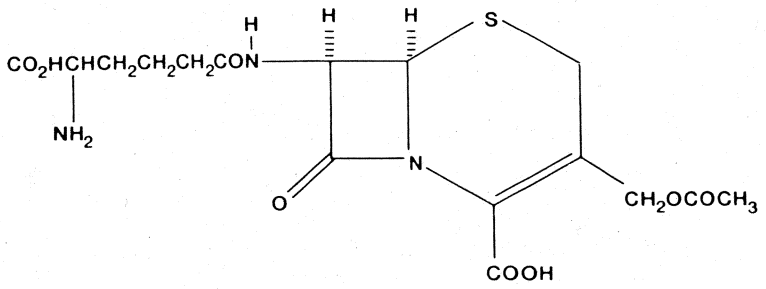
Expedient Antibiotics Production

Final Report

ORNL DWG 87-470



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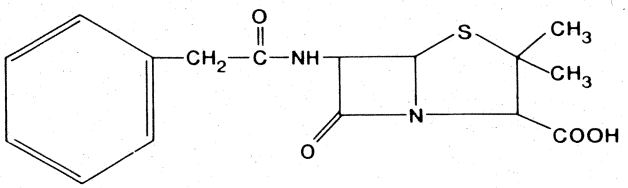

PENICILLIN G

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Expedient Antibiotics Production
Final Report

by
P. R. Bienkowski
C. H. Byers
D. D. Lee

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The literature on the manufacture, separation and purification, and clinical uses of antibiotics was reviewed, and a bibliography of the pertinent material was compiled. Five antimicrobial drugs, penicillin V and G (and amoxicillin with clavulanic acid), Cephalexin (a cephalosporin), tetracycline and oxytetracycline, Bacitracin (topical), and sulfonamide (chemically produced) were identified for emergency production. Plants that manufacture antibiotics in the continental United States, Mexico, and Puerto Rico have been identified along with potential alternate sites such as those where SCP, enzyme, and fermentation ethanol are produced. Detailed process flow sheets and process descriptions have been derived from the literature and documented.

This investigation revealed that a typical antibiotic-manufacturing facility is composed of two main sections: (1) a highly specialized, but generic, fermentation unit and (2) a multistep, complex separation and purification unit which is specific to a

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19. ABSTRACT (continued)

particular antibiotic product. The fermentation section requires specialized equipment for operation in a sterile environment which is not usually available in other industries. The emergency production of antibiotics under austere conditions will be feasible only if a substantial reduction in the complexity and degree of separation and purity normally required can be realized. Detailed instructions were developed to assist State and Federal officials who would be directing the resumption of antibiotic production after a nuclear attack. Initially, all plant managers should be contacted (using the lists provided in this report) to determine if their facility has been destroyed or damaged. If a plant has been damaged, the manager should determine the feasibility of making repairs, the potential capacity of the repaired plant, and the type of antibiotic that can be produced. If enough production cannot be realized from undamaged and slightly damaged plants, the plants from the list of alternate emergency production sites must be contacted, determination of the plant status made, and the managers informed of impending conversion to antibiotic production. If alternate sites must be used, a team of skilled personnel must be assembled to convert the plant to antibiotic production in the austere environment.

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EXECUTIVE SUMMARY

OBJECTIVE

It is desired to determine the most efficient methods for meeting the antibiotic needs of the population for the period beginning 30 days after a nuclear disaster.

BACKGROUND

Meaningful preparedness for catastrophic disasters which would cause mass casualties includes both development of capabilities for reducing the impact of the hazard, and steps to deal with the effects that cannot be prevented. A catastrophic disaster could destroy production capabilities for antibiotics and create, at the same time, conditions under which epidemic diseases and large numbers of injuries occur. The availability of broad-spectrum antibiotics could be of major importance to the survival and recovery of the threatened population. One purpose of this project is to identify several antibiotics for emergency production based on clinical need and efficiency of production and provide documentation on the production of the antibiotics. We also propose modification of antibiotic production equipment, material, and processes that would allow expedient production of broad-spectrum antibiotics under the austere conditions following a nuclear attack.

PROCEDURE

The approach used to obtain the necessary information and develop the desired instructions for the expedient production of antibiotics involve the following:

1. Select antibiotics for emergency production, based on need and production capabilities.

2. Document current production technologies including equipment, materials, and processing steps for the selected antibiotics.
3. Document current U.S. antibiotic production sites, potential capacities, and procedures for expedient contacting of plant managers after a nuclear attack.
4. Identify potential emergency production sites which lie outside of the risk areas, making recommendations as to how these facilities could be used for production of antibiotics.
5. Recommend modifications in current production technology and functional substitutions for certain basic equipment and facilities which would allow rapid conversion of emergency production sites to antibiotic manufacturing.
6. Identify alternative sources of antibiotics.

CONCLUSIONS

1. Antibiotics chosen are penicillin V and amoxicillin (from penicillin G)(and combined with clavulanic acid, an alternative if cephalexin is not able to be produced), cephalexin (a cephalosporin), tetracycline and oxytetracycline (broad-spectrum, widely tolerated, and widely prescribed and available), bacitracin (as a topical antibiotic), and sulfanilamides (sulfamethazine, sulfamethizole, sulfathiazole, etc.) which are chemically produced.
2. The expedient dosage form should be oral. Capsule, tablet, and suspension forms provide enhanced storage stability and decreased need for sterility, purity, and quality control required for injectable forms. Fewer and simpler separation and purification methods can be used. Transportation is simplified and dosage control for oral antibiotics is feasible outside of sterile hospital locations.
3. Several large antibiotic-manufacturing facilities lie outside of the risk areas and probably will not be damaged.

4. Several large antibiotic-manufacturing facilities lie just within the risk areas and may sustain only minor damage.
5. Pharmaceutical companies often maintain a 4- to 16-week inventory of finished products and a 4- to 8-week working inventory of finished products ready for formulation. Distribution facilities for these companies located in major cities throughout the country also may have substantial inventories of antibiotics. Company headquarters should be contacted to determine the status of warehoused antibiotics. Boston, Charlotte, Miami, St. Louis, Louisville, Kansas City, Seattle, San Francisco, Chicago, Dallas, and Atlanta have major distribution facilities.
6. All existing antibiotic-manufacturing facilities should be surveyed immediately after an attack.
   a. Undamaged facilities should be operated at maximum capacity for a selected antibiotic, subject to feedstock and solvent availability.
   b. Rapid assessment should be made of damaged facilities to determine repair feasibility.
7. Design protocols for the conversion of undamaged non-antibiotic production sites to antibiotic production must be determined. A "model" retrofit design to an existing plant (e.g., penicillin V production at the Hutchinson, MN, SCP plant) should be made. The outcome of this model design would be:
   a. Provide State and Federal personnel with very accurate resource estimates of time, manpower, and materials required.
   b. Establish methods of planning for conversion.
   c. Evaluate and determine the best separation method for retrofit under austere conditions.
   d. Provide State and Federal personnel with a retrofit design at once—could also serve as the model for the other retrofits which might be needed.
8. Designated possible expedient antibiotic-production sites

should be surveyed, and their management should be informed of impending conversion to antibiotic production.

9. Personnel who are familiar with a chosen emergency antibiotic-production plant must be brought together. Included are the operating engineer, plant operators, and chemical and biochemical engineers. Also someone who is familiar with the biological needs of the fermentation organisms and the growth and fermentation of microorganisms (such as a biochemist, molecular biologist, microbiologist, etc.) is necessary. Someone with plant construction or modification experience is also desirable to help plan the modifications and implement the desired changes.
10. To assure the availability of personnel who are adequately trained in the use of the equipment and the process, consideration should be given to training military personnel who have a background in this area. A listing of technical experts both within and outside of the military should be assembled and kept current.
11. Antibiotic production couples a relatively small, highly specialized, aerobic fermentation section with a complex multistep separation/purification section. Expedient production at alternate sites will only be feasible if there is a significant simplification in the separation and purification sections of the process. This may be accomplished by reducing the number and complexity of the separation steps by using new separation methods. Other methods that produce a usable product in one or two steps, such as filtration followed by spray drying, may also be used, thereby producing an impure product. Impure products are generally acceptable only for oral use. Primary alternate sites must include an in-place aerobic fermentation system and should contain some existing separation/purification equipment which could be adapted to one of the antibiotic production schemes.
12. The specialized microorganisms used in the various antibiotic fermentations must be procured and kept viable. Many strains

of an organism can be used to produce a given antibiotic, but, if the most efficient strain is not selected and used, plant capacity can be substantially reduced (by ten to one hundred times). The desired strains of the organisms should be stockpiled by freeze drying or alternate long-term storage techniques that would allow the cultures to be immediately available to expedient producers. Consideration should also be given to stockpiling some specialized equipment, finished bulk chemicals (sulfur, 6-APA, etc.), and other critical supplies.

13. Small volume antibiotics with specialized uses (e.g., lincomycin for serious bacterial infections in penicillin-allergic patients) should be stockpiled, and responsible funding agencies should be identified.
14. Many veterinary antibiotics are identical to human drugs, but lower in purity. They could be used with caution in an emergency.
15. Substantial antibiotic capacity is available in Mexico and Puerto Rico. Consideration should be given to importation of the necessary antibiotics from these locations.
16. The raw materials necessary for fermentations are generally available. They include carbohydrates (such as glucose, sucrose, and lactose), nitrogen sources (like corn steep liquor or soybean meal), organic acids, and various inorganic chemicals for pH control and growth. Separation/purification requires organic solvents (amyl or butyl acetate, 1-butanol), filter aid and precoats for broth filtration, and various salts and minerals for salting-out procedures and pH control.
17. General utility requirements for antibiotic production include plentiful steam for sterilization and for solvent recovery and purification. Electricity is required to run the pumps, centrifuges, extractors, mixers, agitation equipment, and chilled water machinery. A source of chilled water is needed to remove heat from the fermenters and to provide cooling for the separation processes that often operate at 0 to 3°C. Basic analyt-

ical and microbiological laboratory facilities are necessary for process control and quality assurance.

18. Several advanced separation technologies hold great promise for making vast reductions in the separation/purification resources required to produce purified antibiotics. These technologies include high performance liquid chromatography (HPLC) using continuous annular chromatography, supercritical fluid extraction, and membrane separation methods.

ABSTRACT

The literature on the manufacture, separation and purification, and clinical uses of antibiotics was reviewed, and a bibliography of the pertinent material was compiled. Five antimicrobial drugs, penicillin V and G (and amoxicillin with clavulanic acid), Cephalexin (a cephalosporin), tetracycline and oxytetracycline, Bacitracin (topical), and sulfonamide (chemically produced) were identified for emergency production. Plants that manufacture antibiotics in the continental United States, Mexico, and Puerto Rico have been identified along with potential alternate sites such as those where SCP, enzyme, and fermentation ethanol are produced. Detailed process flow sheets and process descriptions have been derived from the literature and documented.

This investigation revealed that a typical antibiotic-manufacturing facility is composed of two main sections; (1) a highly specialized, but generic, fermentation unit and (2) a multistep, complex separation and purification unit which is specific to a particular antibiotic product. The fermentation section requires specialized equipment for operation in a sterile environment which is not usually available in other industries. The emergency production of antibiotics under austere conditions will be feasible only if a substantial reduction in the complexity and degree of separation and purity normally required can be realized. Detailed instructions were developed to assist State and Federal officials who would be directing the resumption of antibiotic production after a nuclear attack. Initially, all plant managers should be contacted (using the lists provided in this report) to determine if their facility has been destroyed or damaged. If a plant has been damaged, the manager should determine the feasibility of making repairs, the potential capacity of the repaired plant, and the type of antibiotic that can be produced. If enough production cannot be realized from undamaged and slightly damaged plants, the plants from the list of alternate emergency production sites must be contacted, determination of the plant status made, and the managers informed of impending conversion

to antibiotic production. If alternate sites must be used, a team of skilled personnel must be assembled to convert the plant to antibiotic production in the austere environment.

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1. INTRODUCTION

It is expected that the per capita demand for antibiotics in the postattack environment will rise sharply although total demand may decrease, remain constant, or increase, depending on the extent of the population destroyed. This increased demand will arise from two sources; treatment of wounds and control of communicable diseases. It is anticipated that a large portion of the current antibiotic production capacity will be destroyed because most antibiotic-manufacturing facilities lie within probable nuclear attack zones. At the same time, problems in the control of communicable diseases will rise sharply due to disruptions in the food and water supplies and sewage treatment systems.

The extent to which antibiotic capacity becomes critical in a given postattack environment depends on many factors, some of which are interdependent. Among the variables are:

1. The relative size of population losses and antibiotic capacity losses,
2. The antibiotic requirements per survivor relative to preattack per capita demands, and
3. The sizes of surviving antibiotic stockpiles.

Such determinations are quite complex and beyond the scope of the present study.

This report documents the clinical uses of the various antibiotics and recommends antibiotics for expedient production in a postattack environment. The current antibiotic-manufacturing sites

are identified along with descriptions of the manufacturing technologies for the selected antibiotics. Possible sites for producing antibiotics under austere conditions are also identified. These include in decreasing order of desirability: damaged but rebuildable antibiotic production facilities; animal and plant antibiotics producers; single cell protein producers; enzyme, msg and other molecular biology product makers; citric acid producers; brewers and fuel ethanol producers; the dairy industry; large hospitals and universities with fermentation research facilities; and farm fermentation units.

Commercial production technologies for the selected antibiotics are documented along with recommendations on how these processes can be modified to allow for conversion of alternate production sites to antibiotic production. The processes for manufacturing antibiotics are very difficult to reproduce at alternate facilities due to the unique nature of antibiotic production. A highly specialized fermentation section requiring sterile conditions is coupled to a very large separation and purification system which requires large volumes of solvents and a great deal of energy.

In summary, this report develops the methodologies necessary for expanding antibiotic capacity in a post attack environment. The extent to which these recommendations are implemented will be determined by the actual environment in existence after a nuclear attack. By coupling this study with mathematical models which predict possible post attack environments for assumed attacks, various possible methodologies and recommendations can be explored by FEMA planners.

2. DEFINITION OF AN ANTIBIOTIC

Antibiotics are a class of antimicrobial chemicals which are produced by microorganisms (mainly the saprophytic molds and bacteria of the soil). These substances will inhibit the multiplication of various microorganisms or may even destroy these organisms by either interfering with cell wall development and/or metabolism. Each antibiotic has its own characteristically selective "spectrum" of potency against various microorganisms, which varies not only for the species but also the strain of the infecting organism and other conditions. The term "broad spectrum" is applied to antibiotics which are effective against a wide range of bacteria.[1] Antibiotics are in general ineffective against typical virus diseases except for the tetracyclines which are sometimes effective against large viruses. With the exception of the penicillins and cephalosporins which are produced by molds, all major classes of antibiotics are derived from bacteria.[2]

Other antimicrobial substances are produced by the higher plants. Some are synthetically produced such as p-aminosalicylic acid and the sulfonamides. The sulfonamides were the first major broad spectrum antimicrobial drugs. However current antibiotics are now more potent than the sulfonamides. The sulfonamides were originally active against a wide range of bacteria, but an increasing incidence of resistance in bacteria formerly susceptible to the sulfonamides has decreased their effectiveness (a single mutation in the bacteria allows some organisms to develop resistance).[1] The sulfonamides are still the choice medication in several common bacterial infections.

3. ANTIBIOTICS SELECTED FOR EXPEDIENT PRODUCTION

The following antimicrobial drugs have been targeted for expedient production. They include antibiotics which are taken in capsule or tablet or suspension form—penicillin V, the amoxicillin derivative of penicillin G, the cephalexin derivative of cephalosporin C, tetracycline and oxytetracycline, and the sulfonamides which are produced chemically. Also selected is the topical antibiotic bacitracin.

Penicillins, cephalexin, and tetracyclines were selected for their widespread clinical applications because they are effective in common respiratory and gastrointestinal tract infections and some common opportunistic infections. Also, their share of production capacity and dollar value means that there are many plants producing or capable of producing them. Opportunistic infections are caused by gram positive and gram negative (G+ and G-) organisms such as *E. coli*, some *Pseudomonads* and *Bacteroides* pathogens. They often cause chronic infections after puncture or burn wounds and are often resistant to penicillin but susceptible to cephalexin. A combination of clavulanic acid (a suicide inhibitor of β-lactamase enzymes) with amoxicillin (called augmentin) gives similar or better coverage than cephalexin in many cases. If the production capabilities are available (it requires the same equipment as penicillin, but uses a different organism), it is a viable alternative to cephalexin. Table 1 gives the 1980 and 1985 figures for antibiotic production in the United States.[3]

Figures 1 and 2 show the general structures of the cephalosporins, penicillins, and tetracyclines. Once a manufacturing process is developed for one of these classes of antibiotics, many similar antibiotics can be produced by either very minor changes in the process (i.e., different growth media, precursors, etc.) or by semisynthetic means. For example while there are many penicillins in use, only three are produced naturally by fermentation (penicillin G, O, and V); all of the others are then produced from penicillin

Table 1. U.S. antibiotic production

Antibiotic	Production (1000 lbs)	Sales 1000 lbs)	Value ($1000)
Cephalosporin	977	970	
Penicillin V & G	6514	1974	32,082
Penicillin G for medicinal	2750		
All other uses	3764		
Semisynthetic Penicillins	1768	448	32,246
Amoxicillin	323		
Ampicillin	964		
All Others	481		
Tetracyclines	6562	4122	86,488
Sulfonamides	4841	771	10,884

(International Trade Commission 1980)

Antibiotic	U.S. Production ($ Millions) 1985
Cephalosporins	1,211.
Penicillins	252.
Tetracyclines	280.
All Others	754.
TOTAL	2,497.

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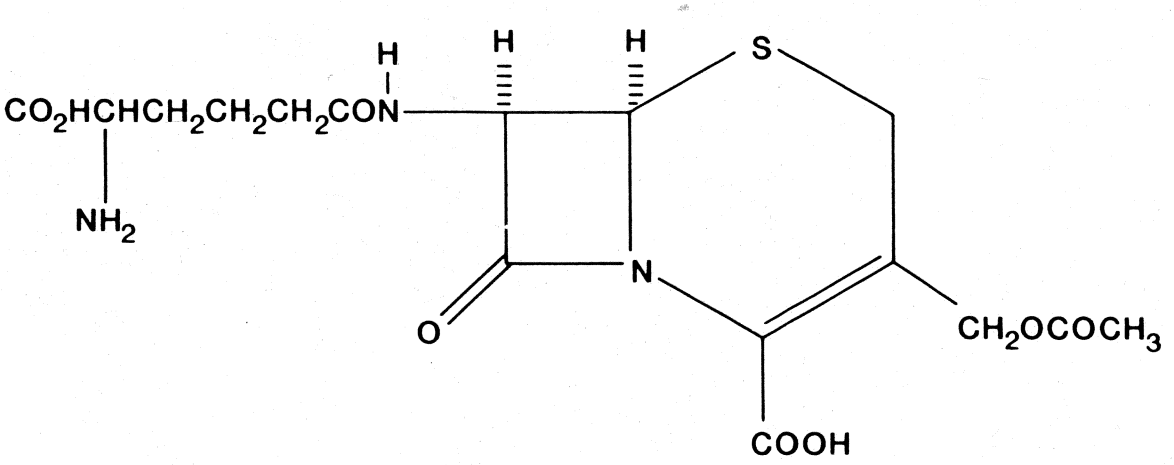
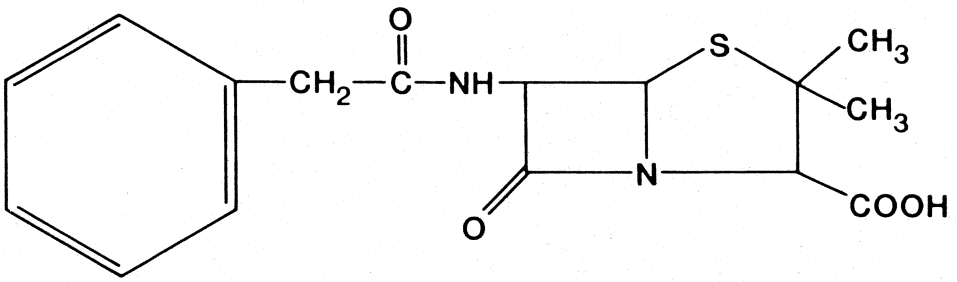


CEPHALOSPORIN C



PENICILLIN G

Fig. 1. Structures of Cephalosporin C and Penicillin G produced in antibiotic fermentations.

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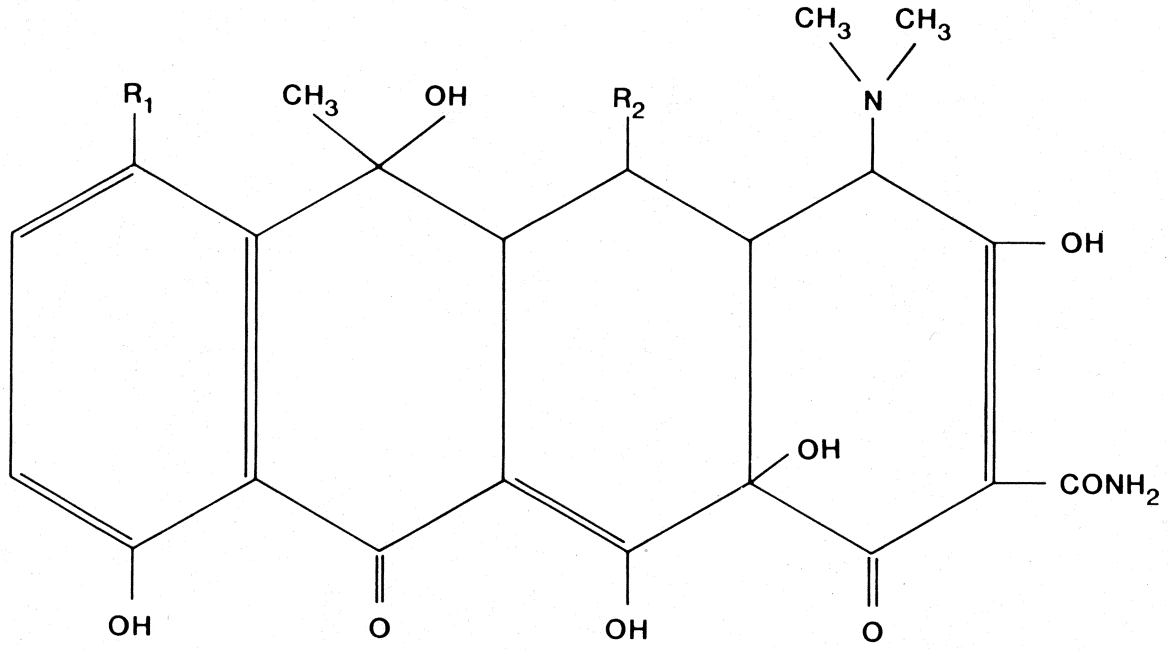

R_1 AND R_2 ARE DIFFERENT IN DIFFERENT ANTIBIOTICS	R_1	R_2
TETRACYCLINE	H	H
CHLORTETRACYCLINE	Cl	H
BROMTETRACYCLINE	Br	H
OXYTETRACYCLINE	H	OH

Fig. 2. Structure of Tetracycline showing substitutions to get related antibiotics.

G by enzymatic (penicillin acylase) (Fig. 3) and/or chemical reactions. The other reaction shown in Fig. 3 is the deactivation reaction which is used by penicillin resistant organisms to confer penicillin resistance. The clinical activity of these three classes of antibiotics covers a "broad spectrum" of uses (Table 2). Table 3 is a list of the various antibiotics and the generally recommended dosages for a course of treatment with the antibiotics.[4] Also listed are the approximate requirements to treat a million patients per month at the recommended treatment rate. This can be used to estimate the total antibiotic required if the affected population can be estimated.

Because of the possibility for a large number of burns and skin wounds, it is desirable to have one topical antibiotic for their treatment. Bacitracin is the most common topical antibiotic, and it has a wide range of clinical applications.

The sulfonamides also represent a family of related drugs. The sulfonamides were the first broad spectrum antimicrobial drugs, coming into widespread use in the 1930s. Due to overuse, the effectiveness of these drugs as "broad spectrum" antibacterials has been reduced over the years as organisms built up resistance, to the point where they do not have the same potency as the fermentation antibiotics.[1] They are still useful and the drugs of choice for urinary tract infections (UTI) and some ear infections. Because the sulfonamides are produced chemically and do not require the complex and specialized equipment necessary for the fermentation antibiotics, they would require less production resources in a postattack environment.

3.1 PENICILLINS

Over 100 different penicillins have been produced by natural fermentations. However, only penicillin G, penicillin V, and, to a small extent, penicillin O are produced in commercial quantities. The other natural penicillins are not therapeutically useful. Penicillins act by specific inhibition of bacterial cell wall synthesis,

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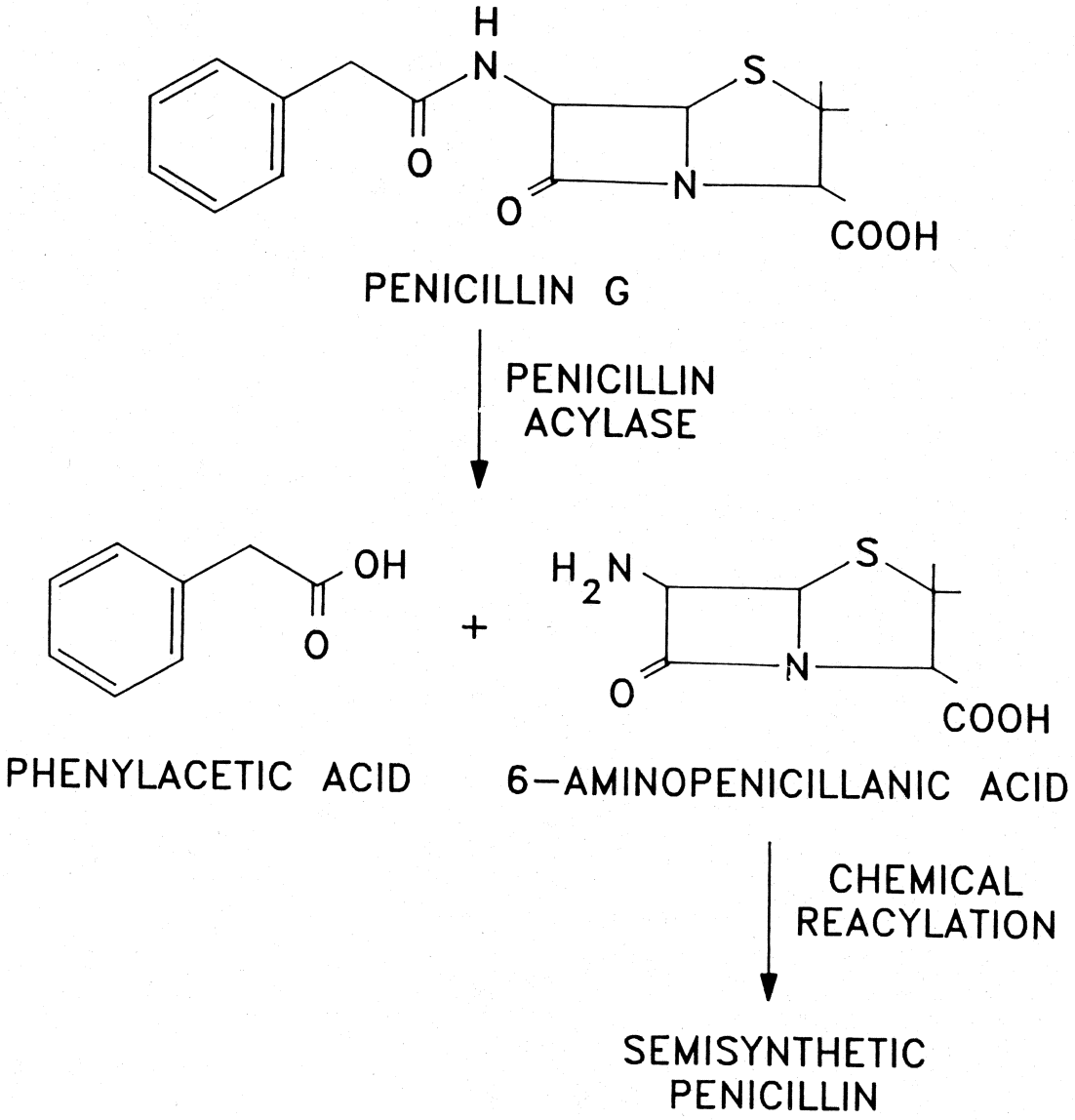

Fig. 3. Chemical modification to penicillin G to produce semisynthetic penicillins.

Table 2. Antibiotics produced in the United States (by classification)

ANTIBIOTIC	PRODUCER	ORGANISM	ACTIVITY	TYPE
--CEPHALOSPORINS--				
Cefaclor, Cefadroxil Cefamandole, Cefoxitin Cephalexin, Cefonicid, Cephaloridine, Cephalothin Cefuroxime, Cephapirin Cephacetrile, Cephradine	3,6,8,11	Cephalosporium acremonium Streptomyces sp.	G+, G-, skin bone, ear, respiratory	ß-Lactam
--SEMISYNTHETIC PENICILLINS--				
Amoxicillin, Phenethicillin Propicillin, Carbenicillin Ticarcillin, Augmentin, Piperacillin, Cyclacillin Epicillin, Ampicillin, Pivampicillin, Bacampicillin, Methicillin, Nafcillin, Oxacillin, Cloxacillin, Dicloxacillin, Flucoxacillin	4,5,9 11,13	Penicillium chrysogenum	G+, G-	ß-Lactam
---NATURAL PENICILLINS---				
Penicillin V Penicillin G	4,6,8,9, 11,13,17	Penicillium chrysogenum	G+	ß-Lactam
---TETRACYCLINES---				
Chlortetracycline, Tetracycline Demeclocycline, Doxycycline Minocycline, Rolitetracycline Methacycline, Oxytetracycline	2,6,13,27	Streptomyces sp.	G+, G- Rickettsia, Mycobacteria Mycoplasma	Polyketide
---BACITRACIN---	10	Bacillus licheniformis B. subtilis		
---SULFONAMIDES---				
Acetyl Sulfisoxazole, Mafenide Acetate, Sulfabenzamide, Sulfacetamide, Sulfadimethoxine Sulfachloropyrazine, Sulfonamide, Sulfathiazole, Sulfamethizole, Sulfasalazine Sulfaquinoxaline, Sulfadiazine Sulfanitran, Sulfisoxazole Sulfamethazine, Sulfachlorpyradizine	2,11,13, 14,15	Chemical Reaction	Uti, Ear	Sulfonamide

PRODUCING COMPANIES

1. Abbott Laboratories
2. American Cyanamide
3. Anheuser-Busch, Inc.
4. Beecham Laboratories
5. Biocraft Laboratories, Inc.
6. Bristol-Myers Company
7. Burroughs Wellcome Co.
8. Eli Lilly and Company
9. Fermenta
10. IMC Chemical Group
11. Merck & Co., Inc.
12. Napp Chemicals, Inc.
13. Pfizer, Inc.
14. Hoffmann-La Roche Inc.
15. Salsbury Laboratories, Inc.
16. SmithKline Beckman Corp.
17. The Upjohn Company

Table 3. Dosage forms and dosage recommendations for expedient antibiotics

Antibiotic	Dosage form	Dosage	Time	Total given
Penicillin G	Injection (powdered for reconstitution)(1 mil u=600mg Pen G sodium)	5-15 mil. u/d	10-15d	80-375g
	Oral tablets	1-3 mil. u/d	10 d	17-50g
	Oral syrup	0.5-3 mil u/d	10 d	8-50g
Penicillin V	Oral tablets	0.6-2 mil u/d	10 d	10-33g
	Oral syrup	0.2-2 mil u/d	10 d	3.3-33g
Amoxicillin	Oral capsules	0.75-1.5 g/d	7-10 d	5.2-15g
	Oral syrup	0.3-1.5 g/d	7-10 d	2.1-15g
Amoxicillin + Clavulanic acid (Augmentin)	Oral tablets	0.75-1.5 g/d amox	7-10 d	5.2-15g
	Oral syrup	+ 0.375 g/d clav. acid		2.6-3.8g
Cephalexin	Oral capsules	1-4 g/d	10 d	10-40g
	Oral syrup	0.5-4 g/d	10 d	5-40g
Tetracycline HCl	Oral capsules	1-2 g/d	7-10 d	7-20g
	Oral syrup	0.25-2 g/d	7-10 d	1.8-20g
	Injection	0.25-2 g/d	4-7 d	1-14g
Oxytetracycline	Oral capsules	1-2 g/d	10 d	10-20g
	Oral syrup	0.5-2 g/d	10 d	5-20g
	Injection	0.25-2 g/d	4-7 d	1-14g
Sulfamethizole or	Oral tablets	2-4 g/d	7-10 d	14-40g
Sulfamethoxazole/ trimethoprim	Oral tablets	1.6g/d	10-14 d	16-22.4g
	Oral syrup	0.4-1.6 g/d	10 d	4-16g
Bacitracin	Topical	2-5 times/d	as needed	14000 u/tube

Penicillin G required per 10^6 treated/month	= 80,000 - 375,000 kg (injection) = 17,000 - 50,000 kg (tablets) = 8,000 - 50,000 kg (syrup)
Penicillin V required per 10^6 treated/month	= 10,000 - 33,000 kg (tablets) = 3,300 - 33,000 kg (syrup)
Amoxicillin required per 10^6 treated/month	= 5,200 - 15,000 kg (capsules) = 2,100 - 15,000 kg (syrup)
Augmentin-- Amoxicillin required per 10^6 treated/month Clavulanic acid	= 5,200 - 15,000 kg (tablets) = 2,600 - 3,800 kg (tablets)
Cephalexin required per 10^6 treated/month	= 10,000 - 40,000 kg (capsules) = 5,000 - 40,000 kg (syrup)
Tetracycline required per 10^6 treated/month	= 7,000 - 20,000 kg (capsules) = 1,800 - 20,000 kg (syrup) = 1,000 - 14,000 kg (injection)
Oxytetracycline needed per 10^6 treated/month	= 10,000 - 20,000 kg (capsules) = 5,000 - 20,000 kg (syrup) = 1,000 - 14,000 kg (injection)
Sulfamethizole needed per 10^6 treated/month	= 14,000 - 40,000 kg (tablets)
Sulfamethoxazole/ trimethoprim needed per 10^6 treated/month	= 16,000 - 22,400 kg (tablets) = 4,000 - 16,000 kg (syrup)
Bacitracin required per 10^6 treated/month	= 14×10^9 units

and kill only growing cells, while not harming resting cells. All three penicillins are produced by the same fermentation process with the constitution of the growth media (the added precursor) determining which is produced.[5] All of the natural penicillins are available in dry, crystalline form and are stable for several years under controlled conditions. Penicillin G is mainly administered parenterally in solution form, and its stability in solution is rather limited. However, over 20 therapeutically useful semisynthetic penicillins are derived from penicillin G.

Penicillin V, O, and G are produced with the submerged culture process in 40,000- to 200,000-l fermenters. Oxygen transfer and the specific culture used are quite important. While many organisms can be used to produce penicillin, the yield is strongly dependent on the strain which is used.[6,7] In general, the concentrations of antibiotics in the fermenter are very low. However, with proper strain selection, penicillin can be produced in concentrations of up to 40 g/l which is 1 or 2 orders of magnitude greater than many other antibiotics.[8] Separation and purification often constitute 90% or more of the steps (especially in the production of injectable antibiotics) in an antibiotic plant, making the concentration in the fermentation broth very important.

