CONTROL OF AERATION AND AGITATION IN ANTIBIOTIC FERMENTATIONS

L. D. SHTOFFER, V. V. BIRYUKOV and V. M. NIKOLUSHKINA

All-Union Research Institute of Antibiotics, Moscow, USSR

ABSTRACT

A method of choosing the aeration and agitation conditions for a fermentation process has been devised which is based on maintaining the maximal rate of oxygen consumption by the culture.

The operation of antibiotic biosynthesis on an aeration and agitation schedule resulting from the method proposed saves energy.

The maximal values of the variable agitation–aeration parameters are those which are regarded as optimal ones for fermentations carried out under constant conditions.

The proposed method of choosing combinations of agitation and aeration conditions is not limited by the design or capacity of fermenters and it can therefore be used under industrial conditions without fear of decline in the product yield.

The algorithm for choosing optimal aeration–agitation conditions can easily be automated and realized technically with the use of any extremal step-functioning controller.

INTRODUCTION

Control of aeration and agitation in the course of antibiotic fermentation can be effected using three substantially different variants:

(i) Control at predetermined agitation and aeration conditions. Generally, operation in this way is not optimal for antibiotic biosynthesis processes distinguished by low reproducibility. This is because of changes in the quality of raw materials and of the inoculum, deviations of actual fermentation conditions from the present ones, changes in the rheological properties of the fermentation medium and in the rate of oxygen dissolution or consumption, etc.

(ii) Control of a certain physicochemical parameter characterizing the efficiency of aeration and agitation with respect to their action on the process. In this case, the parameter which is being controlled is a factor acting upon the process but does not itself result from the process.

A typical example is the control of the concentration of dissolved oxygen or of its change in time[1–10]. Provided a singular relation between the values of this factor and the yield of the desired product exists, such a control system operates successfully. For example, the control of dissolved oxygen is of advantage, provided the producing microorganism may be inhibited or the biosynthesis course can be affected by increased oxygen concentration;

in all other cases it is sufficient to maintain the oxygen concentration above a limiting level. Data are available showing that in antibiotic biosynthesis the concentration of dissolved oxygen does not influence the process productivity within a wide range of oxygen concentrations.

On the other hand, aeration and agitation may affect the process because of changes in the concentration of another gas dissolved in the medium, i.e. carbon dioxide[11–14], because of changes in the size of dead-water zones in the apparatus, the liquid-to-biomass mass transfer, the sizes of the agglomerates of 'semi-rigid' colonies, etc.[15, 16]

Each of these effects, acting on the process simultaneously during changes in aeration and agitation, can be evaluated by its specific parameter, and therefore, the control of aeration and agitation by this variable is transferred into a multi-parameter and a multi-link control system having only two governing actions: the agitator speed, and the rate of air supply for aeration.

(iii) Control of a certain biochemical parameter characterizing the result of the process. The rate of antibiotic production would be an ideal parameter for this purpose, disregarding its rather slow response to changes in fermentation conditions and the difficulty of its continuous measurement. The same applies also to the parameter connected with the production of antibiotics, the biomass growth rate.

Control of the culture respiration intensity, suggested by Shu[17], offers a more promising way, since this parameter—which is a biochemical result of the process—is sensitive to practically all mechanisms of the aeration–agitation action and it can be relatively easily measured by automatic devices. The response of the culture to changes in this parameter during fermentation is fast enough and reversible for short-term disturbances.

A disadvantage of the respiration intensity control system was suggested by Shu *et al.*[18] and it lies in the fact that the programme of respiration intensity changes in time is set rigidly for the whole period of the fermentation.

Actually the intensity of culture respiration is determined at any moment by two independent sets of parameters, (i) the amount and the physiological state of the biomass, including the presence of nutrients and metabolites in the medium (these parameters may be characterized in the aggregate, e.g. by maximal demand of the culture for oxygen, as determined with the use of a Warburg apparatus) and (ii) the 'hydrodynamic' conditions of respiration, i.e. by aeration and agitation conditions. Unavoidable variation in the first set of parameters (initial conditions) means that maintaining a rigid program in respiration actually induces different programmes for the 'hydrodynamic' conditions of fermentation. For example, if a relatively large amount of the inoculum is introduced into the fermenter, the maintenance of the rigid programme in respiration is possible only at the expense of deteriorating the conditions of the culture life activity. This will result in irreversible changes of the culture physiological state, and, consequently, in a decrease in antibiotic production. On the contrary, if the amount of the inoculum is relatively small it is quite possible that no aeration and agitation conditions will provide for the respiration intensity required by the rigid programme.

