Folia Microbiol. 38 (2), 141 - 146 (1993)

Continuous Cultivation of the Yeast *Saccharomyces cerevisiae* at Different Dilution Rates and Glucose Concentrations in Nutrient Media

D. PEJIN and R. RAZMOVSKI

Faculty of Technology, Novi Sad, Yugoslavia

Received May 10, 1992
Revised version December 30, 1992

ABSTRACT. The influence of glucose concentration in nutrient media on the specific growth rate and biomass yield in the course of continuous fermentation of *Saccharomyces cerevisiae* was investigated. An increase of glucose content in media decreased the specific growth rate and the biomass yield. Glucose concentration had significant effects on protein and phosphate contents of cells. However, an increased glucose concentration increased the fermentative power of *S. cerevisiae* (SJA-method). An increase of the dilution rate decreased the cell concentration in the fermentor. Specific growth rate approached the values of the dilution rate. The best agreement has been obtained at a dilution rate of 0.20/h. This dilution rate proved to be most convenient for the investigated microorganism and cultivation conditions (media composition, pH, aeration intensity and temperature). Biomass yield proved to be decreased by an increase of the dilution rate.

Description of the kinetics of microbial growth under continuous conditions can be found in the classical papers of Monod (1942), Málek and Fencl (1966), Noack (1968), Kubitschek (1971), Bergter (1972), Aiba *et al.* (1973) and Pirt (1975). Growth kinetics as well as the microbial growth parameters proved to be defined with mathematical precision. These relations permit the investigators to compare the calculated parameters with the measured ones obtained experimentally. These results enable the investigators to derive conclusions concerning the question whether the investigated microorganisms obey the kinetics and the degree of deviations from the calculated parameters. *Saccharomyces cerevisiae* is an extremely important microorganism for industry (Rose and Harrison 1970), being often used as the model microorganism for studies of eukaryotic cells. Oura (1974) investigated the effects of aeration rate on *S. cerevisiae* metabolism. An increase of aeration rate increases the capability of cells to activate aerobic metabolism. An obvious maximum of aerobic metabolism intensity was observed in the course of aeration of the cultivation system with a mixture of air and oxygen 9:1. In the course of continuous cultivation of microorganisms, dilution rates represent an extremely important variable (Fiechter *et al.* 1987). Therefore, the aim of these investigations was to study the effects of dilution rates on the specific growth rates of *S. cerevisiae*. Besides the growth rate, the effects of dilution rate on the yield, metabolic activities (the activity of carbon dioxide evolution and ability of oxygen consumption) and technological characteristics (fermentative power) have also been investigated. Glucose concentration is one of the basic characteristics of media used for *S. cerevisiae* cultivation, as it is known that it could effect the type of metabolism (Bronn 1986). Sustained oscillations in variables such as biomass concentration, sugar concentration, carbon-dioxide evolution rate, oxygen uptake rate, dissolved oxygen concentration, effects of pH and nitrogen levels have been observed in continuously cultured *S. cerevisiae* under a variety of operating conditions (Borzani *et al.* 1977; Parulekar *et al.* 1986; Porro *et al.* 1988; Chen and Mc Donald 1990). The investigation also included the study of the effects of glucose concentration in media on the composition and characteristics of yeast cells.

MATERIALS AND METHODS

Microorganism. Pure culture of *Saccharomyces cerevisiae* strain DTN from the collection of the *Institute of Microbiological Processes and Applied Chemistry of the Faculty of Technology* in Novi Sad was used. The pure culture was maintained on a slant with the same medium composition as used by Parulekar *et al.* (1986) for continuous cultures plus 1.5 % (*W/W*) of agar, and it was stored at 4 °C. The inoculum was prepared as follows: One loopful of baker's yeast from a slant was transferred to 50 mL of medium (the same composition as used by Parulekar *et al.* 1986), in a 250-mL Erlenmeyer flask. The

flask was incubated with shaking (3.3 Hz) in New Brunswick shaker (Lab-line) at 30 °C for 24 h before it was used to inoculate the fermentor.

Substrate preparation. Assays were carried out by the feed medium used by Parulekar *et al.* (1986), although the glucose concentrations were varied from 10 to 40 g/L. Glucose at the fed concentrations was used as the sole carbon and energy source.

