High Productivity and Good Nutritive Values of Cellulolytic Bacteria Grown on Sugarcane Bagasse

INTRODUCTION

The fermentation of cellulosic wastes by cellulolytic bacteria shows promise as a process for obtaining new low-cost protein sources; at the same time, it solves a problem of waste disposal. At present, many problems remain to be solved before making this process economically profitable: The low productivity due to poor cell density attained,[1,2] and low nutritive value of the biomass produced[3] stand out as two of them.

Humphrey et al.[4] have pointed out that the critical question in the microbial degradation of cellulose is how to optimize the cell yield in order to achieve nearly total cellulose degradation. Bellamy[5] calculated that the rate of cellulose utilization should be at least 1–5 g L^{-1} h^{-1} for the process to be profitable.

The work described here deals with how increases of cell concentration and productivity change the media concentration of a fermentation process using *Cellulomonas* sp., and the determination of the nutritive value of the product.

MATERIALS AND METHODS

Microorganism and Medium

Cellulomonas strain IIbc was used throughout the experiments. Alkali-pretreated sugarcane bagasse was used as the energy and carbon source at 25 and 40 g/L. Alkaline pretreatment of bagasse was performed[6] in 10% NaOH solution at 180°C for 1 h. The medium contained, additionally, per 10 g of pretreated bagasse: KH_2PO_4, 3.5 g; Na_2HPO_4 $12H_2O$, 18.0 g; NH_4Cl, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; and thiamine, 1.3 mg. We also added NaCl at 1.0 g/L to the medium.

Culture Conditions

The fermentation process carried out is aerobic. We used glass and stainless-steel stirred fermenters of 5- and 40-L operating volume, respectively, equipped with pH, temperature, air flow, and stirrer speed automatic controls. The operational parameters were 35°C, pH 6.5, and 1.0 vvm. The fermenters were inoculated with culture at 10% (v/v) from bacteria grown for 48 h in similar conditions. The cell concentration was monitored turbidimetrically.[7] In order to estimate the dry biomass weight, the corresponding value of optical density (OD) must be divided by 1.5.[2]

Analytical Methods

The nitrogen content of the cell was determined by the micro-Kjeldahl method. Sample digestion was carried out according to the procedure described by Choudry.[8] In order to determine the amount of protein, the Biuret method was employed according to Herbert, Phipps, and Strange.[9]

Amino acid analyses were made on lyophilized biomass which had been hydrolyzed in 6*N* HCl under reflux for 24 h using at least 10 mL acid/mg material. The hydrolysates were evaporated in vacuum and analyzed in an automatic amino acid analyzer, model AAA 881 (Mikrotechna, Prague). Determination of DNA and RNA was accomplished according to the Dutch and Parry method.[10]

Animal Experiments

The biological value (BV), net protein utilization (NPU), and N digestibility were determined by feeding three groups of five male weanling albine rats during 10 days with experimental diets.

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These animals received a basal diet including bacterial biomass as the sole source of protein at a dose calculated to provide 100 g crude protein/kg (N × 6.25). In each experiment, in addition to the control (without protein) diet given to one of the groups of rats, a second group was fed on a diet containing casein to provide 100 g crude protein/kg (N × 6.25).

RESULTS AND DISCUSSION

Experimental Fermentation Runs

Figure 1 shows the results of increasing the bagasse concentration in the medium. Table I presents the growth rates, cell concentration, yield, and maximal productivity (a parameter which predicts the maximal value of the volumetric productivity that could be achieved in a continuous culture).

The diauxic growth shown by the culture at lower bagasse concentration (10/L),[7] was not clearly apparent, probably because of the existence of enough available quantities of hemicellulose and amorphous cellulose to allow bacterial growth and simultaneous synthesis of the induced component of cellulase complex for attacking the crystalline cellulose.

The cell concentration was increased up to 13 g/L of dry biomass weight by changing the alkali-pretreated bagasse concentration from 10 to 40 g/L. The growth rate was kept between 0.14 to 0.16, achieving a bagasse conversion efficiency of 0.33-0.36 g dry biomass weight/treated bagasse fed. The value of maximal productivity achieved was significant.

Protein Quality and Nucleic Acid Content

The N content of the biomass was 9.27 ± 0.07 g/100 g dry lyophilized cells or 57.95 ± 0.42% of crude protein (N × 6.25). The estimation of protein content in the biomass by Biuret method was 49 ± 1%.