To optimize the process conditions in a given apparatus it is necessary to maximize the culture respiration intensity at any moment of the fermenta-

tion. Starting from a certain critical value, further increase of the agitator speed and of the air supply rate usually does not result in an increase of the culture respiration intensity. The process was therefore conducted at minimal (critical) values which ensure maximal respiration intensity.

MATERIALS AND METHODS

The experiments were carried out in 100-litre fermenters, 400 mm in diameter (*Figure 1*). The batch volume was 50 litres and the height of the

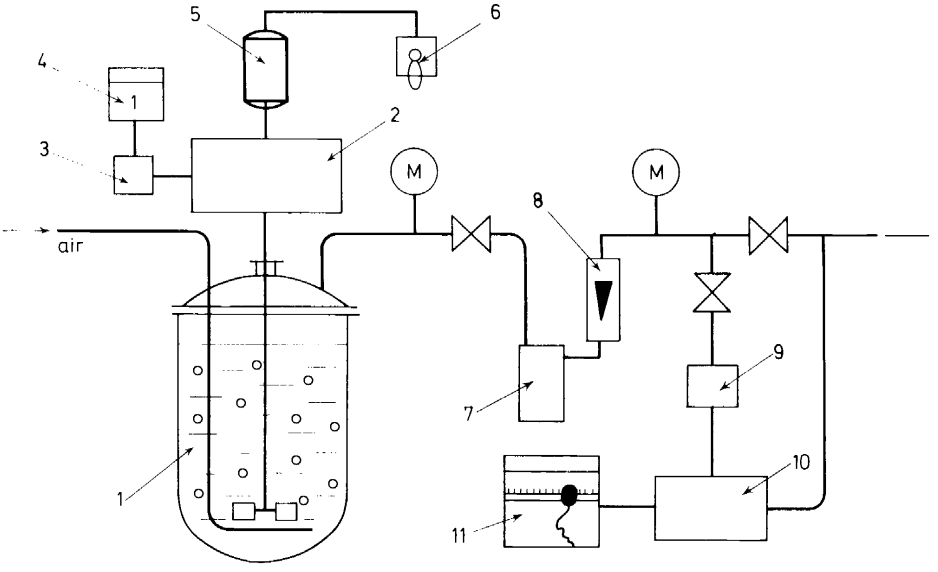

Figure 1. Experimental stand for determining aeration–agitation conditions.
1. Fermenter; 2. Variable speed gear; 3. Tachogenerator; 4. Voltmeter; 5. Motor; 6. Control key; 7. Droplet trap; 8. Rotameter; 9. Drier; 10. Gas analyzer probe; 11. Gas analyzer recorder; 12. Manometer

liquid level was 400 mm. The fermenter was equipped with an open, blade-turbine-agitator with 6 blades, 185 mm in diameter. The agitator speed was varied using variable speed gearing (type VC-1) with remote control The rotation was transmitted from the variable speed gear-box to the agitator shaft via a tapered gear-box and a belt drive. The air supply was measured by a glass rotameter (type RS-5). The oxygen consumption was estimated by analyzing the outlet air with the use of an automatic oxygen analyzer (type MGK-14).

The experimental aeration–agitation conditions were chosen in the following way. Every 12 hours, the dependence of the oxygen consumption rate on the agitator speed at constant air supply, and on the air supply at constant agitator speed, were determined. A step-wise change of the agitator speed or air supply introduced the new rate of oxygen consumption which stabilized in 15 to 20 minutes.

From the results of this experiment we determined the critical values of the agitator speed (n_{crit}) and of the air supply (q_{crit}). Those values were such as to ensure an oxygen consumption dependent on aeration–agitation conditions at steady state conditions of maximal respiration. From *Figure 2* it

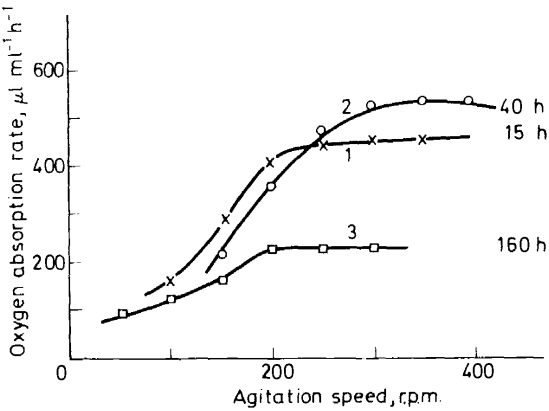

Figure 2. Plot of oxygen consumption rate vs. agitation rate at various periods of oxytetracycline fermentation

can be seen that with an air supply of 25 l min^{-1} the critical speed of the agitator is 250 r.p.m. at the 15th hour of fermentation; 300 r.p.m. on the 40th, and 200 r.p.m. on the 160th hour.

