Damped Oscillations in Continuous Culture of *Lactobacillus plantarum*

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SUMMARY

Lactobacillus plantarum exhibited long-period damped oscillations when grown aerobically with glucose as the rate-limiting substrate. Hydrogen peroxide accumulated in the cultures and its concentration also exhibited damped oscillations. It is suggested that inhibition of growth by hydrogen peroxide is involved in the production of the damped oscillations of microbial population density.

INTRODUCTION

Oscillations of population density, biomass concentration, and environmental variables in pure microbial cultures have been reported.

Finn & Wilson (1954) noted sustained oscillations of pH, light extinction, and other variables in product-limited, continuous and batch cultures of *Saccharomyces carlsbergensis* in nutrient broth with 1 % (w/v) glucose; the period of these oscillations was of the order of 2 h. Similar oscillations were reported by Maxon (1960) for growth of *S. cerevisiae*. Mateles & Goldthwaite (1963), employing the same medium as Finn & Wilson, observed no oscillations, sustained or damped, in pH or sugar concentration in product-limited continuous culture of *S. carlsbergensis* or *Pseudomonas ovalis*; they suggested that their results differed from those of Finn and Wilson because they obtained a continuous record of concentrations whereas Finn and Wilson only had data at discrete sampling times. However, Fiechter & von Meyenburg (1968), using a continuous monitoring device in a study of gas exchange in continuous culture of *S. cerevisiae*, reported sustained oscillations of gas exchange rate with a period of about 2 h.

Damped oscillatory responses of chemostat cultures to upset conditions were reported by Mor & Fiechter (1968) for growth of *S. cerevisiae* (period about one day) and by Zines & Rogers (1970) for growth of *Klebsiella aerogenes* (period < 1 h).

Oscillations observed have been ascribed to various causes: a time lag in the adjustment of yeast growth rate to a change in the pH of the surroundings (Finn & Wilson, 1954); synchrony of reproduction by budding (Fiechter & von Meyenburg, 1968); a time lag, modelled as a second order differential equation, in the adjustment of growth rate to changes in ethanol concentration (Zines & Rogers, 1970).

These reports of short period (of the order of hours or less) oscillations in pure cultures have been criticized by Sinclair *et al.* (1971): 'Most [of these reports] can be explained as being the result of poor sampling techniques or bad practice in the application of pH, foam, temperature or dissolved oxygen control.'

A number of models predict oscillations in continuous cultures of pure microbial

populations. The inhibitor models of Ramkrishna, Fredrickson & Tsuchiya (1967) predict damped oscillations under some conditions. If variable biomass composition is also allowed for, they will predict sustained oscillations under some conditions. Less complex models, such as the modification of the maintenance model of Ramkrishna *et al.* (1966) proposed by Megee *et al.* (1972), will also predict damped oscillations, as will the modification of Monod's model for endogenous metabolism proposed by Herbert (1958). These models suggest that genuine oscillations may occur if inhibition by metabolic products ('staling effects'), consumption of substrate for maintenance, or both, are significant effects in the culture being considered.

We report damped oscillations of long period (of the order of days) that occurred in continuous cultures of *Lactobacillus plantarum* with glucose as the limiting substrate. These effects only occurred with aerobic growth, never under anaerobic conditions.

METHODS

Organism. *Lactobacillus plantarum* ATCC20218 was used. It was carried in stock as a stab culture in medium containing (% w/v): tryptone, 0.5; yeast extract, 2.5; glucose, 1.0; agar, 0.5; pH 7.0 to 7.2. The stock cultures were incubated at 37 °C for 48 h and then held in a refrigerator. They were transferred monthly.

Growth conditions. The chemostats and medium were those described by Lee, Fredrickson & Tsuchiya (1974); the medium was at pH 6.5. Filtered air was sparged through the cultures. Redox potential was not measured. The nutrient supply for the continuous culture was regulated by stainless-steel hypodermic capillary wire. The hydraulic head of the medium reservoir forced the medium into the chemostat. The height of the reservoir was adjustable so that the holding time could be regulated; no pumps were needed. The chemostat was jacketed for temperature control at 27 ± 0.5 °C. To start up a chemostat, the stock culture was first transferred to 100 ml of the medium in a 300 ml Erlenmeyer flask. After incubation for 24 h, this was inoculated into the chemostat which was run batchwise (with no feed) for 24 h and then run with feeding for a minimum of 5 days.

Analytical methods. Bacteria were counted with a model Z_B Coulter counter (Coulter Electronics Inc., Hialeah, Florida, U.S.A.) with a 30 µm aperture tube. Samples were diluted in a special saline as has been reported (Lee *et al.*, 1974).

Glucose was determined by the glucostat method (Worthington *Manual*, 1972). In samples where hydrogen peroxide was also present, it was first destroyed with catalase solution. Catalase was then destroyed by boiling the sample for 30 s.