3.2 CEPHALOSPORINS

Cephalosporins are economically and therapeutically as important as the penicillins. These drugs are broad-spectrum antibiotics, active against respiratory, ear, skin, and bone infections caused by G+ and G- bacteria, and they have low toxicity. In a manner similar to the penicillins, they act by inhibiting bacterial cell wall (peptidoglycan) synthesis, and they kill growing cells but not resting cells.[9] Natural cephalosporin C is the main fermentation product, but is not very therapeutically active, and is converted to cephalexin or other active forms which are therapeutically important. Cephalosporin C is produced by the same fermentation process used for the penicillins but utilizes different growth media and producer organisms. However, final fermentation broth concentrations are much lower (1.0 to

4.0 g/L) and include many similar compounds resulting in a more difficult separation and purification process.[10] The active cephalosporins (cephalexin or others) are normally administered orally in capsule form.

3.3 TETRACYCLINES

Tetracyclines are an important class of broad-spectrum antibiotics that are active against both gram-positive and gram-negative bacteria, as well as rickettsias, mycoplasmas, leptospiras, spirochetes, and chlamydias. Tetracyclines act as inhibitors of protein synthesis with the site of action being the 30S ribosome, where binding of aminoacyl-tRNA to the ribosomal A-site is inhibited. There are three natural and six semisynthetic tetracyclines of economic and therapeutic importance. Like the penicillins and cephalosporins, the tetracyclines are produced by an aerobic fermentation process except that the organism is a bacteria instead of fungi. Presently, there are about 20 different organisms which produce tetracyclines. The yield is low, with maximum published fermentation broth concentrations of only 2.0 g/l.[11-13]

3.4 BACITRACIN

Bacitracin is used as a topical antibiotic, and it has broad-spectrum antibiotic effects against gram-positive and normal streptococcus and staphylococcus skin infection-producing bacteria.[14] While it is very effective when taken internally, this use is severely restricted due to problems with toxicity. The structure of bacitracin is shown in Fig. 4. Bacitracin is produced by conventional aerobic submerged culture fermentations like those of the other antibiotics. Many different strains of bacteria can be used. The most effective produces a fermentation broth concentration of 9 g/l. The extent to which separation and purification control the process depends upon the use.[15] For animal feed uses (as a growth promoter), the separation and purification process is fairly simple. Bacitracin is stored as a fine white powder with good long-term stability.

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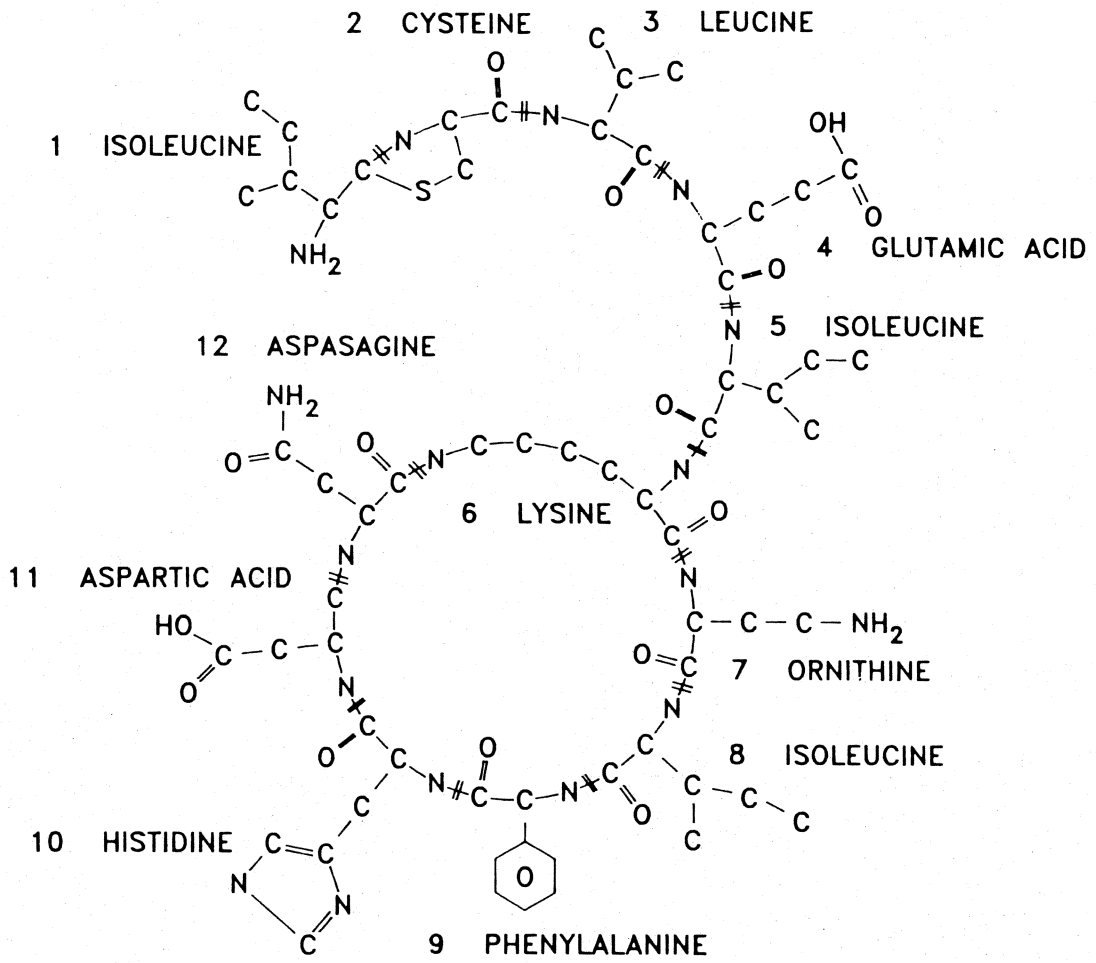

Fig. 4. Structure of Bacitracin A. For convenience, most of the H atoms in the formula have been omitted. Source: Ø. Frøyshov, "The Bacitracins: Properties, Biosynthesis, and Fermentation," pp 665-694 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

3.5 SULFONAMIDES

Sulfonamides are a family of chemically produced anti-infective agents containing a benzene ring with a sulfonamido group ($-SO_2NH-$) and an -R group in the para position. Several examples are shown in Fig. 5. The antibacterial properties are derived from the drug's ability to disrupt the microorganism's metabolism.[16] Sulfonamides are normally administered in capsule form, and they exhibit long-term storage stability when they are stored in light-resistant containers. Adverse effects of sulfonamides are numerous and may involve nearly all organ systems. However, serious or fatal reactions are infrequent. Most adverse reactions appear to be due to hypersensitivity and increase with increasing dosage.[17]

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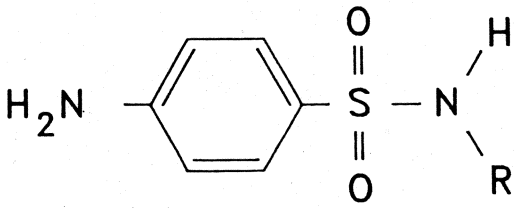
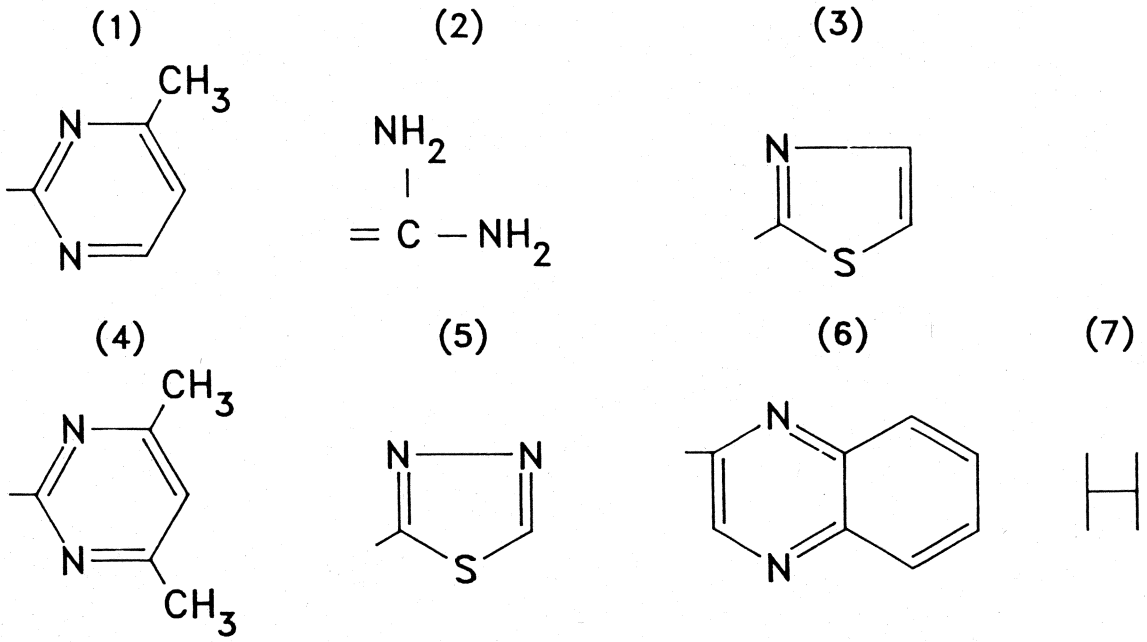


SULPHANILAMIDE
WHERE R IS:



WHERE (1) IS SULFAMETHYLDIAZINE (2) IS SULFAQUANIDINE
(3) IS SULFATHIAZOLE (4) IS SULFAMETHAZINE
(5) IS SULFATHIADIAZOLE (6) IS SULFAQUINOXALINE
(7) IS SULPHANILAMIDE (SULFONAMIDE)

Fig. 5. Structure of sulfonamides.

4. ANTIBIOTIC MANUFACTURERS

The first step to be taken by emergency managers after a nuclear attack is to contact the antibiotic-manufacturing facilities for a damage assessment. Table 4 lists all of the current manufacturing facilities, along with their telephone numbers. As can be seen from Fig. 6, some of these production sites lie outside the risk areas and may not sustain damage.[18] The TR-82 risk areas were used in this analysis. The (current) NAPB-90 planning base was not available when this analysis was done. The difference should not be significant since most antibiotic plants are located in highly industrialized areas assumed at risk in both documents. The analysis here may be a little more conservative (pessimistic). These include: American Cyanamid's facility in Hannibal, Missouri; Lilly's facility in Clinton, Indiana; Burroughs Wellcome's plant in Greenville, North Carolina; and Salsbury Laboratories' plant in Charles City, Iowa. Many facilities in the risk areas are near the edge and may sustain only minor damage; for example, Eli Lilly has a very large plant south of Lafayette, Indiana, which is at the edge of the potential blast zone centered on West Lafayette.

Table 4. Risk assessment for antibiotic producers in the U. S. assuming three different attack scenarios

ANTIBIOTIC PRODUCER COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ---=Clear (TR-82)	ANTIBIOTICS PRODUCED
Abbott Laboratories 1400 Sheridan Rd. North Chicago, IL 60064	800 323-9065 910 235 1584	North Chicago, IL 60064	Lake	XXXX	---	****	EES
American Cyanamid Company One Cyanamid Plaza Wayne, NJ 07470	201 831-2000 130 400	Hannibal, MO 63401	Marion	---	---	---	Tetracyclines
		Willow Island, WV 26190	Pleasants	XXXX	---	---	Tetracyclines
		Bound Brook, NJ 08805	Somerset	XXXX	---	****	Sulfa
		Pearl River, NY 10965	Westchester	XXXX	---	****	Tetracyclines
Beecham Laboratories 501 Fifth St. Bristol, Tenn. 37620	615 764-5141 201 469-5200	Bristol, TN 37620	Sullivan	XXXX	---	XXXX	Semi-synthetic
		Piscataway, NJ 08854	Somerset	XXXX	---	****	Penicillins
Biocraft Laboratories, Inc. 92 Route 46 Elmwood Park, NJ 07407	201 796-3434	Waldwick, NJ 07463	Bergen	XXXX	---	XXXX	Semi-synthetic Penicillins
Bristol-Myers Company P.O. Box 4755 Syracuse, NY 13221-4755	315 432-2000 444 2012 id sls	Syracuse, NY 13221	Onandaga	XXXX	---	XXXX	Semi-synthetic Penicillins
Burroughs Wellcome Co. 3030 Cornwallis Road Research Triangle Park N.C. 27709	919 248-3000	Greenville, NC 27834	Pitt	---	---	---	Acyclovir, trimethoprim
Eli Lilly and Company 307 E. McCarty Indianapolis, IND. 46206	317 261-2000	Indianapolis, IN 46285	Marion	XXXX	---	****	Penicillin V,
		Clinton, IN 47842	Vermillion	---	---	---	Cephalosporins
	317 474-1430	Lafayette, IN 47902	Tippecanoe	---	---	XXXX	Cephalosporins
Fermenta 510 E. Union St. P.O. Box 1808 West Chester, PA 19380	215 436-7500 902517	West Chester, PA 19832	Chester	XXXX	---	---	Penicillin V,G & Semi-Synthetic Penicillins
IMC Chemical Group 666 Garland Place Des Plaines, IL 60016	312 296-0600	Terre Haute, IN 47808	Vigo	XXXX	---	XXXX	Bacitracin

Table 4. (Continued)

ANTIBIOTIC PRODUCER COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ---=Clear (TR-82)	ANTIBIOTICS PRODUCED
Merck & Co., Inc.	201 574-4000	Albany, GA 31705	Dougherty	XXXX	---	XXXX	Sulfa, Thiennimycin
126 E. Lincoln Ave.		Elkton, VA 22827	Rockingham	---	---	---	Cephalexin
Rahway, NJ 07065		Danville, PA 17821	Montour	---	---	---	riboflavin
	314 353-7000	St. Louis, MO 63116	St. Louis	XXXX	XXXX	XXXX	none
Napp Chemicals, Inc.	201 773-3900	Lodi, NJ 07644	Bergen	XXXX	---	XXXX	Sulfa
199-T Main St.	134 649						
Lodi, NJ 07644							
Pfizer Inc.	212 573-2323	Groton, CT 06340	New London	XXXX	---	****	Pen, Tetra, Strepto
235 E. 42nd St.		Terre Haute, IN 47808	Vigo	XXXX	---	XXXX	Tetracyclines, etc.
New York, NY 10017		Milwaukee, WI 53212	Milwaukee	XXXX	---	XXXX	Microbial Products
Hoffmann-La Roche Inc.	201 235-5000	Belvidere, NJ 07823	Warren	XXXX	---	---	Sulfa
340 Kingsland St.	133 417	Nutley, NJ 07110	Essex	XXXX	---	****	Sulfa
Nutley, New Jersey 07110							
Salsbury Laboratories, Inc.	800 247-1833	Charles City, IA 50616	Floyd	---	---	---	Sulfa
2000 Rockford Rd.							
Charles City, IA 50616							
SmithKline Beckman Corp.	215 854-4000	Conshohocken, PA 19428	Montgomery	---	---	XXXX	Cephalosporin
1500 Spring Garden Street	83 4487						
Philadelphia, PA 19101							
E. R. Squibb & Sons	609 921-4000	Kenly, NC 27542	Johnston	---	---	---	
P.O. Box 4000							
Princeton, N.J. 08540							
The Upjohn Company	616 323-4000	Kalamazoo, MI 49001	Kalamazoo	XXXX	---	XXXX	EES , etc.
7000 Portage Road	224 465						
Kalamazoo, Michigan 49001							

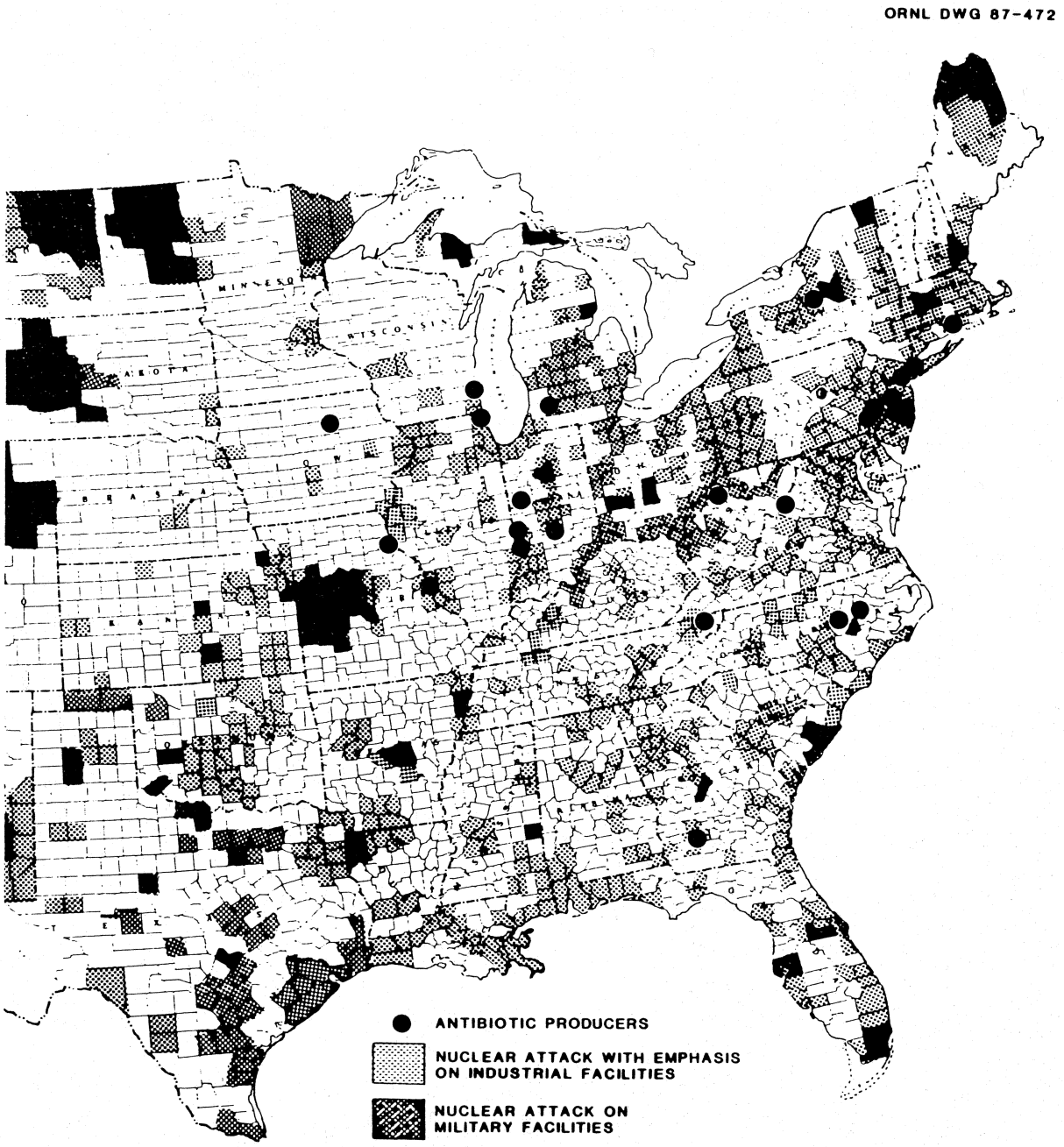


Fig. 6. Antibiotic producer locations superimposed on U. S. map with two types of nuclear attacks.

5. ANTIBIOTIC PRODUCTION TECHNOLOGIES - GENERAL DESCRIPTION

Antibiotic-manufacturing plants contain two main processing segments; a highly specialized fermentation section and a large, energy-intensive separation and purification section. While the fermentation section generally occupies about 50% of the space and accounts for about 50% of the equipment costs, it accounts for only a relatively small portion of the processing steps required for antibiotic production (approximately 10% of total steps are fermentation related) (see Fig. 7).[19] Highly specialized fermentation equipment (including fermenters, air and fluid filters, agitators, heat exchangers, pumps, etc.) is necessary. Operating conditions specify large volumes of sterile air, a sterile environment and growth media, low temperature cooling water or chilled water (in the penicillin fermentation for example, heat removal is critical), etc. The separation and purification section generally employs standard equipment normally found in the chemical processing industries.

The equipment includes centrifuges and filters for separating the microbes from the broth which contains the antibiotic. Solvent extractors (such as the Podbielniak extractor, the first centrifugal unit to gain commercial acceptance during its use in the separation of penicillin) are used to separate antibiotics from the fermentation broth. Distillation columns are used for solvent recovery and purification. This equipment must all be operated in a sterile manner. These sections can contain a large number of steps, similar in size and function to a small oil refinery. Figure 7 does not show the solvent purification section which usually requires distillation columns. The separation and purification sections use large volumes of solvents which must, in turn, be purified and recycled, usually by energy intensive distillation. If the antibiotic is the injectable type, the purification is very exacting and extensive, with very strict quality control, because the antibiotic has to be pure, with no by-products or foreign matter. For these reasons, the orally administered antibiotics are recommended for production during the period which requires expedient antibiotic production techniques.

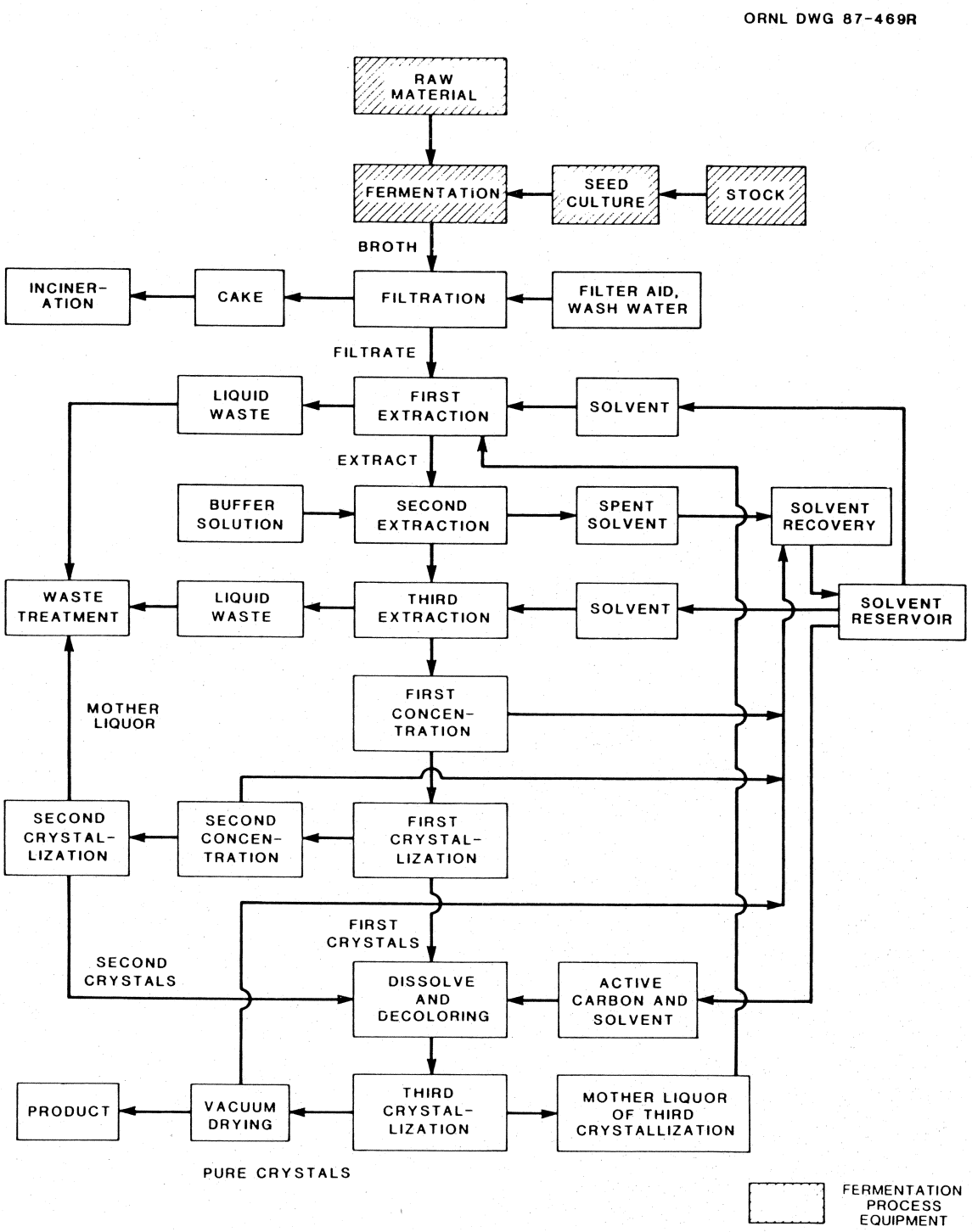


Fig. 7. Block diagram for a general antibiotic production process.

5.1 RAW MATERIALS AND UTILITIES

The most important raw materials are the organisms themselves, because although many different organisms can produce a particular antibiotic, the yield and final fermentation broth concentration are strongly dependent on the particular organism used. For example, at 40 g/liter broth penicillin concentration, 50,000 kg of penicillin would require about 6.3 fermentation batches of 200,000 liters per batch.[5] If the concentration were only 4 g/liter (still high compared to the wild strains), 63 fermentation batches would be required, greatly increasing the separation and purification, energy, and raw material requirements. The time necessary would also increase proportionately. The most efficient organisms for each type of antibiotic are discussed in the appropriate appendices.

The energy and carbon sources required by the organisms along with other nutrients are provided by the growth media. The antibiotic precursors, such as phenylacetic acid for penicillin, are also important in order to produce the desired type of antibiotic. The growth medium is quite important and specific to the particular organism used and the antibiotic desired. Table 5 lists the components of a typical growth media for penicillin in a shake flask or factory. Growth media for the other types of antibiotics are given in the appropriate appendices.

Most antibiotics are separated by filtration from the fermentation broth and further purified by solvent extraction which requires organic solvents, acids, and bases. The specific chemicals required are dependent on the particular antibiotic being produced, the desired purity, and the end use (whether formulated for oral delivery or for injection) (see appropriate appendices).

Utility requirements consist of sterilized air, purified water, cooling and chilled water, electricity, and steam. The exact consumption depends dramatically on the particular antibiotic which is being produced and is covered in the appropriate appendices. Large amounts of cooling and chilled water are required to control the large amount of heat evolved during aerobic fermentations and

Table 5. Growth media for penicillin - shake flask and factory[a]

Component	Corn-steep liquor medium (%)	Calam and Hockenhull's medium (%)
Main carbohydrate-		
shake-flask-- lactose	3.0-4.0	3.0
production-- continuous glucose		
Other carbohydrates		
Glucose	0.0-0.5	1.0
Starch		1.5
Non-reducing polysaccharides	variable	
Specific precursors		
Phenethylamine and other precursors	variable	
Phenylacetic acid	Continuous supply for production	0.05
Organic acids		
Acetic acid	0.05	0.25
Citric acid		1.0
Lactic acid	0.5	
Main nitrogen source		
Amino acids, peptides, amines	Variable	
Ammonium sulphate		0.5
Other nitrogen sources		
Ammonia	Variable	
Ethylamine		0.3
Total nitrogen	0.15-0.2	0.2
Total solids	8.0-9.0	8.5

[a]D. Perlman, "Chemically defined media for antibiotic production," Ann. N. Y. Acad. Sci. 139 258-269 (1966).

because the optimum fermentation temperature is usually near ambient. Consumption of electricity is high due to the large amount of agitation required for oxygen transfer in the aerobic fermentations (5 to 40 hp/1000 gal) and for the production of chilled water. For those antibiotics which are recovered by solvent extraction, large steam consumptions result from the purification of the extracting solvents by distillation for recycle back to the purification section.

5.2 FERMENTATION

Operating an antibiotic fermentation unit requires that special consideration be given to culture preservation, growth media, air and media sterilization, oxygen transfer, and temperature control in the fermenter. Equipment that is capable of being sterilized and maintained in aseptic condition during operation is also essential.

Many different cultures can be used to produce a specific antibiotic; however, the fermenter yield (units of antibiotic per unit fermenter volume), which helps determine plant capacity, varies dramatically with the specific culture used. Long-term preservation of an efficient culture is vital to antibiotic manufacturing. Three methods used for culture preservation: subcultivation, low-temperature storage, and freeze drying (see Appendix I).[20] Each company jealously guards its own production organisms as trade secrets.

The growth medium is an important part of an antibiotic fermentation, influencing the yield and, in some cases, the specific antibiotic which is produced (penicillin G, V, or O depending on the precursor supplied).[5] The growth medium can be rather complex and is different for each antibiotic produced. See Table 5 for a typical growth medium for penicillin.

Antibiotics can be destroyed or inactivated by some microorganisms, and/or microorganisms can invade the fermentation broth and disrupt the pure culture fermentation. In both cases a substantial reduction in yield will result. To prevent contamination, a sterile environment must be maintained. Fermentations require large volumes of air which must be sterilized to remove bacteria which have particle sizes down to 0.5 μ. There are three commercial methods for air

sterilization: (1) heat treatment (whole air supply maintained at ≥100 °C), (2) filtration through fibrous media and filtration through granular media (see appendix J).

In order to maintain an aseptic environment, specialized equipment is required for the fermenter, transfer pumps, etc, that can be easily cleaned and sterilized. The culture must be increased in size several times before a commercial fermenter can be charged (see Fig. 8).[21] Each one of these steps requires a sterile transfer. Steam and chemical sterilization methods are used for in-place sterilization of equipment.[22] Appendices J and K cover specialized equipment and sterilization methods. Antibiotic fermentations are aerobic with optimum operating temperatures in the 20 to 30°C range. Oxygen transfer in the fermentation broth can be a controlling variable with vigorous agitation required. Aerobic fermentations generate a great deal of heat which results in demands for chilled water for cooling. In addition, the vigorous agitation produces foaming problems, which often cannot be dealt with using standard antifoam chemicals because they cause downstream separation problems.[23]

5.3 SEPARATION AND PURIFICATION

All antibiotic fermentations are generic in nature (i.e., they can all use the same equipment). Different antibiotics are produced by using different cultures and growth media, while some alterations in operating conditions are required for optimum performance. The separation and purification sections of an antibiotic plant can differ substantially, depending on the antibiotic which is being produced. The specific separations required for the antibiotics selected for expedient production are delineated in the appropriate appendices. In general, filtration, centrifugation, extraction, adsorption, and crystallization are involved. Some veterinary antibiotic are produced by spray drying the filtration supernatant without the usual extensive

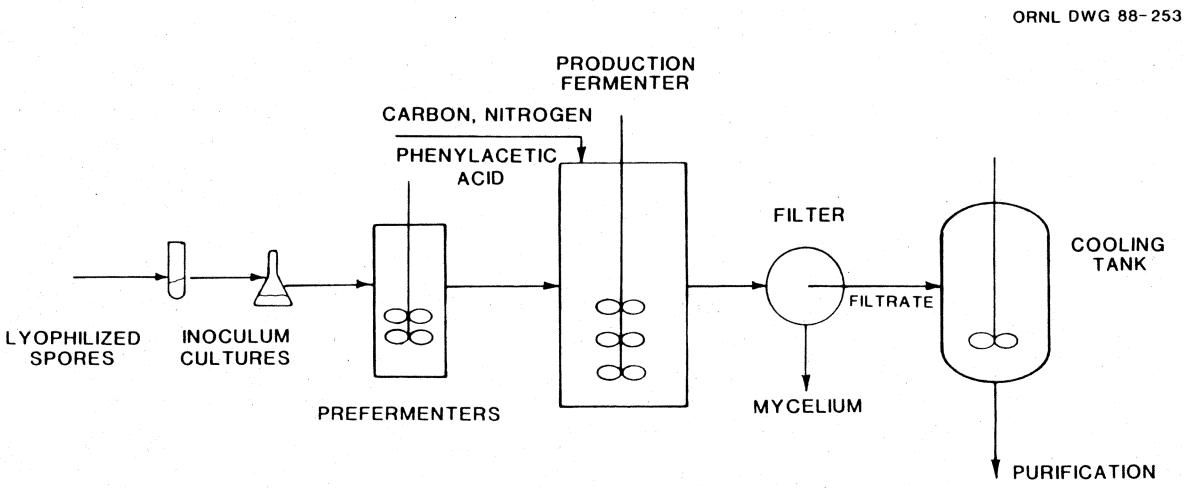


Fig. 8. Procedure and flowsheet for beginning a penicillin fermentation.

purification steps required for human antibiotics.[22,24] Under austere conditions, with reduced purity requirements, spray drying may be an attractive alternative allowing production of a dry product for oral use (impure products cannot be used for injection). Two recent developments in separation technology have the potential to substantially reduce the size of the separation/purification section of antibiotic-manufacturing plants. These are high performance liquid chromatography and supercritical fluid extraction (see Appendix L). These new methods may become increasingly important in the next three to five years as single-step, modular units which can separate specific antibiotics easily and inexpensively.

5.4 ANALYTIC PROCEDURES/QUALITY CONTROL

Chromatography and fluorescence detection are standard methods for assaying antibiotics. High-performance liquid chromatography (HPLC) uses regular and reverse phase and ion exchange packings, with ultraviolet light as the detector source, to analyze for the penicillins and cephalosporins, and recently for the tetracyclines.[25,26] Thin-layer paper and gel chromatography are used for bacitracin and the tetracyclines.[27] HPLC assays are particularly important for the β-Lactam antibiotics in order to detect impurities from degradation products and intermediates which are quite similar to the active antibiotic and result in toxic reaction in humans.[28,29] When given in clinical dosage, the major toxic effect of the pure antibiotics is an allergic reaction and is quite rare. The major toxic reaction from impure antibiotics is an allergic reaction to the intermediates and degradation products and is much more common.

5.5 ADVANCED TECHNOLOGIES

Three relatively new and potentially very powerful separation/purification methods for antibiotics are (1) chromatography (particularly continuous annular chromatography), (2) supercritical fluid (SCF) extraction and (3) membranes.[30-38] If the size of the antibiotic-manufacturing process is to be substantially reduced, this reduction will have to come in the separation and purification

sections of the plant which represent well over half of the total plant size. Chromatography, SCF extraction, and membrane technologies are relatively new in regard to their application to the antibiotic purification process. Most of this work is still in the developmental stages, although some penicillins and cephalosporins are separated commercially using HPLC and/or membranes.[39,40] Several European patents have been filed on the use of SCF extraction for antibiotic separation and purification.[41] These new technologies not only have the potential for a vast reduction in the size of the separation equipment, but also in the total number of processing steps. Normal antibiotic separations require between 40 and 60 processing steps. The new technologies offer much greater selectivity which should translate into a substantial reduction in the number of processing steps required.

While these new technologies could not be used to retrofit an antibiotic plant if a national disaster occurred in the near future, they will greatly simplify such a conversion within the next 3 to 5 years and beyond. It would be possible to make good estimates of the impact of either annular chromatography or SCF extraction on the antibiotic process in a retrofit situation because most of the pertinent information is available in the literature. These new separation technologies could have a similar impact on the separation and purification of antibiotics that submerged culture fermentation had on the fermentation end of the process. In the 1940s, antibiotic plants were very inefficient because surface culture fermentations were used.[5] When, in 1943, the submerged culture process was demonstrated at the USDA Peoria Labs, one 500-gallon fermenter using the submerged culture process outproduced all of the large surface-culture antibiotic plants that were operating in England.[5]

6. EXPEDIENT PRODUCTION OF ANTIBIOTICS

Based on the TR-82,[18] Industrial Emphasis and Military Emphasis attacks, it is anticipated that insufficient antibiotic-production capacity will remain intact to meet the needs of the population. The first expedient production measure to be taken should be a damage assessment of existing antibiotic-manufacturing facilities and a determination of feasibility of timely repair. If antibiotic-production capacity is still insufficient, alternative facilities will have to be converted to antibiotic production.

