponding to DO saturation with respect to air) and R is the chart reading at a time t during the growth phase.

Specific respiration rate. The method of Bandyopadhyay *et al.* (1967) was used to estimate the microbial specific respiration rate (mg O_2/g DW cells/h). Henry's law coefficient for the fermentation medium was calculated according to the method of van Krevelen & Hoftijzer (1948).

Banana pulp and liquor properties. Experiments were conducted in order to determine the moisture content, dry weight and the insoluble solids before and after autoclave sterilization of the bananas. Bananas were cut longitudinally and one-half of known fresh weight (FW) was chopped in a Waring blender after adding a known volume of deionized water. The entire contents of the blender (banana liquor plus insoluble solids) then were transferred to a 1 l Erlenmeyer flask, which was autoclave-sterilized (121°C) for 30 min, then cooled overnight with occasional shaking to prevent solids adhering to the glass surface. The original yellow-coloured solution/suspension turned darkish brown after sterilization and cooling. Solids concentration was determined by taking 50-ml samples before and after sterilization, centrifuging each sample at 34,000 *g* for 20 min, followed by vacuum filtration of the supernatant through 0.45-μm pore size cellulose acetate filters (Sartorius). The total dry weight of the residual solids then was determined gravimetrically. The dry weight of the original banana pulp and the insolubles content of the pre- and post-sterilization banana liquors/suspensions then were correlated with the fresh weight.

Results

Banana and banana liquor properties

The equivalent dry-weight (DW) concentration of banana total solids and the insoluble solids contents of the banana liquor/suspensions before and after sterilization were determined over a range of fresh weight (FW) concentrations (10–40% w/v FW) (Figs 1, 2). Given the expected variation in natural products, there is reasonable correlation of the DW concentration (i.e. the potential amount of substrates for yeast production) achieved in the banana liquors/suspensions prepared from the fresh bananas with the fresh weight (FW) concentration (% w/v) used.

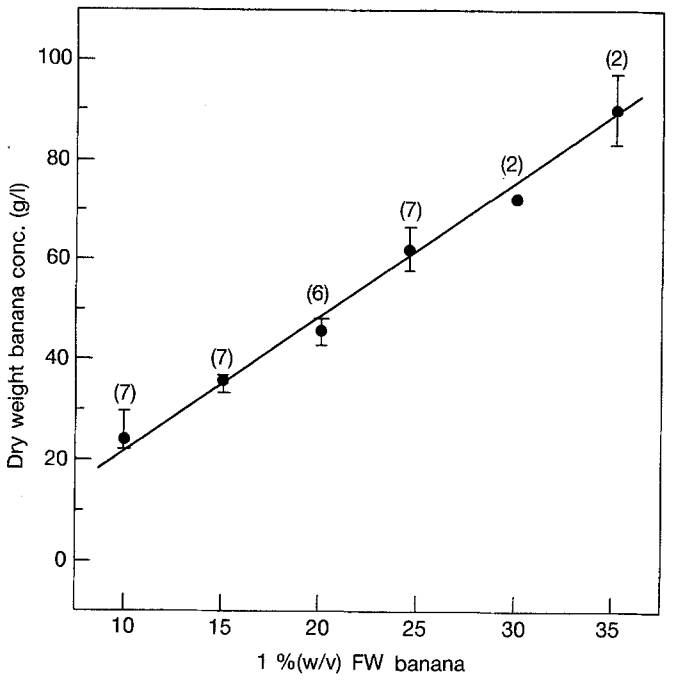

Fig. 1 Relationship between total solids concentration of banana liquors (DW) and the pulp fresh weight concentration (FW% w/v) for *Musa sapientum* var. 'Gros Michel' (colour classification 7). Bars indicate standard deviation of the measurements; number at the top of each bar gives the number of independent samples tested in each case.

Although the results indicate a rather large standard deviation, Fig. 2 shows that the amount of banana insolubles systematically decreased upon sterilization at 121°C for 2 h, except perhaps for the 35% (w/v) FW case where only one pre-sterilization sample was tested. That is, heat treatment of that type results in solubilization of some banana solids which originally were insoluble, e.g., starch. In the case of the 10% FW banana liquor/suspension, about 80% of the total fresh banana solids were solubilized following mixing and autoclaving.

The combined results of Figs 1 and 2 show that before autoclaving, the total soluble solids in the banana liquor average only 17.0 g/l at 10% FW and only 37.9 g/l at 25% FW. However, the total soluble solids in the banana liquor after sterilization ranged from an average of 19.1 g/l with 10% FW banana to 45.1 g/l when starting with 25% FW banana. Thus, the described heat-treatment process increases the soluble banana solids by 12.4 and 19% at the 10 and 25% FW starting levels, respectively, which could lead to equivalent increases in the potential biomass and crude protein contents of the final fermentation broth. If all the soluble solids were fermentable and assuming an average yield of *c.* 0.5 g DW biomass/g substrate consumed, starting with 10 or 35% FW the post-sterilization soluble solids could produce up to about 10–30 g DW cells/l broth or a corresponding crude protein concentration of *c.* 4.5 or 13.5 g/l broth, respectively, assuming a DW biomass crude protein content of 45%.

Growth on glucose

Four replicate experiments were done for growth of *C. utilis* on glucose at initial