Equipment and fermentation. Fermentation was carried out in a fermentor (*Chemap-Pec Volketswil*, Switzerland) with a total volume of 10 L, consisting of a top-driven stirrer, a water-cooled condenser on the air outlet line, and built-in foam, temperature, pH and dissolved oxygen (DO) control systems. Dissolved oxygen and pH were measured with a DO probe (Model 900, New Brunswick) and pH electrode (Ingold), respectively. The working volume for all experiments was 5.0 L. Good mixing was assured by the rotation of two flat-blade agitators set at 10 Hz. The air flow rate was 4 VVM (air volume per liter suspension per minute). Temperature was maintained at 30 °C. The fermentor was equipped with an additional unit that measured the mass (liquid) in the system, whereas the substrate mass in the fermentor was kept constant (chemostat). The in- and outflowing air streams were continuously monitored for oxygen and carbon dioxide concentrations. As measuring apparatus, a gas analyzer (Hartmann and Braun, Frankfurt/Main, Germany) was used. During the cultivation process the flow was regulated to correspond to the selected dilution rate (D), *i.e.* the period spent in the fermentor unit. All liquids were pumped by means of peristaltic pumps.

Analytical methods. During continuous cultivation samples were taken for the determination of dry matter (Rolf *et al.* 1982), of trehalose and glycogen (Grba *et al.* 1975), and of nitrogen and phosphorus (Bronn 1986). The specific oxygen uptake and the specific carbon dioxide production rate, as well as the respiration quotient were calculated.

All chemicals were of analytical grade or of the highest purity available. Fermentative power was determined on SJA fermentograph (well-known analyzer from the International IUPAC test).

RESULTS AND DISCUSSION

Results of the investigations of the effects of dilution rate on the parameters of growth and composition are shown in Table I (the substrate contained 15 g/L glucose).

Table I. Influence of the dilution rate on the specific rate of growth, content of proteins, phosphorus, glycogen and trehalose, with the inflowing nutrient medium containing 15 g glucose per L; (pH = 5.5; DO = 80 %)[a]

Dilution rate, 1/h	μ 1/h	Proteins %	Phosphorus %	Trehalose %	Glycogen %
0.05	0.04	48.8	2.0	4.2	21.7
0.07	0.05	51.6	2.2	3.8	19.8
0.10	0.09	52.7	2.4	3.5	19.2
0.20	0.20	53.9	2.5	3.2	18.5
0.42	0.32	54.2	2.6	3.2	17.0

[a]Mean values of five experiments.

In order to avoid errors, each dilution rate (*i.e.* period spent in the fermentor unit) was examined with five complete exchanges of the fermentor content.

If the results of specific growth rate μ are analyzed and compared with the dilution rate D it is possible to conclude that the specific rate of growth for the chosen strain is lower than all the investigated rates of dilution. Good agreement was obtained only in the case when D = 0.20/h and μ = 0.20/h. The protein content in dry matter increases with dilution rate and reaches its maximum value at D = 0.42/h.

Explanation for this phenomen could be found in the fact that with an increase of the rate of dilution greater amounts of nutrient compounds are brought to the fermentor whereas the cells assimilate greater quantities of nitrogen from the medium and synthesize a greater quantity of proteins in biomass. Increasing protein in biomass increases the content of phosphorus in biomass. A parallel increase of protein (nitrogen) and phosphorus content in cells could be explained by the cells tending to maintain constant nitrogen-to-phosphorus ratio which is a well-known characteristic of *S. cerevisiae* (White 1954). The content of trehalose and glycogen decreases with increasing rate of dilution as can be seen from values shown in Table I. These results are in agreement with those published by Küenzi and Fichter (1972). In most cases, differences in the saccharide composition of yeast were attributed to the influence of medium composition, aeration, strain specificity and growth phase (Panek 1962; Polakis and Bartley 1966). Little attention was paid to the relation between growth kinetics and reserve carbohydrate synthesis. Glycogen and trehalose serve as endogenous carbon and energy sources for budding and are required in addition to the slowly supplied exogenous substrate and allow the cells to carry out the budding process at a constant rate, irrespective of the generation time (Küenzi and Fichter 1969). Under excess exogenous substrate the budding proceeds without degradation of endogenous saccharides. Probably, the glycogen present in cells growing at maximum rates consists of a fraction with the function of a structural saccharide. Several authors have shown that the polysaccharide, determined normally as glycogen in yeast is not uniform in its structure and function (Sjöblom and Stople 1964; Rothman and Cabib 1969).