6.1 ALTERNATE PRODUCTION SITES

The most promising candidates for conversion to antibiotic production are animal or plant antibiotic producers, enzyme or molecular biology product production plants, single-cell protein producers, and fermentation ethanol producers. Tables 6 and 7 list the locations and telephone numbers of these plants.[42] Figures 9 and 10 illustrate the proximity of these plants to the nuclear attack zones. Some possible candidates for conversion are listed in Table 8. These include enzyme, molecular biology product (monc sodium glutamate (MSG), vitamin B-2 and B-12, xanthan, and other polymers) makers, single cell protein producers, citric acid producers, and breweries. Large hospitals with research facilities, universities, mushroom producers, the dairy industry, and farm ethanol fermentation units may provide additional candidates.

6.2 CONVERSION TO ANTIBIOTIC PRODUCTION

Antibiotic production is a two-step process: (1) production of the antibiotic via fermentation of the raw materials in the growth media, and (2) separation of the antibiotic from the fermentation broth and purification to an acceptable level for the required end use. The fermentation section of the process is quite specialized, and would be very difficult to retro-fit to a plant without fermentation capability. For this reason, a plant which is to be converted

Table 6. Risk assessment for enzyme producers in the U.S. assuming three different attack scenarios

ENNZYME PRODUCER COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ---=Clear (TR-82)
Amber Laboratories 433 E. Michigan St. Milwaukee, WI 53202	414 271-6755	Juneau, WI 53039	Dodge	---	---	---
Miles Laboratories, Inc. P.O. Box 932 Elkhart, IN 46515	219 264-8111	Clifton, NJ 07015 Madison, WI 53701	Passaic Dane	XXXX XXXX	--- ---	**** XXXX
Norwich Eaton Pharmaceut. 17 Eaton Ave. Norwich, NY 13815	607 335-2111	Norwich, NY 13815	Chenango	---	---	---
U. S. Biochem.Corp. P.O. Box 22400 Cleveland, OH 44122	216 765-5000 980718	Cleveland, OH 44128	Cuyahoga	XXXX	---	****
Pharmacia P-L Biochem.,Inc. 2202 N. Bartlett Ave. Milwaukee, WI 53202	414 225-2600	Milwaukee, WI 53202	Milwaukee	XXXX	---	XXXX
Novo Laboratories, Inc. 59 Danbury Road Wilton, CT 06897	203 762-2401 964 385	Wilton, CT 06897	Fairfield	XXXX	---	XXXX
Gist-Brocades USA Inc. PO Box 241068 Charlotte, NC 28224	704-527-9000	Kingstree, SC 29556	Williamsburg	---	---	---
Grain Processing Corp. 1600 Oregon St Muscatine, IA 52761	319 264-4211 46 8497	Muscatine, IA 52761	Muscatine	---	---	---
PMP Fermentation Prod.,Inc. 709 N.E. Water St. Peoria, Ill. 61650	414 347-7464	Peoria, Ill. 61650	Peoria	XXXX	---	XXXX
Merck Chem. Mfg. Div. PO Box 196 Danville, PA 17821	201-574-4000	Danville, PA 17821	Montour	---	---	---

Table 6. (Continued)

ENNZYME PRODUCER COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ----=Clear (TR-82)
Atomergic Chemetals Corp. 100 Fairchild Ave. Plainview, NY 11803	516 349-8800 14 4612	Plainview, NY 11803	Nassau	XXXX	---	****
SmithKline Beckman Corp. 6200 El Camino Real Carlsbad, Calif. 92008	714-871-4848	Carlsbad, Calif. 92008	San Diego	XXXX	---	+++
The Upjohn Co. 7000 Portage Road Kalamazoo, MI 49001	616 323-4000 224 465	Kalamazoo, MI 49001	Kalamazoo	XXXX	---	XXXX
Revlon, Inc. PO Box 511 Kankakee, IL 60901	212-572-5000	Kankakee, IL 60901	Kankakee	---	---	---
Accurate Chem.&Sci.Corp. 300 Shames Drive Westbury, NY 11590	516 433-4900 800 645-6264 14 4617	Westbury, NY 11590	Nassau	XXXX	---	****
CPC Internat'l Inc. PO Box 347 Argo, Il 60501	201-894-4000	Argo, IL 60501	Macon	---	---	****
American Hoechst Corp. 10933 Torry Pine RD. La Jolla, CA 92037	619 450-9600	La Jolla, CA 92037	San Diego	XXXX	---	****
Pfizer Inc. 4253 N. Port Washington Ave. Milwaukee, WI 53212	414 332-3545	Milwaukee, WI 53212	Milwaukee	XXXX	---	XXXX
Chem. Dynamics Corp. 3001 Hadley Road South Plainfield, NJ 07080	201 753-5000 883 447	S. Plainfield, NJ 07080	Somerset	XXXX	---	****
Anheuser-Busch, Inc. One Busch Place St. Louis, MO 63118	314 577-2000	St. Louis, MO 63118	Saint Louis	XXXX	XXXX	XXXX

Table 7. Risk assessment for fermentation ethanol producers in the U.S. assuming three different attack scenarios

FERMENTATION ETHANOL COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ---=Clear (TR-82)	Capacity (million gal/yr)
Archer Daniels Midland Co.	319 398-0600	Cedar Rapids, IA 52413	Linn	XXXX	---	XXXX	
PO Box 1470	217 424-5200	Decatur, IL 62500	Macon	---	---	XXXX	}220
Decatur, IL 62525	309 673-7828	Peoria, IL 61600	Peoria	XXXX	---	XXXX	
Georgia-Pacific Corp. 133 Peachtree St. NE Atlanta, GA 30303	404 521-4000	Bellingham, WA 98225	Whatcom	---	---	---	6
Grain Processing Corp. 1600 Oregon St Muscatine, IA 52761	319 264-4211 46 8497	Muscatine, IA 52761	Muscatine	---	---	---	60
Midwest Solvents Co. Inc.	913 367-1480	Atchison, KS 66002	Atchison	---	---	---	22
1300 Main St	309 353-3990	Pekin, IL 61554	Tazewell	XXXX	---	XXXX	9
Atchison, KS 66002							
Nabisco Brands, Inc.(CCPC)	319 242-1141	Clinton, IA 52732	Clinton	XXXX	---	---	8
600 Three First National Plaza Chicago, IL 60602	312 269-5300						
Pekin Energy Co. PO Box 10 Pekin, IL 61554	309 346-1121	Pekin, IL 61554	Tazewell	XXXX	---	XXXX	60
A. E. Staley MFG. Co.	217 423-4411	Loudon, TN 37774	Loudon	XXXX	---	---	60
2200 East Eldorado St		Decatur, IL 62525	Macon	---	---	XXXX	
Decatur, IL 62525							
Amber Laboratories 433 E. Michigan St. Milwaukee, WI 5320	414 271-6755	Juneau, WI 53039	Dodge	---	---	---	10
South Point Ethanol Inc.		South Point, OH 45680	Lawrence	XXXX	---	XXXX	60
Alcohol Energy Corp.		Staley, NC 27355	Randolph	---	---	---	<10
Baca Food and Fuel Coop.		Campo, CO 81029	Baca	---	---	---	<10
Bornhoft, Paul		Merino, CO 80741	Logan	XXXX	XXXX	****	<10
A. Smith Bowman Dist.Corp.	703 528-9091	Reston, VA 22090	Fairfax	XXXX	---	****	<10

Table 7. (Continued)

FERMENTATION ETHANOL COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ---=Clear (TR-82)	Capacity (million gal/yr)
Charmel Energy Co.		Muleshoe, TX 79347	Bailey	---	---	---	<10
Coburn Enterprises, Inc.		Sherman, SD 57060	Minnehaha	XXXX	---	---	<10
Colorado Gasohol Inc.		Walsh, CO 81090	Baca	---	---	---	<10
Crystal Fuels, Inc.		Bonaparte, IA 52620	Van Buren	---	---	---	<10
Ecological Energy, Inc.		Roca, NE 68430	Lancaster	---	---	---	<10
Food and Energy, Inc.		Litchfield, ME 04350	Kennebec	---	---	---	<10
Kentucky Agricultural Energy Coop.		Franklin, KY 42134	Simpson	XXXX	---	---	21
Lenox Grain Fuels, Inc.		Lenox, IA 50851	Taylor	---	---	---	<10
Marlin Car Care		Marlin, TX 76661	Falls	---	---	---	<10
A. E. Montana, Inc.		Amsterdam, MO 64723	Bates	XXXX	XXXX	****	<10
Raven Alcohol Distillery		Selma, CA 93662	Fresno	XXXX	---	---	<10
Southern Distilleries Co.		Ashford, AL 36312	Houston	XXXX	---	---	<10
Spudcohol		Pingree, ID 83262	Bingham	XXXX	---	---	<10
Syncorp, Inc.		Roberta, GA 31078	Crawford	---	---	---	<10
U. S. Gasohol Corp.		Lockeford, CA 05237	San Joaquin	XXXX	---	---	<10
White Flame Fuels, Inc.		Van Buren, AR 72956	Crawford	---	---	****	<10

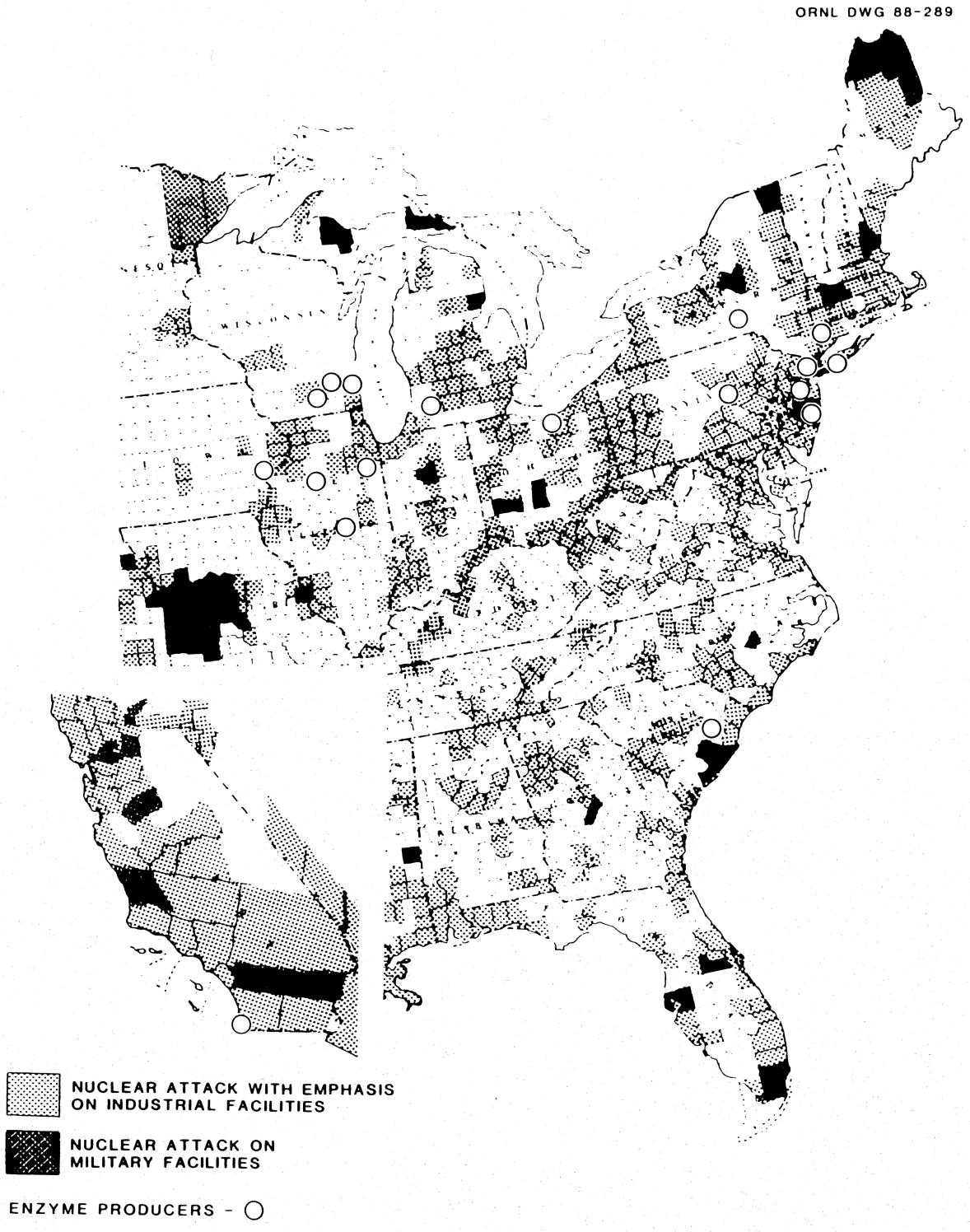


Fig. 9. Enzyme producer locations superimposed on U.S. map with two types of nuclear attack.

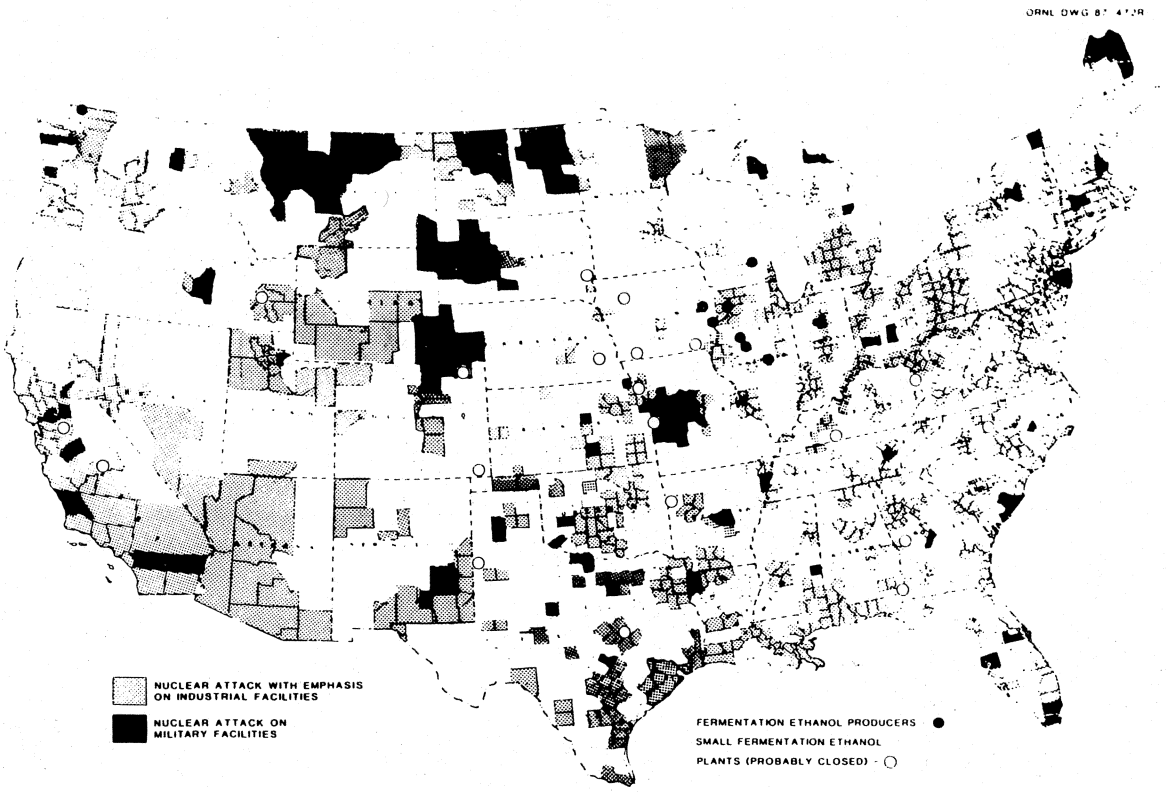


Fig. 10. Fermentation ethanol producer locations superimposed on U.S. map with two types of nuclear attack.

Table 8. Other candidates for conversion to antibiotic production

OTHER BIOPRODUCT MAKERS COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ---=Clear (TR-82)
Acetic acid (vinegar) producers						
H. J. Heinz Co., Pittsburgh, PA 15230	412-237-5757	Pittsburgh, PA 15230	Allegheny	XXXX	---	****
Standard Brands, Inc. 625 Madison Ave. New York, NY 10022	212-759-4400					
Beatrice/Hunt-Wesson Foods 1645-T W. Valencia Dr. Fullerton, CA 92643	714-680-1000	Fullerton, CA 92643	Orange	XXXX	---	****
Citric acid producers						
Miles Laboratories Citro-Tech Products PO Box 932 Elkhart, IN 46515	219-262-7453	Elkhart, IN 46515	Elkhart	---	---	XXXX
Mayo Chemical Co. 5544 Oakdale Rd., S.E. Smyrna, GA 30080	404-696-6711	Smyrna, GA 30080	Cobb	XXXX	---	XXXX
American International Chemical, Inc. 209 W. Central St. Natick, MA 01760	617-655-5805	Natick, MA 01760	Middlesex	XXXX	---	+++
Bartlett Chemicals Inc. PO Drawer 10710 Jefferson, LA 70181	504-734-1971	Jefferson, LA 70181	Jefferson	XXXX	---	XXXX
Mallinckrodt, Inc. 2nd & Mallinckrodt sts. St. Louis, MO 63147	800-325-7155 800-325-8888	St. Louis, MO 63147	St. Louis	XXXX	XXXX	XXXX
Gallard-Schlesinger Chemical Mfg. Corp. 584 Mineola Ave. Carle Place, NY 11514	516-333-5600	Carle Place, NY 11514	Nassau	XXXX	---	****
Pfizer Chemical Div. Pfizer, Inc. 235-T E. 42nd St. New York, NY 10017	212-573-2323	Groton, CT 06340	New London	XXXX	---	****
		Terre Haute, IN 47808	Vigo	XXXX	---	XXXX
		Brooklyn, NY	Kings	XXXX	---	XXXX
		Southport, NC	Brunswick	XXXX	---	XXXX

Table 8. (Continued)

OTHER BIOPRODUCT MAKERS COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ---=Clear (TR-82)
Gluconic and glutamic acid producers						
PMP Fermentation Prod.,Inc PO Box 2219 Milwaukee, WI 53201	414-347-7467	Milwaukee, WI 53201	Milwaukee	XXXX	---	XXXX
Ashland Chemical Co. PO Box 2219 Columbus, OH 43216	614-889-3333	Columbus, OH 43216	Franklin	XXXX	XXXX	XXXX
Napp Chemicals, Inc. 199 Main St. Lodi, NJ 07644	201-773-3900	Lodi, NJ 07644	Bergen	XXXX	---	XXXX
Stauffer Chemical Co. Cowles Chem. Div. 3737-T Park East Dr. Cleveland, OH 44122	216-831-0200	Cleveland, OH 44122	Cuyahoga	XXXX	---	XXXX
Stauffer Chemical Co. Food Ingredients Nyala Farm Road Westport, CT 06881	203-222-3000	Westport, CT 06881	Fairfield	XXXX	---	XXXX
Vitamin B-2 or B-12 producers						
Pfizer Inc. New York, NY 10017	212-573-2323	Milwaukee, WI 53212	Milwaukee	XXXX	---	XXXX
Rhone-PoulencInc.Chem.Div. PO Box 125 Monmouth Junction, NJ 08852	201-297-0100	Monmouth Junction, NJ 08852	Monmouth	XXXX	---	****
Merck & Co.,Inc. Danville, PA 17821	201-574-4000	Danville, PA 17821	Montour	---	---	---
Merck & Co., Inc Elkton, VA 22827	201-574-4000	Elkton, VA 22827	Rockingham	---	---	---
Hoffmann-La Roche Inc. 340 Kingsland St. Nutley, NJ 07110	201-235-5000	Nutley, NJ 07110	Essex	XXXX	---	****

Table 8. (Continued)

OTHER BIOPRODUCT MAKERS COMPANY HEADQUARTERS	TELEPHONE/ TELEX	MANUFACTURING LOCATION	COUNTY	INDUSTRIAL ATTACK XXXX=HIT ---=Clear	MILITARY ATTACK XXXX=HIT ---=Clear	XXXX=BLAST +++=FALLOUT ****=BOTH ---=Clear (TR-82)
Xanthan and other gum producers						
American Cyanamid Co. Indust.Chem.Prod. Wayne, NJ 07470	201-831-2000	Wayne, NJ 07470	Bergen	XXXX	---	XXXX
Pharmacia, Inc. 800-T Centennial Ave. Piscataway, NJ 08854	201-457-8000	Piscataway, NJ 08854	Somerset	XXXX	---	****
Hercules Inc. Hercules Plaza Wilmington, DE 19894	302-594-6500	Wilmington, DE 19894	New Castle	XXXX	---	****
Merck & Co.,Inc., Kelco Div., 8355-T Aero Dr. San Diego, CA 92123	619-292-4900	San Diego, CA	San Diego	XXXX	---	****
Merck & Co.,Inc. Kelco Div., Okmulgee, OK 74447	619-292-4900	Okmulgee, OK 74447	Okmulgee	XXXX	---	---
Rhone-Poulenc Inc. Chem. Div., POB 125 Monmouth Junction, NJ 08852	201-297-0100	Monmouth Junction, NJ 08852	Monmouth	XXXX	---	****

to expedient antibiotic production must have a sterilizable fermentation section in place. The specific fermentation equipment required for the production of the different antibiotics is similar. The equipment in an aerobic fermentation unit should be able to produce any of the desired antibiotics. The requirements are (1) the organism to produce the antibiotic, (2) sterile conditions for culture transfers, (3) ability to sterilize all equipment, (4) sterile air supply, (5) fermenter agitation and cooling and chilled water capacity (with either internal coils or vessel jacket), and (6) basic instrumentation for process control (oxygen tension, pH, temperature, pressure, etc.).

The separation and purification section of an antibiotic plant is usually large (of comparable size and cost to the fermentation section), contains many steps, and is specific to the antibiotic being produced. In addition, the degree of purity required varies with the application and delivery form of the antibiotic. The major retrofit required to convert an existing fermentation plant to antibiotic production is the addition of a separation and purification section. For the conversion to be feasible under the constraints of the time limits involved, a substantial reduction in size and complexity of the separation and purification unit must be achieved. The most likely way to accomplish this is to make substantial reductions in the purity of the final antibiotic product. Reduced-purity products can only be tolerated in antibiotics which are taken orally. Impure products can be made by spray-drying the filtered broth, thereby greatly simplifying the production procedure. Spray-drying is commonly used in the enzyme, SCP, and veterinary antibiotic industries, and it is a readily available technology requiring only a large source of clean, hot, dry gas and a large cylindrical vertical chamber into which the material can be sprayed in the form of small droplets. Spray-dried antibiotics could only be given as oral antibiotics because of the lack of complete purity and some adverse reactions could be expected. If existing antibiotic-producer plants are intact or easily repaired, however, injection quality antibiotics could be produced at these locations.

Other possibilities for reducing the size and complexity of expedient separation and purification equipment involve employing either HPLC or supercritical fluid (SFC) extraction to perform the separation and purification from the filtered or unfiltered fermentation broth.[35,43] These new separation methods could substantially reduce the size and complexity of an antibiotic plant without sacrificing product quality. However, both HPLC and SCF extraction are presently in the developmental stage for antibiotic separations.

6.3 SINGLE-CELL PROTEINS

Facilities that produce single-cell protein are probably the best candidates for conversion to anitbiotic production following a nuclear disaster. These plants have fermentation facilities which operate under highly aerobic and sterile conditions to grow microorganisms which are similar to those used to produce antibiotics. Both the antibiotic and SCP fermentation processes deal solely with pure cultures, while most other fermentation industries deal with the much simpler mixed culture fermentations. The sterilization equipment is in place for sterilizing the air, media, nutrients, and the downstream separation processing equipment. The production of antibiotics is highly aerobic requiring large quantities of compressed sterile air, and SCP plants have this capacity. These plants also have all of the necessary laboratory facilities required for chemical, biochemical, microbiological, and quality assurance analyses required during the production of sterile products. The equipment necessary to operate at the subambient conditions of antibiotics plants (0-25°C) is also present. Some separation equipment is also available, especially filters and centrifuges for separating cells from the media, spray- drying facilities to produce a dry stable product, and often ultrafiltration units for producing very pure macromolecular products (like antibiotics).

The SCP plant located at Hutchinson, Minnesota, has been identified as a top candidate for conversion to antibiotic production after a national disaster. This plant is outside any of the nuclear attack zones and contains two 75,700-liter (20,000-gallon) state-of-

the-art fermenters. The surrounding area can provide most of the raw materials required, and a constant source of 10°C cooling water is present. The cooling water source is quite important because of the large chilled water requirements in antibiotic plants. If chilled water has to be produced by refrigeration, it represents a large energy requirement. Detailed information on the Hutchinson SCP plant is given in Appendix H.

7. ALTERNATE SOURCES OF ANTIBIOTICS

Under conditions when it becomes impossible to produce enough antibiotics in the postattack environment, two additional alternatives exist. There is substantial antibiotic-manufacturing capacity in Mexico and in Puerto Rico. Table 9 lists the headquarters and telephone numbers of the four antibiotic manufacturers in Puerto Rico and the Mexican manufacturers. Consideration should be given to importing antibiotics from these locations after a nuclear attack. Small planes can carry significant quantities of dry crystalline antibiotics.

Antibiotics can be stored for many years in bulk crystalline form at low temperatures. In planning for a nuclear attack, consideration should also be given to building stockpiles of antibiotics stored in a manner which will ensure a shelf life of many years. In any case several low volume antibiotics which have unique properties should be stockpiled. For example, lincomycin, tobramycin, and spectinomycin are used for the treatment of some severe infections in penicillin allergic patients.

Table 9. Alternate antibiotic production sites outside of the continental U.S

Antibiotic producer company headquarters	Telephone/ telex	Manufacturing location	Antibiotics produced
Puerto Rico			
Abbott Laboratories 1400 Sheridan Road North Chicago, IL 60064	(800) 323-9065 (910) 235-1584	Barceloneta 00617	Erythromycin and its salts
Eli Lilly and Company 307 E. McCarty Indianapolis, IN 46206	(317) 261-2000	Carolina 00630 Mayaguez 00708	Tobramycin sulfate Erythromycin estolate, Cephalexin
The Upjohn Company 7000 Portage Road Kalamazoo, MI 49001	(616) 323-4000	Arecibo 00612	Clindamycin, Lincomycin
E. R. Squibb & Sons P.O. Box 4000 Princeton, NJ 08540	(609) 921-4000	Humacao 00661	Nystatin, Amphotericin b
Mexico			
Orsabe, S. A.		Cuernavaca, Mexico	Synthetic Penicillins
Beneficiadora e Industrializadora S.A. de C.V.		Carretera Mexico-Laredo, Mexico	Gentamicins
Fermentaciones y Syntasis, S.A.		Mexico	Cephalosporin C Synthetic Penicillins
Fermic S.A. de C.V.		Ixapalapa, Mexico	Cephalosporin C, EES, Synthetic Penicillins Tetracyclines
Quinonas de Mexico		Mexico City, Mexico	Synthetic Penicillins

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APPENDIX A

Production of Penicillin Antibiotics

Appendix A. PRODUCTION OF PENICILLIN ANTIBIOTICS

The production of penicillin begins from a master stock of the production organism from which vegetative mycelium is prepared in a series of inoculum development steps to increase the concentration of fungal mycelium. The inoculum development is carried up through shake flasks and then into an agitated vessel with a medium containing sufficient fermentable carbohydrate, an organic nitrogen source, calcium carbonate as a buffer to prevent low pH values, and various inorganic salts as in Tables A-1 and A-2. The presence of about 1% carbon dioxide appears essential for germination of spores, and the carbon dioxide must be kept below 4% to prevent strong inhibition during penicillin production. A critical spore concentration ($\approx 5 \times 10^9$ spores/m^3) occurs above which more filamentous mycelium are formed, enzyme levels are high and penicillin production is good in the production reactor. Below this concentration, dense pellets are formed, glucose utilization is less efficient and enzyme levels and penicillin production are lower. The spore concentration is only important in the first shake flask inoculation. An inoculum volume of 5 to 10% of the production fermenter (at about 20 kg mycelium dry weight/m^3 product from the inoculum reactor) is required to give a high specific penicillin production rate in the production fermentation. It is very important that the culture still be in the uninhibited growth phase (log growth) when the transfer is done.

The present fermentation process is a fed-batch process or a repeated fed-batch process (a portion of the broth is withdrawn at intervals). A fermenter of 150 to 200 m^3 is used at about 80% full (120 to 160 m^3 of broth) with 3- to 4-kW/m^3 power input. Oxygen is supplied by air flow at 30 to 60 m^3/m^3 broth per hour (0.5 to 1.0 VVM) at 300 kPa (43 psia) pressure with a fermenter pressure of 135 to 170 kPa (19.5 to 24.5 psia) and an oxygen uptake rate of 25 to 50 mol/m^3 broth per hour. The initial mycelium concentration in the fermenter is 1 to 2 kg dry weight/m^3 The other conditions include broth temperature of $\approx 25°C$, pH of 6.5, and a continuous feed glucose concentration of 500 kg/m^3 solution with a glucose feed rate of 1.0 to 2.5

Table A-1. Raw materials needed for penicillin production

(120-160 m^3 batch-yielding ~ 28 kg Pen G Na salt/m3 ~ 3100-4200 kg)
(~20,000-25,000 units per ml)

Material	Quantity (approximate)	Used for
Glucose	2.5kg/m^3 broth per hour(200 hr ~56,000 kg/batch	Fermentation
Phenylacetic acid		Fermentation
Acetic and/or Lactic acid	~0-6kg/m^3 ~ 500 kg/batch	Fermentation
Amino acids, peptides,amines		Fermentation
Ammonia		Fermentation
Corn-steep liquor solids	40-50kg/m^3 ~ 5000 kg/batch	Fermentation
Antifoam-consumable oil	Variable by continuous feed	Fermentation
Minerals for defined medium		Fermentation
Filter-aid		Filtration
Amyl or butyl acetate	30-40 m^3 (30-40,000 L/8-10,000 gal)	Solvent-extraction
Activated charcoal		Purification
Potassium or sodium acetate		Crystallization
Isopropyl or butyl alcohol		Crystallization
Sulfuric or phosphoric acid		pH adjustment

Table A-2. Alternate materials for defined medium for penicillin production

Component	Concentration (kg/m^3)	Batch requirement (kg)
Na_2SO_4	0-1	up to 160 kg
$(NH_4)_2SO_4$	0-10	up to 1600 kg
NH_4NO_3 or KNO_3	0-8	up to 1280 kg
KH_2PO_4, NaH_2PO_4, K_HPO_4	0.4-8	up to 1280 kg
$CaCO_3$	0-5	up to 800 kg
$MgSO_4 \cdot 7H_2O$	0.25-0.8	up to 128 kg
$FeSO_4 \cdot 7H_2O$ /$Fe(NH_4)_2(SO_4)_2$	0.04-0.4	up to 64 kg
$ZnSO_4 \cdot 7H_2O$	0.01-0.05	up to 8 kg
$CuSO_4 \cdot 5H_2O$	0.005-0.01	up to 1300 g
$MnSO_4 \cdot 4H_2O$	0.01-0.06	up to 9600 g
$CoSO_4 \cdot 7H_2O$	0-0.005	up to 800 g
$CaCl_2$	0-0.05	up to 8000 g

NOTES: Alternate carbon sources--Lactose, Ethanol, Acetic acid or acetate, Pentoses, Hexoses, disaccharides (maltose, etc.), polyols, dextrins, starch hydrolysates, and molasses. (Ethanol or fatty oils cause a higher oxygen demand and heat generation than glucose or other sugars.)

Alternate nitrogen sources--Cottonseed meal, brans with phytic acid and phosphorus, Pharmamedia, Penicillium mycelium, and ammonia, ammonium sulfate.

Precursor Sources--1-phenyl-n-decane and 1-phenyl-n-dodecane.

kg/m^3 broth per hour (1.8 kg/m^3 per hour optimum) and a total fermentation duration of 180 to 220 hours. Tables A-1 and A-2 give the approximate requirements for the chemicals needed for the fermentation. Figure A-1 shows the overall process.[23]

Productivity in the penicillin fermentation depends upon the genetic capability of the culture, biomass concentration, limited mycelial growth rate, and mycelial mass during the rapid growth phase ending at the optimum mass limit of about 80% of the maximum. Also, starting at the optimum glucose concentration for the fermentation, maintaining the optimum glucose and nutrient feed rate during the fermentation, and having the appropriate precursor affect productivity. Specific oxygen uptake rates and carbon dioxide evolution rate are also associated with productivity. Temperature control is important for obtaining the maximum production rate of penicillin, and the metabolic heat and the mechanical heat generated by agitation and aeration must be removed from the reactor. However, this becomes more difficult as the viscosity of the mycelial mass increases during the fermentation.[2,5,8,35,44]

Several control algorithms for the carbon feed have been developed, based on dissolved oxygen, pH, carbon dioxide evolution, the growth curve, and the specific growth rate. The uptake rates for the other nutrients besides carbon (glucose) are shown in Table A-3. Shown in Fig. A-2 in a typical concentration profile of the fed-batch penicillin fermentation with some of the control parameter profiles shown. Tables A-4 and A-5 give some of the common yields for penicillin G and V from glucose, and the distribution of glucose into penicillin, mycelium, and maintenance energy during a fermentation.

