SHORT COMMUNICATION

Glycogen Synthesis by Glucose-limited *Candida utilis*

By C. L. SCHWARTZKOFF¹ AND P. L. ROGERS^{2*}

¹ Department of Chemical Engineering, University of Sydney, N.S.W. 2006, Australia

² School of Biotechnology, University of New South Wales, Kensington, N.S.W. 2033, Australia

(Received 1 March 1982)

Candida utilis NRRL Y11868 was grown at several dilution rates in a glucose-limited chemostat. Protein and total carbohydrate contents of the cells varied markedly with dilution rate, primarily because significant glycogen synthesis occurred at the higher growth rates.

INTRODUCTION

Previous reports have described the growth of *Candida utilis* in carbon-limited chemostat conditions, mainly in connection with an evaluation of the potential of this yeast for single-cell protein production (Brown & Rose, 1969; Alroy & Tannenbaum, 1973; Herbert, 1976). The effect of growth rate on macromolecular composition has received considerable attention in these studies; the wide variations in composition which have been observed appear to be related to the complex or incomplete growth media employed in most experiments reported. As part of a programme involving the utilization of renewable feedstocks for the production of food-grade yeast, we have undertaken a detailed examination of the effect of growth rate on *C. utilis* in defined, glucose-limiting medium.

METHODS

Candida utilis NRRL Y11868 was grown at 35 °C, pH 4.5, in a Porton-type chemostat with a 2-litre working volume. One litre of growth medium contained 20 g glucose (autoclaved separately), 1.3 g K₂SO₄, 0.79 g H₃PO₄, 0.34 g MgSO₄·7H₂O, 0.11 g Na₂SO₄, 20 mg CaCl₂·2H₂O, 7.6 mg FeSO₄·7H₂O, 2.8 mg ZnSO₄·7H₂O, 0.76 mg MnSO₄·4H₂O, 0.15 mg CuSO₄·5H₂O, 0.15 mg CoCl₂·6H₂O, 0.15 mg Na₂MoO₄ and 0.11 mg H₃BO₃.

Ammonia gas was supplied as the nitrogen source and pH control agent. This resulted in a steady-state residual concentration of 0.2 g ammonium nitrogen l⁻¹ at each dilution rate. The ammonium concentration was estimated according to the phenol–hypochlorite method of Weatherburn (1967). The medium was glucose limiting with residual substrate concentration being less than 5 mg l⁻¹ at all but the highest dilution rate tested. Lowering the inlet glucose concentration by 25% resulted in a precisely proportional decrease in cell concentration with no significant change in carbon:cells or carbon:CO₂ ratios, specific oxygen uptake rate (Q_{O_2}), specific CO₂ production rate (Q_{CO_2}), or residual glucose concentration. Filter-sterilized air was sparged into the culture, the flow rate being adjusted to provide a dissolved oxygen tension of 50% air saturation. A minimum of 10 culture volumes was allowed to pass through the fermenter before sampling each steady state.

Cellular protein and total carbohydrate concentrations were estimated using the biuret and anthrone methods respectively (Herbert *et al.*, 1971). Cells were analysed for DNA using the Burton method and for RNA using the orcinol method as described by Herbert *et al.* (1971). Glycogen analyses were performed according to the amyloglucosidase method of Becker (1978), and glucan, mannan and trehalose were analysed by the method of Trevelyan & Harrison (1956). Residual glucose in the culture broths was determined on samples from which the yeast had been removed by membrane filtration within 5 s of sampling from the fermenter. Hexokinase test kits obtained from Boehringer-Mannheim were used in this assay. The sensitivity of the method was improved to better than 1 mg l⁻¹ by using the supplied buffer undiluted, which allowed the analysis of larger volumes of culture

filtrate. For the calculation of the carbon utilization results, the inlet glucose concentration was determined using the Lane and Eynon general volumetric method (Horwitz, 1970), the carbon content of washed and dried cells was estimated by Australian Microanalytical Services (University of Melbourne), and biomass concentration in the fermenter was determined by weighing the washed and dried pellets after centrifugation of 10.0 ml samples. An accurately calibrated rotameter was used to determine inlet air flow rates, with a rotary gas meter being used as a check on exit gas flow rate. Exit gas CO₂ concentration was monitored using a Hartmann and Braun Uras 2T infra-red analyser.

