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TECHNOLOGY FOR THE PRODUCTION OF TETRACYCLINES AND ERYTHROMYCIN*

by

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1. PRODUCTION

Tetracycline is a broad-spectrum antibiotic produced by certain varieties of Streptomyces rimosus. Oxytetracycline and chlortetracycline are also produced by other varieties of these micro-organisms, two or more antibiotics of the group (especially tetracycline and chlortetracycline) being sometimes produced simultaneously. In the last 15 years, strains of high biosynthetic specificity have been developed which make possible the isolated production of each of the three antibiotics (e.g., the production of tetracycline alone even when large quantitites of chlorides are present in the fermentation medium).

Any process involving the fermentation of secondary metabolites is extremely delicate, and this is particularly true in the case of the production of tetracyclines, where one is dealing with highly productive strains, which consequently have very strict metabolic requirements.

The management of the cultures comprises basically two cycles:

(a) Maintenance and improvement of the strains

This consists of periodic transplantings of the strains to solid media and the growth of isolated clones which on maturity are selected according to their macroscopic morphology. For propagation purposes, these clones are transferred to solid spore-producing media from which suspensions of spores, all derived from the original spore, are obtained.

These suspensions may be freeze-dried, absorbed in inert media or preserved as such for months under refrigeration (+3-+5°C), and constitute the point of departure for the fermentation process. Prior to production, the suspensions are usually checked for productivity, purity of the resultant antibiotic, and sterility.

It is important to note that strict "monoculture" conditions must be maintained and that, as a consequence, a laboratory for handling these strains must be equipped with air filtration and environmental sterilization systems.

(b) Preparation of the cultures

This is carried out on the basis of the spore suspensions, a small portion of which is transferred to culture flasks containing a suitable medium for the development of the biomass (usually a medium rich in

carbohydrates and poor in proteins). The cultures are agitated in rotary or alternating agitators at a controlled temperature (normally 25°C) for a variable period of between 30 and 40 hours. Upon completion of this stage, the developed culture is placed in a pre-fermentation tank for the industrial propagation of the biomass.

Normally, these pre-fermentation tanks are stainless steel tanks equipped with systems for temperature control, constant agitation and air distribution (the air must be completely sterilized through filtration. The culture medium (normally like the kind used in laboratories) and the tank together with its fittings must be sterilized in advance. Sometimes, when developing the biomass under plant conditions, the process must be repeated twice.

As the pre-fermentation process continues, continued checks are made not only of growth but also of "sterility" (monoculture conditions) and other physical parameters (pH, viscosity, etc.). Development ends after 20-25 hours at variable temperatures of between 29° and 25°. At this point, the culture is transferred to the pre-sterilized fermentation tank containing a protein-rich production medium. Fermentation may take between 150 and 200 hours (semi-continuous), yielding a final antibiotic concentration of between 25 and 30 grams/litre.

The fermentation tank is of stainless steel and is equipped, basically, with devices to provide constant agitation and control of pH, temperature and air; systems for the control of froth and dissolved oxygen are also desirable.

With fermentation complete, the antibiotic-rich broth (following treatment to kill the micro-organism) is discharged into the purification section where the solids (basically mycelium, which can provide a valuable nutrient) are eliminated by filtering, after which the crude antibiotic is precipitated in the filtered broth. Normally, in the case of tetracyclines the entire purification process takes place in an aqueous medium and consists of a series of crystallizations (occasionally as many as six), taking advantage of the fact that these antibiotics are amphoteric and can thus be dissolved in either an acid or an alkaline medium. Purification processes employing solvents are still used, but less frequently, since the total yields in water are high (approximately 80 per cent of the amount of antibiotic present in the fermentation medium).

The most important products are oral tetracycline hydrochloride, base tetracycline, oral chlortetracycline hydrochloride and oral base oxytetracycline. There are also sterile preparations for parenteral use (above all the hydrochlorides and base oxytetracycline). In addition to these products, there exist other, less widely consumed salts, such as complex tetracycline phosphate, calcium oxytetracycline and such derivatives as pyrrolidinomethyltetracycline and its salts (derived chemically from the base molecule).

The last ten years have seen the development of demethylated products (demethylchlortetracycline, demethyldeoxytetracycline), which are obtained by the fermentation of specialized strains; however, it is generally thought that for the present and for a relatively long time ahead the outlook is for increasing production of the base molecules (tetracycline and oxytetracycline) in view of their therapeutic properties.

Erythromycin is a medium-spectrum antibiotic produced by fermentation of Streptomyces erythreus. The essential features of the laboratory handling of this strain are basically the same as for the tetracyclines, although there are a few differences (longer growing times and higher temperatures: 30-37°C).

Pre-fermentation is carried out in a single stage at a temperature of about 35°C in an aerated system. Fermentation is also carried out at high temperatures and involves the addition of precursors (normally sources of C_3). Fermentation times fluctuate betwee 150 and 300 hours, the average final antibiotic concentration being 5-8 grams/litre.