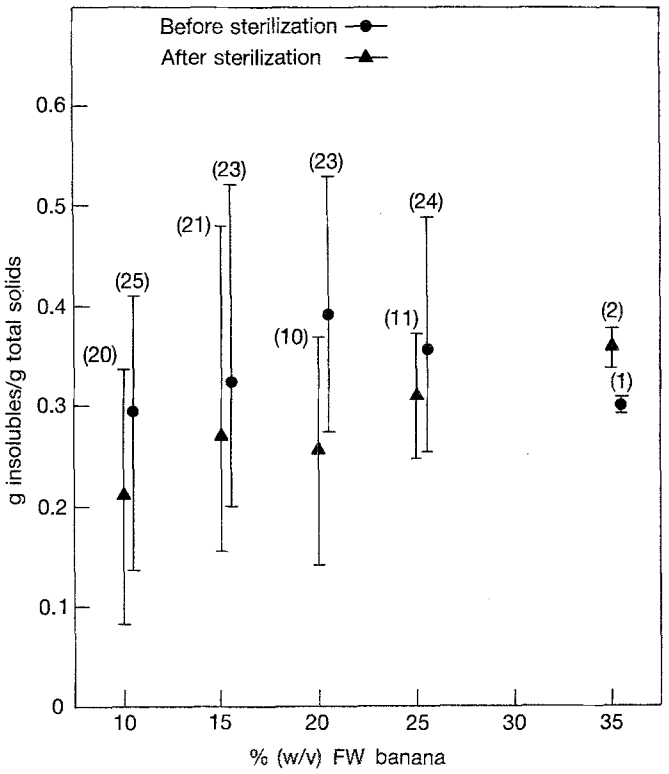

Fig. 2 Insoluble solids fraction (g insolubles per g total solids) of banana liquors/suspensions derived from *Musa sapientum* var. 'Gros Michel' of colour classification 7 (artificially ripened). Sterilization was at 121°C for 30 min. Bars indicate standard deviation of the measurements; the number of independent samples tested is indicated at the top of each bar.

concentrations of *c.* 23 g/l; typical results are shown in Fig. 3. In the stationary phase of experiments 3 and 4, the final cell dry-weight concentration reached *c.* 10.5 g DW/l, giving a biomass-from-substrate-utilized yield factor of $Y_{x/S} = 0.45$.

Linear regression of data in the exponential growth phases of Fig. 3 shows that $\mu_{max}(x)$ was 0.383 and 0.363 h^{-1} for experiments 3 and 4, respectively. A semi-logarithmic plot of OD versus time (Fig. 4) yielded maximum specific growth rates μ_{max}(OD) which were consistent with those calculated from the dry-weight measurements (see Table 1). However, as shown by the different representative slopes of the OD and the $(R_O - R)/R_O$ data in Fig. 4, the dissolved oxygen history method of Hill & Robinson (1974) in these cases yielded values of μ_{max}(DO) which were 17 or 25 to 35% higher than the corresponding values based on x or OD, respectively (Table 1). These discrepancies are likely due to the fact that the specific respiration rate of the yeast during the exponential growth phase was not sufficiently constant (Table 2), as was assumed for the development of the DO history model (Hill & Robinson 1974).

The specific respiration coefficients (Q_{O_2}) and the corresponding volumetric oxygen uptake rates (OUR) for the batch fermentation of experiment 3 are shown in Table 2.

Samples of fermentation broth were taken for ethanol analysis (Sigma Chemical enzymatic analysis kit and procedure; Anon. 1974). No ethanol was detected in any of the samples. The lack of ethanol production also is confirmed by the absence of any

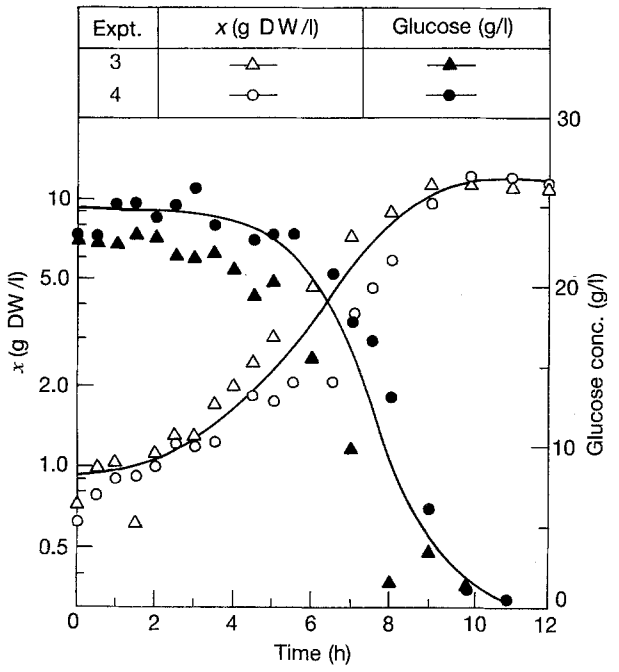

Fig. 3 Cell production, x, expressed as dry weight biomass concentration, and substrate uptake for growth of *C. utilis* (ATCC 9226) on glucose at 35°C and pH 4.5. Data of experiments 3 and 4.

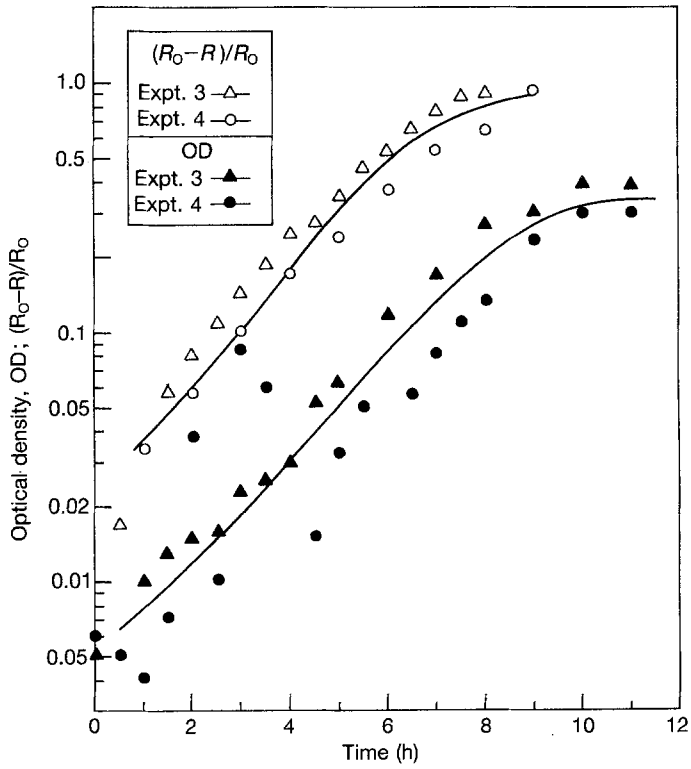

Fig. 4 Determination of the maximum specific growth rate by the optical density (OD) and the dissolved oxygen history $[(R_O-R)/R_O]$ methods for the batch growth of *C. utilis* (ATCC 9226) on glucose at 35°C and pH 4.5. Data of experiments 3 and 4.