Product Recovery Processes

The recovery of penicillin from a fermentation broth takes about 15 hours. Gist-Brocades' state-of-the art process is described below, and shown in Fig. A-3. The first step of the separation is the filtration of the mycelium from the broth on a rotary vacuum filter using filter aid or precoats and on which the mycelium is

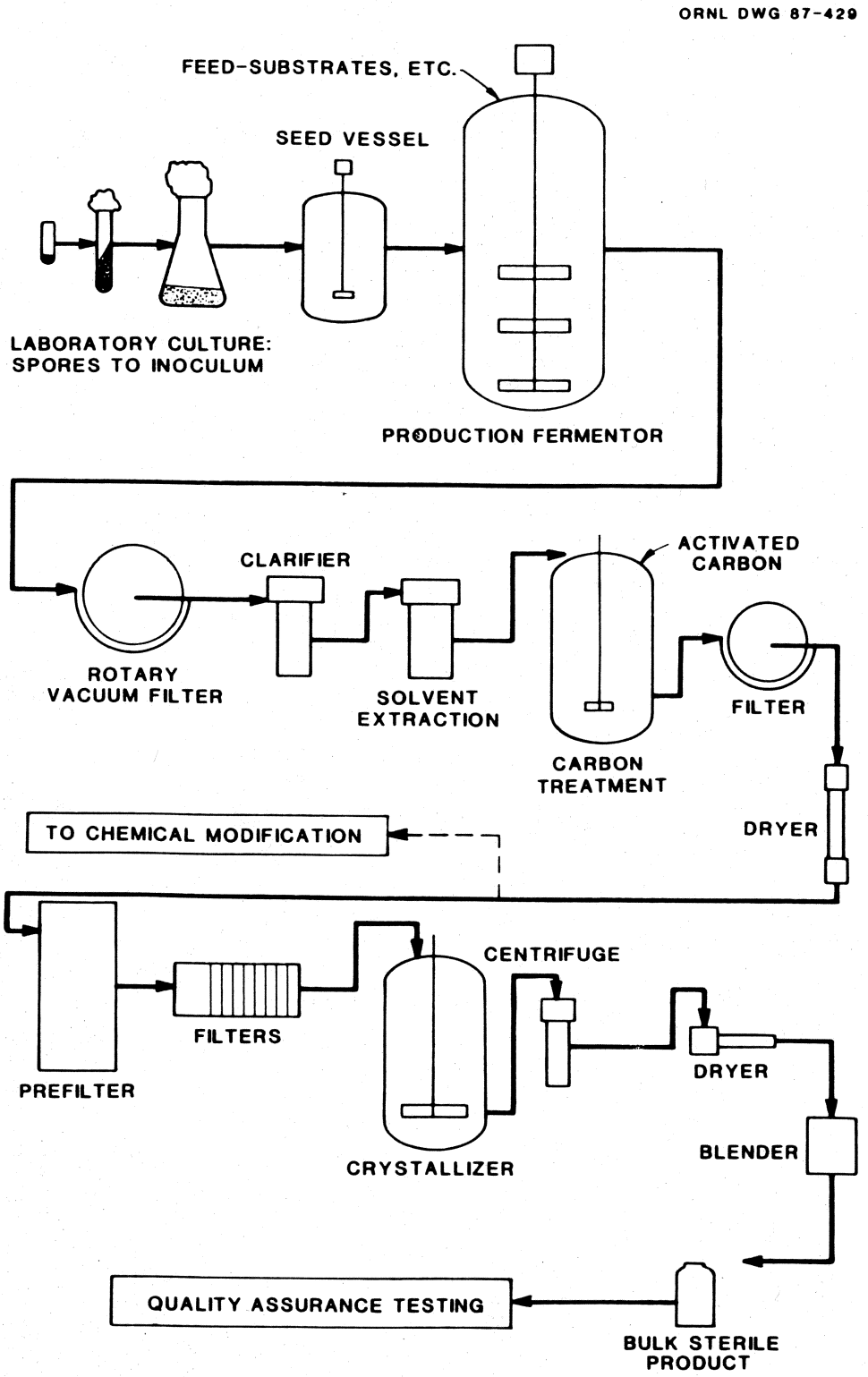

Fig. A-1. General production scheme for penicillin production.

Table A-3. Specific uptake rates for nutrients during a penicillin fermentation[a]

Nutrients and precursor	Specific uptake rates (at μ=0.015 hr[b]; in mmol/g mycelium dry weight/h)
Carbon (sugar)	0.33
Oxygen	1.60[b]
Nitrogen (as NH_3)	0.12
Phosphorus (as PO_4^{-3})	0.006
Sulfur (as SO_4^{-2})	0.029
Precursor (Phenylacetic acid)	0.013

a D. D. Y. Ryu and J. Hospodka, "Quantitative physiology of Penicillium chrysogenum in penicillin fermentation," Biotechnol. Bioeng. 22 289-298 (1980).

b For maintenance, growth and penicillin production.

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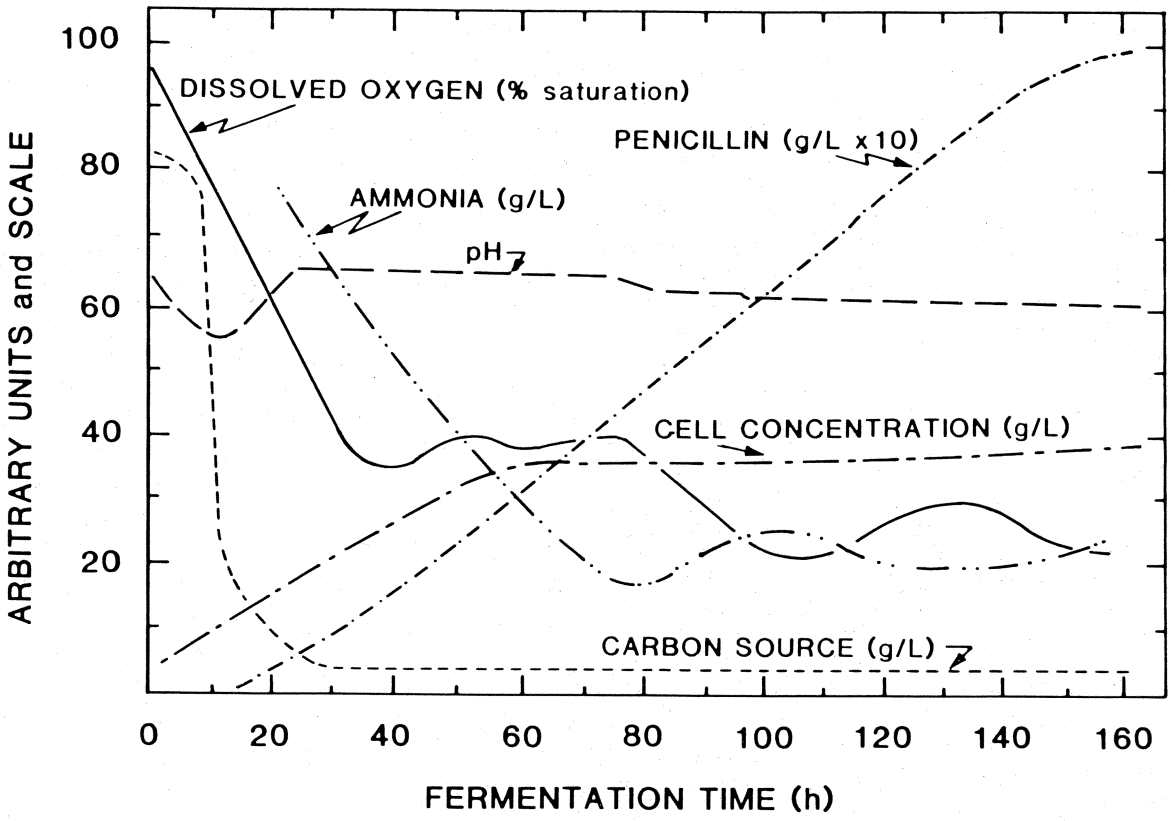

Fig. A-2. **Typical concentration profile of fed-batch penicillin fermentation.** Source: D. D. Y. Ryu and J. Hospodka, "Quantitative physiology of Penicillium chrysogenum in penicillin fermentation," Biotechnol. Bioeng. 22 289-298 (1980).

Table A-4. Carbon distribution into penicillin, mycelium, and maintenance energy.[a]

Percentage of carbon fed that is consumed for			
Penicillin	Mycelium	Maintenance	Remarks
6	25-29	65-69	140 hr fermentation
10-11	20-28	61-70	200 hr fermentation aminoadipic acid recycled
16	27	57	aminoadipic acid discarded

aD. D. Y. Ryu and J. Hospodka, "Quantitative physiology of Penicillium chrysogenum in penicillin fermentation," Biotechnol. Bioeng. 22 289-298 (1980).

Table A-5. Maximum and real yields of penicillin from glucose[a]

Method	Units pen G or V (per mg glucose)	Mole pen G or V (per mole glucose)	g pen G-Na (per g glucose)
Maximum from model calculation	700-2198	0.207-0.667	0.410-1.32
Model calculation	95-200	0.029-0.061	0.057-0.12
Experimental	80-212	0.024-0.064	0.048-0.115

[a]G. J. M. Hersbach, C. P. van der Beek, and P. W. M. van Dijck, "The Penicillins: Properties, Biosynthesis, and Fermentation," pp 45-140 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

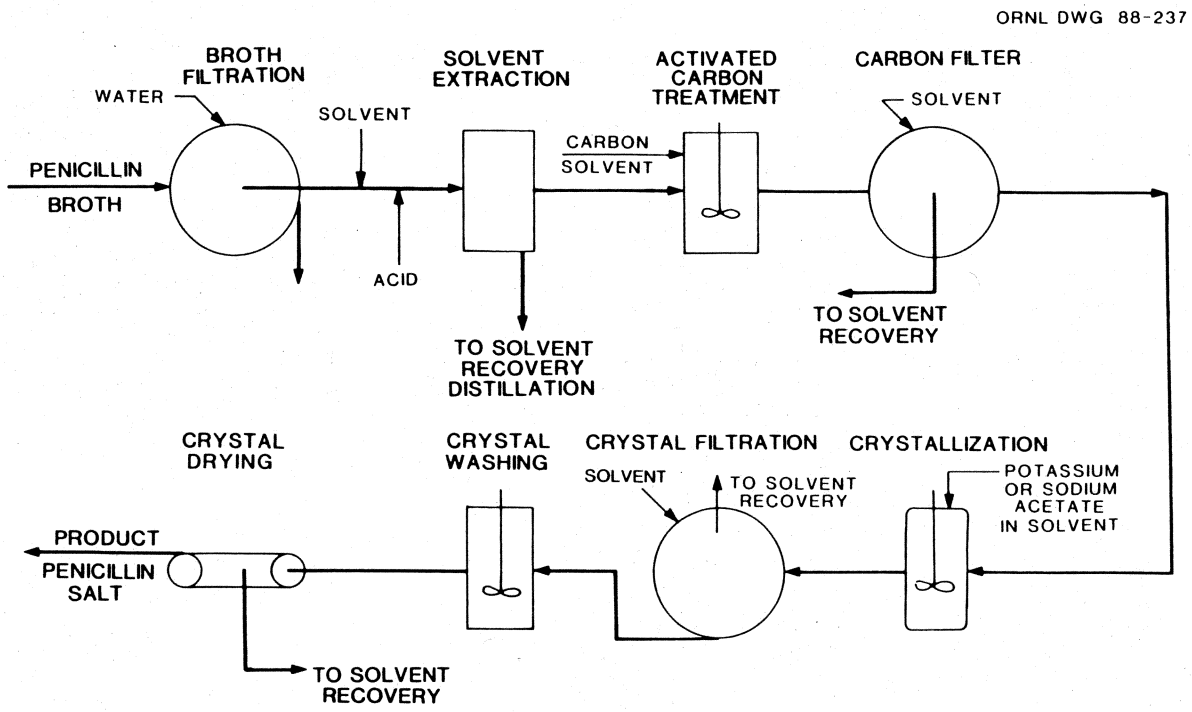

Fig. A-3. Penicillin purification process of Gist-Brocades.
Source: G. J. M. Hersbach, C. P. van der Beek, and P. W. M. van Dijck, "The Penicillins: Properties, Biosynthesis, and Fermentation," pp 45-140 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

also washed. Penicillin is extracted in the acid form from the filtrate under very carefully controlled conditions of temperature, pH, and sterility to minimize chemical and enzymatic degradation of the penicillin in the pumps, vessels, and the extractor. The extractant is amyl acetate in a continuous countercurrent multistage centrifugal extractor at pH 2.5 to 3.0 and at 0 to 3°C. Centrifugal contactors such as the Podbielniak extractor with throughputs up to 98,540 L/h (26,000 gal/h) are used. Partition of penicillin between organic and water phases is shown in Figures A-4 and A-5. Demulsifying agents are used to prevent emulsion formation and to obtain a high separation efficiency, and dilute sulfuric or phosphoric acid are used to control the pH. The water phase and the organic are fed to the extractor at a 4:1 ratio, using short residence times to prevent degradation of the penicillin under the acid conditions of the extraction. It is also possible to extract the whole broth from the fermenter with the solvent without going through a filtration step. A problem of this method is that the components of mycelium will also be extracted and will contribute impurities that must be removed during the purification steps.

Following extraction, the penicillin-containing solvent is treated with a slurry of activated charcoal, filtered on a precoated rotary vacuum filter, and washed with solvent. Potassium or sodium acetate is added to the solvent, and penicillin G or V is crystallized as the salt from the solvent phase, with critical parameters including potassium or sodium concentration, pH value, penicillin concentration, and temperature. The crystals are separated on a rotary vacuum filter. The crystals are washed and predried with anhydrous isopropyl alcohol, butyl alcohol, or other volatile solvent and then treated with fresh solvent on a horizontal vacuum belt filter and dried with warm air, resulting in a 99.5% pure penicillin. Spent solvents are processed for solvent purification by washing, distillation, and drying. Table A-6 describes the steps in the traditional purification process and Fig. A-6 shows the stages in a typical extraction.[5,44]

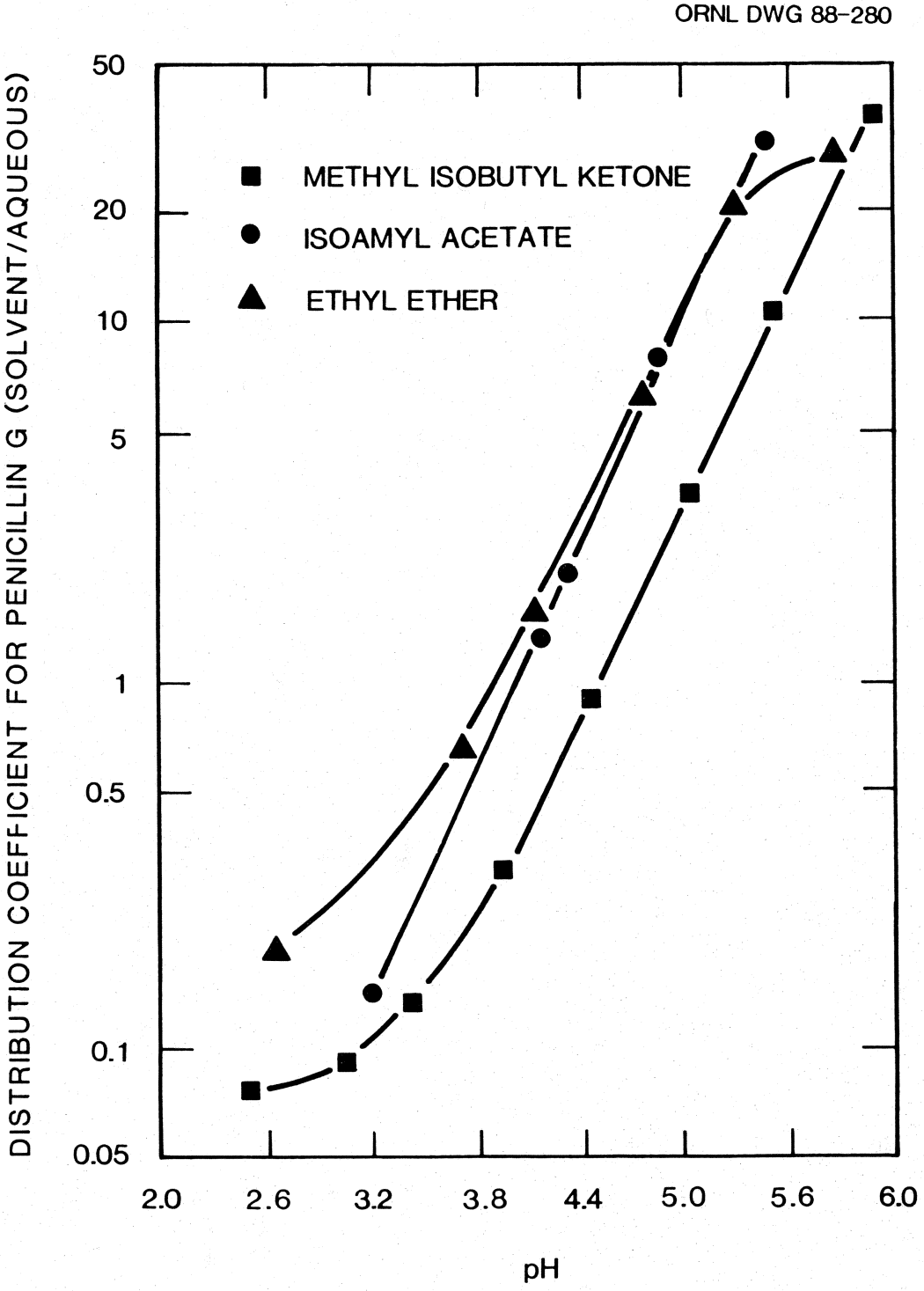

Fig. A-4. Distribution coefficients vs. pH of penicillin G between water and solvents. Source: R. L. Feder, Recovery of Penicillin--Diostribution Coefficients and Vapor-Liquid Equilibria, M.S. Thesis, Polytechnic Institute of Brooklyn, (June, 1947).

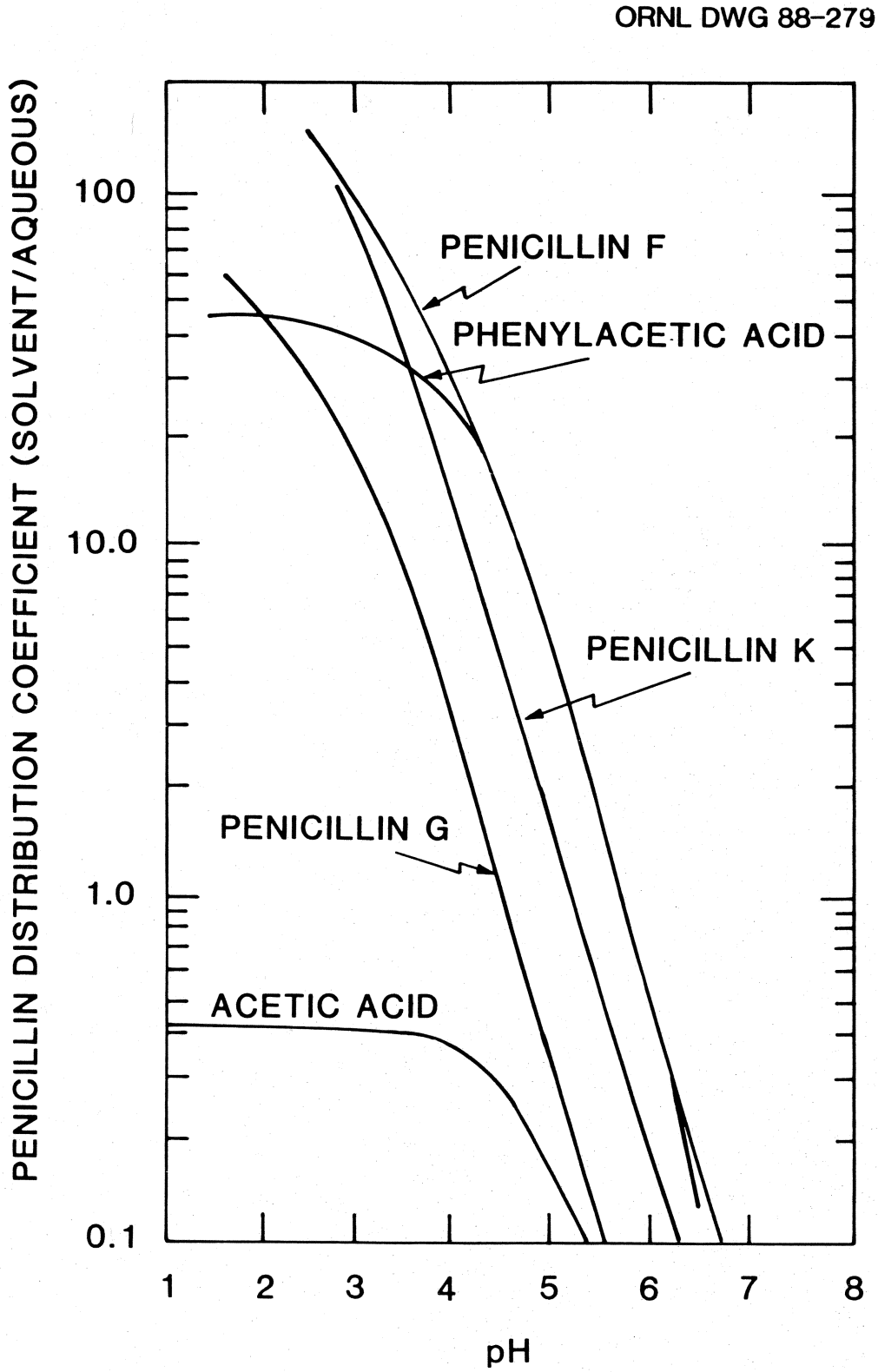

Fig. A-5. Partition of penicillins as a function of the pH of the extraction solvent (isoamyl acetate). Source: M. Souders, G. J. Pierotti, and C. L. Dunn, "The Recovery of penicillin by extraction with a pH gradient,' pp 39-42 in The history of penicillin production, Chemical Engineering Progress Symposium Series, Vol. 66, No. 100, American Institute of Chemical Engineers, New York, 1970.

Table A-6. Traditional penicillin purification

Step	Purpose	Equipment	Basis	Size
Filtration	Separation of mycelia from penicillin-containing broth	Continuous, rotary vacuum drum filter		to 148 m^2 (1600 ft^2)
Extraction	Separation of penicillin from other soluble components	Continuous, multistage countercurrent extractors	Differential extraction: when pH>pK_a, penicillin more soluble in organic phase; when pH,pK_a, penicillin more soluble in aqueous phase	to 98540 l/hr (26000gal/hr)
Crystallization	Further purification and stabilization	Tanks and gravitational separators	Via addition of sodium or potassium ions	
Drying	Stabilization	Vacuum or warm-air driers	Anhydrous solvent	

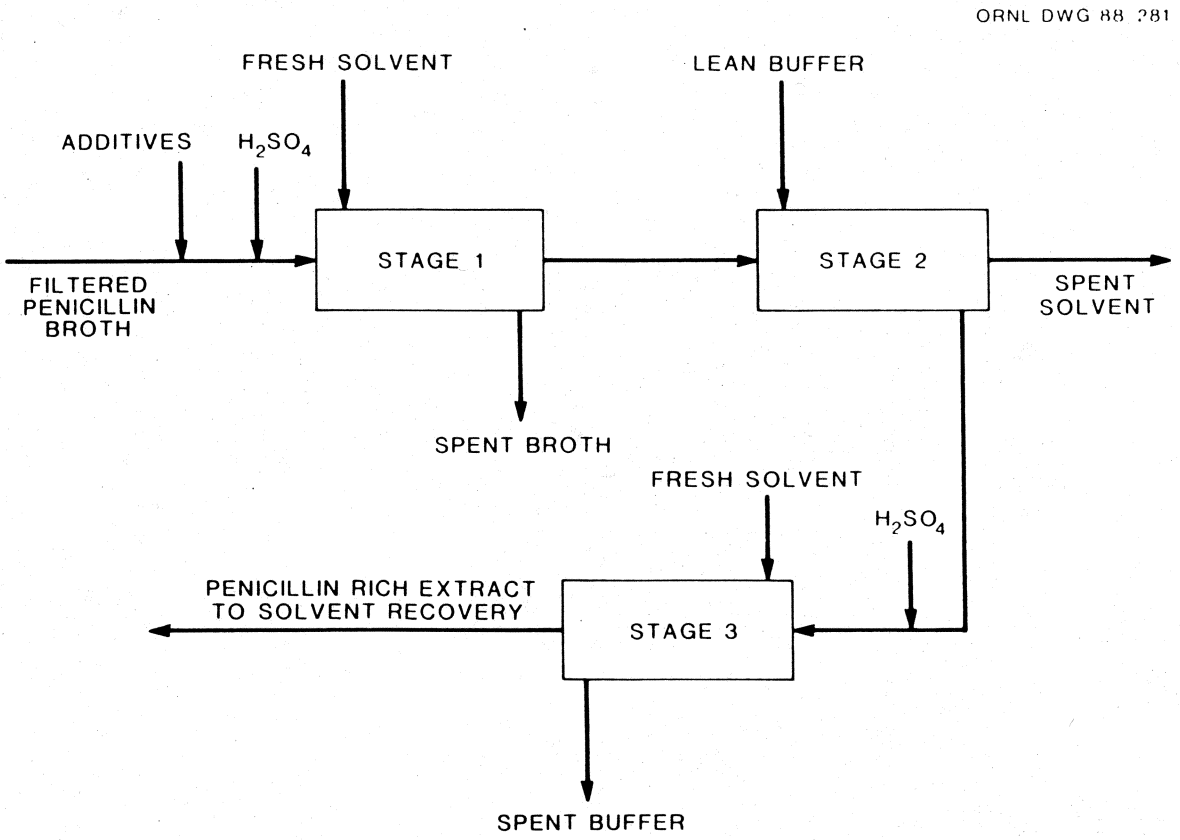

Fig. A-6. Traditional three-stage extraction of penicillin. Source: S. Queener and R. Swartz, "Penicillins: Biosynthetic and semisynthetic," pp 35-122 in Economic microbiology, Vol. 3. ed A. H. Rose. Academic, New York, 1979.

Three types of indirect assay methods have been used for penicillin quality control. They the iodometric techniques, mercury imidazole and the ferric hydroxamate methods. These have been improved through the years so that automatic analyses can be done. Direct ultraviolet absorption is used to detect penicillins as they are separated on reverse-phase HPLC columns containing octyl and octadecyl bonded material as the stationary phase. HPLC is able to separate the side-chain precursor, penicillin, and penicillin by-products or degradation products in one analysis. Because of this, it is a fast, easy to learn, reliable method for quality assurance. Immobilized penicillinase can also be used in combination with a pH electrode or a thermistor to measure the concentration of the penicillin in the fermenter or fermentation broth.

Modification of Penicillin G to Produce Semisynthetic Penicillins and Cephalosporins.

The semisynthetic penicillins are produced with 6-aminopenicilanic acid (6-APA) as the starting material by chemical or enzymatic deacylation of penicillin G. Some cephalosporins can be produced using penicillin G as the starting material. The chemical synthesis of 6-APA is shown in the Fig. A-7. The enzymatic route is usually accomplished using acylase enzymes bound to supports and operating continuously in columns to convert the penicillin G to 6-APA. The 6-APA is converted by chemical acylation with appropriate acid chlorides and results in some of the structures shown in Fig. A-8. Enzymatic methods are also available to convert the 6-APA to the semisynthetic penicillins, but such methods cannot compete economically with the chemical conversion. Other semisynthetic penicillins such as oxacillin, cloxacillin, dicloxacillin, and flucloxacillin also exhibit lactamase inhibitory properties. Clavulanic acid (produced along with cephalosporins by *Streptomyces clavuligerus*) and olivanic acid (produced by *Streptomyces olivaceus*) are produced by fermentation and are potent irreversible (suicide) inhibitors of β-lactamases but have no or very weak antibiotic function on their own. They are used in conjunction with one of the other semisynthetics such as amoxycillin to give a much broader spectrum product so that the penicillin

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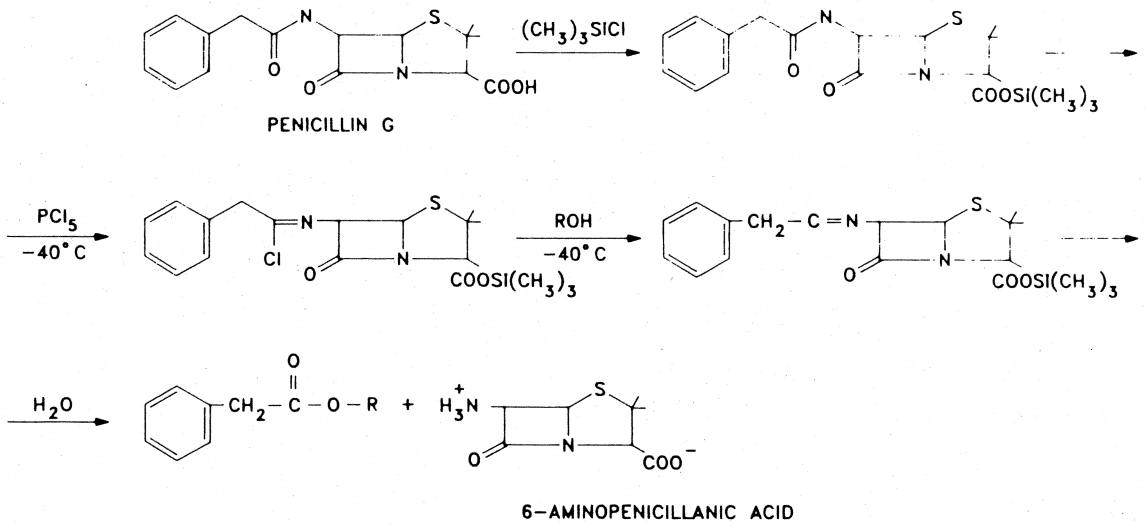

Fig. A-7. Chemical synthesis of 6-aminopenicilanic acid (6-APA). Source: G. J. M. Hersbach, C. P. van der Beek, and P. W. M. van Dijck, "The Penicillins: Properties, Biosynthesis, and Fermentation," pp 45-140 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

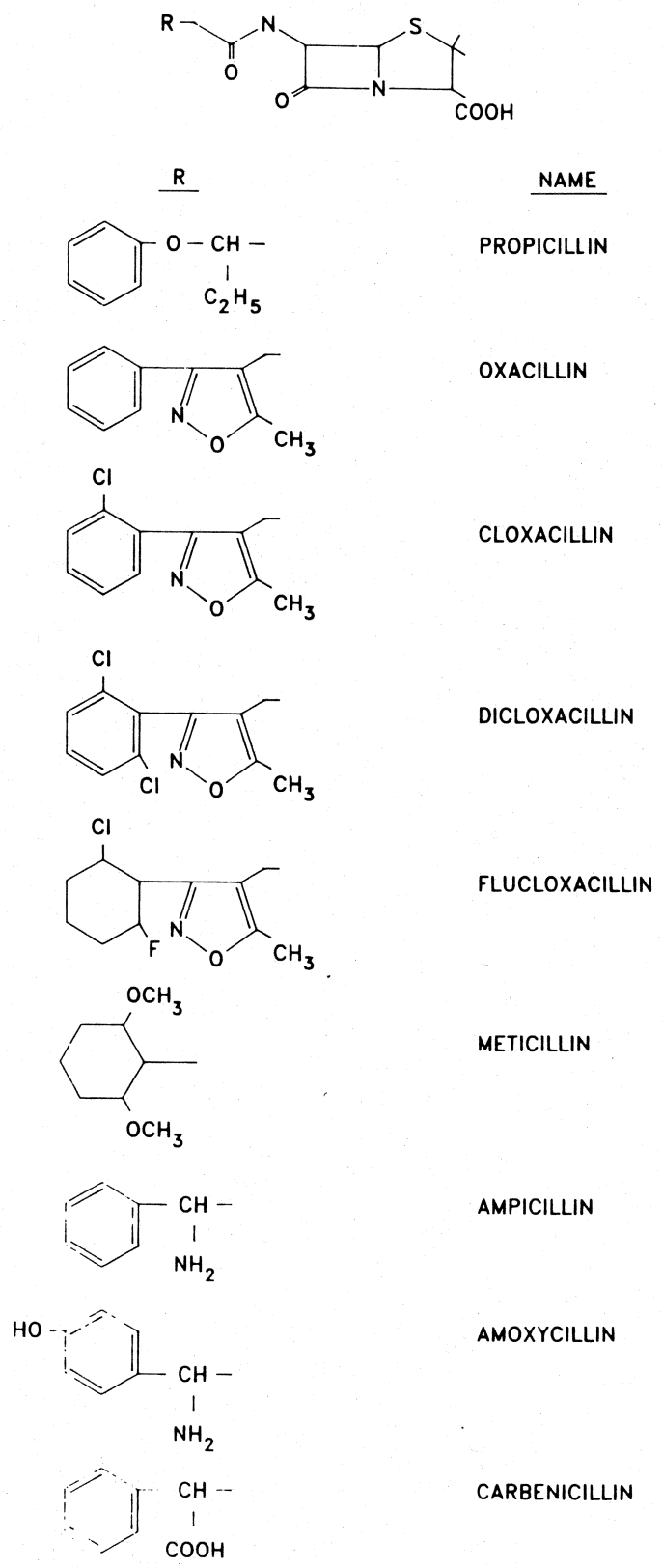

Fig. A-8. Chemical structures of some semisynthetic penicillins. Source: G. J. M. Hersbach, C. P. van der Beek, and P. W. M. van Dijck, "The Penicillins: Properties, Biosynthesis, and Fermentation," pp 45-140 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

is effective even against bacteria which are normally resistant to the β-lactam antibiotics such as certain resistant Gram-positive bacteria and Gram-negative bacteria including Pseudomonas sp.

Penicillin G can also be converted to 7-aminodeacetoxycephalosporanic acid (7-ADCA) in about a 70% yield by oxidation to its sulfoxide, conversion of the sulfoxide to the corresponding deacetoxycephalosporanic acid with transient protection of the carboxyl group, and subsequent deacylation as shown in Fig. A-9. The semisynthetic cephalosporins which can be formed by this route are shown in Fig. A-10.