The most commonly used extraction system employs a water-insoluble solvent, although in the last five years considerations of cost have led to the development of purification systems using water. The solvent (concentrate) yields base erythromycin or certain of its salts (e.g., the oxalate or lactate), which constitute the point of departure for the production of the commercial raw materials. These are, in order of importance, erythromycin estolate, stearate, ethyl carbonate, base erythromycin and erythromycin lactobionate.

Prospects for the production of this antibiotic are for continued growth in view of its efficacy and low toxicity, coupled with possible increases in the consumption of the stearate and - owing to its harmful effects on the liver, which have been the subject of much discussion - a decline in the sales of the estolate.

2. PROBLEMS IN THE ESTABLISHMENT OF A TETRACYCLINE AND ERYTHROMYCIN PRODUCTION INDUSTRY

The problems most often encountered in efforts to begin production of tetracycline and erythromycin are of three kinds:

2.1. Inputs;

2.2. Equipment;

2.3. Personnel.

2.1. One of the most critical problems concerns the availability of inputs of constant and optimum quality. Tetracycline production is directly linked to the quality of the "corn steep", there being virtually no substitute for this source of protein. In general, there is a great deal that is not known regarding the characteristics of the corn steep and also regarding the metabolic requirements of the micro-organism, so that, in general, it is only on the basis of experience that one can judge whether the raw material is suitable or not. It is normally necessary to be able to rely on maize of consistent quality from the outset, since the producing strains adapt to certain characteristics in the raw materials.

The remaining agricultural raw materials (starch, corn meal and other protein sources) do not pose as difficult a problem (except for the presence of pesticides or toxins, which are anyway easily recognizable), while materials of animal origin (lard) or inorganic materials (source of nitrogen, calcium carbonate, oligo-elements) are less critical.

Our experience indicates that from the outset one must keep close touch with the raw materials suppliers so that they understand the problems of fermentation. As a general rule, raw material consignments must be checked in a laboratory or pilot plant to ascertain their productivity in the light of possible interrelations with the other components of the medium.

Even so, and despite all the precautions that may be taken, a certain period of adaptation of the strains to the available raw materials must be expected.

While the production of tetracycline is linked to products derived from maize cultivation, erythromycin production is almost totally dependent on the cultivation of soya, this being the principal source of protein.

Here also, strict quality control standards must be instituted, as otherwise there will be great variability in the results. On the basis of our experience, the raw material problem can be solved only gradually as the suppliers become sensitized and the strain adapts itself to the materials.

Another important input is water, which must be of optimum quality, if possible cold (in order to ensure a lower bacterial content and provide an effective coolant, since it should be remembered that very sizable amounts of heat are released during the fermentation process) and very abundant. In any case, water with a high dissolved-salt content must be avoided, as it may be harmful to fermentation.

Also of major importance is the quality of the air. Our experience indicates that it is best to avoid areas where the air is heavily contaminated with bacteria because of dust or high humidity. The presence of contaminated air greatly complicates the problem of fermentation tank contamination and makes necessary additional outlays for filters. (Air is the principal nutrient and is required in large amounts - around one litre of air per litre of broth per minute).

Electric energy is also an essential input because of the need to keep the medium constantly aerated and agitated. If, as in our case, one cannot rely on a constant energy supply, an alternative source must be installed. To give an idea of the importance of aeration and agitation, it may be recalled that when the production process is under way a fermentation tank has a dissolved-oxygen reserve of only about 10 seconds, after which asphyxia begins.

2.2. The most important engineering problem concerns the selection of the air compression and filtration equipment.

The fermentation units (pre-fermentation and fermentation tanks) are normally standard and universal (in actual practice, apart from some minor modifications, a conventional stainless-steel fermentation tank equipped with an agitation system may be used for virtually any antibiotic fermentation process), as is also the extraction equipment (filters, press, centrifuges, liquid separators, and continuous-operation extractors).

The economic size for a fermentation tank is normally more than 30,000 litres total capacity, with a trend towards tanks of up to 100,000 litres.

In our experience, the use of oil-less compressors (screw-type or centrifugal) coupled with filtration systems based on absolute filters provide the most suitable air-compression systems.

2.3. Personnel selection and training is another problem area of the greatest importance, as the processes in question require a high level of technical skill and care.

In fact, when one considers that even the simplest processes require about one month (and sometimes even three months) from the time they first begin in the strain preparation laboratory until the release of the product for sale, and that the slightest oversight during this entire period may result in problems not only in the yield, but also in the quality of the final product, the importance of the level of personnel training becomes immediately apparent.

In our case the situation has gradually improved, as far as professional staff are concerned, thanks to the organization of university courses and study groups which have led to a substantial improvement in their level of technical competence.

The problem continues, however, to be very serious in the case of middle-level technicians and workers, for whom, in our situation, in-plant training is still the best approach.