Table 1 Maximum specific growth rates (μ_{max}) and biomass yield coefficients ($Y_{x/S}$) for growth of *Candida utilis* (ATCC 9226) on various carbon sources at pH 4.5 and 35°C. Polypropylene glycol P400 antifoam, except where noted

Carbon source	Initial conc. (g/l)	Expt. No.	μ_{max} (h^{-1})				$Y_{x/S}$
			x^a	OD[a]	DO[b]	CP[a]	
Glucose	23	1	–	–	0.395	–	–
		2	–	0.321	0.469	–	–
		3	0.383	0.332	0.447	–	0.45
		4	0.363	0.340	0.474	–	0.45
Fructose	20–22	5[c]	0.269	0.451	0.443	–	0.44
		6	0.364	0.572	0.427	0.568	0.51
		7	0.378	0.637	0.527	0.491	0.56
Sucrose	20	8	0.519	0.500	0.509	0.489	0.46
		9	0.502	0.543	0.536	0.465	0.58
Mixed sugars							
glucose	7.33						
fructose	4.00	10	0.431	0.478	0.473	0.473	(0.61)[d]
sucrose	4.83	11	0.430	0.560	0.469	0.476	(0.56)[d]
	16.16						
Whole banana liquor							
glucose	9.93						
fructose	5.44	12	–	–	0.515	0.500	(0.62)[e]
sucrose	4.59						
	19.96						

Notes: (*a*) slope of semi-log plots of cell dry weight (x), optical density (OD) and crude protein (CP) versus time; (*b*) slope of ln $[(R_O-R)/R_O]$ versus time from dissolved oxygen (DO) history; (*c*) ethylene glycol antifoam; (*d*) based on total initial carbohydrate concentration; (*e*) based on final corrected crude protein conc. of 5.55 g/l, assuming cells are 45% DW CP, and on initial total sugar conc.

diauxic growth patterns in Figs 3 and 4. Similar conclusions can be made about all the fermentations on the other carbon sources, the results of which are presented in the following sections. Thus, within the detectable limits of analysis, the yeast exhibited fully-aerobic metabolism for all the fermentations reported in this paper.

Growth on fructose

The biomass and substrate concentration data for three experiments (5–7) are shown in Fig. 5. These were run under identical conditions, except that for experiment 5 the antifoam used was ethylene glycol, whereas in experiments 6 and 7 the usual polypropylene glycol P400 was used to suppress foaming. It is evident from Fig. 5 that there are significant differences between both the cell growth and the substrate utilization histories for fermentations using these two different antifoams, which differences also are reflected by the corresponding data of Table 1. When ethylene glycol was used (experiment 5), a lag phase of some 5 h was observed, in contrast to the nearly immediate start (lag phase $\leq$ 1 h) of growth and substrate consumption for the case of polypropylene glycol P400 antifoam (experiments 6 and 7).

As shown in Table 1, the growth rate of *C. utilis* on fructose in the exponential phase was apparently strongly inhibited by the ethylene glycol antifoam, based on the comparative μ_{max} values calculated from the dry weight (x) and optical density (OD)

versus time data. This antifoam also appears to have somewhat reduced the biomass yield ($Y_{x/S}$) compared to those observed with polypropylene glycol P400, as indicated in Table 1.

The fructose data of Table 1 for experiments 6 and 7, both of which used the same antifoam (polypropylene glycol P400) as in all other experiments excepting No. 5, indicate greater differences between the values of the maximum specific growth rates calculated by the various methods than was the case for growth on glucose (experiments 3 and 4, Table 1). For fructose $\mu_{max}(x)$ was consistently and significantly lower than the corresponding values obtained from the other three methods (OD, DO, CP); in particular, the large differences between the μ_{max} values based on $x(t)$ and OD(t) data are rather surprising. None the less, from the overall comparative

Table 2 Specific respiration rate coefficients (Q_{O_2}) and volumetric oxygen uptake rates (OUR) for batch growth of *C. utilis* (ATCC 9226) on various carbon sources at pH 4.5 and 35°C

Carbon source[a]	Expt. No.	Ferm. Time (h)	Q_{O_2} (mgO_2/g DW/h)	OUR[b] ($molO_2/m^3/h$)
Glucose	3	4.5	154	11.8
		6	72	10.6
		7	21.3	4.8
		9	35.8	12.6
Fructose	6	4.5	136	11.2
		5.5	98	11.4
		6	68	9.5
		6.5	15	2.5
		7.5	8.6	2.2
	7	4.5	124	8.9
		5	120	10.1
		5.5	105	10.6
		6	83.7	10.0
		6.5	72.2	8.7
		7	34	5.8
Sucrose	8	4.5	323	16.8
		5	173	11.8
		5.5	121	10.3
		6	63.1	7.4
		6.5	38.7	5.7
	9	3.5	297	10.0
		4	235	11.5
		4.5	173	11.2
		5	146	11.2
		5.5	82	8.8
		6	48	6.5
		6.5	24	4.1
Mixed sugars (glucose + frucose + sucrose)	10	3	340	5.7
		4	336	12.6
		5	132	9.0
		6	50	6.1
		7	21	4.2
	11	3.5	257	9.9
		4.5	155	10.2
		5.5	65	6.9

Notes: (*a*) Initial (total) concentration *c.* 20 g/l (see Table 1); (*b*) OUR = $Q_{O_2}\, x/32$, where x = biomass conc., g DW/l.