Hydrogen peroxide was determined immediately after filtration of the sample with a standard peroxidase method using dianisidine as the substrate (Worthington *Manual*, 1972).

Lactic acid was measured by a modified Barker & Summerson (1941) method.

RESULTS

In the experiments (Fig. 1), chemostat holding time (the reciprocal of the dilution rate) was varied, and glucose concentration, hydrogen peroxide concentration and cell density (i.e. bacterial count) were monitored. Glucose was fed at 0.85 g l⁻¹; the temperature was held at 27 °C in all experiments.

When the holding time was 3.5 h (Fig. 1*a*), glucose fell to a level below 10⁻³ g l⁻¹ immediately and stayed there throughout the experiment. The cell density initially fell, and after a brief undershoot settled down to a steady state level. The concentration of H₂O₂ sometimes showed an initial slight undershoot, but quickly settled down to a steady state level.

Fig. 1. Transient data of aerobic, continuous culture of *L. plantarum* at holding times of (a) 3.5 h, (b) 7.0 h, (c) 15.2 h and (d) 38.0 h. Sugar feed 0.85 g l⁻¹; pH 6.5; temperature 27 °C. Δ , Hydrogen peroxide; $\square$, glucose ($< 10^{-3}$ g l⁻¹); $\circ$, cell density.

When the holding time was 7.0 h (Fig. 1b), glucose quickly fell to a level below 10⁻³ g l⁻¹. It is not altogether clear whether the cell density oscillated and then damped out. The concentration of H₂O₂ may also have shown damped oscillations. The toxicity of high concentrations of H₂O₂ to bacteria needs no comment.

Damped oscillations were apparent when the holding time was 15.2 h (Fig. 1c). Glucose fell to its usual level but definite oscillations in cell density could be seen. Similarly, damped oscillations were seen in the concentration of H₂O₂. As the cell density began to attain steady state, the hydrogen peroxide concentration also seemed to steady.

Finally, when the holding time was held at 38 h (Fig. 1d), the glucose concentration steadied at less than 10⁻³ g l⁻¹ but the cell density and concentration of H₂O₂ underwent damped oscillations. The period and amplitude of oscillations generally increased as the holding time increased.

Two chemostats were then set up in series, the effluent from one being the influent to the second. Glucose was fed at 1.0 g l^{-1} , the pH was 6.5, and aerobic conditions prevailed. The holding time was 3.5 h in the first chemostat and 14.5 h in the second. This was accomplished at the same feed rate by controlling the size of the chemostats. Glucose in the first chemostat fell quickly to its usual value. Lactic acid accumulated in these chemostat cultures at steady state conditions. The concentration of H_2O_2 steadied after initially oscillating slightly. The concentration of bacteria in both chemostats gradually stabilized. The holding time of the second chemostat should have been enough to have resulted in oscillations in organism numbers and the concentration of H_2O_2 . However, essentially steady state conditions prevailed. Presumably the conditions in the first chemostat are controlling.

When the holding times were 12 h in the first chemostat and 6 h in the second, the glucose value quickly settled to its usual value but cell density, H_2O_2 , and lactic acid began to oscillate.

Finally, an experiment was set up during which the holding time was increased from 3.8 to 12.5 h. When the holding time was 3.8 h, glucose quickly settled to its usual concentration. Hydrogen peroxide concentration oscillated but did not exceed 20 mg l^{-1} . At this concentration the H_2O_2 is not sufficient to inhibit *L. plantarum*, so that no oscillations of organism numbers were found. When the holding time was raised to 12.5 h, however, oscillations developed in both the cell density and the concentration of H_2O_2 .

DISCUSSION

The oscillatory phenomena reported here differ from most of those held to be spurious by Sinclair *et al.* (1971): their period is of the order of days rather than hours. This is much longer than the period of the sole control device, a thermostat, which might have imposed external periodicity on the culture (no feed pumps, pH controllers, foam controllers, or dissolved oxygen controllers were used in the experiments). It is also much longer than the sampling period. These facts lead us to believe that the oscillatory phenomena reported here are not driven by external periodic processes but are a consequence of the imposition of an upset, such as a changed holding time, on the growth, metabolic and regulatory processes of the population.

The damped oscillations occur with *L. plantarum* under aerobic conditions, pH about 6.5, at longer holding times, and at glucose levels of 1.0 g l^{-1} . They do not occur with short holding times.

The mechanism which produces the oscillations observed is not altogether clear. The observed production of H_2O_2 , the well-known inhibitory effects of this compound on lactobacilli, and the observed oscillations in its concentration all suggest that it may play a major role in producing the oscillations. The damping out of H_2O_2 is a characteristic feature of this organism. Either the H_2O_2 damps out in concentration (it is unstable in the presence of organic matter), or the organisms adapt to it.

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