RESULTS AND DISCUSSION

The percentage of substrate carbon incorporated into cellular material and CO₂ during the growth of *C. utilis* NRRL Y11868 on glucose is shown in Fig. 1 (a). Cell carbon content was approximately 45%, and varied little with dilution rate. Within experimental error, these products accounted for all the substrate carbon consumed, consistent with the findings of Herbert (1976) on the growth of *C. utilis* NCYC 321. Residual glucose was present at very low concentrations (Fig. 1 a) except at growth rates close to the maximum specific growth rate (0.5 h⁻¹), typical of the pattern conventionally observed in glucose-limiting conditions.

Some unusual variations in macromolecular composition were observed (Fig. 1 b). The protein content decreased markedly at higher growth rates, coinciding with an increase in total carbohydrates. Whilst there is little published information on changes in the carbohydrate content of *C. utilis*, several authors have examined the variation in protein content with growth rate (Brown & Rose, 1969; Alroy & Tannenbaum, 1973; Herbert, 1976). Most of these reports have shown a pattern fairly consistent with Herbert's original work on *Aerobacter* (Herbert, 1961), where protein content varied only slightly with dilution rate, in marked contrast to the present results. Nucleic acid content in *C. utilis* NRRL Y11868 showed no unusual features (Fig. 1 b).

In view of the unexpected variation in total carbohydrates, the nature of the extra carbohydrate present at higher dilution rates was examined. Whilst glucan, mannan and trehalose contents varied only slightly (accounting for approximately 6%, 11% and 2% of dry mass respectively), the glycogen content of the cells increased markedly at higher growth rates, thereby accounting for the observed increase in total carbohydrate content (Fig. 1 b). In subsequent experiments we have observed similar glycogen accumulation in another strain, *C. utilis* NRRL Y900 (unpublished results).

Following the studies of Herbert (1961) on *Aerobacter*, and subsequent research on other bacteria and yeasts, it has generally been accepted that the cell protein content changes little as the growth rate of micro-organisms is varied in carbon-limited chemostats. This principle has been the basis for virtually disregarding protein content as a significant variable in many discussions on the productivity of single-cell protein processes. The present work illustrates the dangers of this assumption in the case of *C. utilis*.

The accumulation of significant quantities of glycogen in a glucose-limited culture is of fundamental interest because this carbohydrate is generally regarded as a reserve material synthesized in carbon-excess environments (Dawes & Senior, 1973). A deficiency of one of a number of nutrients may lead to glycogen synthesis in micro-organisms (Dicks & Tempest, 1967), although most observations have been made on cultures at low or zero growth rates in carbon-excess, nitrogen-deficient conditions (Trevelyan & Harrison, 1956; Holme, 1957; Wilkinson & Munro, 1967). In particular, Herbert (1958, 1961) demonstrated the accumulation of glycogen at low and zero growth rates in nitrogen-limited *C. utilis*. Prior to the present work, extensive glycogen synthesis during steady state carbon-limited growth has been reported only with *Saccharomyces cerevisiae* (Küenzi & Fiechter, 1972). In *S. cerevisiae*, glycogen deposits are found maximally at low growth rates, in contrast to the pattern in *C. utilis*. Küenzi & Fiechter (1972) suggested that glycogen was synthesized in *S. cerevisiae* during the single-cell phase of the cell cycle for subsequent use as a reserve carbon-energy supply during budding.

Fig. 1. Growth and composition of glucose-limited *C. utilis* NRRL Y11868. (a) Utilization of substrate: ○, substrate carbon incorporated as biomass; ●, substrate carbon forming CO_2 ; □, total substrate carbon accounted for as cells plus CO_2 ; ■, residual glucose concentration in the medium. (b) Macromolecular composition of the cells: ○, protein; ●, total carbohydrate; □, RNA; ■, DNA; △, glycogen.