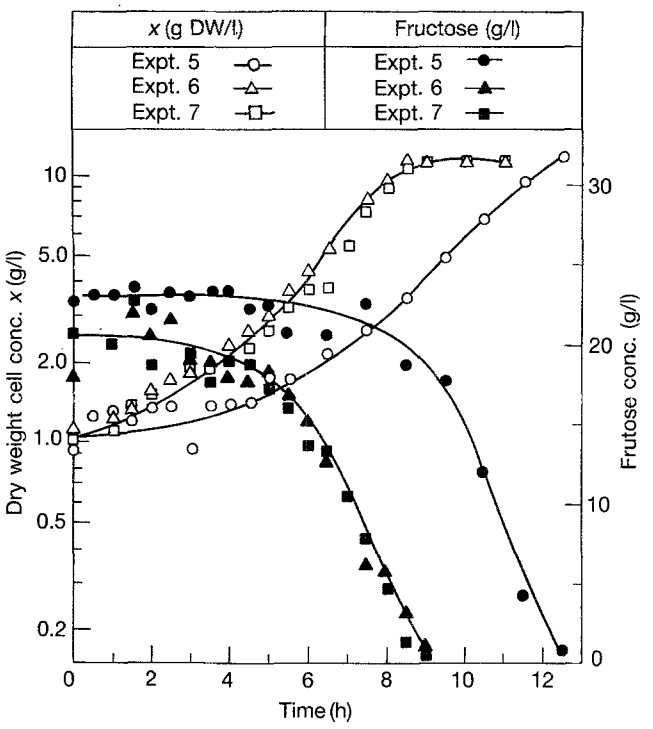

Fig. 5 Cell growth and substrate consumption for cultivation of *C. utilis* (ATCC 9226) on fructose at 35°C and pH 4.5. All experimental conditions were identical, except for the type of antifoam used: ethylene glycol for experiment 5, but polypropylene glycol P400 for experiments 6 and 7.

data, it can be concluded that *C. utilis* growth under the indicated conditions metabolizes fructose at an equivalent (x basis) or somewhat higher (OD, DO, CP bases) rate than glucose.

As shown in Table 2, the fermentation oxygen demands for *C. utilis* essentially are equivalent for growth on either glucose or fructose at otherwise identical conditions.

Growth on sucrose

For the case of 20 g/l initial sucrose concentration, calculated maximum specific growth rates and fermentation oxygen demands are also given in Tables 1 and 2, respectively (experiments 8 and 9). No significant differences were observed between the maximum specific growth rates calculated from the four different methods (Table 1), with the possible exception of the CP-based results for experiment 9. Looking at the overall results, the kinetics of *C. utilis* aerobic batch growth on sucrose and fructose appear to be similar, whereas growth on glucose is somewhat slower.

The specific respiration rates (Q_{O_2}) in the early- to mid-log phase of the cultures using sucrose as the sole carbohydrate are noticeably greater than those for fructose, which in turn are somewhat greater than those for the glucose-based fermentations (Table 2). This behaviour parallels that of the respective maximum specific growth rates, as discussed above, and the corresponding carbohydrate uptake rates (metabolic rates). As the same series of metabolic pathways (glycolysis, tricarboxylic acid cycle, electron-transport chain) are used in each case for the energy-yielding bio-

oxidation of the substrates, such parallelism between growth and oxygen uptake rates is not unexpected.

Growth on mixed sugars

Sucrose uptake by yeasts is in the form of its hydrolysis products, glucose plus fructose, formed by the enzymatic action of wall- or membrane-bound invertase (β-D-fructofuranosidase). The activity of the latter may be inhibited by monosaccharides (Elorza *et al.* 1977). With mixtures of sucrose, fructose and glucose (such as would exist in banana liquors), it is thus possible that cell growth might not be uniphasic under all conditions. Therefore, preliminary multi-substrate growth studies were done using specified mixtures of pure sugars: sucrose, 4.83, glucose, 7.33 and fructose, 4.00 g/l initial concentrations before sterilization, which relative amounts approximate the potential concentrations and ratios in banana liquor produced from 10% (w/v) fresh weight ripe banana.

HPLC difficulties prevented reliable analyses of the individual sugars in the samples taken from these experimental runs. However, the DW measurements (raw data not shown) and those of OD, DO and CP (Fig. 6) enabled evaluation of μ_{max} and $Y_{x/S}$ for these cases (experiments 10 and 11 in Table 1). Examination of the plots on Fig. 6 of the OD and CP data and the $x(t)$ data show that there was essentially no lag phase and that there were no apparent discontinuities in the overall exponential growth phase,

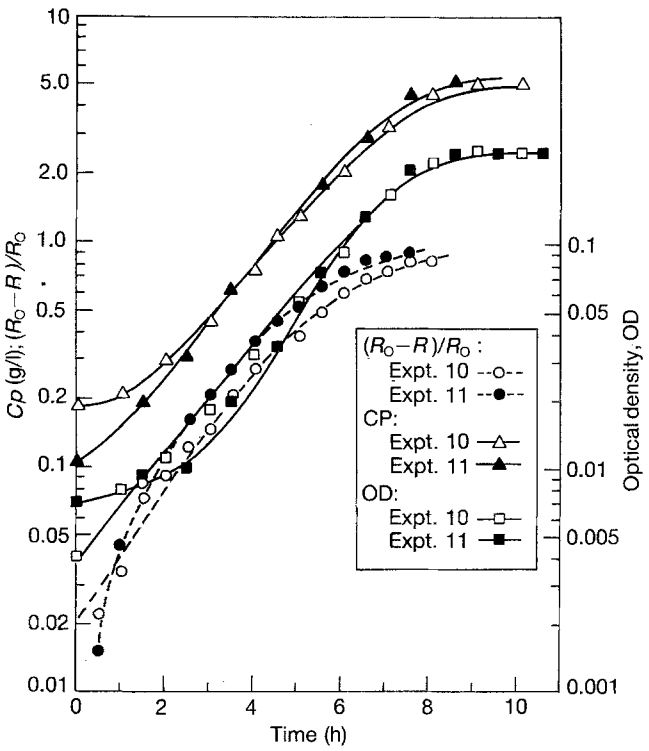

Fig. 6 Variation of optical density (OD), crude protein concentration (CP) and dissolved oxygen history parameter $[(R_O-R)/R_O]$ with batch cultivation time of *C. utilis* (ATCC 9226) on mixed sugars at 35°C and pH 4.5. Initial sugar concentrations (g/l) before sterilization: glucose, 7.33; fructose, 4.00; sucrose, 4.83.