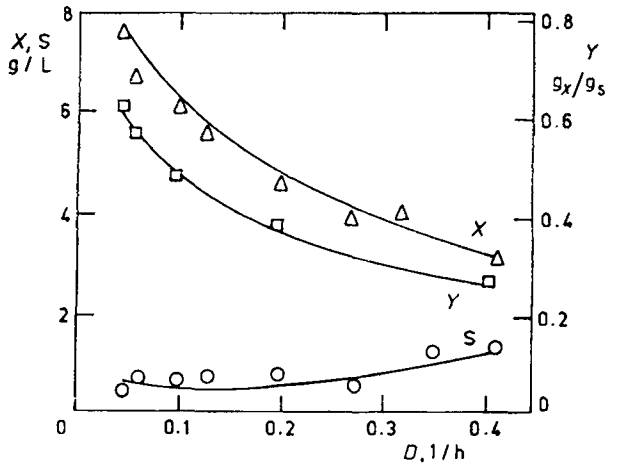

Fig. 1. Dependence of the content of biomass (X), sugar (S) and yield (Y) on the dilution rate (D); *circles* – glucose content, *triangles* – biomass, *squares* – yield. Glucose content in substrate 15 g/L, pH = 5.5, DO = 80 %.

The dependence of the content of biomass, glucose and yield on the rate of dilution is shown in Fig. 1. The content of glucose in the fermentor increases but biomass concentration, as well as biomass yield per substrate consumed, decreases. These results are in agreement with those published by Wasungu and Simad (1982) and Guerts *et al.* (1980). In continuous working systems it is usually assumed that the yield constant Y is actually constant as a function of the specific growth rate μ. However, this is often not the case. Fig. 1 shows an example of this exception corroborated by our experiments. At low dilution rates ($D < 0.1$/h) a yield approximating the theoretical was obtained. With increasing the rate of dilution, the yield decreases and at a dilution rate of 0.4/h, a 50 % of the theoretical yield was obtained. An explanation could be probably found in the fact that the transfer rate of substrate carbon to cells decreases with increasing dilution (Martin *et al.* 1966). The reverse transfer rate behaves similarly. As shown in Table I and in Fig. 1, these variations coincide with decreasing cell dry matter and decreasing carbon

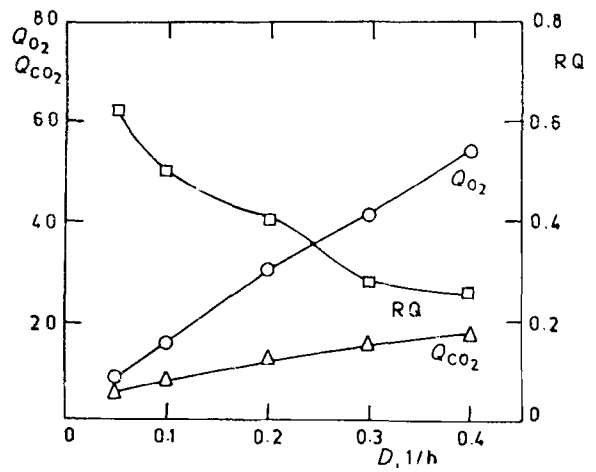

Fig. 2. Dependence of the specific oxygen uptake rate (Q_{O2}), specific CO_2 production rate (Q_{CO2}, both in mL g^{-1} h^{-1}) and respiratory quotient (RQ) on the dilution rate (D); *circles* – specific oxygen uptake rate, *triangles* – specific CO_2 production rate, *squares* – respiration quotient. Glucose content 15 g/L; pH = 5.5; DO = 80 %.

content of the cell. Consequently, the substrate S content (glucose) increases with increasing rate of dilution D (Fig. 1).