The reason why extensive glycogen deposits have not been observed in previous studies on the growth of *C. utilis* in carbon-limiting conditions appears to be that no measurements of glycogen were made. Only Herbert (1976) has provided information on carbohydrate contents of cells cultivated in defined, complete medium; he noted that total carbohydrate content in strain NCYC 321 was similar at dilution rates of 0.05 h^{-1} and 0.45 h^{-1} . Whilst it is possible that strain variations could account for the different effects observed in the present study, it seems likely also that environmental factors may have been responsible. Preliminary studies on strain NCYC 321 by the authors indicate that the steady-state residual ammonium level in the medium may be particularly significant (unpublished results). The effect of non-limiting nutrient concentrations on *Aerobacter* was noted by Dicks & Tempest (1967), who observed a critical effect of the ratio between extracellular K^+ and NH_4^+ on the macromolecular composition.

REFERENCES

- ALROY, Y. & TANNENBAUM, S. R. (1973). The influence of environmental conditions on the macromolecular composition of *Candida utilis*. *Biotechnology and Bioengineering* **15**, 239–256.
- BECKER, J. U. (1978). A method for glycogen determination in whole yeast cells. *Analytical Biochemistry* **83**, 56–64.
- BROWN, C. M. & ROSE, A. H. (1969). Effects of temperature on composition and cell volume of *Candida utilis*. *Journal of Bacteriology* **97**, 261–272.
- DAWES, E. A. & SENIOR, P. J. (1973). The role and regulation of energy reserve polymers. *Advances in Microbial Physiology* **10**, 135–266.
- DICKS, J. W. & TEMPEST, D. W. (1967). Potassium–ammonium antagonism in polysaccharide synthesis in *Aerobacter aerogenes* NCTC 418. *Biochimica et biophysica acta* **136**, 176–179.
- HERBERT, D. (1958). Some principles of continuous culture. In *Recent Progress in Microbiology*, Symposium of the 7th International Congress on Microbiology, pp. 381–396. Edited by G. Tunevall. Stockholm: Almqvist & Wiksell.
- HERBERT, D. (1961). The chemical composition of micro-organisms as a function of their environment. *Symposia of the Society for General Microbiology* **11**, 391–416.
- HERBERT, D. (1976). Stoichiometric aspects of

- microbial growth. In *Continuous Culture 6, Applications and New Fields*, pp. 1–30. Edited by A. C. R. Dean, D. C. Ellwood, C. G. T. Evans & J. Melling. Chichester: Ellis Harwood.
- HERBERT, D., PHIPPS, P. J. & STRANGE, R. E. (1971). Chemical analysis of microbial cells. *Methods in Microbiology* **5B**, 209–344.
- HOLME, T. (1957). Continuous culture studies on glycogen synthesis in *Escherichia coli* B. *Acta chemica scandinavica* **11**, 763–775.
- HORWITZ, W. (editor) (1970). *Official Methods of Analysis of the Association of Official Analytical Chemists*. 11th edn, pp. 532–533. Washington: Association of Official Analytical Chemists.
- KÜENZI, M. T. & FIECHTER, A. (1972). Regulation of carbohydrate composition of *Saccharomyces cerevisiae* under growth limitation. *Archiv für Mikrobiologie* **84**, 254–265.
- TREVELYAN, W. E. & HARRISON, J. S. (1956). Yeast carbohydrate fractions. Separation from nucleic acid, analysis and behaviour during anaerobic fermentation. *Biochemical Journal* **63**, 23–33.
- WEATHERBURN, M. V. (1967). Phenol–hypochlorite reaction for determination of ammonia. *Analytical Chemistry* **39**, 971–974.
- WILKINSON, J. F. & MUNRO, A. L. S. (1967). The influence of growth limiting conditions on the synthesis of possible carbon and energy storage polymers in *Bacillus megaterium*. In *Microbial Physiology and Continuous Culture*, pp. 173–185. Edited by E. O. Powell, C. G. T. Evans, R. E. Strange & D. W. Tempest. London: H.M.S.O.