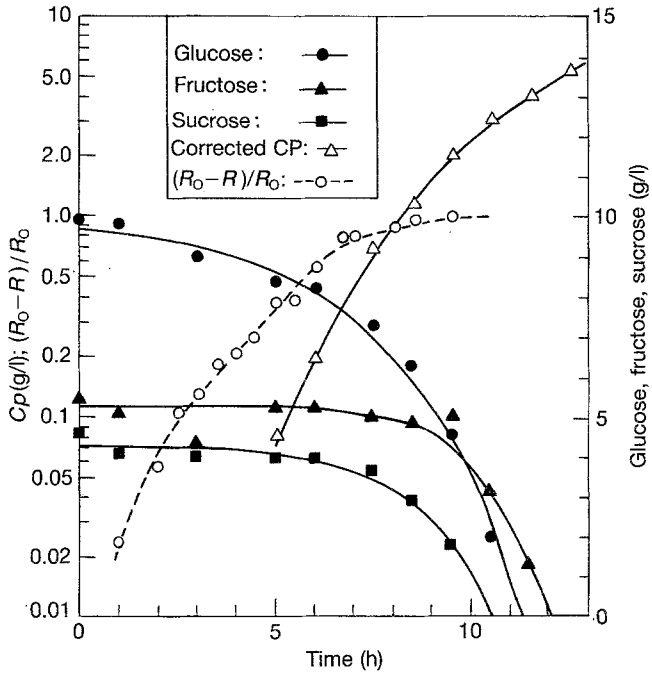

Fig. 7 Time course of fermentation of *C. utilis* (ATCC 9226) on whole banana liquor at 35°C and pH 4.5. Variation of soluble sugar concentrations, corrected crude protein (CP) and DO-method maximum specific growth rate parameter $(R_O-R)/R_O$ for experiment 12.

such that cell growth can be described as being uniphasic (although the substrate uptake pattern may have been sequential, i.e. glucose/fructose followed by sucrose, the latter uptake beginning after the monosaccharide(s) concentration(s) had been decreased to a sufficiently low level) (see Fig. 7). The plots of the DO history data [parameter $(R_O-R)/R$] for the multi-substrate systems of experiments 10 and 11 (Fig. 6) are non-linear, which undoubtedly reflects significant variations in Q_{O_2} values during the fermentation period as the organism presumably shifts from growing mainly on monosaccharides in the early log phase, to growth principally on the disaccharide sucrose in later log phase. Judging by the Q_{O_2} values (Table 2) for experiments 10 and 11, the yeasts appear to have been metabolizing mainly sucrose by the third hour of fermentation.

The calculated maximum specific growth rates for experiments 10 and 11 are given and compared in Table 1. All eight values listed should be considered as being 'apparent' ones which reflect the time-average growth behaviour resulting from whatever substrate uptake pattern was existent, and as being valid in practice only for the particular sugar concentrations and ratios used. The significant non-linearity of the DO history plots in Fig. 6, for reasons discussed above, makes application of the Hill & Robinson (1974) model uncertain in these particular cases; thus, the apparently good agreement of the μ_{max}(DO) values in Table 1 for experiments 10 and 11 with those computed from the other three methods may only be fortuitous. Comparison of the μ_{max} values calculated from the x, OD and CP data for growth on the mixed-carbon source medium (which are in good agreement) with those for single-substrate

growth cases (Table 1) indicates that the average maximum specific growth rate for the sucrose–glucose–fructose case lies between those observed for the two pure monosaccharides and that observed for pure sucrose.

Growth on whole banana liquor

Growth of *C. utilis* on whole banana liquor treated with potassium alum was investigated in experiment 12 using a glucose-grown inoculum. The sterilized fermentation medium contained (by HPLC analysis) glucose, fructose and sucrose in concentrations of 9.93, 5.44 and 4.59 g/l, respectively. The medium also contained suspended solids at a concentration of about 0.5% (w/v); therefore, optical density and cell dry weight concentrations could not be determined by the conventional methods and, hence, the yield coefficient ($Y_{x/S}$) also could not be calculated in the usual manner for this fermentation. Samples were taken for crude protein (CP) and soluble-sugar analyses; in addition, the dissolved oxygen (DO) tension history was recorded. The time course of this fermentation is shown in Fig. 7. The CP and DO data were used to estimate values of μ_{max} (Table 1).

The sugar-consumption patterns (Fig. 7) indicate that glucose was assimilated preferentially. Fructose and sucrose uptakes began at about the same time and when the glucose concentration had decreased to *c.* 6–7 g/l. Samples of broth taken at the start of the whole banana liquor fermentation and before there was any significant change in the biomass concentration from that provided by the inoculum, had crude protein concentrations averaging 1.0 g CP/l. This base value was subtracted from all further CP analyses (i.e., CP data for $t \geqslant 5$h, Fig. 7) to yield the 'corrected' CP concentration arising from biomass growth in the fermenter. The maximum specific growth rate calculated from the corrected CP(t) data for the banana liquor fermentation was comparable to that previously observed in the synthetic glucose–fructose–sucrose medium, as may be seen by comparing the Table 1 values for experiments 10 to 12.

The plot of $(R_O - R)/R_O$ in Fig. 7 exhibits non-linearity, as was the case for the corresponding plot in Fig. 6. As discussed previously, the apparent excellent agreement between μ_{max}(DO) and μ_{max}(CP) values for experiment 12 (Table 1) may only be fortuitous.

As is also shown in Table 1, an apparent biomass yield factor may be calculated for the growth of *C. utilis* on whole banana liquor by assuming that all the crude protein of the solids fraction of the medium was microbial in origin and that the crude protein content of the yeast cells was 45% on a dry weight basis. The apparent yield factor so calculated is $(Y_{x/S})_{app} = 0.62$ which is reasonable by comparison with the values calculated for growth on the synthetic multi-sugar medium (experiments 10 and 11, Table 1).

Discussion

The fermentable sugars' content of bananas depends on species and growth conditions as well as on the state and conditions of ripening. For *Musa cavendishii* (yellow skin freckled with brown dots), Poland *et al.* (1938) reported sugar contents of 17.5, 13.4 and 50.1 g/100 g DW pulp for glucose, fructose and sucrose, respectively. Sgarbieri & Figueiredo (1971) measured 30 g/100 g DW pulp reducing sugars and 62.6 g/100 g DW

pulp total sugars for *M. cavendishii* ripened at 25°C under natural conditions and for samples taken 12 days after the total yellow condition.

