CHANGING APPROACHES TO ANTIBIOTIC PRODUCTION

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As our knowledge of the nutritional and cultural conditions needed for antibiotic production has increased, so has it been necessary to modify the techniques employed. Most antibiotics are formed after the cell has ceased to divide. All such cultures have, therefore, a dual function—the reproduction of new cells and the synthesis of antibiotic by the mature organism.

This process might be envisaged in a simple idealized surface culture as the formation of a mycelial mat and its subsequent elaboration to produce antibiotic. By definition, however, this ideal situation can never be realized. Establishment is not complete in a single reproductive phase; nor does all multiplication cease abruptly in favour of antibiotic production. Autolysing cells release nutrient; this supplements the unused original medium constituents and thus supports new growth to replace in part the spent mycelia.

One may draw an illustration from the early history of penicillin manufacture. Originally the medium employed was chemically defined and extremely simple. This was superseded by media based on complex sources of nitrogen, such as casein digest or the more practically useful corn steep liquor. Later on, the easily utilized sugars, sucrose or glucose, gave place to less available oligo- and polysaccharides. Of these, lactose gave the greatest improvement in yield. Hindsight clearly indicates that these nutrients broke down to the proximal intermediates, ammonia, carbohydrate and fatty acids, at steadier and more appropriate rates over longer periods. Thereby, they provided for the replacement of spent cells and a longer productive life. Incidentally, their presence also made it possible for the organism to create the stability of environment required for a long and productive life. The accumulation of toxic levels of ammonia from lysing cells was mitigated and a suitable pH range persisted longer. Failure to appreciate this aspect of the metabolism of complex nutrients has led to a great deal of experiment with media based on mixtures of amino-acids: the apparently specific stimulation by certain of these might well be explained by their relative rates of deamination and by their "ketogenic" or "antiketogenic" nature.

It is clear, then, that even at this primitive stage of development a continuous process was going on in the culture, although it was not long maintained. Attempts to prevent this by admitting new nutrient under the pre-formed felt were only successful in the laboratory, for the mechanical difficulties in large-scale production were insuperable.

At this time, the limitations of surface culture were only too apparent.

The use of thin layer culture meant that the production of adequate quantities of broth required acres of fermentation area. Moreover, the rate of production was limited by the slow diffusion of air through the mycelial mat and by the diffusion of solids upwards through the liquid layers. It was obvious that the organism should be more rapidly brought into contact with its oxygen and nutrient supplies.

The submerged culture of moulds had already been introduced, for the same reasons, in the production of gluconic acid and, after a suitable design of reactor had been developed, it rapidly superseded the surface culture method of penicillin production. This change, however, brought about new and considerable engineering problems. Large volumes of sterile air had to be provided at measured rates; new dispersing and mixing techniques were required to assure the best culture conditions; constant temperature maintenance was imperative. Further, since the decay phase was also accelerated, regular checking of pH and nutrient status became routine and one often had to rely on such information to determine the best harvest time.

In the early days of submerged culture, the medium employed was essentially similar to that used for surface growth. Chalk was then added to control fluctuations in hydrogen ion concentration. Soon afterwards animal or vegetable oils and fats were found beneficial. Although these were first employed merely to mitigate foaming, which was then a serious problem, it was found that they also increased titre. Although many explanations have been put forward for this activity, it is still not clear as to how much they contribute by affecting the proximal systems engaged specifically in the biosynthesis and how much they work through their effect on cell growth.

Productive capacity was further improved by use of the precursor, phenylacetic acid. This not only contributed the acyl group of benzylpenicillin; over ninety per cent of it also underwent oxidation to CO_2. To avoid much of this waste, phenylacetic acid was added continuously at such a rate as to supply the needs of penicillin synthesis without the accumulation of excess and, consequently, with a minimum loss from oxidation.

In 1953, Johnson[1] and his group published a description of equipment for this purpose. They produced penicillin by the continuous addition of glucose and phenylacetic acid under controlled conditions of pH. Gradual addition of glucose was fully as effective as including lactose in the batch initially. This technique has been widely adopted but the criteria for determining addition rates vary.