Dependence of the specific oxygen uptake rate (Q_{O2}), specific CO_2 production rate (Q_{CO2}) and respiration quotient (RQ) are given in Fig. 2. The values for specific oxygen uptake and specific carbon dioxide release are linearly dependent on the dilution rate as expected. The respiration quotient (RQ) decrease shows that the oxidative way of glucose degradation is intensified since the respiration quotient is the value of the ratio between energy production and efficiency of biosynthesis. This quotient appears to be a function of the citrate cycle and respiratory chain activity (Dekkers *et al.* 1981).

The fermentative power is one of the most important technological characteristics of *S. cerevisiae*. The results of the determination of this fermentative power at different rates of dilution (glucose content in the substrate 15 g/L) are shown in Table II. It may be concluded that an increase of the rate of dilution increases the fermentative power. The explanation of the slight increase of fermentative power can be found in the fact that the protein content of yeast cells increases.

Table II. Influence of the dilution rate on the fermentative power (inflow as in Table I)[a]

Dilution rate 1/h	Fermentative power[b] mL CO2[c]
0.05	1200
0.07	1320
0.10	1450
0.20	1520
0.42	1550

[a]Mean values of five experiments.
[b]SJA method.
[c]Per g dry matter.

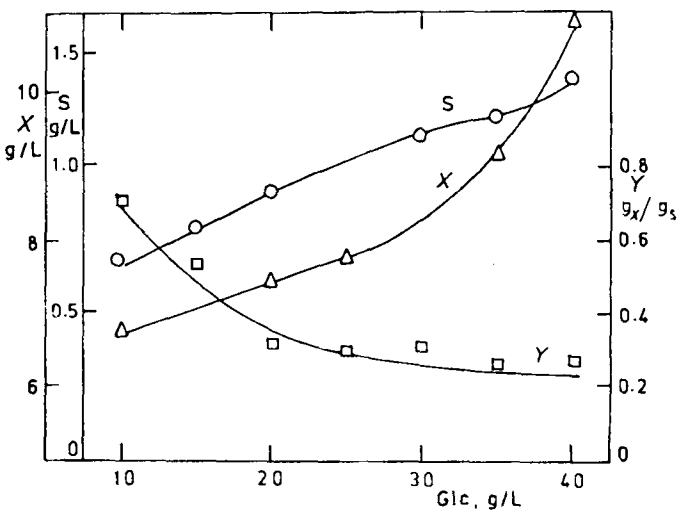

Fig. 3. Dependence of the content of biomass (X), glucose (S) and yield (Y) on glucose concentration (Glc) in substrate added during continuous cultivation; *circles* – glucose content, *triangles* – biomass, *squares* – yield. Dilution rate D = 0.125/h, DO = 80 %, pH = 5.5.

Table III. Influence of glucose concentration in the inflowing nutrient medium on the specific rate of growth, content of protein, phosphorus, glycogen and trehalose (D = 0.125/h; pH = 5.5; DO = 80 %)

Glucose g/L	μ 1/h	Proteins %	Phosphorus %	Trehalose %	Glycogen %
10	0.153	52.3	2.9	3.0	16.9
15	0.154	52.1	2.6	3.4	17.5
20	0.135	51.1	2.3	3.8	18.2
25	0.131	50.4	2.2	4.1	19.3
30	0.128	49.6	2.1	4.6	20.9
35	0.121	49.5	2.0	4.7	21.3
40	0.092	48.3	2.0	5.0	22.5

The results of the specific rate of growth, content of protein, phosphorus, trehalose and glycogen, at different glucose concentrations, at a constant rate of dilution of 0.125/h are shown in Table III. Glucose concentration has a significant effect on the specific rate of growth. At a concentration of 10 g glucose per L the value of the specific growth rate was 0.153/h and at 40 g glucose per L the specific growth rate was 0.092/h. Besides, the content of glucose in the substrate also influenced the composition of yeast dry matter. At 10 g glucose per L, the contents of protein, phosphorus, trehalose and glycogen were 52.3, 2.9, 3.0 and 16.9 %, respectively, while at 40 g glucose per L, the contents were 48.4, 2.0, 5.0 and 22.5 %, respectively. These results could be explained by a repression of respiration causing a Crabtree effect. The lowered energy from glucose degradation caused the decrease of specific growth rate. These results indicate that the rules applying to discontinuous growth of *S. cerevisiae* are also valid for its continuous growth.