A detailed study of the carbohydrate content of *Musa sapientum* var. 'Gros Michel' bananas picked green and ripened under tropical conditions was conducted by Barnell (1941). He found the soluble carbohydrates to be (in g sugar/100 g DW pulp): glucose, 30; fructose, 27; and sucrose, 10 as average values for bananas between the ripe and over-ripe stages. On the other hand, the sugar content of the ripe pulp of *Musa paradisiaca* species grown in Nigeria was only 17% dry weight (Ketiku 1973).

In general, starch is first synthesized and then later is hydrolysed to sugars during development and ripening of the banana fruit. There is a complex interaction of various sectors of the plant's overall metabolism, in which invertase together with β-amylase and other enzymes play a crucial role (Shukla *et al.* 1973). The overall result is that the banana rapidly becomes richer in di- and monosaccharides (sucrose; glucose and fructose).

A mass balance on the results of this work obtained from analyses of the whole banana (*M. sapientum* var. 'Gros Michel') fermentation medium before inoculation shows that the bananas used had fermentable sugar concentrations (as g sugar/100 g DW banana) of 44.1, 24.2 and 20.4 for glucose, fructose and sucrose, respectively. Considering the differences in ripening conditions used in the two different cases, our experimental values are in reasonably good agreement with those reported by Barnell (1941), except for the glucose content, which was significantly greater in our case. Considering that our potassium alum-treated, mildly-acidic pre-fermentation medium was autoclave-sterilized (121°C) for 2 h, our higher glucose value may be due to partial hydrolysis of the starch content of the banana pulp. This postulate is further supported by the observed 28% reduction in insoluble solids after sterilization for 30 min under more acidic conditions in the 10% (w/v) FW banana pulp experiments (Fig. 2).

The specific respiration rates of *C. utilis* (ATCC 9226) grown on mixed sugars medium simulating banana pulp liquor derived from *M. sapientum* var. 'Gros Michel' (experiments 10 and 11, Table 2) and the corresponding volumetric oxygen uptake rates are not particularly high throughout the batch cultivation time. Thus, the agitation and aeration power input requirements for an industrial-scale process should not be uneconomic. The Q_{O_2} results obtained for our test organism are directionally consistent with those obtained by others using different strains of *C. utilis*. Rickard *et al.* (1971) studied the oxygen uptake requirements of *C. utilis* var. *major* cultured in a laboratory-scale stirred tank fermenter at constant pH (5.0 ± 0.1) and using an initial glucose concentration of 61 g/l and with oxygen control. At the mid-point of the exponential growth phase (51 g/l residual glucose), Q_{O_2} was 333 mg O_2/g DW/h, which decreased to 198.4 mg O_2/g DW/h in the stationary phase (glucose exhausted). The possible ethanol concentrations in this experiment were not reported (Rickard et al. 1971); however, in another study, Rickard & Hewetson (1979) indicated that the same yeast grown on 20 g/l glucose showed no apparent aerobic fermentation patterns.

De Deken (1966) reported that glucose concentrations up to 60 g/l did not result in the onset of the Crabtree effect (substrate concentration repression of aerobic metabolism) in aerated shake-flask cultures. Thus, initial sugar concentrations of considerably greater than 20 g/l (equivalent to 10% (w/v) FW ripened banana liquor) may be tolerated by *C. utilis* (ATCC 9226) while maintaining the totally aerobic

metabolism which maximizes the yield of cells (and their contained crude protein). None the less, and in order to be conservative in the design, we present below a summary of a preliminary microbial protein production flowsheet based on using banana liquors prepared from only 10% (w/v) FW banana concentrations.

Microbial protein process

Based on the results of this study, a preliminary process flowsheet has been developed (Aker 1981) for a batch fermentation plant to process 20,000 kg/day FW cull banana pulp for the production of *C. utilis* microbial protein, based on using banana pulp liquors prepared from 10% (w/v) fresh weight banana. Assuming that there is a continuous supply of bananas throughout the year, this is about the daily equivalent of the pulp content of the reported 15.4 t/a of bananas culled in St. Lucia (LIFE 1972), a relatively small production source in the world-wide banana economy.

As shown in the summary process flow sheet (Fig. 8), four 50 m^3 working liquid volume fermenters are required to be operated in parallel. If the total fermentation cycle (cleaning, sterilizing, inoculation, growth and harvesting) of each fermenter is chosen to be out of phase with those of the others, the overall process becomes a semi-

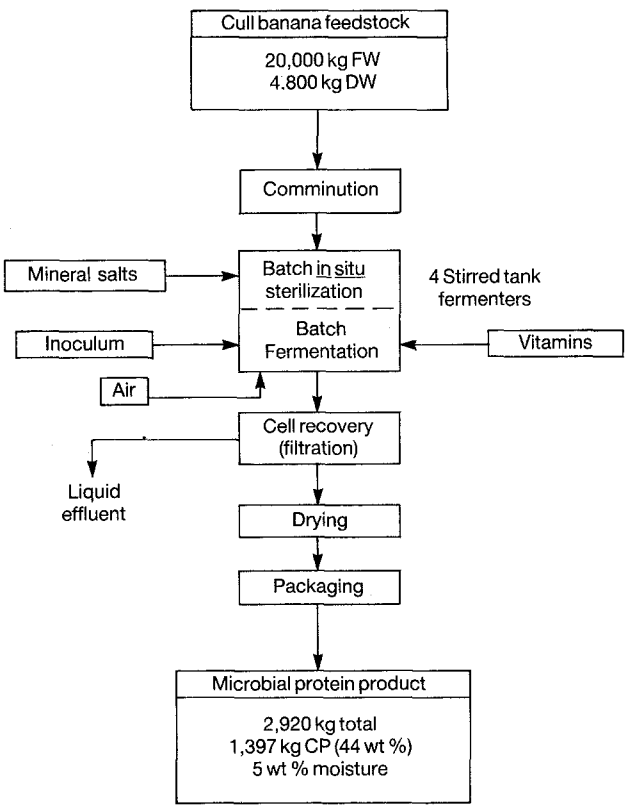

Fig. 8 Process flow schematic for the batchwise production of *C. utilis* microbial protein from cull *M. sapientum* var. 'Gros Michel' banana pulp. Sugars content as specified in the text. Design conditions for each of the four fermenters are summarized in Table 3.