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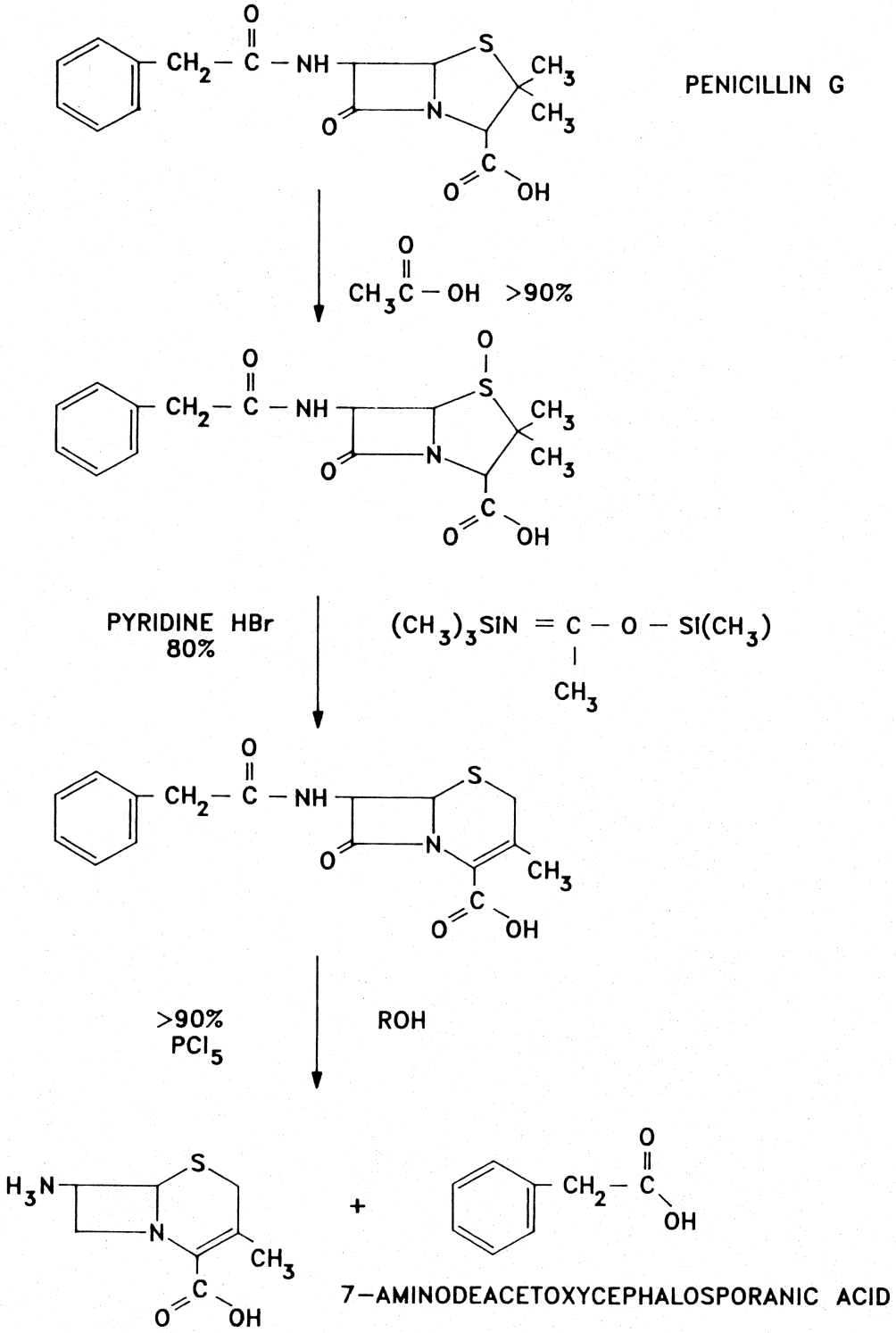

Fig. A-9. Conversion of penicillin G into 7-aminodeacetoxycephalosporanic acid (7-ADCA). Source: G. J. M. Hersbach, C. P. van der Beek, and P. W. M. van Dijck, "The Penicillins: Properties, Biosynthesis, and Fermentation," pp 45-140 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

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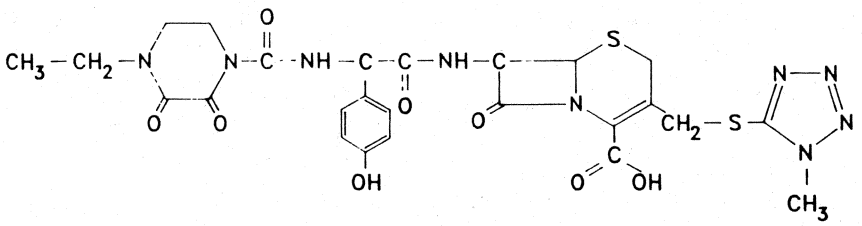

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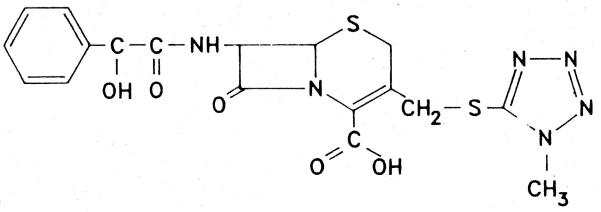

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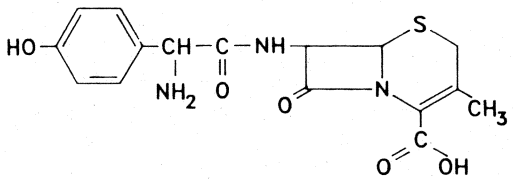

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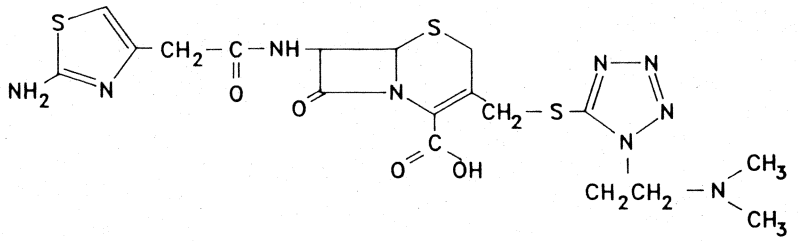

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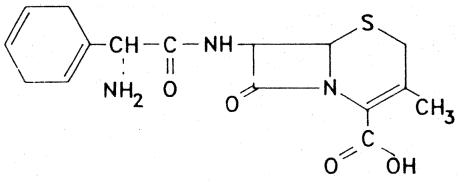

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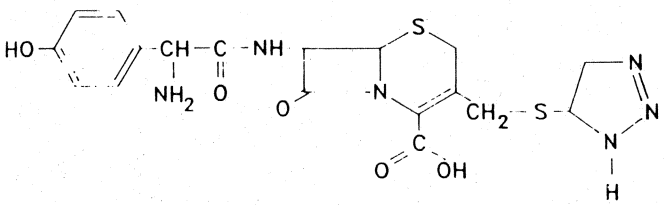

CEFATRIZINE

Fig. A-10. Chemical structures of some semisynthetic cephalosporins. Source: G. J. M. Hersbach, C. P. van der Beek, and P. W. M. van Dijck, "The Penicillins: Properties, Biosynthesis, and Fermentation," pp 45-140 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

APPENDIX B

Production of Cephalosporin Antibiotics

Appendix B. PRODUCTION OF CEPHALOSPORIN ANTIBIOTICS

Cephalosporins are produced by a fermentation process which is very similar to that used in the production of penicillin. The differences are in the growth media, microorganism used and the final fermentation broth concentration of the antibiotic. Table B-1 lists four typical growth media for cephalosporin fermentations taken from the patent literature.[10] The production media is a complex media with corn steep liquor, meat meal, sucrose, glucose, and ammonium acetate used. Several cephalosporin producing organisms (C-2237 and C-3009) can produce the antibiotic while growing on a paraffin-based growth media as shown in Table B-2. The fermenter is normally operated at 22°C and a pH of 6.9 (5 to 7.3) with an air rate of 0.8 to 1.2 volumes per volume of medium per minute. The overall fermentation takes about 114 hours. Figures B-1 and B-2 give the chemical structure of some clinically important cephalosporins while Tables B-3 and B-4 provide information on the clinical activity of the cephalosporins against a wide variety of organisms.

Cephalexin was one of the first cephalosporins produced and is one of the most often used. It has now come off of patent restrictions and is available in generic form. Cephalexin is produced by chemically splitting of the cephalosporin C to make 7-minocephalosporanic acid (7-ACA) and then a chemical reacylation (see Fig. B-3).

Cephalosporins are produced by classes of microorganisms known as fungi and actinomycetes. Within these classifications, a large number of strains have been found which produce the antibiotic, and Table B-5 provides a listing of some of these organisms and the particular cephalosporins which they produce. Unlike the penicillin fermentation, the final fermentation broth concentration is quite low, 0.8 to 4.0 g/L depending upon the particular cephalosporin produced and the organism employed.[10]

Structurally, the cephalosporins are very similar to the penicillins and several are being produced commercially from penicillin G by biosynthetic means. Figures B-2 and B-3 illustrate the general pathway. One advantage of this process is that the penicillins can

Table B-1. Cephalosporin production media[a]

Component	Percent			
	Medium A	Medium B	Medium C	Medium D
Peanut meal	4.0	4.0	4.0	4.0
Soybean meal	2.0	2.0	2.0	2.0
Beet molasses	3.45	3.45	3.45	3.45
Methyl oleate	0.575	0.575	0.575	0.575
Lard oil[b]	6.0	6.0	6.0	6.0
Sodium sulfate	0.4	0.3	---	---
Ammonium sulfate	---	0.1	---	---
Methionine	---	---	0.4	---
Calcium carbonate	0.2	0.2	0.2	---
Calcium sulfate	---	---	---	0.4
Yield: μg Cephalosporin/ml	3240	3560	4190	3920

[a]D. A. Preston and W. E. Wick, "Laboratory Evaluation of Cephalosporin Antibiotics," Developments in Industrial Microbiology Volume 16, American Institute of Biological Sciences, Washington, D.C. (1975).

[b]Lard oil is rarely used now as it causes problems in the separation and purification steps; continuous carbohydrate feed is now commonly used.

Table B-2. Carbon utilization for cephalosporin production[a]

Strain[b]	Carbon Source	Conc. %	Growth[c]	Cephalosporins produced[d] (μg/ml)		
				DACPC	DCPC	CPC
C-2237	n-Paraffin[e]	8	+++	120	25	110
	Sodium acetate[f]	5	++	25	--	5
	Soy bean oil	5	+++	15	--	10
	Glycerol	5	+++	85	5	60
	Glucose	8	+++	160	35	120
C-3009	n-Paraffin[d]	8	+++	18	3	15
	Ethanol	5	+++	15	--	5
	Sodium acetate[e]	5	++	10	--	15
	Soy bean oil	5	+++	6	--	5
	Glycerol	5	+++	8	--	5
	Glucose	8	+++	50	15	30

[a]D. A. Preston and W. E. Wick, "Laboratory Evaluation of Cephalosporin Antibiotics," Developments in Industrial Microbiology Volume 16, American Institute of Biological Sciences, Washington, D.C. (1975).

[b]*P. persicinus* C-3009 and *P. carneus* C-2237.

[c]Symbols; +++, very good; ++, good; +, poor growth.

[d]DACPC-Deacetoxycephalosporin C; DCPC-Deacetylcephalosporin C; CPC-Cephalosporin C.

[e]Component; C12=22.3%, C13=49.9%, C14=27.5%, C15=0.4%

[f]A mixture of one part acetic acid and two parts sodium acetate. A 2% mixture in the medium and 1% fed at 72, 96, and 120 hours.

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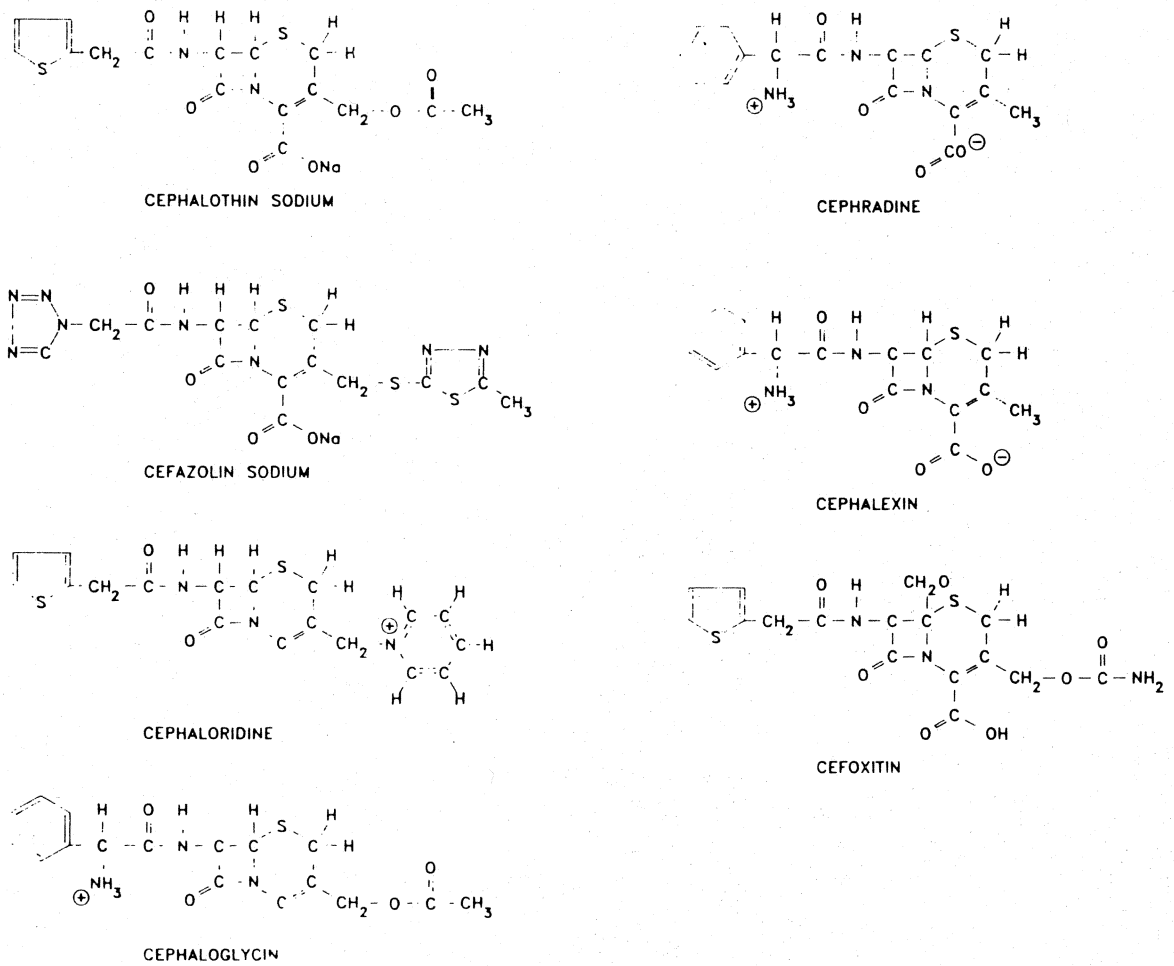

Fig. B-1. Structures of many of the cephalosporin antibiotics. Source: D. A. Preston and W. E. Wick, "Laboratory Evaluation of Cephalosporin Antibiotics," Developments in Industrial Microbiology, 16, American Institute of Biological Sciences, Washington, D.C. (1975).

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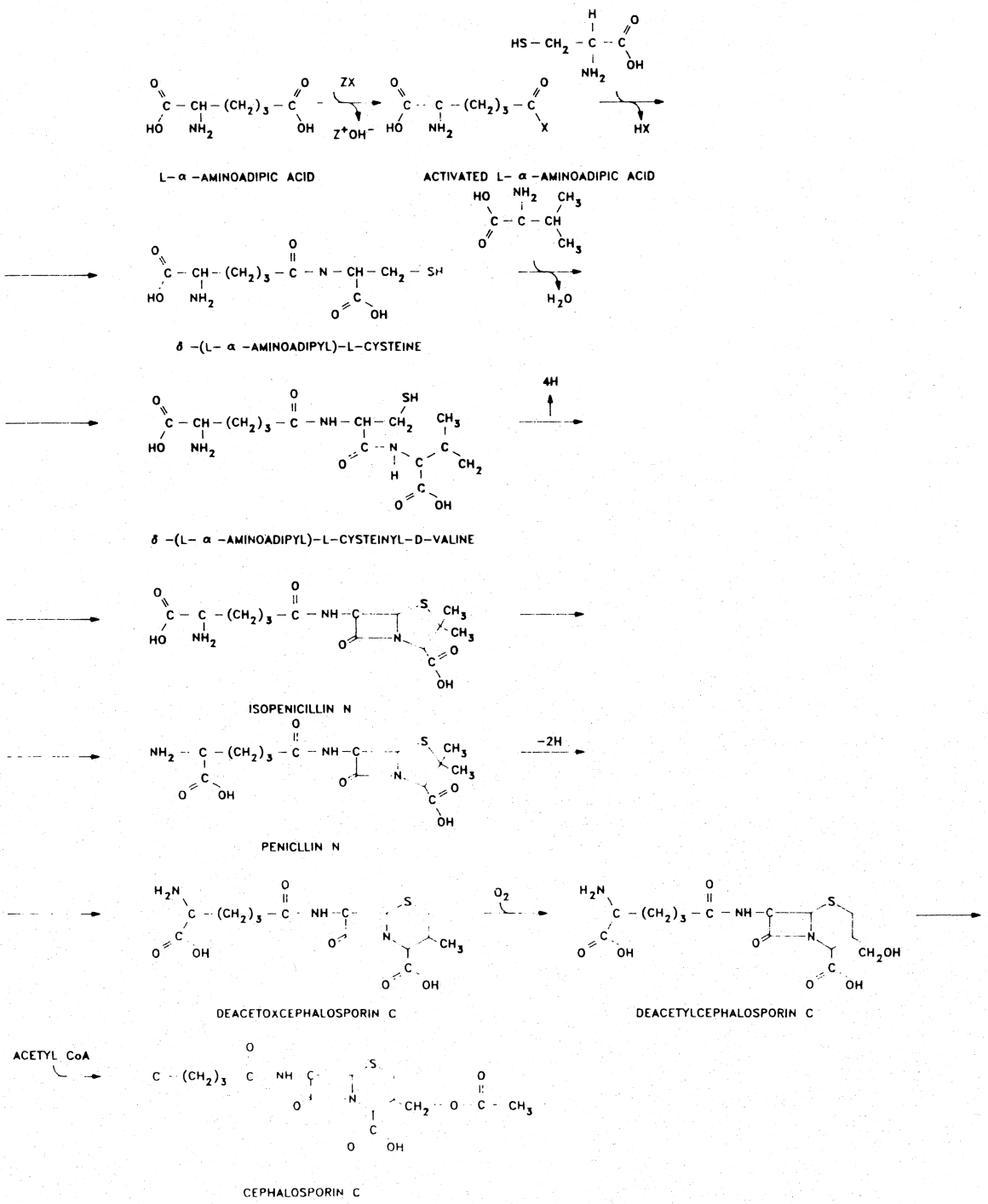

Fig. B-2. Biosynthetic pathway for the formation of cephalosporin.

Table B-3. Clinical characteristics of common cephalosporin antibiotics[a]

Characteristic[b]	Cephalothin	Cephaloridine	Cephaloglycin	Cephalexin	Cefazolin
In vitro activity against gram+ cocci (except group D strep)	+++	++++	+++	++	++
In vitro activity against cephalosporinase-negative gram neg[c]	++	+++	++++	++	++
In vitro activity against cephalosporinase-producing gram neg[d]	--	--	+	--	±
In vitro activity against Pseudomonas	--	--	--	--	--
Route of administration	Parenteral	Parenteral	Oral	Oral	Parenteral
Relative peak concentration in mouse blood after 20 mg/kg	++	+++	±	++++	++++
Relative duration	+	+++	++	++	++++

[a]D. A. Preston and W. E. Wick, "Laboratory Evaluation of Cephalosporin Antibiotics," Developments in Industrial Microbiology Volume 16, American Institute of Biological Sciences, Washington, D.C. (1975).

[b]Symbols: ++++ = very effective; +++ = effective; ++ = moderately effective; + = little effect; ± = marginal; -- = no effect.

[c]e.g., E. coli, Proteus mirabilis, Klebsiella pneumoniae, Salmonella sp., Shigella sp.

[d]e.g., Indole-producing Proteus sp., Enterobacter sp., Serratia sp.

Table B-4. Characteristics of cephalosporins of clinical interest

Name	Method of administration		Specific activity (minimum inhibitory concentration, μg/ml) against the following:	
	Injection	Oral	*S.aureus*	*Pseudomonas*
Cefamandole	+		0.9	0.8
Cefazolin	+		0.8	2
Cefoxitin	+		2	6
Cephalexin		+	5	9
Cephaloglycin		+	2	2
Cephaloridine	+		4	9
Cephalothin	+		0.4	17
Cephapirin	+		0.6	13
Cephacetrile	+		2	8
Cephradine		+	5	20

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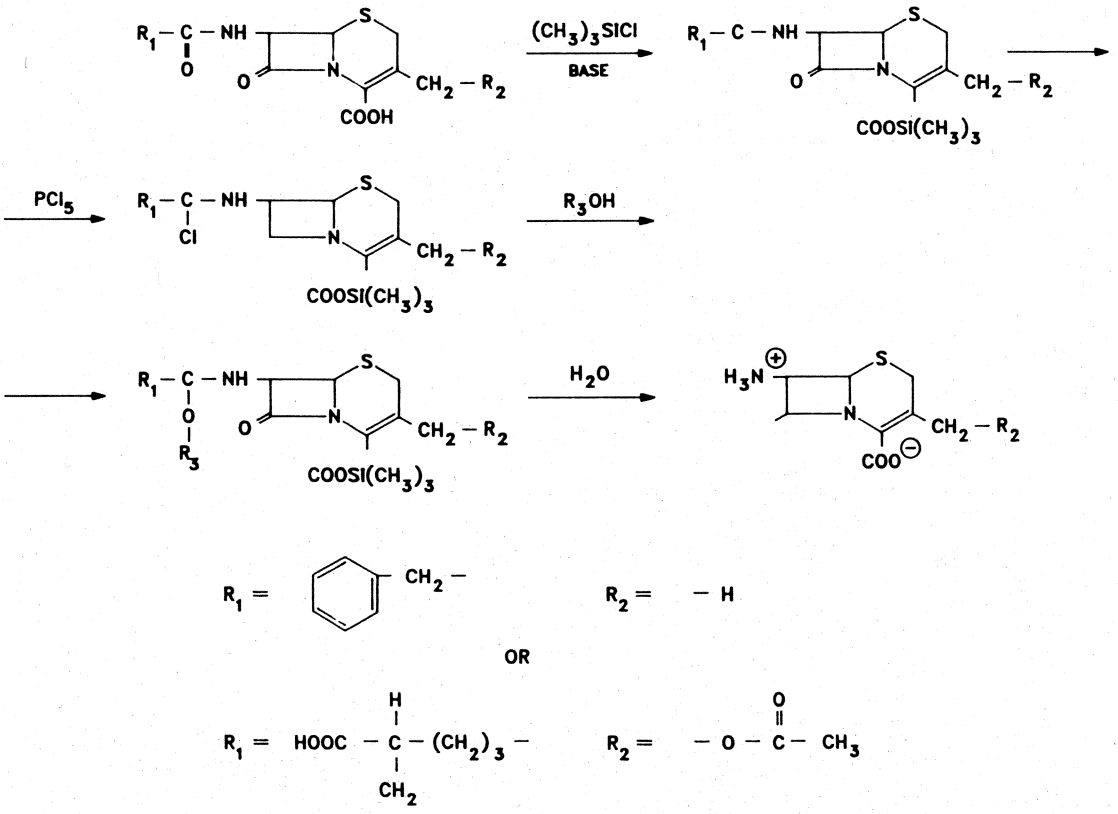


Fig. B-3. Chemical deacylation of cephalosporin C.

Table B-5. Microorganisms which produce various cephalosporins[1]

Compound	Microorganism
Cephalosporin C	*Cephalosporium acremonium*, *C. polyaleurm*, *Arachnomyces minimus*, *Anixiopsis peruviana*, *Spiroidium fuscum*, *Paeciiomyces persicinus*, *Paec. carneus*.
Deacetylcephalosporin C	Mutants of *C. acremonium*, *C. polyaleurum*, *Ar. minimus*, *An. peruviana*, *Sp. fuscum*, *Paec. persicinus*, *Paec. carneus*
Deacetoxycephalosporin C	Mutants of *C. acremonium*, *C. polyaleurum*, *Ar. minimus*, *An. peruviana*, *Sp. fuscum*, *C. chrysogenum*, *C.* sp., *Emericellopsis* sp., *Paec. persicinus*, *Paec. carneus*, *Diheterospora chlamydosporia*, *Scopulariopsis* sp., *Streptomyces lipmanii*, *Str. clavuligerus*
A 16886 A	*Str. Clavuligerus*
A 16884 A	*Str. lipmanii*
Cephamycin C or A16886 B	*Str. clavuligerus*, *Str. lactamdurans*
Cephamycin B	*Str. griseus*, *Str. chartreusis*, *Str. cinnamonensis*, *Str. fimbriatus*, *Str. halstedii*, *Str. rochei*, *Str. viridochromogenes*
Cephamycin A	Same as Cephamycin B producers.
F1	Mutants of *C. acremonium*
C2	Mutnats of *C. acremonium*
Penicillin N	All strains which produce cephalosporins

[1]Source: T. Kanzaki and Y. Fujisawa, "Recent Progress in Cephalosporin Fermentations,"J. Takeda. Res. Lab. 34 (2) 324 (1975).

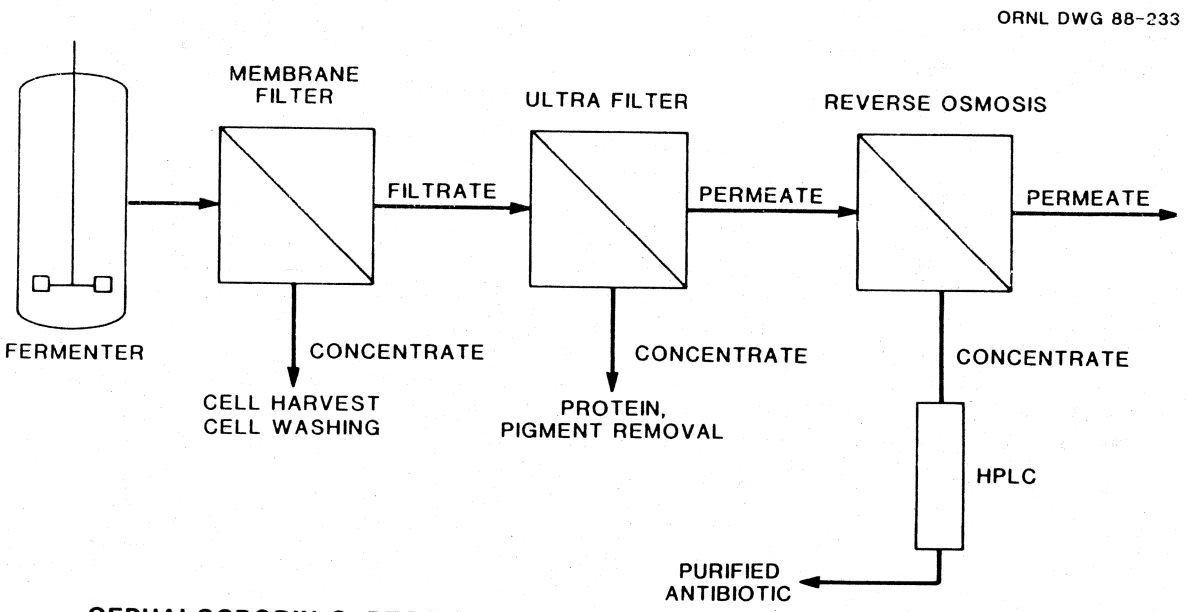
Table B-5. (Continued)

6-Aminopenicillanic acid	*C. acremonium*
Tripeptide P 3 (δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine)	*C. acremonium*
Tetrapeptide P 2 (α-aminoadipic acid, cystein, valine and glycine)	*C. acremonium*
Tetrapeptide P 1 (α-aminoadipic acid, cysteine, β-hydroxyvaline, and glycine)	*C. acremonium*
Peptide S 1 (Dimer of δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine)	Mutants of *C. acremonium*
Peptide S 2 (Disulfide of δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine and methanthiol)	Mutants of *C. acremonium*

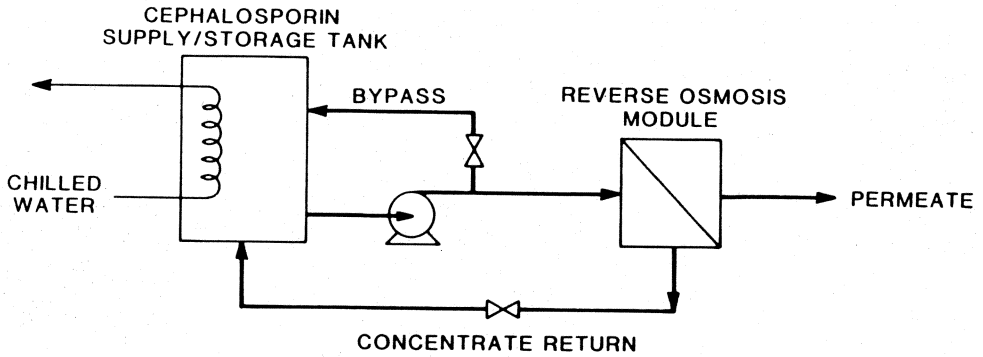
be produced at a lower cost than the cephalosporins. Cephalosporins can also be produced chemically; however, the large-scale commercialization of the process is not feasible because of the operating conditions. Some steps require temperatures of -74°C, while other steps occur at 120°C, and expensive and toxic chemicals are required (such as benzene, toluene, lead tetraacetate, phosgene, pyridine, methylene chloride, L-(+)cysteine (precursor), t-butanol, and sodium acetate). However, all of these compounds can be found in quantity at many university chemistry departments and could be used to serve the needs of a small area. The step-by-step syntheses is given by Woodward et al.[45] and is available in any chemistry department library. A mathematical model for the fermentation of cephalosporin C is given by Matsumura et al.[46]

Product recovery

Cephalosporins are extracellular fermentation products, allowing a bulk filtration to be used to separate the solid matter from the antibiotic containing fermentation broth. Recovery from the filtrate is difficult due to the low concentration of product and the need to remove high molecular weight (1000 MW) biological compounds which can lead to allergic and toxic manifestations when the drug is administered.[40] This is not as important for the oral product as for the products for injection. Many different separation and purification schemes are employed, including conventional solvent extraction, ion exchange resins, liquid chromatography with conventional resins and salting out procedures. Fig. B-4 gives a general process flow sheet for the recovery and purification of cephalosporins. The general procedure is to filter the fermentation broth at acidic pH (5.0), followed by adsorption of the filtrate on activated carbon, removal of the adsorbed antibiotic by contacting the carbon with a mixture of water and a polar organic solvent, contacting the eluate with an anion exchange resin, and eluting the resin with a salt solution at a pH of from 5.5-10.0. Frequently, the carbon adsorption steps are replaced with a precipitation step. Many different precipitations are possible. Among the more common are[47]



CEPHALOSPORIN C. RECOVERY PROCESS FROM FERMENTATION BROTH



REVERSE OSMOSIS CONCENTRATION OF CEPHALOSPORIN C

Fig. B-4. Schematics for recovery and concentration of cephalosporin C. Source: M. Kalyanpur, W. Skea and M. Siwak, "Isolation of Cephalosporin C from Fermentations Broths Using Membrane Systems and High-Performance Liquid Chromatography," Developments in Industrial Microbiology, 26 455 (1985).

1. Crystallization of the potassium or sodium salt from purified aqueous solution of the cephalosporin by concentration and/or addition of large volumes of a miscible solvent.

2. The zinc salt (also copper, nickel, lead, cadmium, cobalt, iron, and manganese) can be crystallized from purified aqueous solutions.

3. Insoluble derivatives such as the N-2,4 dichlorobenzoyl cephalosporin and tetrabromocarboxybenzoyl cephalosporin are crystallized as the acid from aqueous solutions.

4. Sodium-2-ethyl hexanoate will precipitate the sodium salt of N-derivatized cephalosporins from solvents.

Figure B-5 gives a typical flow diagram for the purification of cephalosporins using ion exchange resins.

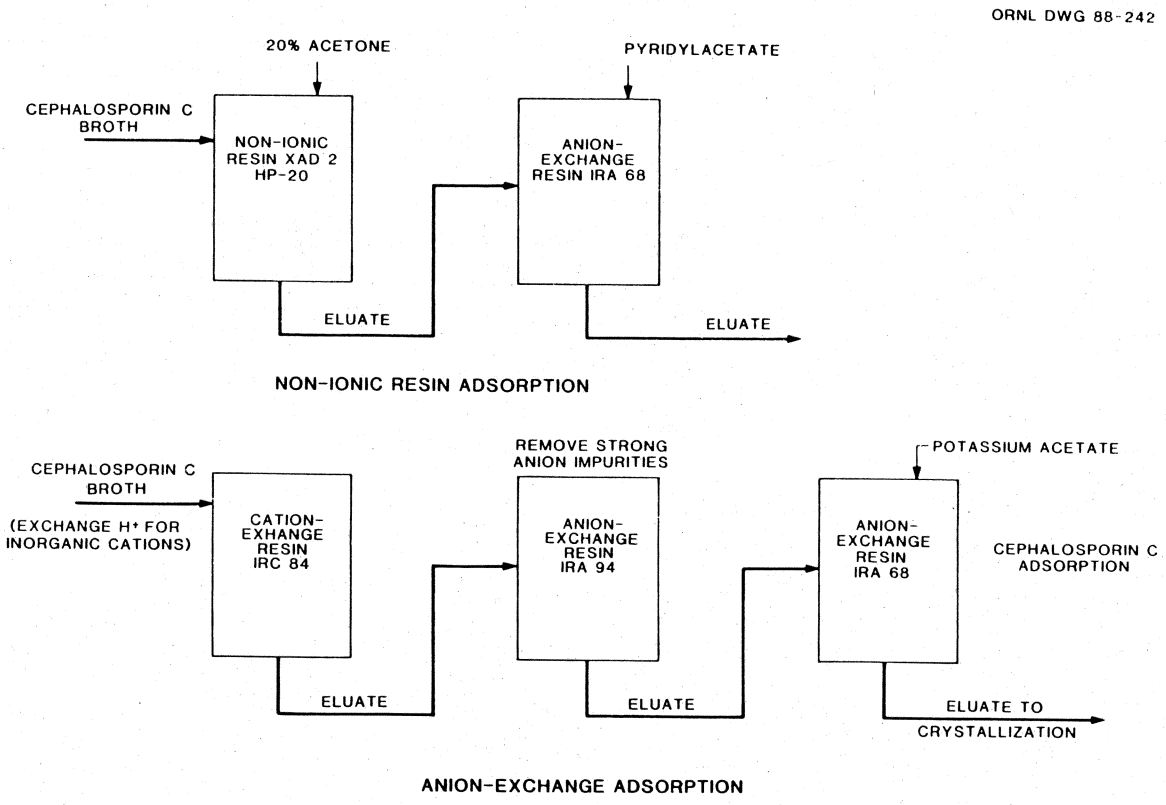


Fig. B-5. Cephalosporin C purification from broth by adsorption. Source: M. E. Wildfeuer, "Approaches to Cephalosporin C Purification from Fermentation Broth," Purification of Fermentation Products, 156 (1985).

APPENDIX C

Production of Tetracycline Antibiotics

Appendix C. PRODUCTION OF TETRACYCLINE ANTIBIOTICS

Tetracycline antibiotics production utilizes preinoculation, inoculation, and fermentation tanks similar to those of penicillin. The processing scheme is shown in Fig. C-1, and the procedure is shown in Fig. C-2. Oxygen must be provided for oxygen transfer and aeration rates of 1 volume of air per volume of fermenter per minute (VVM) are customary. Oxygen enriched air provides faster growth and higher yields. The fermenter can have a 100- to 150-m^3 working volume with three open turbines that are 1450 to 2100 mm in diameter with maximum speeds of 80 rpm and a power input of 300 kW (400 Hp) at the shaft (up to 3 kW/m^3). Oxygen supply continuity is very important because interruption of the oxygen supply for more than 10 min results in the stopping of tetracycline production.

Strains are kept either in a freeze-dried or liquid-nitrogen state as spore stock. A culture is started by sporulation on an agar slant, followed by the second sporulation in a stationary culture on millet in flasks. The first step requires 7 to 10 days, and the second 14 days. About 10^5 to 10^6 spores/cm^3 are inoculated into the first submerged culture fermentation in the preinoculation tank and allowed to grow for 24 to 26 hours. The contents of the first fermentation are transferred to the inoculation tank, allowed to grow for 18 to 20 hr, and then this material (about 5 % of the fermentation vessel volume) is added to the fermentation vessel with the nutrients and allowed to grow for 100 to 200 hr with tetracycline production. All growth takes place at 29°C.