Table II shows the essential amino acid contents of *Cellulomonas* strain IIbc along with the ideal amino acid pattern suggested by FAO and the profile of those products which might conceivably be competitors to the protein from the IIbc strain, which has as their limiting amino

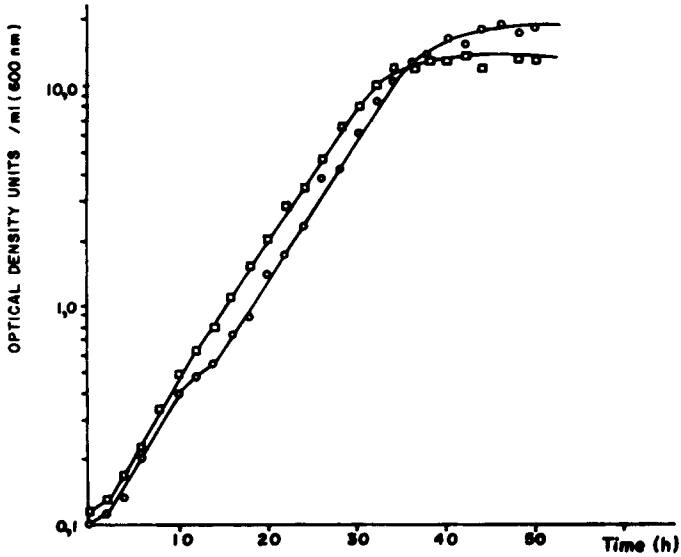

Fig. 1. Effect of bagasse concentration on cell density, measured as optical density units per milliliter. Bagasse concentration is (□) 25 g/L and (○) 40 g/L.

TABLE I[a]
Results of the Experimental Fermentative Runs

Bagasse concentration (g/L)	Growth rates u_{max1} (h^{-1})	Growth rates u_{max2} (h^{-1})	Dry biomass (g/L)	Yield	Maximal productivity (g L^{-1} h^{-1})
8[b]	0.18	0.16	2.8	0.36	0.5
25	0.16	0.14	2.0	0.36	1.4
40	0.16	0.15	13.2	0.33	2.1

[a]Note that "Yield" equals dry biomass/bagasse concentration and that maximal productivity equals the product of the dry biomass and the growth rates.
[b]Previously reported in ref. 2.

acid methionine at 0.61%.[11,12] The nucleic acid contents of the biomass was ca. 15%, showing that the RNA fraction was 9.6 ± 0.6% and the DNA fraction was 5.5 ± 0.4%.

Animal Experiments

In experiments with rats, the biological value (BV) of the bacterial proteins, calculated on the basis of the total N content of the diet, was 62. The N digestibility was 90%, which indicates a good utilization of the bacterial proteins. The net protein utilization (NPU) of 56 was calculated from the BV and N digestibility.

In the determination of the nutritive value of microorganisms, consideration must be given to the effect of N in compounds other than proteins, peptides, and amino acid—namely, the nucleic acids and polyhexosamines. In bacteria, by far the greater contribution to non-α-NH_2 is from nucleic acid, whose N represents more than 10% of total N contents of biomass. In order to avoid this interference, the calculations can be based on α-amino N, but the difficulties inherent

TABLE II
Essential Amino Acid Content of SCP from *Cellulomonas* Strain IIbc in Comparison with Reference Proteins and Other Sources

Amino acid	Ideal AA pattern FAO[a]	Cellulomonas IIbc	*Cellulomonas* strain[a]	Yeast BP process[b]	Soybean protein[a]
Lys	4.20	4.21	7.87	5.38	6.60
His	[c]	3.98	2.91	1.45	2.50
Arg	[c]	7.82	7.43	3.45	7.00
Thr	2.80	3.88	5.20	3.73	3.90
Val	4.20	4.91	7.30	4.00	5.20
Met	2.20	0.61	2.16	1.10	1.10
Iso	4.20	2.85	4.46	3.66	5.80
Leu	4.80	6.68	9.76	5.38	7.60
Phe	2.80	3.25	4.26	3.31	4.80
Tyr	2.80	3.09	[c]	2.76	3.20

[a]From ref. 11.
[b]From ref. 12.
[c]Not reported or specified.

in this method are so great that it is preferable to base them on the total N content of the diet. At present, it has a greater practical value. On this basis, our figures compare favorably with those of Yang[3] and Dunlap and Callihan,[11] who obtained an N digestibility lower than 80% for a *Cellulomonas* strain. One very important observation from these experiments was that no toxic effects were found with rats fed with IIbc strain biomass at a level of 80% of the diet for 10 days.

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