continuous one in spite of each separate fermenter being operated batchwise, achieving more efficient utilization of the downstream processing equipment. Table 3 lists the key information relating to the design and operating conditions of each fermenter. For each 20,000 kg FW banana pulp (*M. sapientum* var. 'Gros Michel', colour classification 7) processed, 2,970 kg of microbial protein solids (5 wt% moisture) of 44 wt% crude protein would be produced under these conditions. More-detailed design calculations are given elsewhere (Aker 1981). Similar, but not identical, results would be expected for the use of naturally-ripened or over-ripe *M. sapientum* var. 'Gros Michel' cull bananas as available at the production site, and for other varieties (e.g. *M. cavendishii*) of similar fermentable sugar content.

Table 3 Fermenter design and operating conditions for process schematic of Fig. 8

Parameter	Volume
Fermenter capacity/dimensions	
Liquid volume	50 m^3
Diameter	4 m
Height	6 m
Agitation/aeration	
Impeller	Standard 6-flat blade turbine
Impeller rotational speed	1 rev/sec
Aeration rate	0.15–0.25 vol/vol/min
Mechanical agitation power input	17.5 kW
Max. required volumetric oxygen transfer coefficient	0.023 s^{-1}
Fermentation conditions	
Temperature	35°C
pH	4.5
Maximum OUR	16.8 mol $O_2/m^3/h$

Acknowledgements

This work was supported, in part, by the Natural Sciences and Engineering Research Council, Canada. The authors are also grateful to Dr Dagmar Vlach, Jana Otruba and Arlene Lamptey for performing the crude protein analyses.

References

AEGERTER, P. & DUNLAP, C. 1980 Culture of five commonly used acid-producing bacteria on banana pulp. *Applied and Environmental Microbiology* **30,** 937–942.

AGAR, D.W. 1985 Microbial growth rate measurement techniques. In *Comprehensive Biotechnology*, Vol. 4, ed. Moo-Young, M., Robinson, C.W. & Howell, J.A. pp. 305–328. Oxford: Pergamon Press.

AKER, K.C. 1981 Growth of *Candida utilis* on Soluble Carbohydrates and Waste Banana Liquor. M.A.Sc. Thesis, University of Waterloo, Waterloo, Canada. 181pp.

ANON 1974 Sigma Technical Bulletin No. 331-UV. Sigma Chemical Company, St. Louis, MO, USA.

BANDYOPADHYAY, B., HUMPHREY, A.E. & TAGUCHI, H. 1967 Dynamic measurement of the volumetric oxygen transfer coefficient in fermentation systems. *Biotechnology and Bioengineering*, **9,** 533–544.

BARNELL, H.R. 1941 Studies in tropical fruits, XI. Carbohydrate metabolism of the banana fruit during ripening under tropical conditions. *Annals of Botany* **5,** 217–247.

CHUNG, S.L. & MEYERS, S.P. 1979 Bioprotein from banana wastes. *Developments in Industrial Microbiology* **20,** 723–732.

DE DEKEN, R.H. 1966 The Crabtree effect: A regulatory system in yeast. *Journal of General Microbiology* **44,** 157–165.

DUBOIS, M., GILLES, K.A., HAMILTON, J.K., REBERS, P.A. & SMITH, F. 1956 Colorimetric method for the determination of sugars and related substances. *Analytical Chemistry* **28,** 350–356.

ELORZA, M.V., VILLANUEVA, J.R. & SENTANDREU, R. 1977 The mechanism of catabolite inhibition of invertase by glucose in *Saccharomyces cerevisiae. Biochimica et Biophysica Acta* **475,** 103–112.

FAO (Food and Agricultural Organization). 1975 *Handbook of Agricultural Statistics.* New York: United Nations Organization.

FAO (Food and Agricultural Organization). 1978 *Production Yearbook.* **32**: 179. New York: United Nations Organization.

HILL, G.A. & ROBINSON, C.W. 1974 Measurement of aerobic batch culture maximum specific growth rate and respiration coefficient using a dissolved oxygen probe. *Biotechnology and Bioengineering* **16,** 331–338.

KETIKU, A.O. 1973 Chemical composition of the unripe (green) and ripe plaintain (*Musa paradisiaca*). *Journal of the Science of Food and Agriculture* **24,** 703–707.

LIFE (League for International Food Education). 1972 Banana Agribusiness: Future Challenges. July.

LEFRANÇOIS, L. 1970 De la production de levure on d'aliments levurés à partir des déchets des industries fruitières. *Fruits* **25**. 112–114.

MILLER, G.L. 1959 Use of dinitrosalicylic acid reagent for the determination of reducing sugar. *Analytical Chemistry* **31,** 426–428.

POLAND, G.L., MANION, J.T., BRENNER, M.W. & HARRIS, P.L. 1938 Sugar changes in the banana during ripening. *Industrial and Engineering Chemistry*, **30,** 340–342.

RICKARD, P.A.D., MOSS, F.J., PHILLIPS, D. & MOLE, T.C.K. 1971 The effects of glucose and oxygen on the cytochromes and metabolic activity of yeast batch cultures, II. *Candida utilis. Biotechnology and Bioengineering* **13,** 169–184.

RICKARD, P.A.D. & HEWETSON, J.W. 1979 Effects of glucose on the activity and synthesis of fermentative and respiratory pathways of *Candida utilis. Biotechnology and Bioengineering* **21,** 2337–2339.

SGARBIERI, V.C. & FIGUEIREDO, I.B. 1971 Transformacoes bioquimicas da banana durants o amadurecimento. *Revista Brasileira de Technologia* **2**. 85–94.

SHUKLA, R.N., SINGH, S., DAS, N., BAIJAL, M. & SANWAL, G.G. 1973 Carbohydrate metabolism in *Musa paradisiaca. Phytochemistry* **12**. 979–985.