The medium for tetracycline production includes sucrose, glucose or molasses, 2.5% wt/vol, organic nitrogen sources (soybean meal or corn-steep liquor, 1.7%), a buffer (calcium carbonate, 0.2 to 0.3%), inorganic salts (NaCl and KH_2PO_4), and vegetable oil (0.2%). Chloride ions serve as precursors of chlor-tetracycline, and benzylthiocyanate is added as an inhibitor of undesirable metabolic pathways during lack of oxygen. The culture is monitored for pH, residual sugar,

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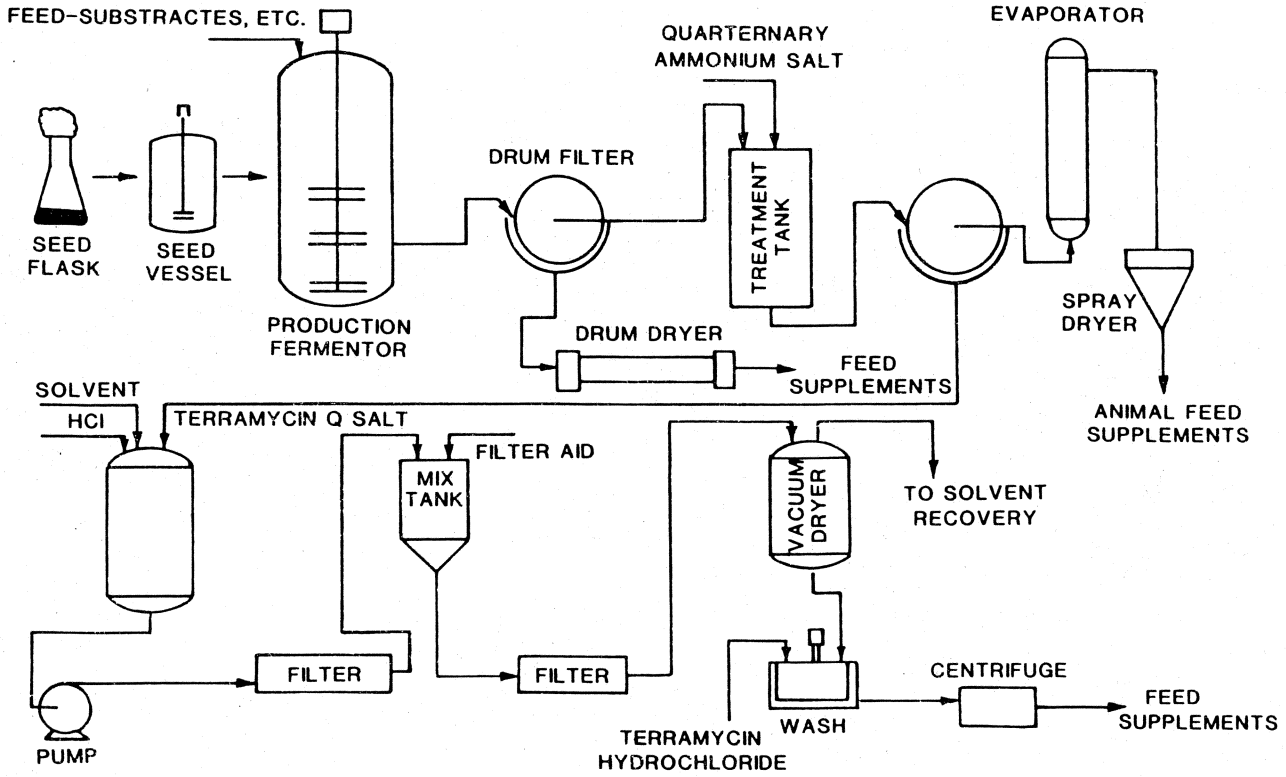


Fig. C-1. Production scheme for oxytetracycline.
Source: D. J. D. Hockenhull, The Fermentation of the Tetracyclines, Interscience Publishers Inc., New York (1959).

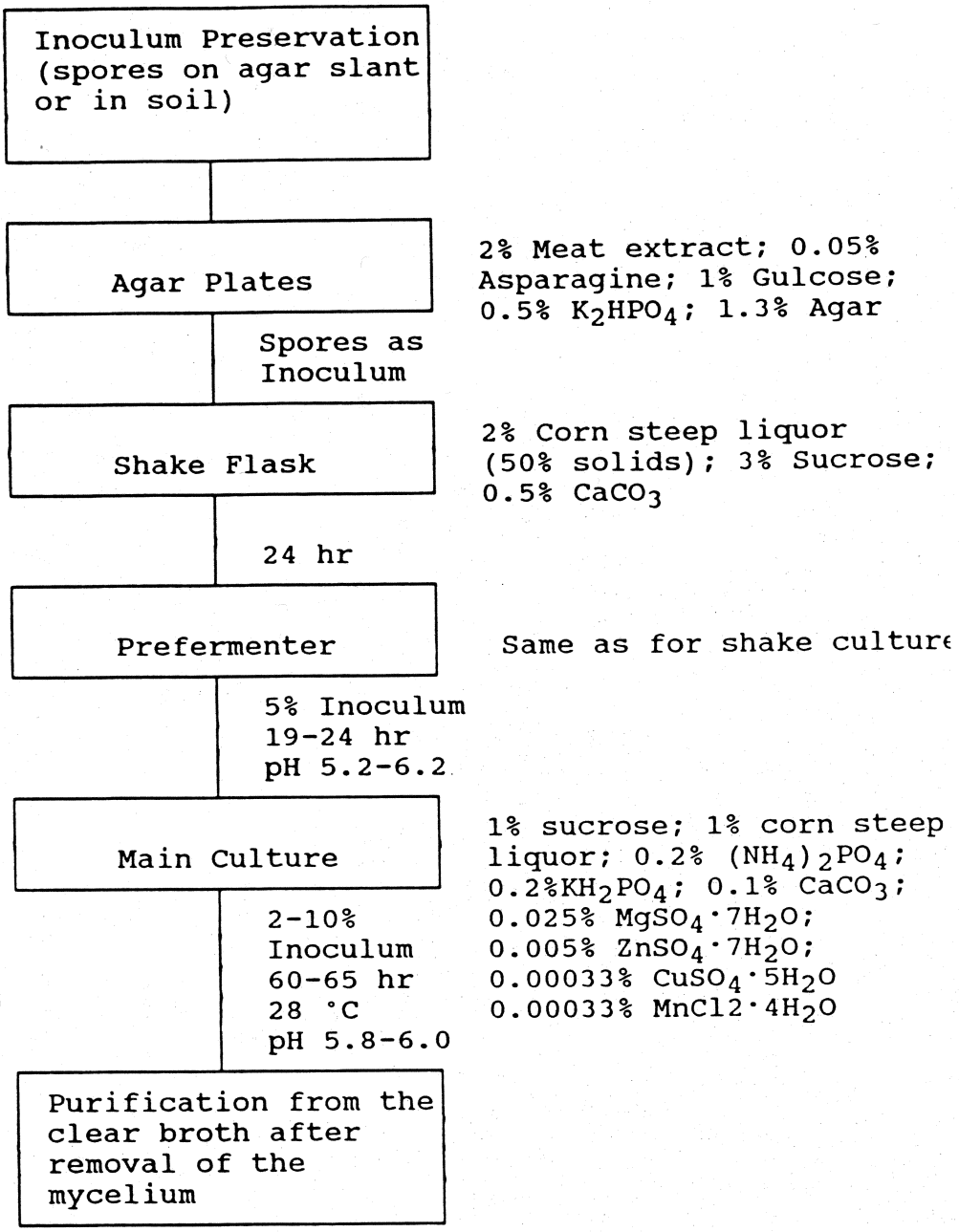


Fig. C-2. Production chart for chlortetracycline.

Source: D. J. D. Hockenhull, The Fermentation of the Tetracyclines, Interscience Publishers Inc., New York (1959).

respiration (CO_2 concentration), increase of biomass in concentration and in volume, and morphology.

Nutrients are sterilized at 120°C for 40 min or at 140°C for 1 min with C and N sources sterilized separately. Aeration ranges up to 0.8 medium vol/min and oxygen overpressure of up to 0.1 MPa are used. The pH, oxygen content of the medium (at least 20% saturation) and the CO_2 in the exhaust air are monitored continuously.

Product Recovery and Purification

Isolation methods for tetracyclines must take into account their amphoteric nature and the possibilities of their polymerization or rearrangement. Several methods have been used to recover and purify tetracyclines and some of these are listed below.[11]

1. Adsorption on diatomaceous earth or active charcoal with subsequent chromatography or selective extraction.
2. Extraction from acid or alkaline medium. The most frequently used extraction agent is 1-butanol, owing to its suitable partition coefficient and economic availability.
3. Direct mash extraction based on solubilizing the antibiotic by acidification, precipitation of Ca^{2+} with ammonium oxalate, addition of quaternary ammonium compounds as carriers, and extraction of the metabolite with an organic solvent, usually one of the methylalkyl ketone type.
4. Precipitation (dry salt) process based on precipitation the antibiotic from dilute aqueous solution of aryl azosulfonic acid dyes. Tetracyclines are precipitated as complexes with alkaline earth metal compounds or with primary and secondary alkyl amines.
5. Solvent extraction of the antibiotic with salt, based on salting out (NaCl) the antibiotic from the aqueous to the organic phase (1-butanol). This method is also suited for refining a crude product.

Oxytetracycline is purified by combining the release of oxytetracycline into the medium by acidification, precipitation of ballast compounds with $K_2Fe(CN)_6$ and $ZnSO_4$, extraction of liquid fractions

with butyl acetate, and precipitation of Ca^{2+} with oxalic acid. After adjusting the filtrate with EDTA, Na_2SO_3, and citric acid, the crystalline base of oxytetracycline is obtained.

Further purification is carried out by crystallization as salts (e.g., hydrochlorides) or bases. Particularly efficient is crystallization from boiling solvents, such as lower alcohols, ketones, or aliphatic ethers of ethylene glycol, which yield nonhygroscopic preparations. Residual amounts of antibiotic in the mother liquor are increased by oxalate and chloride anions, while sulfate anions have the opposite effect. Crystallization is most efficiently performed at 2°C for 3 hours.[48]

Semisynthetic tetracyclines are prepared from oxytetracycline by chemical synthesis. Methacycline, doxycycline, minocycline and rolitetracycline can be prepared. Methacycline is made by a three-step synthesis and is isolated as a sulfosalicylate which is converted to a hydrochloride of methacycline by crystallization from an acid mixture of acetone and methanol in a 62% yield.

Analytical Methods

Chemical assays of tetracyclines based on metal complexes utilize the characteristic absorption of fluorescence of the complex formed. Using this method, one can analyze the principal tetracyclines both in biological material and in commercial preparations. Polarographic assay of tetracyclines is based on their reduction in a boric acid-sodium borate buffer. During the fermentation and isolation process, paper chromatography, thin-layer chromatography, and gel chromatography can be used for assay. Recently, HPLC, using both regular and reverse phase packings and ion exchange packings, has been used for analyses of the tetracyclines.[27]

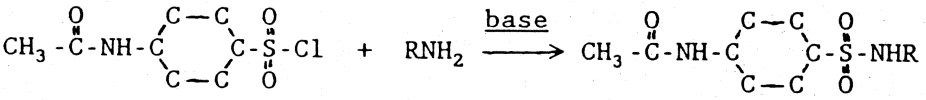
APPENDIX D

Sulfonamides

Appendix D. SULFONAMIDES

The sulfonamides (sulfa drugs) represent a large class of antimicrobial drugs which are produced by chemical reaction. The sulfonamides came into common usage in the mid 1930's and are still produced in quantity today. From the base compound, many clinically active derivatives have been produced, and there are over 5,000 different sulfonamide derivatives. Figure D-1 presents the chemical structure of the most important sulfonamides. The processing steps necessary to produce purified sulfonamides are common to the organic chemical and dyestuffs industries and are described briefly below.[16]

The usual method of sulfonamide preparation is the reaction of N-acetylsulfanilyl chloride (ASC) with the appropriate amine.



Extra amine or base is used to neutralize the hydrochloric acid freed in the reaction. The acetyl product is treated with aqueous alkali to the free amino compound. ASC is obtained by chlorosulfonation of acetanilide. The drugs are white powders melting above 150°C, and azo and nitro derivatives may be yellow. The sulfas are sparingly soluble is cold water but are somewhat soluble in alcohol or acetone. Assays for the sulfa drugs are usually done by titration with nitrous acid and paper or thin-layer chromatography.[49]

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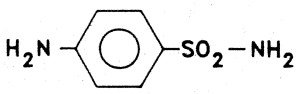
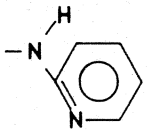
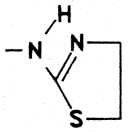
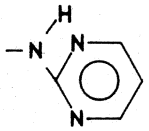
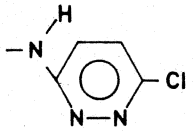
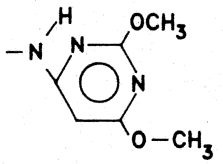
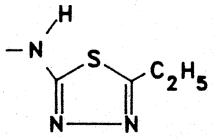
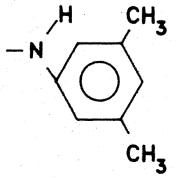
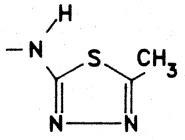
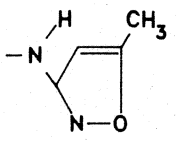
SULFANILAMIDE	
SULFAPYRIDINE	
SULFATHIAZOLE	
SULFADIAZINE	
SULFACHLOROPYRADAZINE	
SULFADIMETHOXINE	
SULFAETHIDOLE	
SULFAMETHAZINE	
SULFAMETHAZOLE	
SULFAMETHOXAZOLE	

Fig. D-1. Therapeutically important sulfa drugs and sulfones. Source: Encyclopedia of Chemical Technology, Third Edition, ed. Kirk-Othmer, Interscience, New York (1978).

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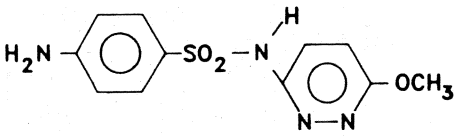
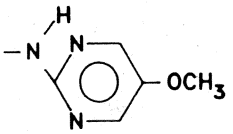
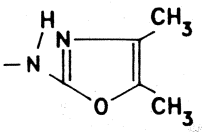
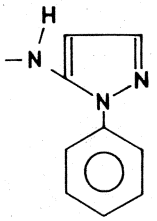
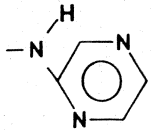
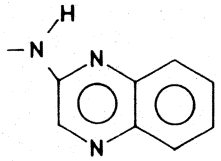
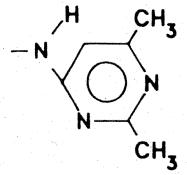
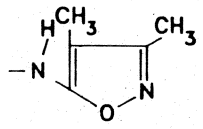
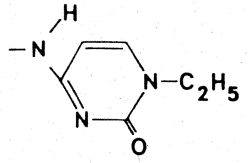
SULFAMETHOXYPYRIDAZINE	
SULFAMETER	
SULFAMOXOLE	
SULFAPHENAZOLE	
SULFAPYRAZINE	
SULFAQUINOXALINE	
SULFISOMIDINE	
SULFISOXAZOLE	
SULFACYTINE	

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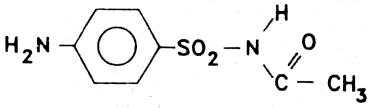
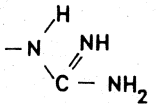
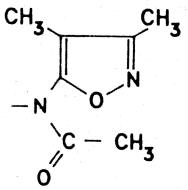
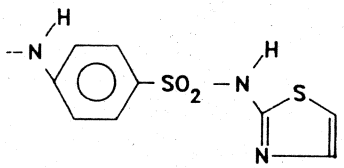
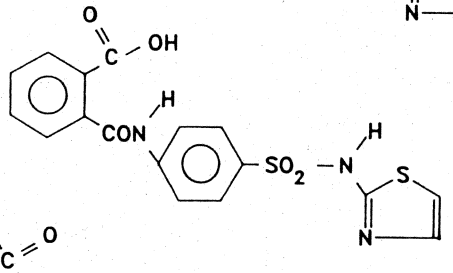
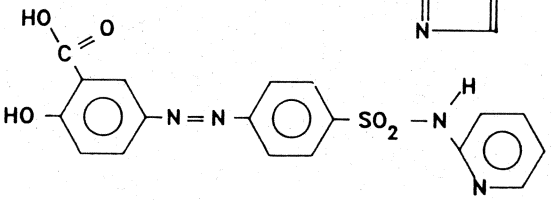
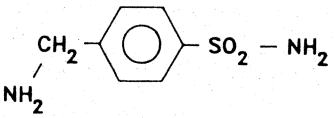
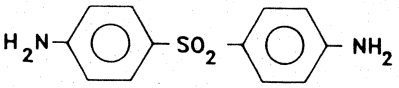
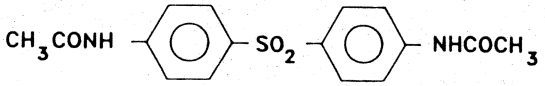
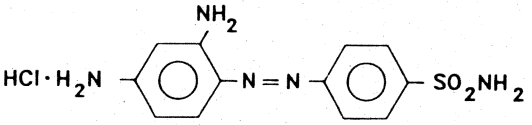
SULFACETAMIDE	
SULFAQUANIDINE	
N'—ACETYLSULFISOXAZOLE	
SUCCINYLSULFATHIAZOLE	
PHTHALYLSULFATHIAZOLE	
SALICYLAZOSULFAPYRIDINE	
MAFENIDE	
DAPSONE	
ACEDAPSONE	
PRONTISIL	

APPENDIX E

Bacitracin Production

Appendix E. BACITRACIN PRODUCTION

Bacitracin has been produced using several different types of media. They include (1) synthetic media containing amino acids as carbon and nitrogen sources, (2) rich media containing beef extracts or tryptone broth, and (3) high-producing media with soybean meal and starch or dextrines/sugars as nutrients. Bacitracin production levels are on the order of 400 to 600 IU/ml where 74 IU (international units) corresponds to 1 mg of the Second International Standard of bacitracin or 5 to 9 g/liter.

The bacitracin-producing strains of *Bacillus licheniformis* are kept on agar slants or in the spore form in dry sterile sand. The bacitracin production process is shown in Fig. E-1 and E-2. Fig. E-3 shows the flow scheme and gives the media compositions used in the various steps of the process. A suitable amount of the culture is used to inoculate shake flasks of tryptone or peptone broth. An intermediate fermenter is usually used as a seed tank for the main fermenter. The fermenters are equipped with an agitator and a jacket for steam or water. The medium is sterilized either by batch or continuous sterilization. The fermentation process is usually monitored by continuous pH and O_2 measurements. The fermentation is carried out at 30 to 37°C, for 24 to 30 hr. Recently, bacitracin has been produced by cells of *B. licheniformis* immobilized in polyacrylamide gel, but this is not commercial.[14]

Recovery and Purification of Bacitracin from Fermentation Broth

Bacitracin is prepared by direct precipitation form the clarified fermentation broth by salts of divalent cations, primarily zinc, but nickel, manganese and cobalt have also been used. Table E-1 gives the solubility of bacitracin, zinc bacitracin, and bacitracin F in various solvents, and Table E-2 lists some precipitation agents for bacitracin. Zinc bacitracin is used primarily as an animal feed-grade product and is probably the most simple separation. After fermentation, salts such as methylene disalicylic acid, lignin sulfonic acid, hydroxymethane sulfonic acid, and alkylbenzene sulfonic acid

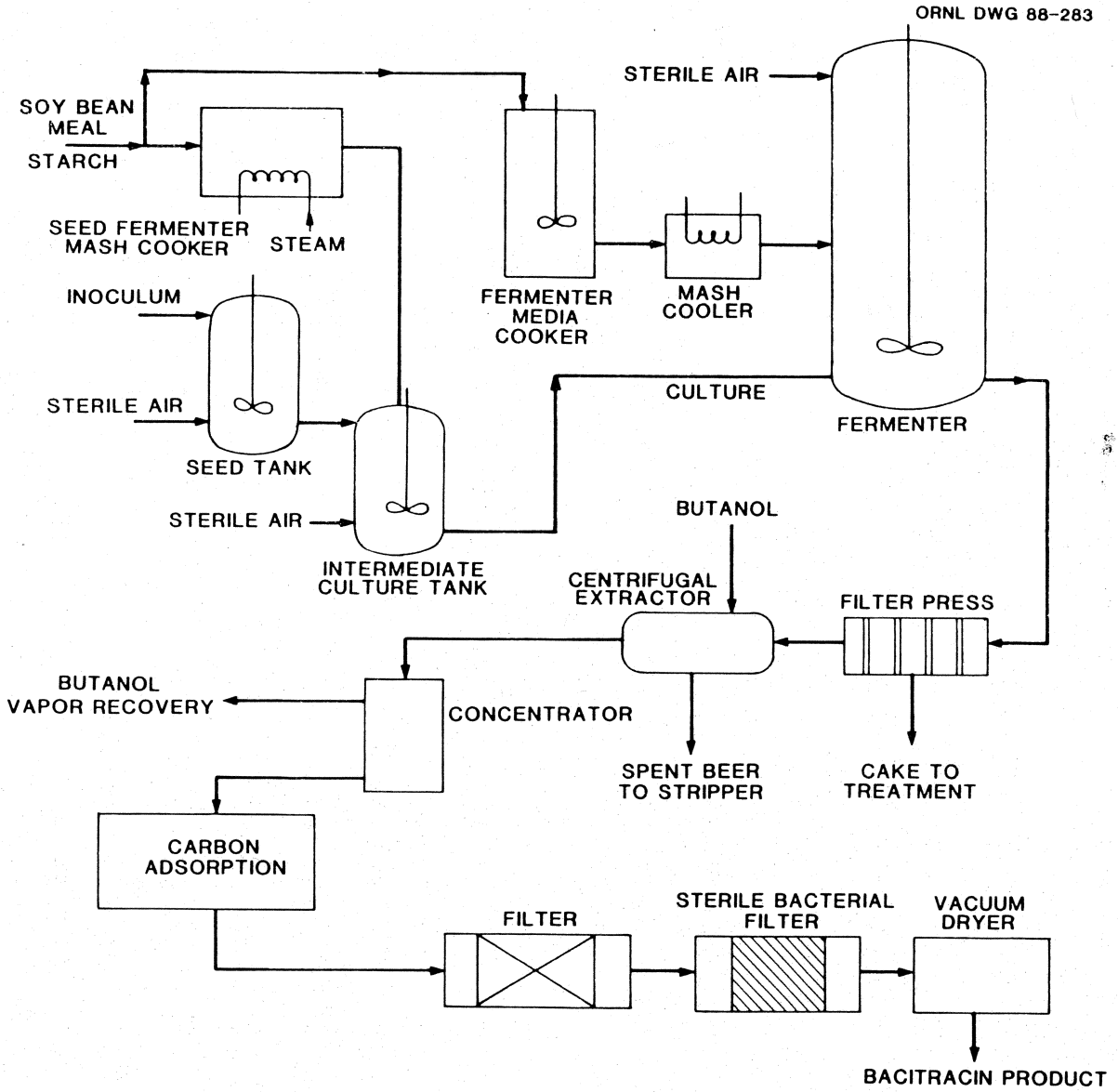


Fig. E-1. Bacitracin production flow and equipment scheme.
Source: H. J. Peppler and D. Perlman, Microbial Technology Volume 1, Second Edition, Academic Press, New York (1979).

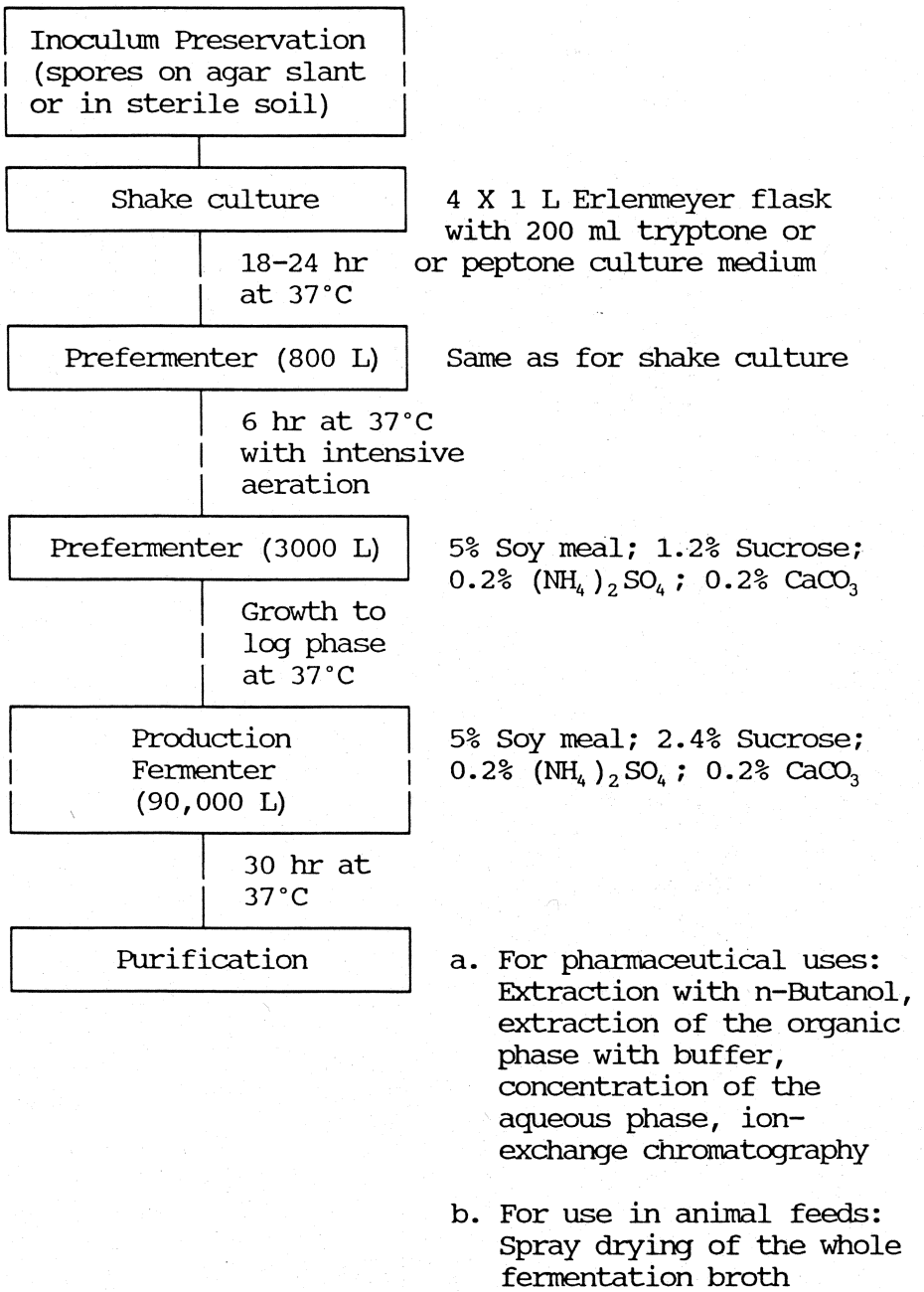


Fig. E-2. Block diagram for the production of bacitracin.

Table E-1. Solubility of bacitracins in various solvents[a]

Solvent	Solubility[b] (mg/ml)		
	Bacitracin	Zinc Bacitracin	Bacitracin F
Petrolether (bp 100-120°C)	--	--	0.002
Petrolether (bp 40-60°C)	--	--	0.007
Carbontetrachloride	0.19	0.009	0.37
Toluene	--	--	--
Diethylether	--	0.002	--
Chloroform	0.006	0.07	0.24
Pyridine	17.0	16.1	11.4
1-Butanol	4.2	0.68	>15.
Acetone	0.08	0.06	0.09
Methanol	>40.	17.3	>15.
Acetonitrile	0.005	0.005	0.07
Water	>40.	10.1	11.2

[a] Ø. Frøyshov, "The Bacitracins: Properties, Biosynthesis, and Fermentation," pp 665-694 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

[b]The solubility of bacitracin (pharmaceutical grade), zinc bacitracin (pharmaceutical grade), and bacitracin F (oxidized bacitracin) were tested at 25°C.

Table E-2. Precipitating agents for bacitracin.[a]

Ammonium molybdate	Molybdic acid
Ammonium rhodanilate	Phosphomolybdic acid
Azobenzene p-sulfonic acid	Phosphotungstic acid
Benzoic acid	Picric acid
Divalent metal ions (Zn, etc.)	Picrolonic acid
Furoic acid	Salicylic acid
Methylene disalicylic acid	

[a] Ø. Frøyshov, "The Bacitracins: Properties, Biosynthesis, and Fermentation," pp 665-694 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

have been used for precipitation of the bacitracin salts. Bacitracin can be adsorbed/precipitated on solid carriers like bentonite and celite. After precipitation, bacitracin is concentrated by filtration, centrifugation, or evaporation, and then dried by drum or spray drying.

For pharmaceutical-grade bacitracin, solvent extraction from the clarified fermentation broth with butanol, or ion exchange followed by solvent extraction is practiced. The precipitation of bacitracin is accomplished with divalent metal ions, primarily zinc.

Assay methods for bacitracin include microbiological (in which inhibition of a test organism is compared to a standard bacitracin), paper and thin-layer chromatography, electrophoresis, isoelectric focusing, and pulse polarographic techniques. The assay method of choice is HPLC using reversed-phase packing and programmed gradient elution or an isocratic system. A chromatogram is shown in Fig. E-4. HPLC has been used for monitoring the fermentation broth during fermentation and during the purification process.[50]

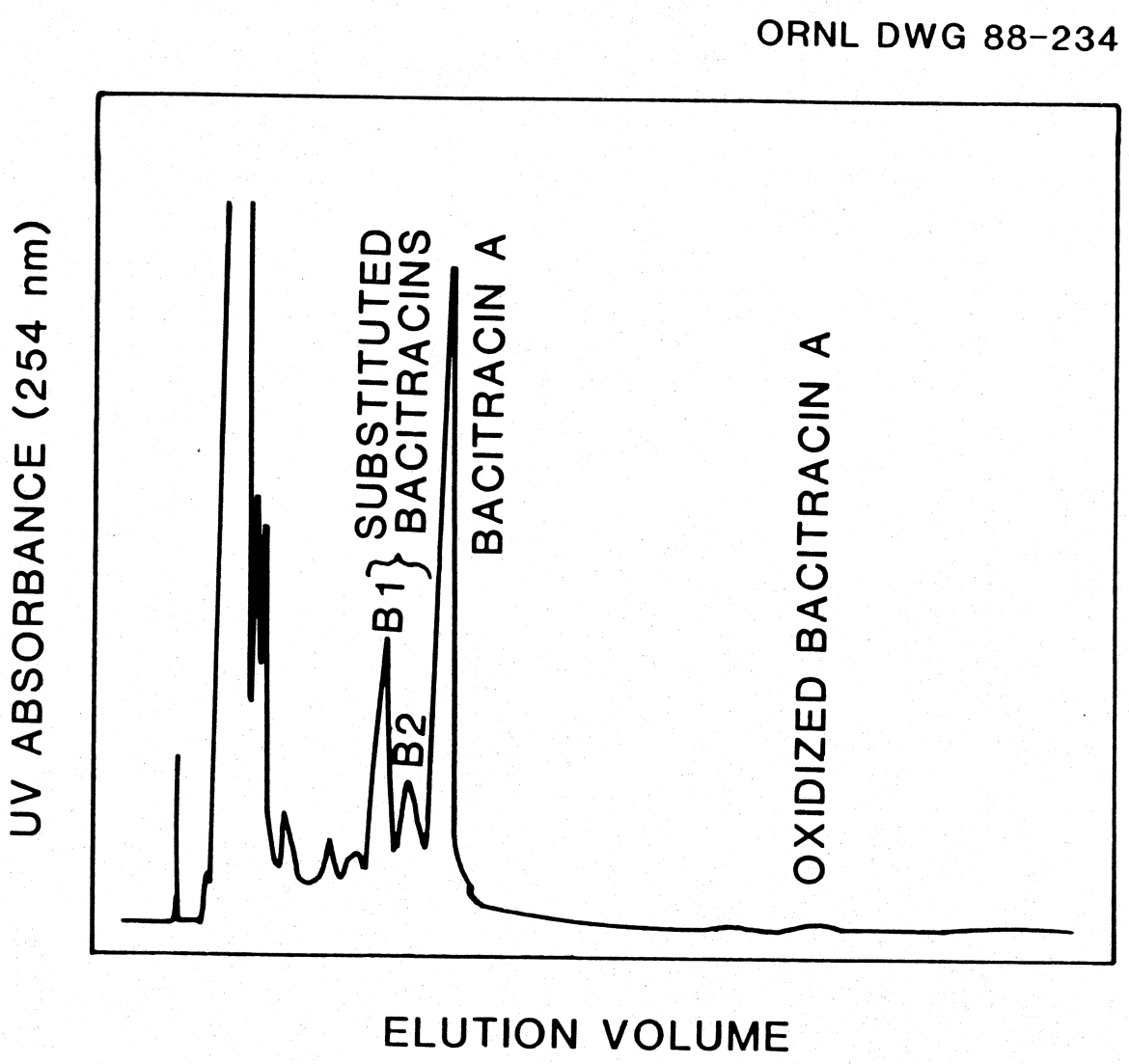


Fig. E-3. HPLC chromatogram of purified bacitracin.
Source: Ø. Frøyshov, "The Bacitracins: Properties, Biosynthesis, and Fermentation," pp 665-694 in Biotechnology of Industrial Antibiotics ed E. J. Vandamme, Marcel Dekker, Inc., New York, 1986.

APPENDIX F

Organic Product Fermentations

Appendix F. ORGANIC PRODUCT FERMENTATIONS

Ethanol process

The process for production of fermentation ethanol is similar in its initial steps to antibiotic production in that a stirred-tank batch fermenter (similar to Fig. F-1) is used to produce a fermentation product which must be separated and purified before use (Figs. F-2, 3, and 4). The ethanol fermentation is fundamentally different, though, because it is essentially an anaerobic process. Some oxygen is required for proper growth, and the aeration equipment may be in place in the fermenters to allow for the sparging that is required for an antibiotic fermentation. The heat exchange and agitation equipment may not be adequate for full- scale antibiotic fermentation, because during an ethanol fermentation, broth viscosity changes very little, and fermentations occur at a higher temperature (35°C vs 23 to 25°C). Separation/purification equipment will generally not be adequate because the ethanol process only includes filtration and distillation and azeotropic distillation (benzene) equipment. Some distillation towers could possibly be used as extraction columns to obtain some separation.

Organic acids

Acetic acid is now often produced in a submerged-culture batch fermentation using a stainless steel fermenter with a high-speed agitator on the bottom of the fermenter for high efficiency oxygen transfer. The vinegar product is filtered using filter aids and fining agents for purification. In the submerged-culture citric acid process, aeration and agitation requirements are similar to antibiotic production needs, and glass- or plastic-coated or 316 stainless steel fermenters are used (adaptable to sterilization and antibiotics fermentations). The purification steps include filtration, precipitation, activated carbon and ion exchange columns, crystallization, and centrifugation (which readily adapt to antibiotic purification needs). Gluconic acid is also produced in a submerged fermentation at 30°C using air sparging in batch mode.