The author has extended this application to the production of griseofulvin. The physiological condition of the mould is maintained by continuous addition of sugar at rates such as to keep the pH down to a pre-arranged control value. By this means the productive phase may be extended to as much as 18 days. This shows that the requirements for steady state culture have been largely met though the nutrient consists only of sugar and a little KCl as griseofulvin precursor. The nitrogen required is adequately supplied by a high initial level, which copes with both cellular re-growth and dilution by nutrient feed.

In industrial practice, this type of process has advantages over the continuous fermentation according to most theoretical treatments since concentration of the product in the harvest broth is maximal and also it makes the fullest use and re-use of metabolites. The mycelial mass reaches a steady level throughout the fermentation until discarded after harvest. This compares favourably with the waste of mycelium and unused food in the conventional type of continuous culture, where the harvest broth has a lower potency (though this is dependent upon throughput rate and stage number). Our own experiments in the field lead us to believe that the maximum yield per hour per unit of occupied fermenter space is likely to be constant; thus, the higher the throughput rate, the greater the nutrient loss, mycelial wastage and product dilution. Furthermore, the dissociation of growth and production phases, as in the multi-stage continuous process, has not yet been shown to increase rates of synthesis, so that one hope of gain is unlikely to be rewarded.

However, with all "steady state" fermentations I have so far encountered, the accretion rate appears to be set during the first phase of growth and mycelial establishment. Unless this is optimal, it is extremely unlikely that the best yield will be attained and far more likely that the accretion rate will remain well below its potential in spite of all efforts to alter it. This "imprinting", whereby the culture as a whole takes on a higher organizational character than the organisms that make it up, is not unfamiliar in biology. In it one can see a pale intimation of that more complex biochemical interaction between cells that makes the ovum gastrulate and differentiate into its separate tissues. This quality of organization on a higher plane than the individual makes it necessary to treat the cultural process as an organic whole in its temporal context, or "gestalt" framework, in contrast to its immediate aspect as a series of balancing rate processes with no history. This is often not appreciated by the keener protagonists of continuous culture for industrial use.

The improvement of mechanical techniques has made possible the production of antibiotics from very simple nutrients such as ammonia, its salts, nitrate, glucose and neutral fats. Notwithstanding this, the complete adoption of synthetic media seems unlikely, since any resultant gain in simplicity and cheapness is offset by the need for more sophistication in operation. Exact timing and regulation of the supply of nutrient at different rates throughout the process becomes critical. The ability to control more variables brings with it the responsibility to do so, and the process has often to be specified more precisely. For practical purposes, it is usually better to establish the organisms on a complex medium before beginning the steady state nutrition with simpler adjuvants.

The changing pattern of culture also affects one's approach to the selection of improved strains. When the course of the fermentation was almost entirely predetermined at inoculation, two criteria were applied, the ability to produce antibiotic more quickly and the ability to maintain the original rate longer, thus making better use of the medium. For example, most of the penicillin producing mutants selected by Farrell[2] seem to have fallen into the second group. As continuous nutrition becomes a commonplace, it will clearly be more useful to select on a basis of better rate constants; although economic efficiency must not be neglected, this type of mutant is often more efficient under sub-optimal conditions.

As well as leading to numerous developments in equipment design, continuous feeding techniques have also altered the logistics of factory operation. Special systems for the supply of sterile nutrients have been set up and connected by a multiplicity of pipes to different culture vessels. This naturally has led to standardization and has thus reduced the flexibility of plant for the introduction of new nutrients, as there is a limit to the number of feed types available at any one time. In fact, because the nutrition is more flexible in terms of rate, it is more critical and more difficult to alter arbitrarily.

Notwithstanding its drawbacks, the concept of antibiotic production as a steady state system based on an understanding of rate processes concerned with assimilation of simple nutrients is here to stay. As instrumentation and control equipment are developed to deal with its specific problems, it is bound to replace entirely the old "batch it and bash on" techniques.

References

[1] K. Singh and M. J. Johnson. *J. Bacteriol* **56**, 339 (1948).
[2] L. Farrell. *Can. J. Med. Sci.* **31**, 512 (1953).