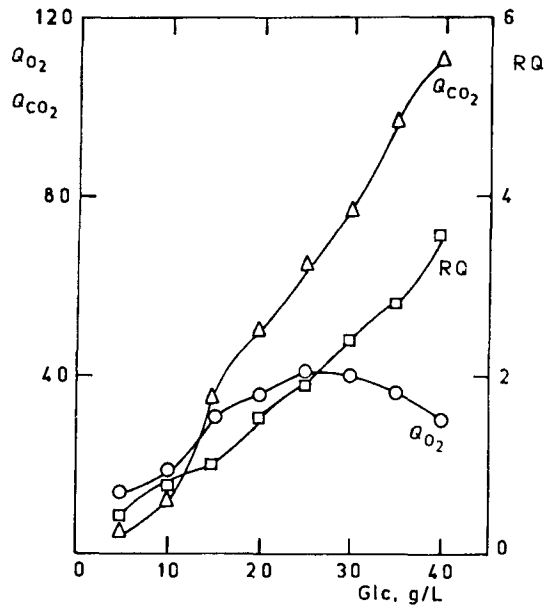

Fig. 4. Dependence of the specific oxygen uptake rate (Q_{O2}), specific CO_2 production rate (Q_{CO2}, both in mL g^{-1} h^{-1}) and respiratory quotient (RQ) on glucose concentration (Glc) in substrate added during continuous cultivation; *circles* – specific oxygen uptake rate, *triangles* – specific CO_2 production rate, *squares* – respiration quotient. Glucose content 15 g/L; pH = 5.5; DO = 80 %.

Table IV. Influence of glucose concentration in the inflowing nutrient medium on the fermentative power (D = 0.125/h; pH = 5.5; DO = 80 %)

Glucose g/L	Fermentative powera mL CO2b
10	1490
15	1450
20	1390
25	1330
30	1300
35	1280
40	1270

[a]SJA method.
[b]Per g dry matter.

Fig. 2 shows the dependence of biomass concentration in the fermentor on glucose concentration in the medium. An increased glucose concentration decreases the biomass yield per substrate consumed. The results in Fig. 4 could be the explanation for this phenomenon. The increase of glucose concentration in the inflowing substrate increases the specific CO_2 production rate (Q_{CO2}) and the respiration quotient (RQ), while the specific oxygen uptake rate (Q_{O2}) increases slightly (up to a glucose concentration of 25 g/L) and after that it decreases. When the glucose content is above 15 g/L, glucose degradation is fermentative which causes a yield decrease irrespective of a high air flow (Fig. 2).

The fermentative power of substrate with different glucose concentrations is shown in Table IV. The fermentative power decreases due to the decrease of protein in yeast cells (Table III).

REFERENCES

AIBA S., HUMPHREY A., MILLIS N.: *Biochemical Engineering*, 2nd ed., pp. 128–151. Academic Press, New York 1973.

BERGTER F.: *Wachstum vom Mikroorganismen*, pp. 87–95. G. Fiescher Verlag, Jena 1972.

BORZANI W., GREGORI R., VAIRO M.: Some observations on oscillatory changes in the growth rate of *Saccharomyces cerevisiae* in aerobic continuous undisturbed culture. *Biotechnol.Bioeng.* **19**, 1363–1374 (1977).

BRONN M.: Möglichkeiten der Substitution von Melasse durch andere Rohstoffe für die Backhefeherstellung. *Branntweinwirtschaft* **14**, 218–223 (1986).

CHEN C., MC DONALD K.: Oscillatory behavior of *Saccharomyces cerevisiae* in continuous culture. I. Effects of pH and nitrogen levels. *Biotechnol.Bioeng.* **36**, 19–27 (1990).

DEKKERS K., DE KOK H., ROELS I.: Energetics of *Saccharomyces cerevisiae* CBS 426: Comparison of an anaerobic and aerobic glucose limitation. *Biotechnol.Bioeng.* **23**, 1023–1035 (1981).

FIECHTER A., KÄPPELI O., MEUSSDOERFFER F.: Batch and continuous culture, pp. 99–129 in A.H. Rose, J.S. Harrison (Eds): *The Yeasts*, Vol. 2 (2nd ed.) *Yeast and the Environment*. Academic Press, London–Orlando–New York 1987.