2.4. Miscellaneous problems

Generally speaking, it must be remembered that pilot equipment is always required for the study of operating conditions, the introduction of new strains, etc.

Normally, any technology in this field will require adaptation - and sometimes very extensive adaptation - before the production levels of the country of origin can be attained. This is due to local conditions as regards supply, the skill levels of the technicians and the location of the producing plants.

It must always be borne in mind that, even with the strictest precautions, contamination losses for certain antibiotics (especially the medium-spectrum category) may be very high (as much as 20 per cent of the total). The situation in the case of broad-spectrum antibiotics is significantly more favourable (losses of 1-2 per cent of the total).

3. CONCLUSIONS

In the light of the considerations discussed above, this type of production of raw material for the pharmaceuticals industry may be said, for a country like Mexico, to represent a typical example of production integration since a large portion of the inputs (in some cases as much as 100 per cent) is of domestic origin. For this same reason, production will generally be internationally competitive in terms of costs. We have also found that, as our experience has increased, the quality of our products has opened the doors to international markets.

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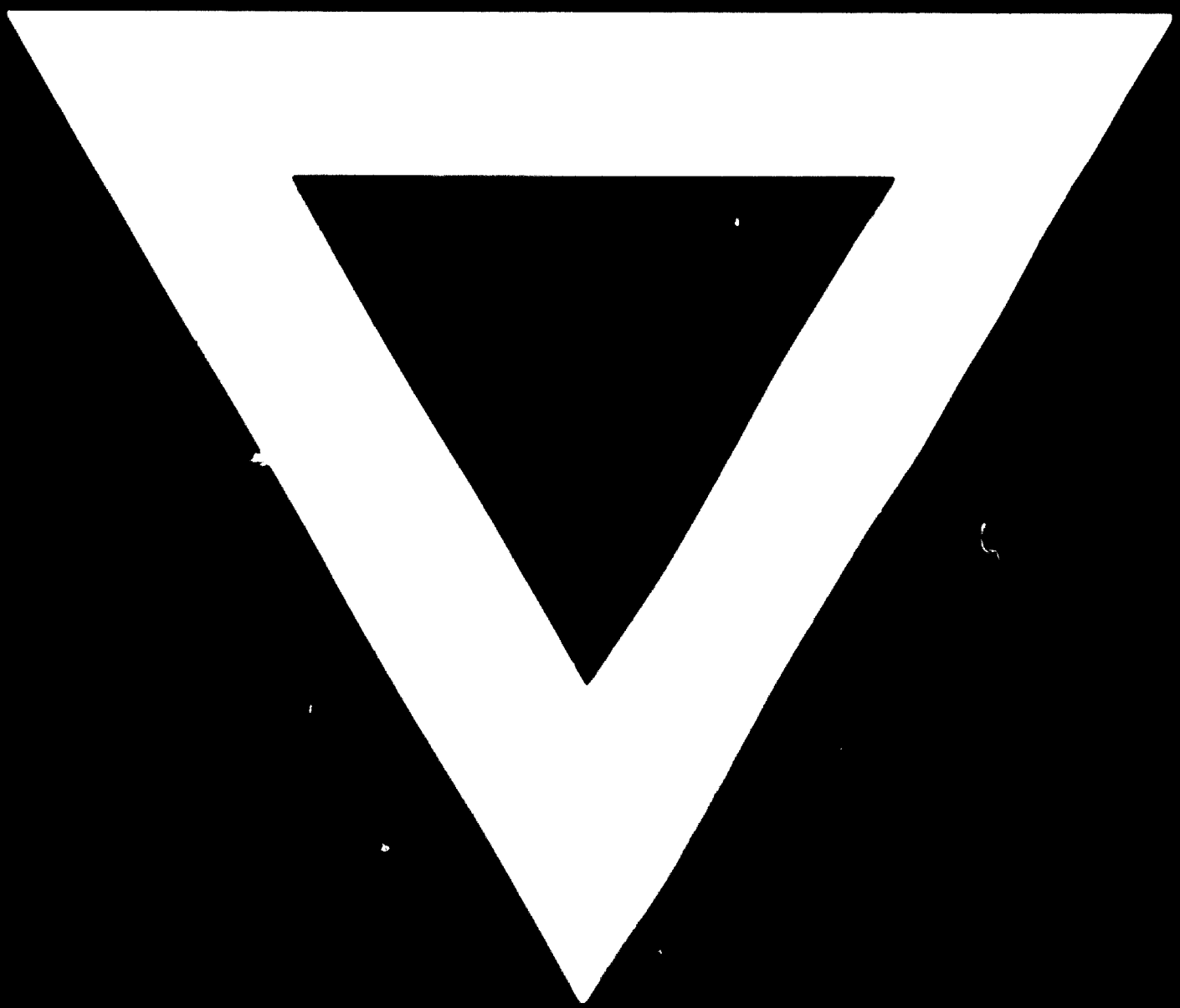

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