The experimental aeration–agitation conditions were established at a level somewhat (by 10 to 15 per cent) higher than the critical one considering possible drift of the 'fermentation conditions–oxygen consumption' relation between each two determinations, in order to prevent irreversible changes in the process course.

The experiments were carried out with oxytetracycline (strain *Actinomyces rimosus* No. 907; using a medium enriched with starch) and oleandomycin production (strain *Actinomyces antibioticus*; medium was enriched with soya-bean meal). For blank experiments on oxytetracycline fermentation a fermenter of the same type as the experimental one was used, where the fermentation conditions were maintained constant at the accepted optimal level (330 r.p.m., 50 l min^{-1}). The latter level was determined earlier by the method of 'sorting out' of stationary aeration–agitation results obtained during a great number of operations in several fermenters. Identical optimal constant conditions in the blank fermenter were used in experiments with oleandomycin fermentation to find the variations in antibiotic production since no preliminary experiments on finding out optimal stationary conditions were carried out in that case.

RESULTS AND DISCUSSION

By using the methods described above we have obtained an experimental stepwise aeration–agitation programme. As regards the oxytetracycline

fermentation a typical picture of agitator speed changing in the course of the process with a constant air supply of 25 l min^{-1} is presented in *Figure 3*. The difference in the final activity of the fermented broths in the experimental and blank fermenters was $\pm$ 10 per cent, which indicates the absence of any

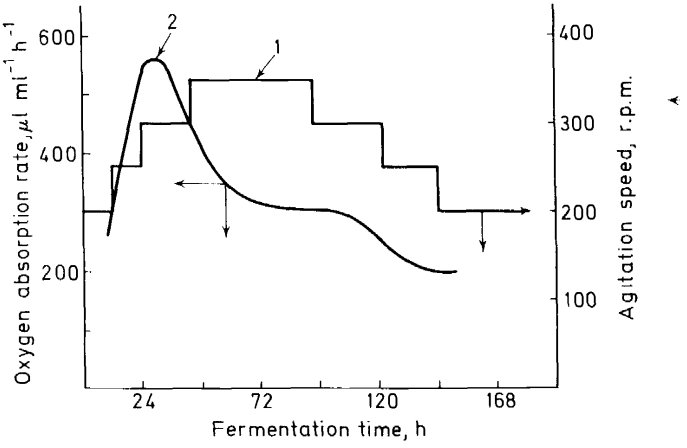

Figure 3. Oxygen consumption rate and experimental conditions of oxytetracycline fermentation. 1. Stepwise agitation; 2. Oxygen consumption.

substantial difference in antibiotic production in the experimental and in the blank equipment taking due account of the accuracy of the analysis and the process reproducibility.

The maximal agitator speed in the experimental fermenters (300 to 350 r.p.m.) coincided with the formerly-determined optimal one (for the constant conditions). It followed, however, that the fermentation could be conducted for a considerable time interval using a less intense agitation (200 to 250 r.p.m.). The air-supply range did not substantially influence the oxygen consumption rate. As a result of the experiments it had been found that a two-fold or even greater decrease of the air supply, compared with values used in the production process (i.e. from 50 down to 25 or even 15 l min^{-1}), did not affect the oxytetracycline yield. The use of step-wise agitation and air-supply reduction changes allow one to reduce substantially the power consumption.

A typical picture of the use of step-wise agitation in the case of oleandomycin fermentation with a fixed air supply (50 l min^{-1}) is shown in *Figure 4*. The maximal agitator speed in the course of the fermentation process was 420 to 470 r.p.m., that is, a speed considerably exceeding that maintained in the blank fermenter (330 r.p.m.). The antibiotic production level in the experimental fermenters exceeded that in the blank tanks by 10 to 15 per cent. A possibility of using limiting conditions in apparatus with constant agitation rate has been tested, these conditions being achieved at maximal oxygen consumption rates. In one of the blank fermenters operating at constant n,

the agitator speed corresponding to the maximal one in a step-wise operated experimental fermenter has been found (i.e. 450 r.p.m.). In this case the course of the biosynthesis and the antibiotic production level in both fermenters were analogous.