GURTS G., DE KOK H., ROELS I.: A quantitative description of the growth of *Saccharomyces cerevisiae* CBS 426 on a mixed substrate of glucose and ethanol. *Biotechnol.Bioeng.* **22**, 2031–2043 (1980).

GRBA S., OURA E., SUOMALAINEN H.: On the formation of glycogen and trehalose in baker's yeast. *Eur.J.Appl.Microbiol.* **2**, 29–37 (1975).

GRBA S., OURA E., SUOMALAINEN H.: Formation of trehalose and glycogen in growing baker's yeast. *Finn.Chem.Lett.* **1**, 61–64 (1979).

KÜENZI M., FIECHTER A.: Changes in carbohydrate composition and trehalose activity during the budding cycle of *Saccharomyces cerevisiae*. *Arch.Mikrobiol.* **64**, 396–407 (1969).

KÜENZI M., FICHTER A.: Regulation of carbohydrate composition of *Saccharomyces cerevisiae* under growth limitation. *Arch.Mikrobiol.* **84**, 254–265 (1972).

KUBITSCHEK H.: *Introduction to Research with Continuous Culture*, pp. 76–79. Prentice Hall, Biological Techniques Series, *Englewood Cliffs*, New York 1971.

MÁLEK I., FENCL Z.: *Theoretical and Methodological Basis of Continuous Culture of Microorganisms*, pp. 15–18. Verlaghaus der Technischen Akademie der Wissenschaften, Prague 1966.

MONOD J.: *Recherches sur la Croissance des Cultures Bacteriennes*, 2nd ed., p. 9. Herman, Paris 1942.

NOACK D.: *Biophysikalische Prinzipien der Populationsdynamik in der Mikrobiologie*, pp. 58–62. Thieme Verlag, Leipzig 1968.

OURA E.: Effect of aeration intensity on the biochemical composition of baker's yeast. I. Factors affecting the type of metabolism. *Biotechnol.Bioeng.* **16**, 1197–1225 (1974).

PANEK A.: Synthesis of trehalose by baker's yeast (*Saccharomyces cerevisiae*). *Arch.Biochem.* **98**, 349–355 (1962).

PANEK A.: Function of trehalose in baker's yeast (*Saccharomyces cerevisiae*). *Arch.Biochem.* **100**, 422–425 (1963).

PARULEKAR S., SEMONES G., ROLF M., LIEVENSE J., LIM H.: Induction and elimination of oscillation in continuous cultures of *Saccharomyces cerevisiae*. *Biotechnol.Bioeng.* **28**, 700–710 (1986).

PIRT S.: *Principles of Microbe and Cell Cultivation*, pp. 29–42. John Wiley & Sons, New York 1975.

POLAKIS E., BARTLEY W.: Changes in dry weight, protein, deoxyribonucleic acid and reserve and structural carbohydrate during aerobic growth cycle of yeast. *Biochem J.* **98**, 883–887 (1966).

PORRO D., MARTEGANI E., RANZI B., ALBERGHINA L.: Oscillations in continuous cultures of budding yeast: A segregated parameter analysis. *Biotechnol.Bioeng.* **32**, 411–417 (1988).

ROLF M., HENNIGAN P., MOHLER R., WEIGARD W., LIM H.: Regulation of glycogen synthesis in the intact yeast cell. *Biotechnol. Bioeng.* **24**, 1191–1193 (1982).

ROSE H., HARRISON J.: *The Yeasts, Vol. III, Yeast Technology*, pp. 350–371. Academic Press, London–New York 1970.

ROTHMAN L., CABIB E.: Regulation of glycogen synthesis in the intact yeast cell. *Biochemistry* **8**, 3332–3341 (1969).

SJÖBLOM L., STOLPE E.: Studies on baker's yeast. I. The carbohydrates of yeast and their changes on storage. *Acta Acad. Aboensis, Ser.B* **24**, 1–28 (1964).

WASUGNU K., SIMAD R.: Growth characteristics of baker's yeast in ethanol. *Biotechnol.Bioeng.* **24**, 1125–1134 (1982).

WHITE J.: *Yeast Technology*, pp. 13–26. Chapman and Hall, London 1954.