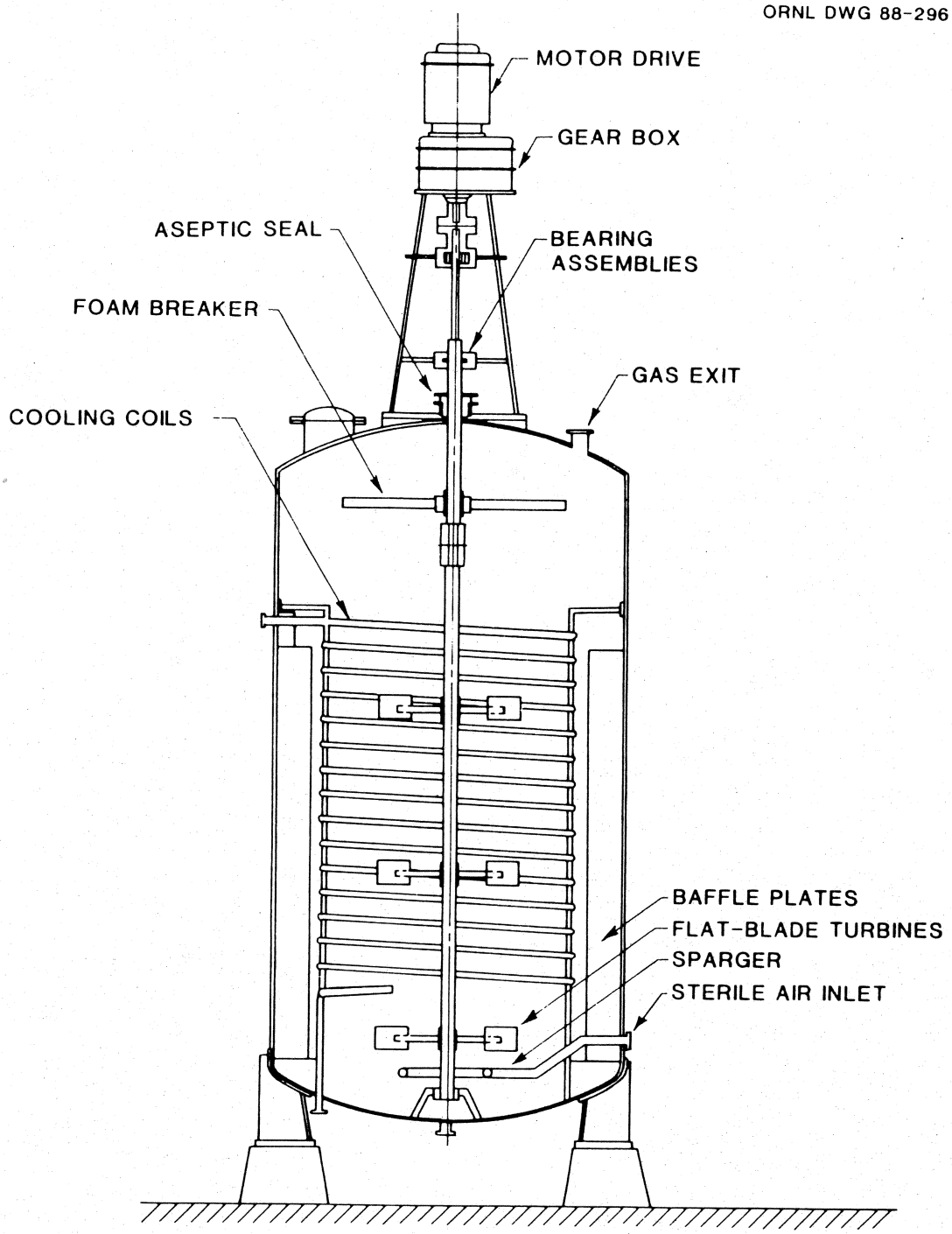


Fig. F-1. Common stirred-tank batch fermenter for ethanol production. Source: S. Aiba, A. E. Humphrey, and N. F. Millis, Biochemical Engineering, Academic Press, Inc., New York (1973).

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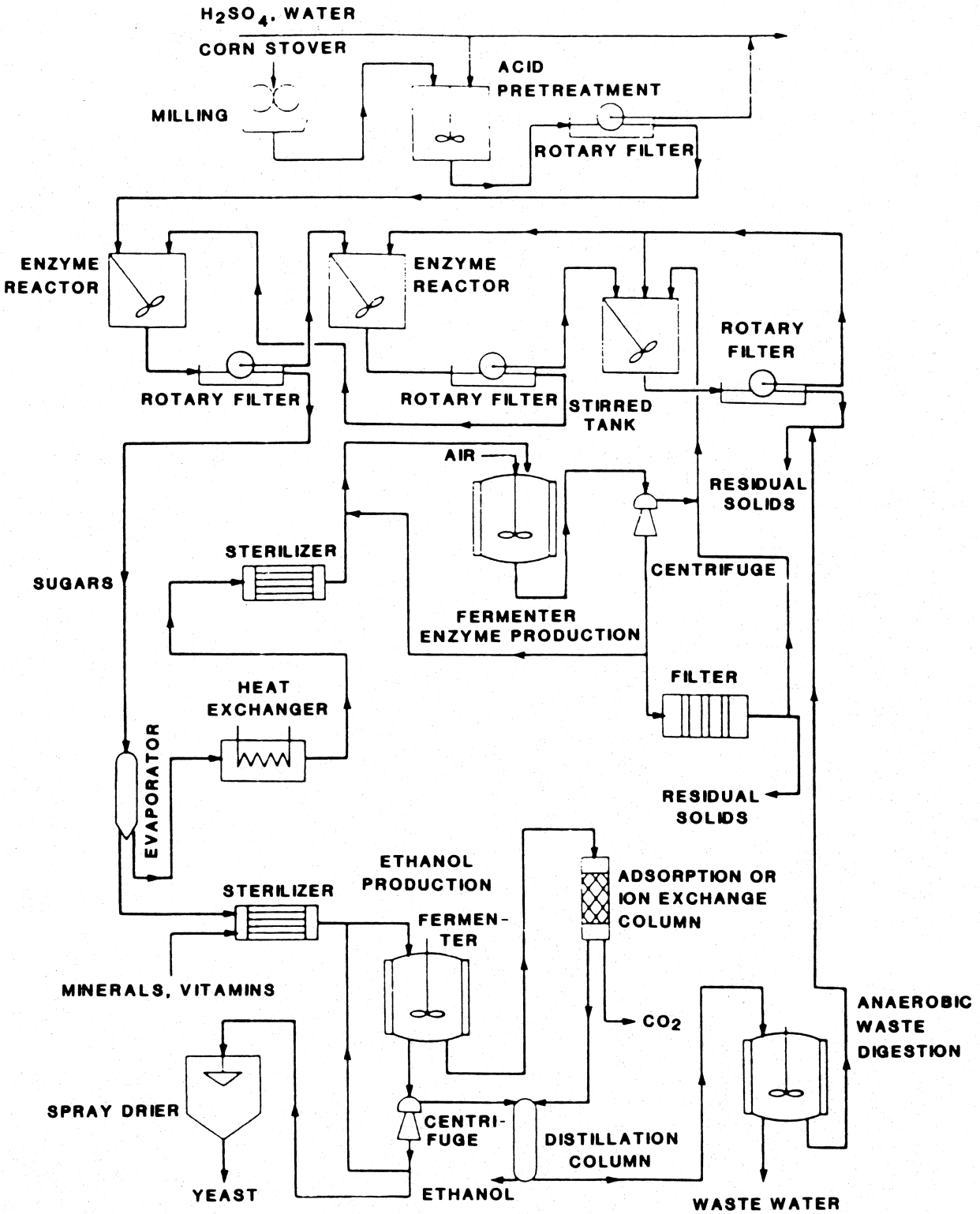

Fig. F-2. Equipment used in the production of ethanol from corn stover. Source: B. Atkinson and F. Mavituna, Biochemical Engineering and Biotechnology Handbook, Nature Press, London (1983).

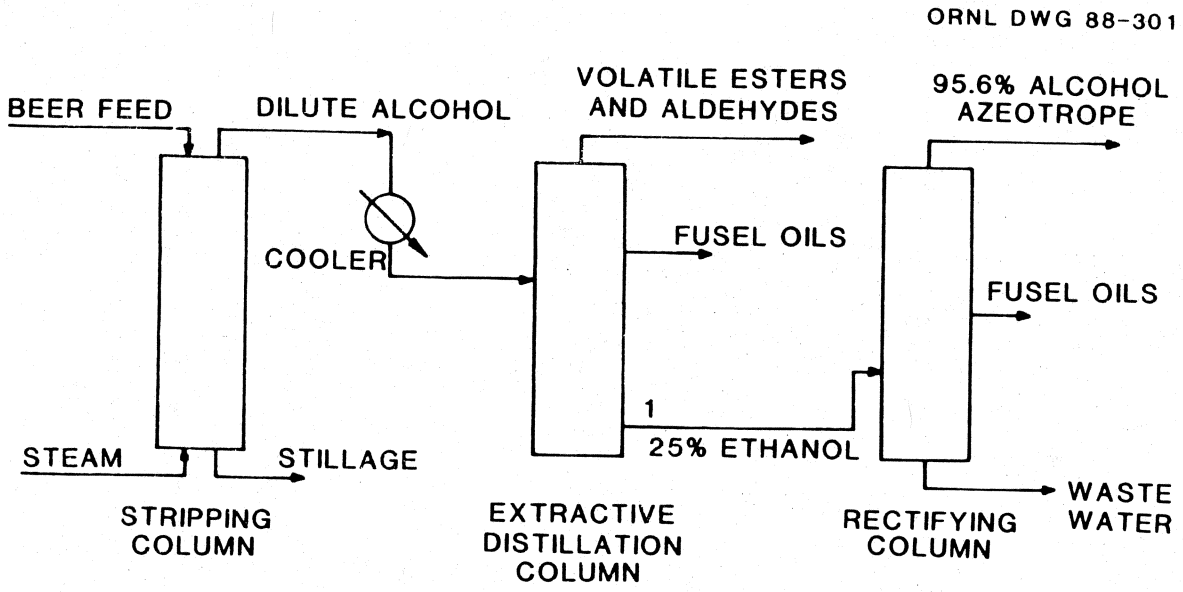

Fig. F-3. Purification equipment for 95% ethanol distillation. Source: B. Atkinson and F. Mavituna, Biochemical Engineering and Biotechnology Handbook, Nature Press, London (1983).

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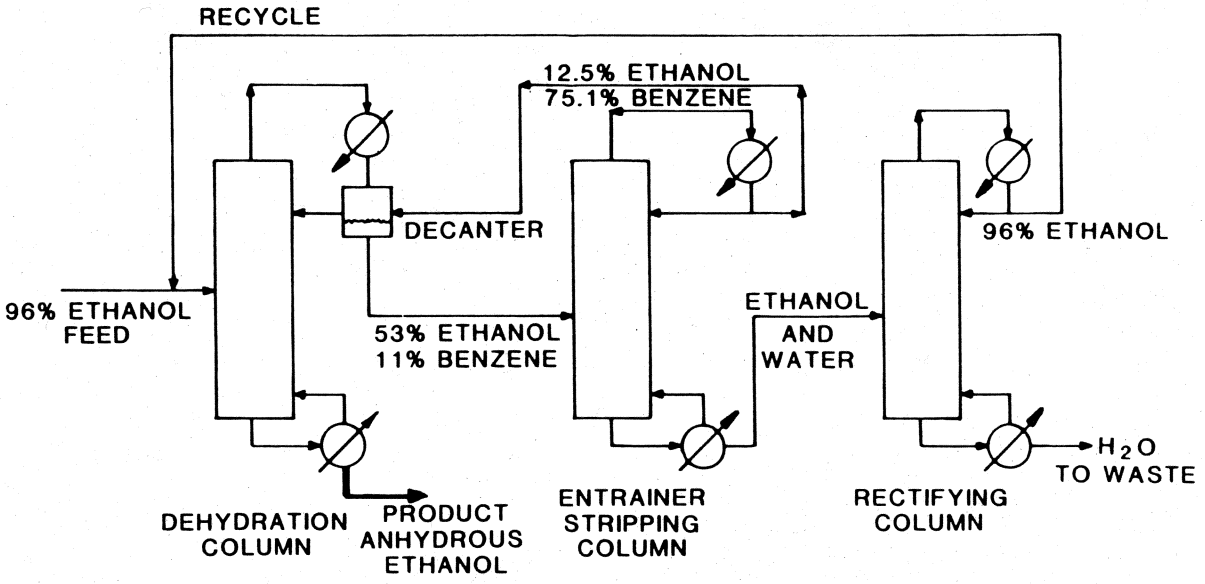

Fig. F-4. Purification equipment for anhydrous ethanol production. Source: B. Atkinson and F. Mavituna, Biochemical Engineering and Biotechnology Handbook, Nature Press, London (1983).

Purification equipment includes filtration, evaporation, crystallization, centrifugation, and drying. Extracellular polysaccharides such as xanthan gum, dextran, and Zanflo, are produced in batch fermentations followed by precipitation, solvent extraction, and combination of these. The fermentations require oxygen, heavy duty agitation due to the high viscosity of the broth as the fermentation proceeds, but very little heat exchange. The fermentation of microorganisms utilizes stirred, jacketed, sparged fermenters, followed by centrifugation, extrusion, drying, and leaching. Cyanocobalamin (vitamin B-12) is produced in a submerged, aerobic, stirred fermentation followed by high-speed centrifugation, heat treatment to release the product, ion exchange, and activated carbon adsorption. Another separation method uses an extraction with phenol or other solvent and precipitation or crystallization. Riboflavin (vitamin B-2) is produced by fermentation performed at about 29°C with agitation and aeration in a jacketed fermenter. The product is recovered by heating the broth followed by centrifugation, chemical reaction, and filtration or adsorption and elution (all very adaptable and necessary for most antibiotic production procedures). Glutamic acid is another organic acid produced by fermentation, which is very oxygen-transfer-dependent. The fermentation of glutamic acid and penicillin are very similar, and both fermentations can and do use the same fermentation and product-recovery equipment.

APPENDIX G

Enzyme Production Technology

Appendix G. ENZYME PRODUCTION TECHNOLOGY

Enzyme production is based on the fermentation of a microorganism to produce the complex enzyme which is then purified by various processing steps to yield a stable preparation. Semisolid or liquid media are used, but only the liquid submerged process is applicable for the discussion of antibiotic production in enzyme equipment. The process flowsheets shown in Figs. G-1, G-2, and G-3 detail the various equipment including the fermenter and associated culture starting tanks, sterilization equipment, feed tanks, sterile air supply, and the filtration and purification equipment for different enzymes. The separation and purification equipment is very much the same as that found in an antibiotic production facility, and very little change would be required for the plant to produce certain of the antibiotics on short notice. The basic differences are in the various solvent extraction requirements and equipment, which in an enzyme process involve only a single- or two-stage extraction, compared to the multiple-stage, multiple-solvent processing required for most antibiotic production schemes. Enzymes are usually 1 to 2 orders of magnitude larger than antibiotics, giving rise to the different separation schemes used for enzymes such as ultrafiltration, chromatography, and dialysis.

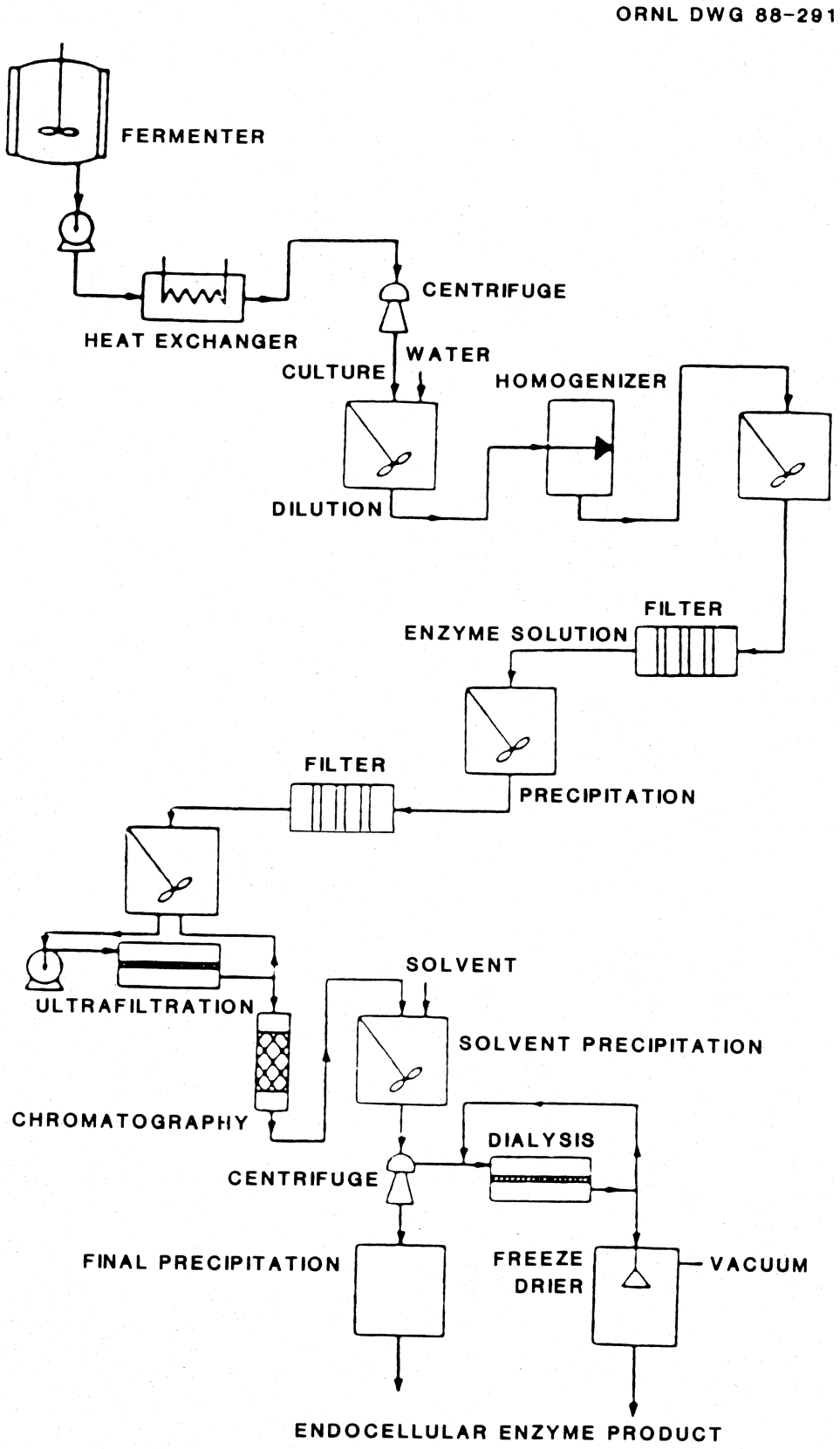

Fig. G-1. Intracellular enzyme purification flow sheet. Source: B. Atkinson and F. Mavituna, Biochemical Engineering and Biotechnology Handbook, Nature Press, London (1983).

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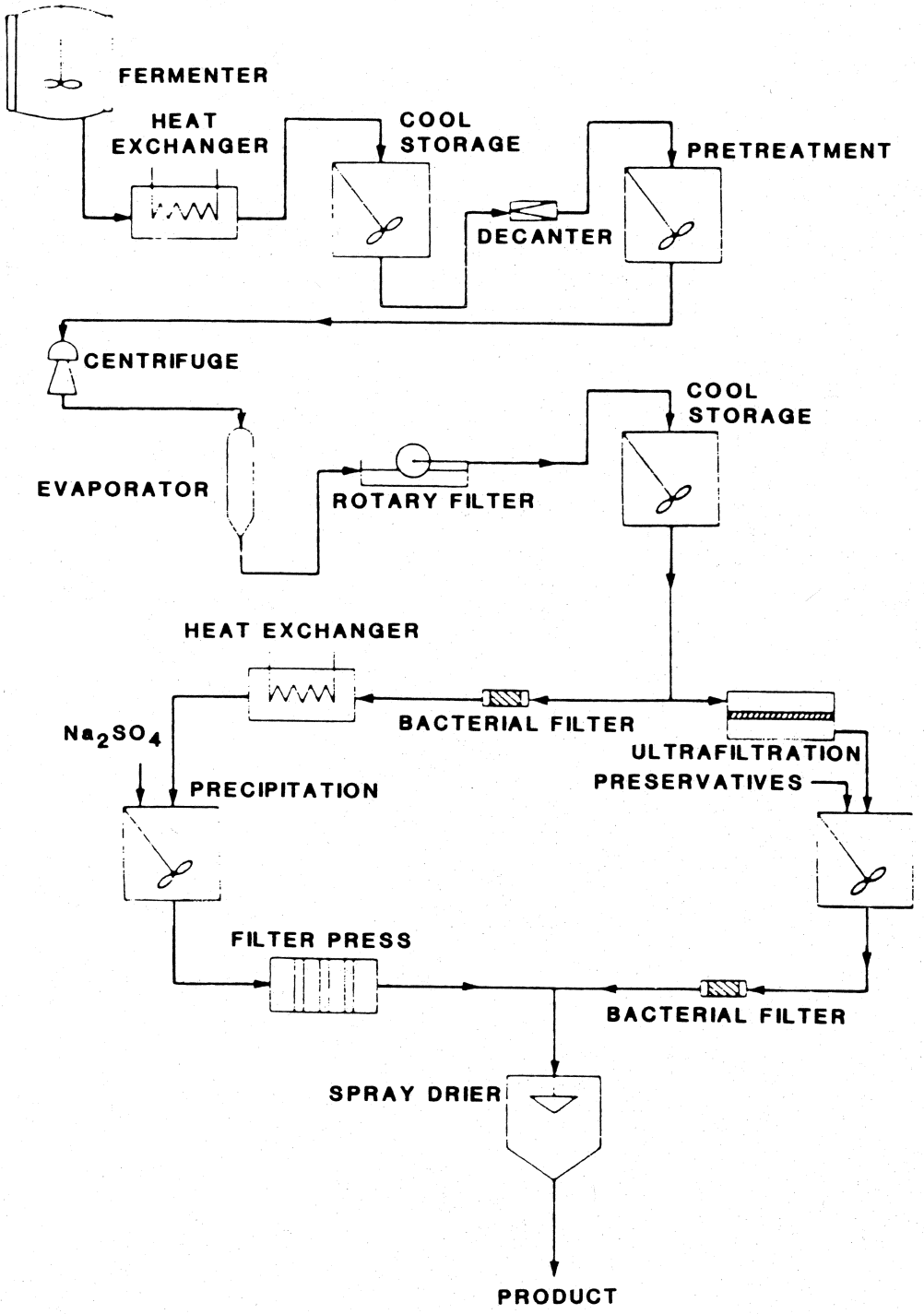

Fig. G-2. Alternate enzyme recovery processes.
Source: B. Atkinson and F. Mavituna, Biochemical Engineering and Biotechnology Handbook, Nature Press, London (1983).

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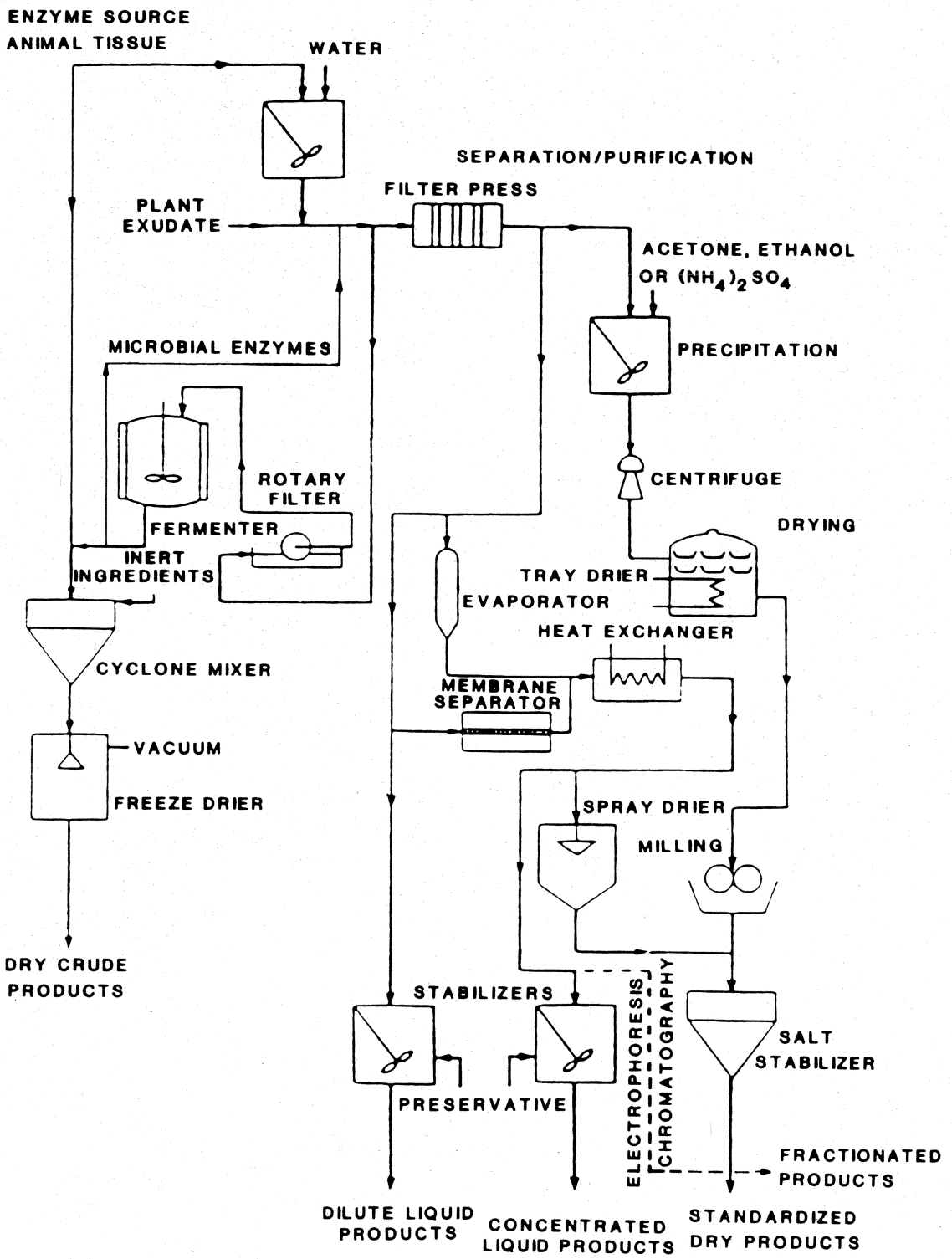

Fig. G-3. Common commercial enzyme process.
Source: B. Atkinson and F. Mavituna, Biochemical Engineering and Biotechnology Handbook, Nature Press, London (1983).

APPENDIX H

Single Cell Protein Production

Appendix H. SINGLE-CELL PROTEIN PRODUCTION

Single-cell proteins are various groups of microorganism that have been considered for food or feed use, including algae, bacteria, yeasts, molds, and higher fungi. The dried cells of these organisms are collectively referred to as single-cell proteins (SCP). In the early 1960s, many major oil companies began researching the possibility of using microorganisms to upgrade refinery streams with the aim of reducing the cost of refining. Certain groups of microorganisms are capable of selectively consuming specific components from these streams which could then be returned to the refinery for further processing. The resulting protein-rich biomass (yeast and bacterial cells) might serve as a source of animal feed or even as a potential feed source to supplement agriculturally produced human foods.

The Pure Culture Products SCP plant in Hutchinson, Minnesota, produces 15.0 million lbs/yr of SCP for human consumption. The plant has FDA approvals for the product (torula yeast, *Candida utilis*) and utilizes a pure culture continuous fermentation under sterile conditions. The entire plant operates under aseptic conditions with facilities for steam sterilization and clean-in-place (CIP). The main components of the plant consist of facilities for raw materials storage and preparation, nutrient sterilization, fermentation with two 20,000-gal continuous pressurized state-of-the-art fermenters, facilities for air compression and sterilization, a chilled water system for fermenter and product cooling, and a separation and product recovery system. Figure H-1 presents a block diagram of this manufacturing facility.

The chilled water system utilizes 10°C-year-round well water for a major portion of the cooling duty, thus saving a great deal of energy. The heat transfer facilities consist of the fermenter cooling baffles, plate and frame heat exchangers for the cooling system, and tri-pipe exchangers for the pasteurization system. The separation system uses centrifuges to separate the microorganisms from the fermentation broth and a large spray dryer for drying the final product. In addition a 540-unit Romicon ultrafiltration unit is in

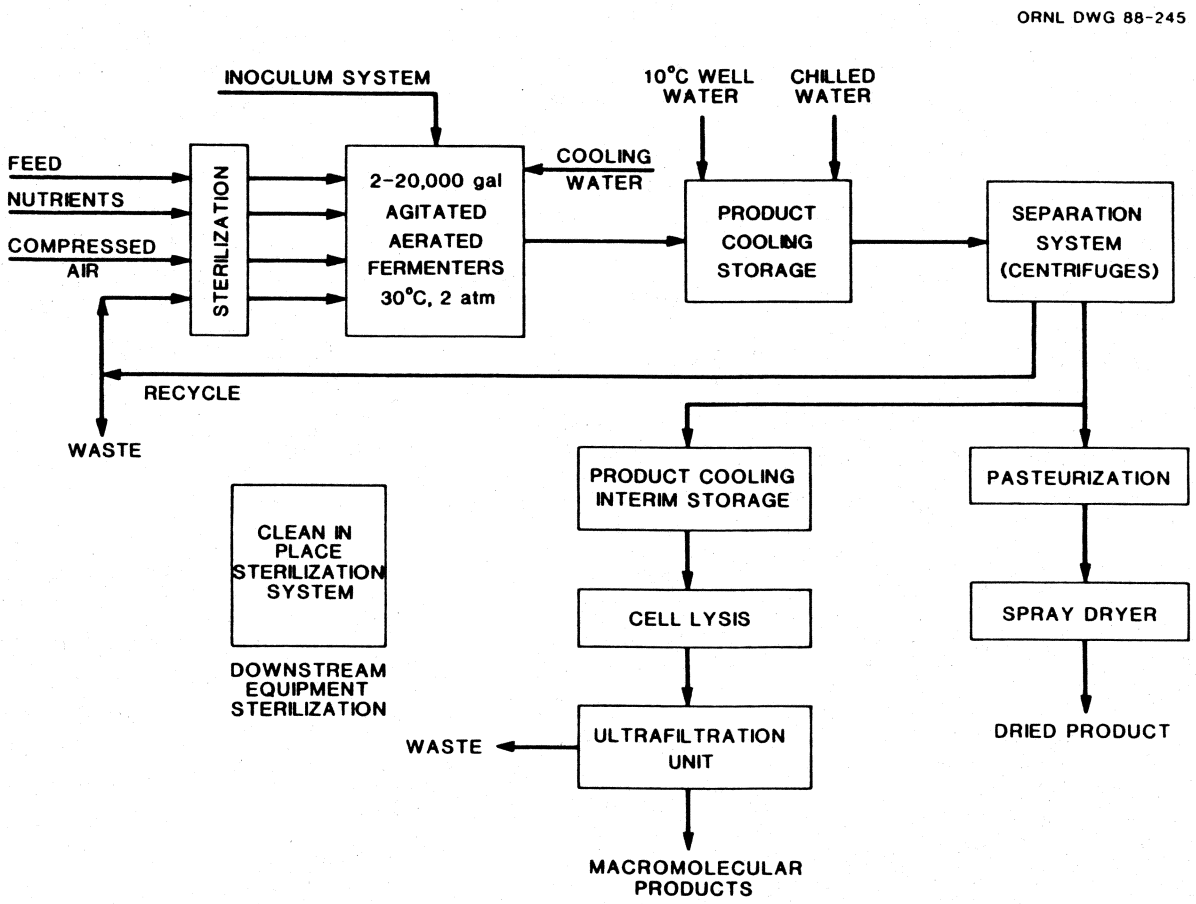

Fig. H-1. Block diagram of the Hutchinson, MN, SCP production facility.

place for the recovery of macromolecules (like antibiotics) from the autolyzed fermentation broth.

The fermentation system operates under highly aerobic conditions with hydraulic drives and turbine agitators to ensure high oxygen transfer rates. The process is highly exothermic and operates at 30 °C with 10°C chilled water circulation for temperature control. The fermenter is sterilized with 121°C, 15-psig steam and has operated for up to 75 days under aseptic conditions while maintaining a pure culture. The facility can also be operated in a batch mode as required for various antibiotics production processes.

The Hutchinson plant was designed to also serve as a research facility and thus has a complete fully equipped laboratory with state-of-the-art analytical capabilities that would be required for antibiotic production. The plant is adjacent to a small river which would facilitate waste removal. The surrounding farming area would be capable of providing most of the raw materials required for antibiotic production.

APPENDIX I

Inoculum Preservation

Appendix I. INOCULUM PRESERVATION

The preservation of production strains of antibiotic-producing organisms over a long period is a basic requirement for a practical fermentation. Not only their survival but also their capability for product formation must by preserved. High-yielding strains have often been damaged in primary metabolism during the strain selection process, and they frequently degenerate during successive transfers. The objective of preservation is to maintain strains as long as possible without cell division. The three most common techniques for preservation are low temperature (2 to 6°C), frozen (-18°C or-80°C in freezers or -196°C under liquid nitrogen), and lyophilization (freeze-drying) with the addition of protective agents such as skim milk or sucrose. Lyophilization is the preferred method for long-term storage.[20]

The preserved culture is revived by growth in a shake flask liquid culture or on solid medium for spore formation. Standard growth times for cultures are

Lyophilized cultures	4-10 days
Frozen cultures	
Bacteria	4-48 hours
Actinomycetes	1-5 days
Fungi	1-7 days
Refrigerated cultures	
Bacteria	4-24 hours
Actinomycetes	1-3 days
Fungi	1-5 days

Following the first flask culture, a second series of shake cultures is made in more flasks from the first flask. For a spore crop, the culture can be cultivated in specially devised liquid media, on agar, or on a solid substrate in 2- to 10-liter glass vessels for 8 to 24 days on bran, peat, rice, or barley, with sterile aeration and daily shaking. The total spore culture is suspended with a surface-

active agent like Tween 80® and transferred to the fermenter. Then, depending on the volume of the production fermenter, fermenter precultures must be made. During all steps, the nutrient medium, temperature of growth, inoculum age, and induction or repression phenomena must be controlled for optimum production. Culture transfers to the larger fermenters should be made while the culture is in the log growth phase to minimize culture lag time at the next stage.

Simple apparatus are available for preservation of cultures by freeze drying, from companies like Virtus, HETO, Labconco, and Stokes Div. of Pennwalt. Figure I-1 shows the survival of a culture of of *Mycobacterium tuberculosis* var. BCG versus the temperature of the freeze drying. The temperature vs time profile is important to minimize damage to the cells in both the freeze and thaw processes. The freezing rate should be about 1°C/min down to -20°C, after which the rate should be as fast as possible down to the storage temperature which should be below -55°C. The freeze drying should take place at a constant temperature below -30°C. Thawing should be as rapid as possible. The cultures should be kept down to small volumes and in containers which allow for rapid heat transfer, (2.0-ml thin glass ampules are recommended). The electrolyte content of the suspending medium should be kept low. The addition of glycerol (10 to 20%), glucose (10%) or sucrose (10%) to the suspending medium will aid cell survival. Temperature fluctuations should be avoided during storage. If the storage temperature is permitted to rise and fall above -20°C, the cells will suffer extensive damage.