VAN KREVELEN, D.W. & HOFTIJZER, P.S. 1948 Sur la solubilité de gaz dans les solutions aqueuses. *Chimie et Industrie: Numéro spéciale du XXIe congrès internationale de chimie industrielle, Bruxelles.*

Summary

Large quantities of cull bananas are available world-wide, and often in regions lacking indigenous sources of high-quality protein animal feedstuffs. One possible method of utilizing these wastes is to upgrade them to microbial protein by means of bioconversion of the fermentable sugars of the ripe or over-ripe pulp by the yeast *Candida utilis*, which is nutritionally well-accepted. Studies of ripe *Musa sapientum* var. 'Gros Michel' bananas were conducted in order to obtain both the microbial and the engineering data necessary for the development of such an aerobic fermentation process. For banana pulp fermentation liquors in the concentration range 10 to 35% (w/v) fresh weight (FW) banana, the corresponding dry weight banana concentrations and the fraction of insoluble solids before and after sterilization (121°C, 30 min) have been measured and are reported. In addition to fermentation studies of banana liquor prepared from 10% (w/v) fresh whole banana, comparative kinetic studies of the batch growth and substrate assimilation pattern were performed on media containing as the sole soluble carbohydrate source either one of the major banana sugars (glucose, fructose, sucrose)

or a mixture of the three. Wherever feasible, the maximum specific growth rate (μ_{max}) was evaluated by four different methods: dry weight biomass, optical density, crude protein content of broth insoluble solids and dissolved oxygen history. In addition to the comparative μ_{max} results, values of the biomass yield coefficient and the specific respiration rates for each case are presented and discussed. A preliminary process design of the fermentation section of a moderate-scale microbial protein production plant based on 20,000 kg FW banana/day feedstock is presented. The results of this study indicate that such a plant would yield about 2,900 kg/day of protein-enriched product containing 5 wt% moisture and 44 wt% (*c.* 1,400 kg/day) crude protein.

Résumé

Croissance de Candida utilis *sur substrats glucidiques simples et complexes et sur déchets de bananes en vue de la production de protéines d'organismes uni-cellulaires*

De grandes quantités de refus de bananes sont disponibles dans le monde, souvent dans des régions dépourvues de ressources protéiques indigènes pour l'alimentation animale. Une possibilité d'utilisation de ces déchets est leur enrichissement en protéines microbiennes par conversion des sucres fermentescibles de la pulpe mûre ou blette, grâce à la levure *Candida utilis*, lequelle est bien acceptée nutritionnellement. Des essais ont été effectués avec des bananes mûres, *Musa sapientium* var. Gros Michel, dans le but de disposer des informations microbiologiques et biotechnologiques nécessaires pour développer ce procédé de fermentation aérobie. On a utilisé des milieux de fermentation contenant 10 à 35% (poids/volume) de bananes fraîches et on a déterminé le poids sec de banane et les teneurs en matière solide insoluble avant et après stérilisation (121°C, 30 min). Simultanément avec les études sur la fermentation de milieux contenant 10% (p/v) de bananes fraiches, on a procédé comparativement à des études de croissance en batch et d'assimilation du substrat dans des milieux de fermentation contenant comme seule source soluble de carbone soit un des sucres majeurs de la banane (glucose, fructose, saccharose), soit un mélange des trois. Chaque fois que cela a été possible, on a mesuré le taux maximum de croissance (λ_{max}) par quatre méthodes différentes: poids sec de la biomasse, densité optique, teneur en protéine brute de la fraction insoluble, et mesure de l'oxygène dissous. Le rendement en biomasse et le taux spécifique de respiration ont été déterminés dans chaque cas particulieur. Un projet préliminaire d'usine pilote de dimension moyenne (20.000 kg de bananes/jour) est présenté. Les résultats de cette étude montrent qu'une telle usine pourrait fournir environ 2.900 kg par jour d'un produit enrichi en protéines, ayant une humidité de 5% et contenant 44% de protéine brute, soit environ 1.400 kg/jour de protéines.

Resumen

Crecimiento de Candida utilis *para la obtención de proteinas celulares en medios con uno o varios azucares y en extractos de banana*

La obtención de grandes cantidades de bananas en mal estado es relativamente sencilla en muchos lugares, a menudo en regiones que carecen de fuentes autóctonas de proteínas de calidad para piensos animales. Una forma de aprovechar estos desechos es incrementar su valor biológico obteniendo proteínas microbianas, nutricionalmente bien aceptadas, mediante bioconversion de los azucares fermentables de las bananas, maduras o pasadas, por la levadura *Candida utilis*. Se han realizado estudios en bananas maduras (*Musa sapientum* var, Gros Michel) afin de obtener los datos precisos, tanto microbiológicos como técnicos, para el desarrollo de este proceso de fermentación aeróbica. Se han medido el peso seco de las bananas utilizadas y la fracción de sólidos insolubles antes y despúes de la esterilización, para los extractos fermetables preparados con un 10% hasta un 35% (p/v) de bananas pesas frescas (FW). Además de los estudios sobre la fermentación en extractos de banana (10% de banana fresca), se han llevado a cabo estudios cineticos comparativos sobre el crecimiento exponencial y sobre el patrón de asimilación de sustrato, en medios que contenían como único carbohidrato soluble uno de los principales azucares de la banana (glucosa, fructosa, saccarosa) o bien una

mezcla de los tres. Siempre que fue posible se evaluó la tasa de crecimiento específico máximo (μ_{max}) utilizando cuatro métodos distintos: peso seco de la biomasa, densidad óptica, contenido de proteína cruda del residuo insoluble del caldo, y, evolución del oxigeno disuelto. Además de los resultados comparativos de μ_{max}, se presentan y se discuten el coeficiente de producción de biomasa y la tasa de respiración específica. Se describe un diseño preliminar de la sección fermentative de una planta de producción de proteínas microbianas para piensos, de tamaño medio, basada en 20 000 kg FW de bananas/día. Los resultados de este diseño indican que la producción de una planta de este tipo oscilaria alrededor de 2900 kg/día de producto enriquecido con proteinas que contendría un 5% de humedad y un 44% (equivale a 1400 kg/día) de proteina cruda.