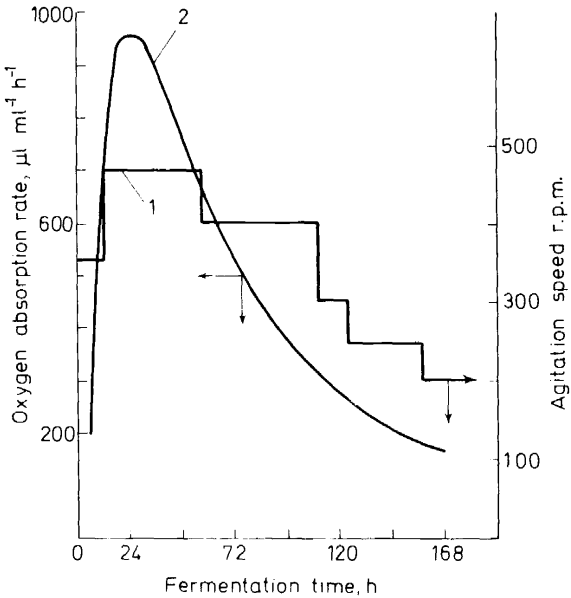

Figure 4. Oxygen consumption rate and experimental agitation conditions of oleandomycin fermentation

It is necessary to note that while choosing an aeration–agitation system by the method suggested above one may obtain several different schedules giving maximal oxygen consumption rate at different ratios of the agitator speed to the air supply. For example, at the 120th hour of oleandomycin fermentation the n_{crit} is 400 r.p.m. at an air supply of 15 l min^{-1} and 250 r.p.m. at an air supply of 50 l min^{-1}. The problem of choosing the proper one of the possible schedules must be solved with consideration of additional criteria, e.g. total economic cost of aeration and agitation, foaming and fermented broth filterability.

REFERENCES

[1] G. Terui and N. Konno, *Techn. Rep. of the Osaka Univ.* **11**, 447 (1961).
[2] S. D. Siegell and E. L. Gaden, *Biotechnol. Bioeng.* **4**, 345 (1962).
[3] D. G. MacLennan and S. J. Pirt, *J. Gen. Microbiol.* **45**, 289 (1966).
[4] Y. Hirose, H. Sonoda, K. Kinoshita and H. Okada, *Agr. Biol. Chem.* **31**, 1217 (1967).
[5] J. Kanzaki, Y. Sumino and H. Fukuda, *J. Ferm. Technol.* **46**, 1031 (1968).
[6] D. G. Kilburn and F. C. Webb, *Biotechnol. Bioeng.* **10**, 801 (1968).
[7] D. A. Self, D. G. Kilburn and M. D. Lilly, *Biotechnol. Bioeng.* **10**, 815 (1968).
[8] D. G. Kilburn, M. D. Lilly, D. A. Self and F. C. Webb, *J. Cell Sci.* **4**, 25 (1969).
[9] F. J. Moss, P. A. D. Rickard, G. A. Beech and F. E. Bush, *Biotechnol. Bioeng.* **11**, 561 (1969).

[10] C. T. Feren and R. W. Squires, *Biotechnol. Bioeng.* **11** 583 (1969).
[11] Z. Lengyel and L. Nyiri, *Biotechnol. Bioeng.* **8**, 133 (1966).
[12] L. Nyiri and Z. Lengyel, *Biotechnol. Bioeng.* **10**, 133 (1968).
[12] I. P. Uvarov, I. Ya. Veselov, E. R. Davidov, and A. D. Gololobov, *Prikl. Biokhim. Mikrobiol.* **7**, 8 (1971) (in Russian).
[14] A. Ishizaki, Y. Hirose and T. Shiro, *Agr. Biol. Chem.* **35**, 1860 (1971).
[15] E. S. Bylinkina, E. A. Ruban, in *Inzhenerskie problemy mikrobiologicheskogo sinteza*, p. 198, Moscow (1969) (in Russian).
[16] V. V. Biryukov and L. D. Shtoffer, *Prikl. Biokhim. Mikrobiol.* **7**, 12 (1971) (in Russian).
[17] P. Shu, *Ind. Eng. Chem.* **48**, 2204 (1956).
[18] A. L. Jensen, T. S. Shultz and P. Shu, *Biotechnol. Bioeng.* **8**, 525 (1966).