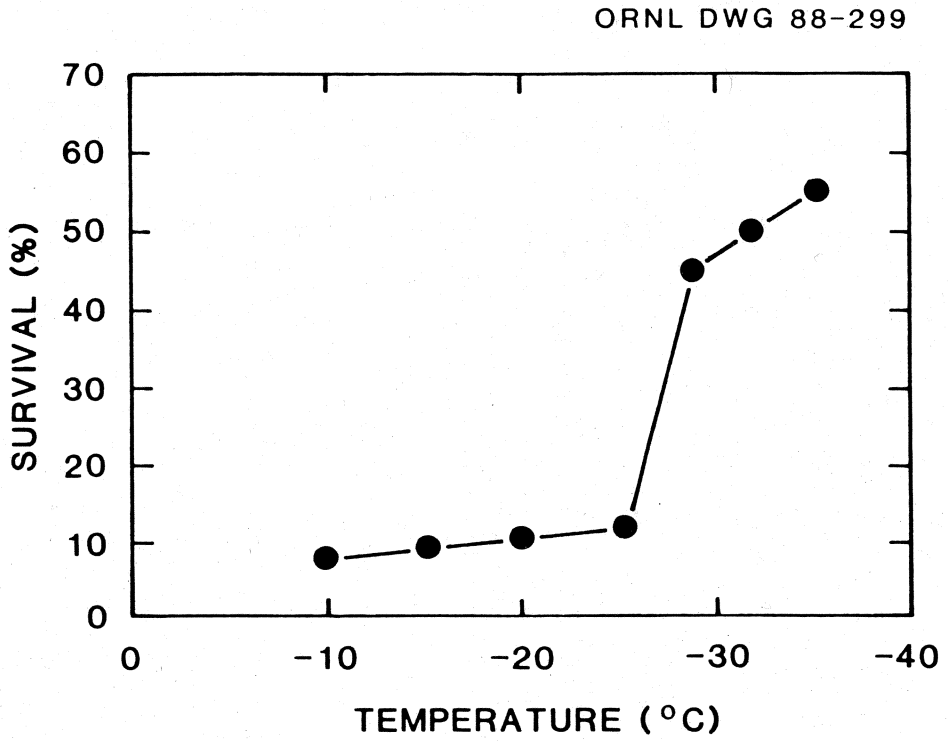

Fig. I-1. The effect of drying temperature on survival of freeze dried cells. Source: P. W. Muggleton, "The Preservation of Cultures," Progress in Industrial Microbiology, 4 191 (1962).

APPENDIX J

Sterilization

Appendix J. STERILIZATION

This appendix will discuss three areas of sterilization which are vital to the successful operation of an antibiotic plant—the sterilization of air, medium, and process equipment.

Sterilization of air implies the complete removal or destruction of all viable particles ranging in size down to the smallest bacterial cell, including bacteriophage and large viruses (0.5-micron). Only three methods have been applied to the sterilization of air in commercial fermentation processes. These are heat, filtration through fibrous media, absolute filter media, and granular media. The most commonly used method to clean intake air is filtration through beds of fibrous material such as glass wool and slag wool.

Figure J-1 shows the general process flow sheet for piping a fermenter with an air filter. Many kinds of fibrous material can be packed into the filtration vessels. Among the more common are cotton wool, slag wool, glass wool, and asbestos/cellulose fibers. The bed depth should be at least 2.5 to 7.5 cm (usually more, and up to 2 to 3 m deep for some commercial fermentations), and it should be designed to produce a superficial velocity in the bed of 15 cm/s (0.5 ft/s). The fiber diameter should be below 6 microns. This will produce filtration efficiencies in the range of 99.7 to 100.0% after 2 to 5 hours of operation. Filters can be regenerated every 2 to 5 hours by sterilizing with "dry" heat or steam at 160 to 180°C for 2 hours, but they usually are regenerated only at the start of each batch along with the fermenter.

Granular material, especially activated carbon and alumina, can be used to sterilize air. Particle sizes are typically in the 30- to 50-mesh range with filtration bed pressure drop up to 10 psi. The efficiency will depend on the bed depth, air velocity, and degree of contamination of the air. In some plants, after every 2 to 5 hours of operation, the filtration bed is taken off line and sterilized with dry heat. In other plants, the bed is sterilized only before the fermentation begins. The filtration mechanism for either granular

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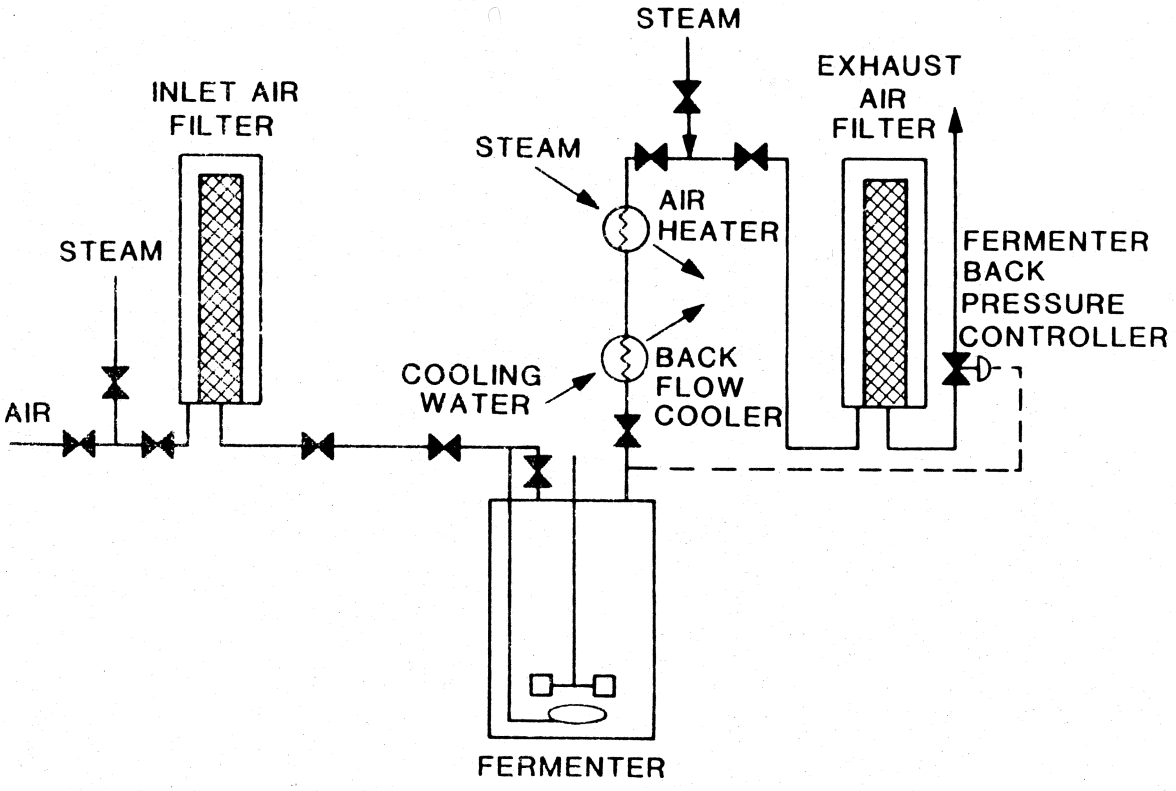


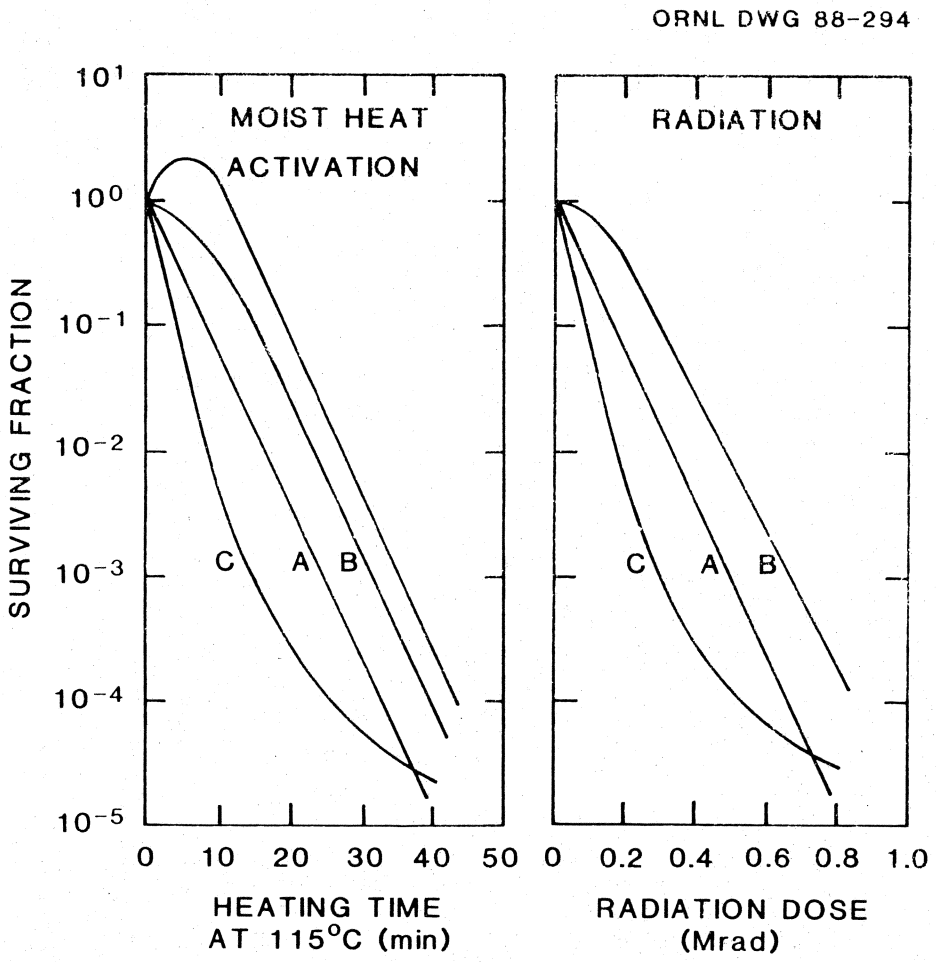
Fig. J-1. Piping diagram for fermenter air filtration.
Source: G. B. Cherry and S. D. Kemp, "The Sterilization of Air," Progress in Industrial Microbiology, 4 37 (1963).

or fibrous bed is a combination of several mechanisms operating simultaneously: (1) diffusion effects due to Brownian motion, (2) electrostatic attraction between particles and the bed material, (3) direct interception of a particle by the bed material, (4) interception as a result of inertial forces acting on the particle and causing it to collide with the bed material, and (5) settling or gravitational effects.

Sterilization of air by heating is technically feasible and is probably the most certain method; however, it is often not applied for economic reasons. At temperatures as low as 170 to 200°C, there is an approximate exponential relation by which the time taken to kill microorganisms decreases as the temperature to which they are exposed increases. At 300°C, a 3-second exposure is sufficient.

The medium and equipment are normally sterilized with heat by using steam. Sterilization with steam is a time vs temperature optimization, as shown in Figure J-2 which presents several time-survival curves for steam sterilization. Usually 121°C for 15 minutes is sufficient, giving a probability of finding one viable spore in 1000 sterilizations if each started with 10^5 spores. The alternative cycle of 115°C for 30 minutes is only marginally adequate due to an F_0 Factor of 7.5 (unit of lethality in the food industry equivalent in minutes at 121°C of all heat considered with respect to its capacity to destroy spores or vegatative cells of a particular organism) giving the probability that 50% of the 1000 batches above would have a viable spore. The fermentation broth medium is normally sterilized in the fermenter before introduction of the inoculum. Figure J-3 gives the two most common methods for controlling a sterilization unit. Sterilization of the main fermenter is accomplished with 145°C steam with a total turn around time of 10 hours.

Continuous sterilization of culture medium is practiced for adding nutrients to ongoing fermentations and for heat sensitive nutrients. Continuous sterilization can be done by injection of steam or by using heat exchangers. Sterilization with steam injection (Figure J-3) is done by injecting steam into the nutrient solution



A = LINEAR CURVE - CHARACTERISTIC OF MOST ORGANISMS

B = CURVE WITH SHOULDER FOLLOWED BY LINEAR REGION - CHARACTERISTIC OF MANY BACTERIAL SPORES

C = STEEP INITIAL CURVE FOLLOWED BY SHALLOWER CURVE AT LONGER TIMES

ACTIVATION = OCCURS WHEN SPORES ARE GERMINATED AT SHORTER TIMES (MOIST HEAT ONLY)

Fig. J-2. Survival curves for sterilization of spores. Source: W. B. Hugo and A. D. Russell, Pharmaceutical Microbiology, Blackwell Scientific, London (1977).

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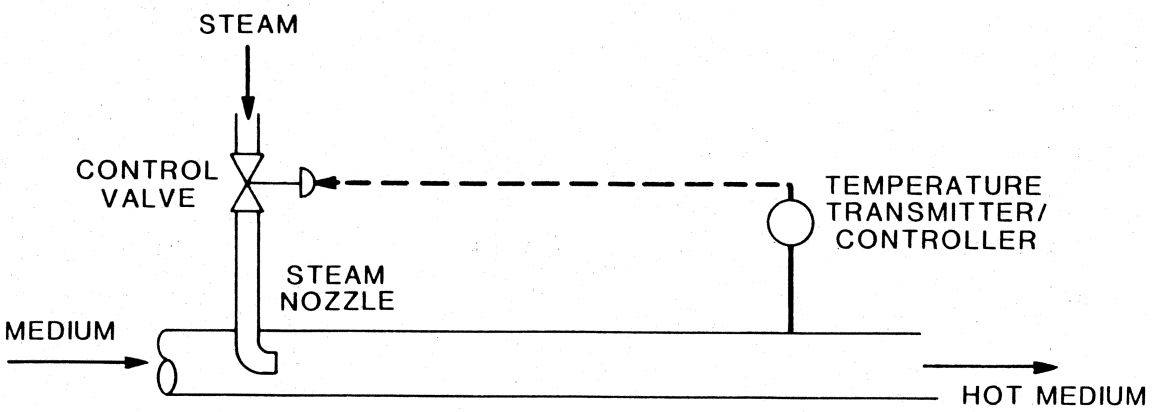
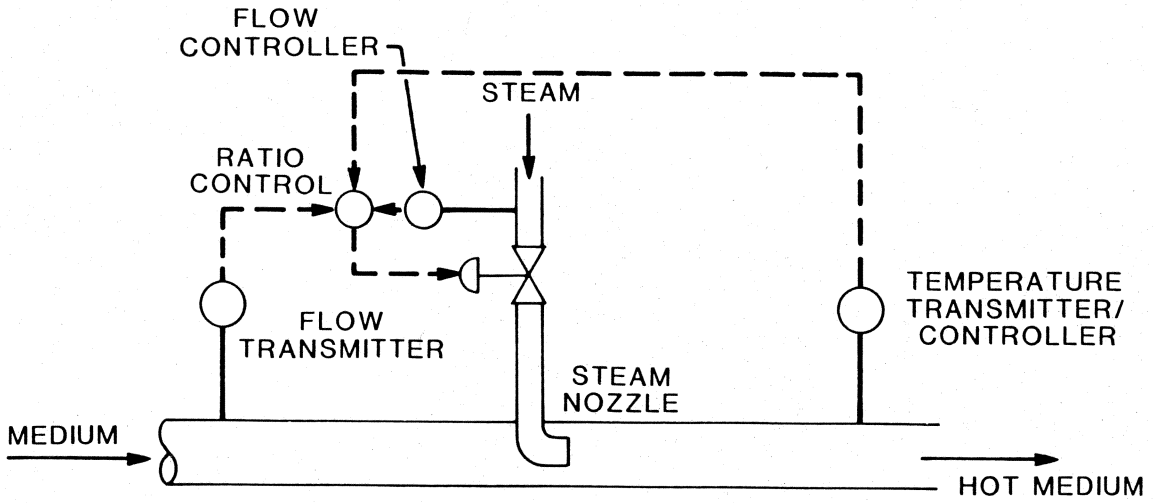


CLOSED LOOP CONTINUOUS STERILIZER CONTROL SYSTEM



FEEDFORWARD-FEEDBACK, CONTINUOUS STERILIZER CONTROL SYSTEM

Fig. J-3. Continuous steam sterilization of medium feed.
Source: G. R. Wilkinson and L. C. Baker, "Modern Trends in Steam Sterilization," Progress in Industrial Microbiology 5 231 (1964).

where the temperature is quickly raised to 140°C and maintained for 4 to 120 seconds. The added moisture is then removed by vacuum; however, the process is sensitive to changes in viscosity of the medium and to pressure variations. The procedure for using heat exchangers is shown in Figure J-4. Over 90% of the energy input is recovered, and the whole process takes only 3 to 4 minutes. Some deposits do form on the heat transfer surfaces and are removed by cleaning agents and the heat exchangers are resterilized.

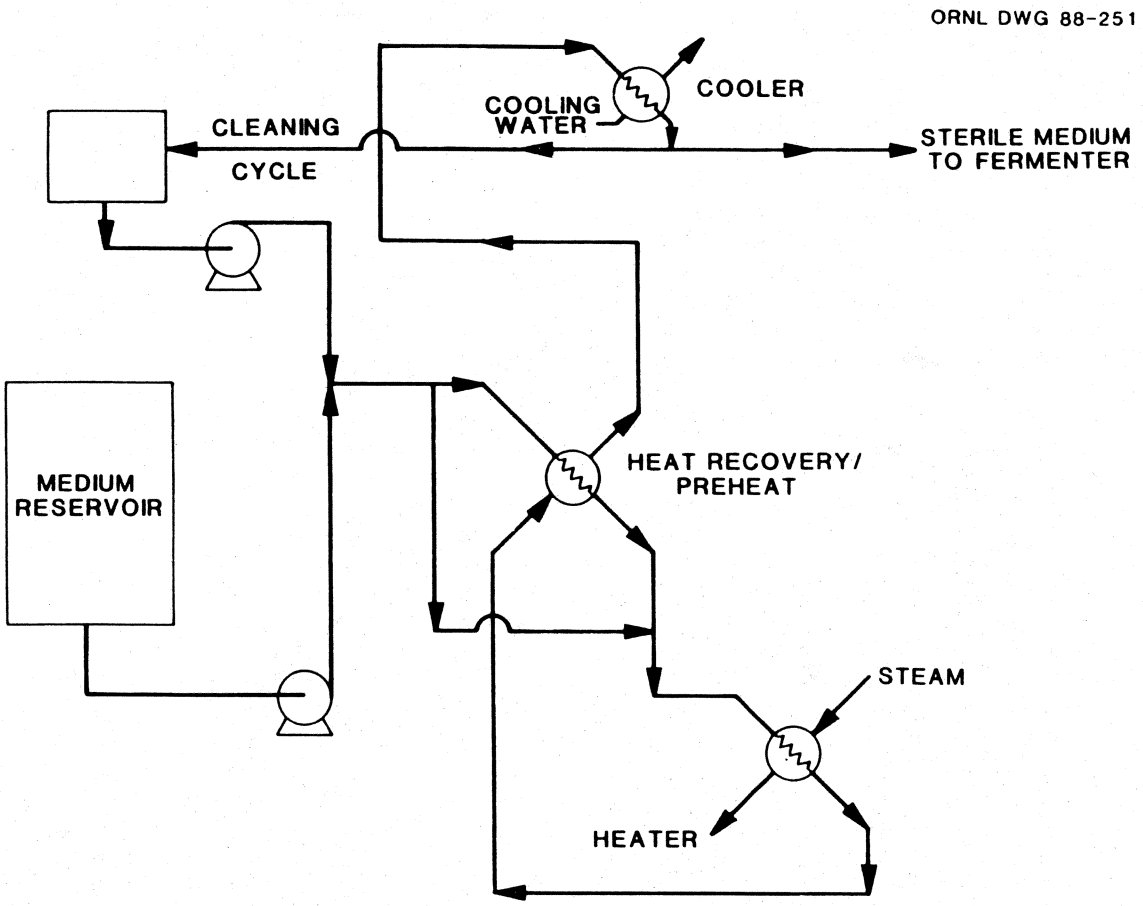


Fig. J-4. Continuous sterilization using heat exchangers. Source: G. R. Wilkinson and L. C. Baker, "Modern Trends in Steam Sterilization," Progress in Industrial Microbiology 5 231 (1964).

APPENDIX K

Equipment

Appendix K. EQUIPMENT

Antibiotic production equipment

This section addresses the specialized equipment required for an antibiotics plant. Specifically included are the main fermenter and auxiliary piping, aseptic metering pumps, agitator seals, and specialized instrumentation.

A conventional fermenter (Fig. K-1) usually consists of an upright cylindrical tank fitted with four baffles, a jacket or coil for heating and cooling, an air sparger, a device for mechanical agitation, and an air filter (see appendix J). Basic instrumentation consists of temperature, pH, and dissolved oxygen measurement and control devices. While fermenters are constructed from many different materials, 316 stainless steel clad vessels are almost always used in the antibiotics industry. Figure K-2 illustrates a typical piping arrangement for sampling and inoculation of a fermenter employed in the production of antibiotics.

Oxygen transfer is very important in antibiotic fermentations, and this is normally accomplished with mechanical agitation. Figure K-3 illustrates several geometric configurations for a typical fermenter. It is essential, for aseptic operation, to seal the agitator shaft as it enters the fermenter. This is usually accomplished with a mechanical seal (Figs. K-4 and K-5). The stuffing box is usually packed with graphite-asbestos and lubricated with silicone, mineral oil, or antifoam oil. Since it is difficult to sterilize the packing, there is an advantage to locating the stuffing box at least partially within the fermenter to gain better heat transfer.

Aseptic metering pumps present some problems for long-term operation. Peristaltic pumps will provide aseptic operating conditions; however, the flow is not continuous and the metering accuracy is not great. Either valved pumps with the valves operated by cams and springs, electromagnetic (mechanically linked to open and close) gear, or rotary sleeve pumps are recommended.

Three areas of instrumentation which are crucial to the operation of an antibiotic plant are dissolved oxygen concentration, pH, and

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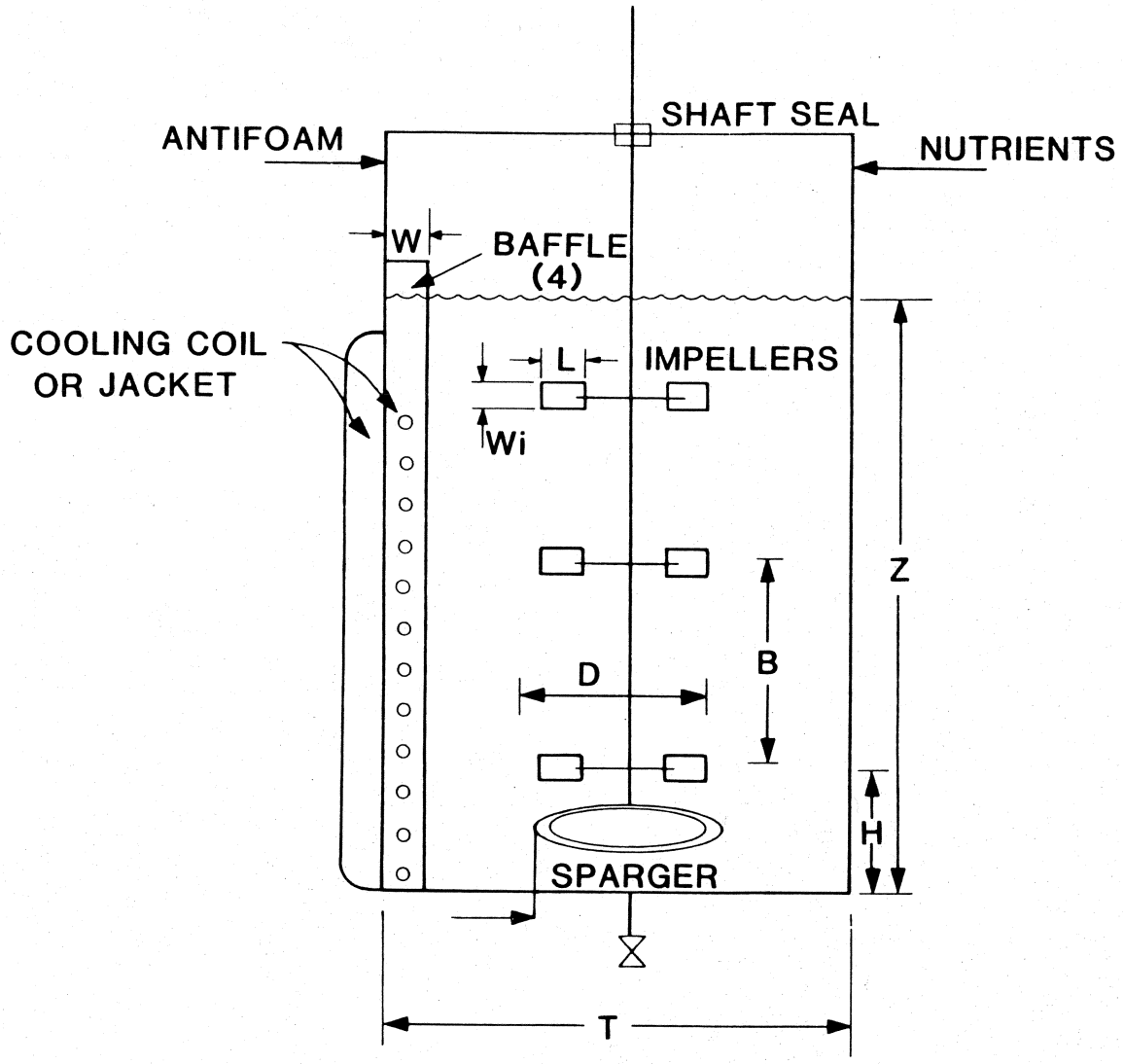

D/T= 0.30–0.50 (.33)

(UNGASSED) Z/T=1.0–2.0

L/D=0.25

Wi/D=0.2

W/T=0.08–0.12

B/D=1.0–2.5

H/D=0.5–1.0

Fig. K-1. Standard fermenter diagram.

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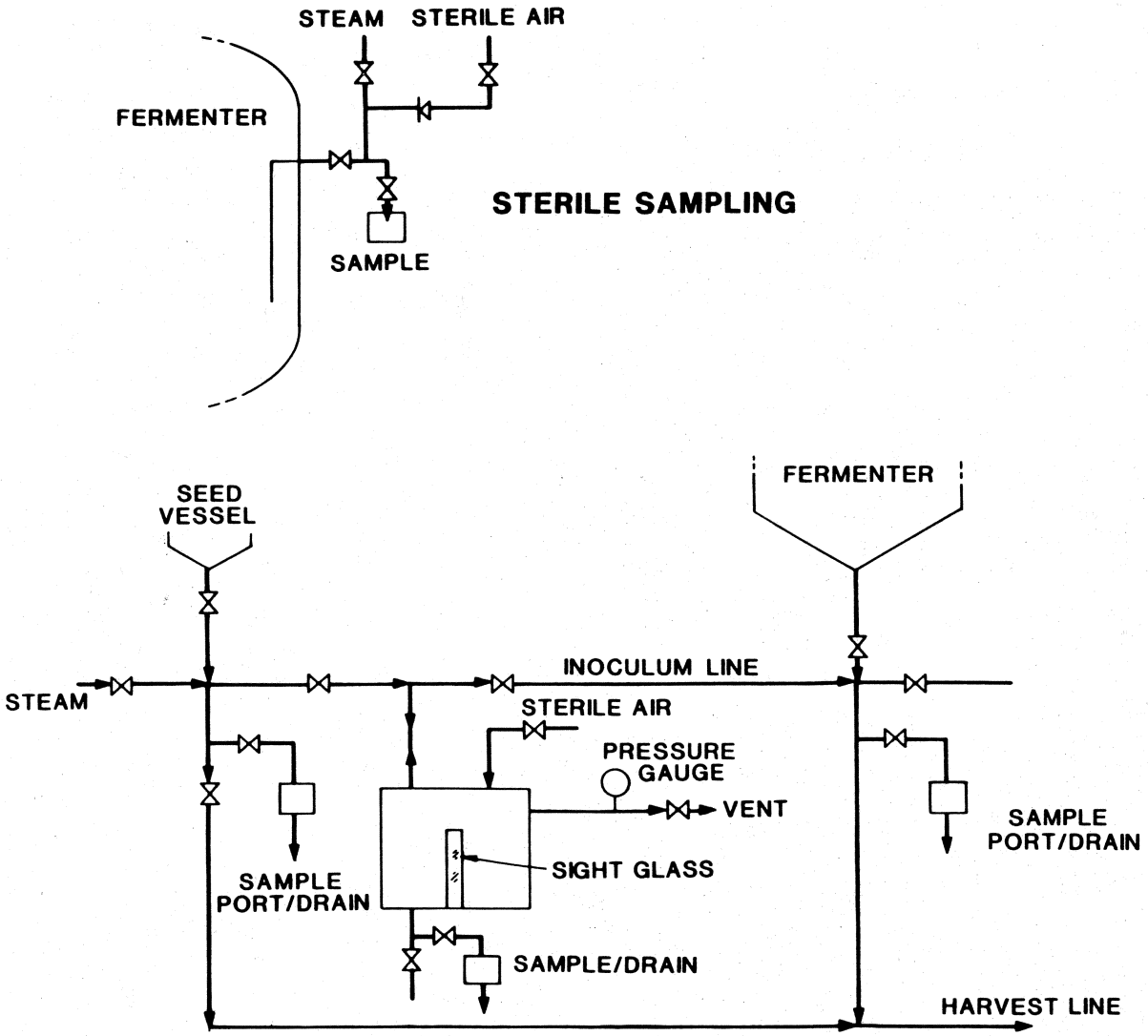

Fig. K-2. Typical piping arrangement for sampling and inoculation.

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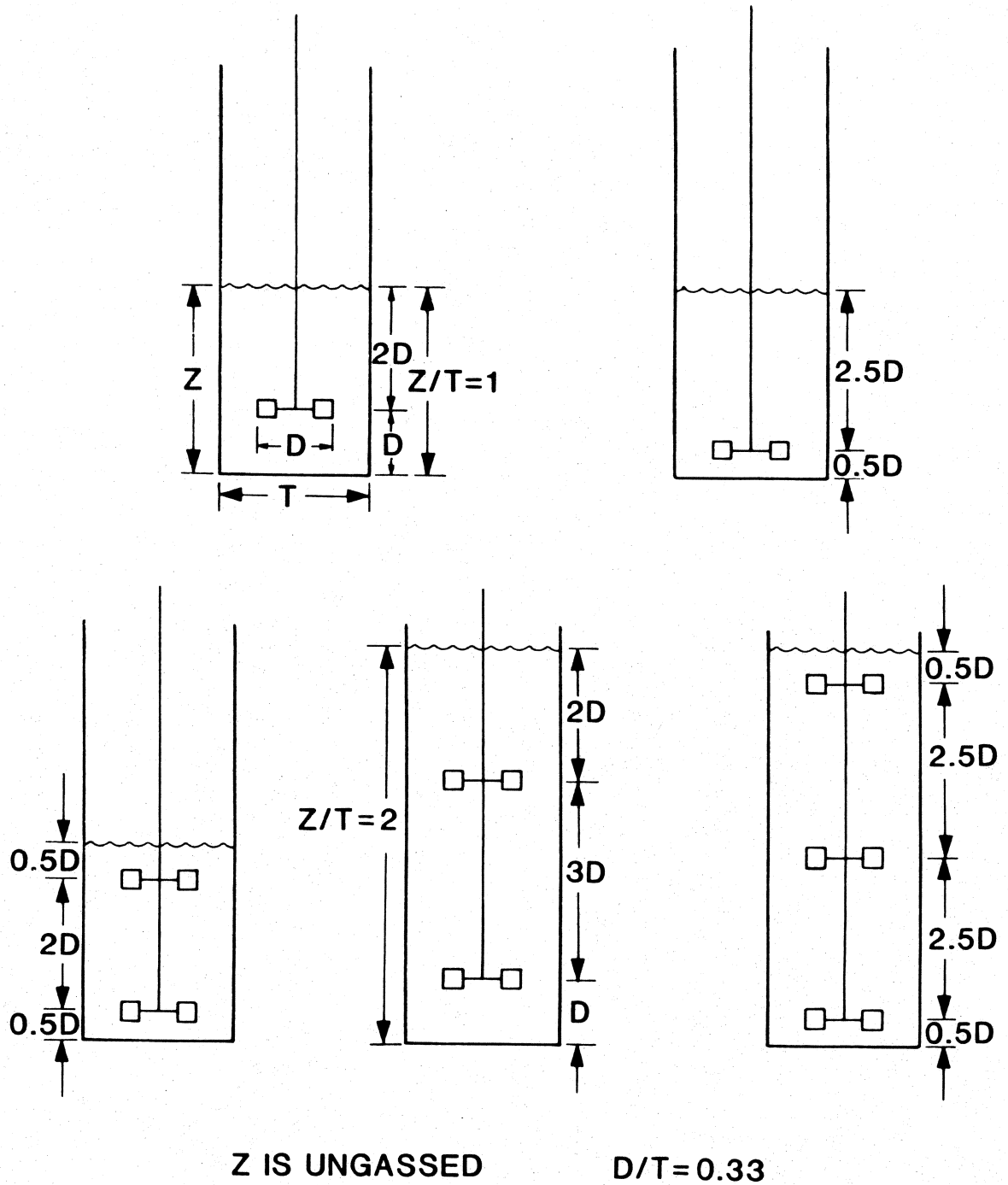

Fig. K-3. Geometric configuration for agitator placement in a typical fermenter.

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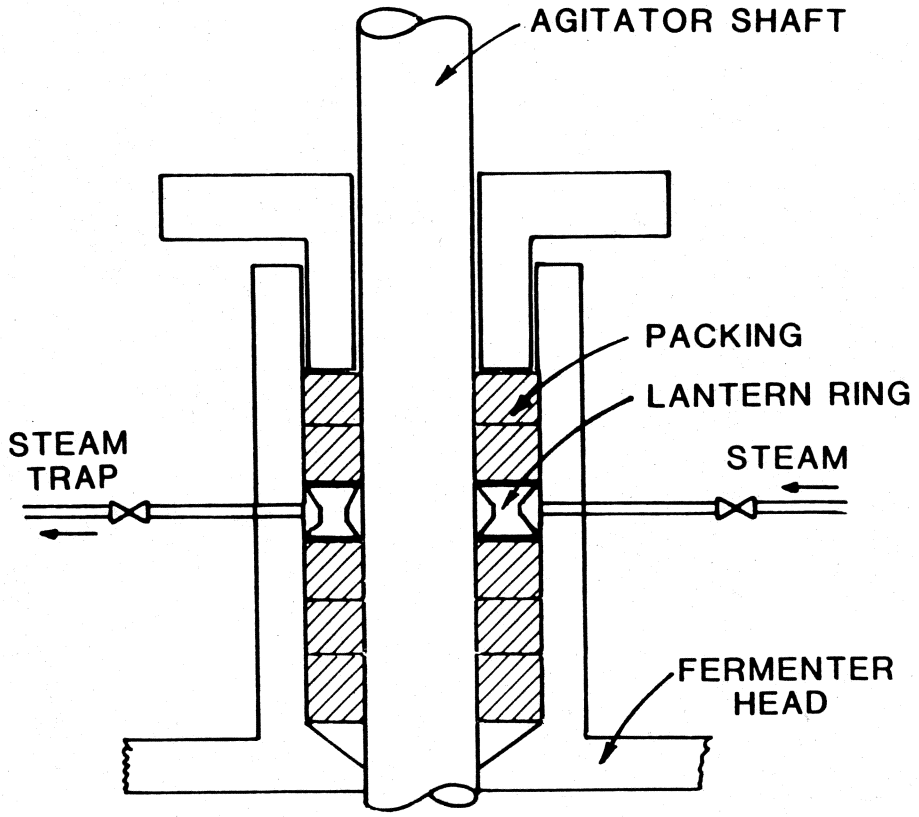

Fig. K-4. Agitator shaft seal for a fermenter.
Source: S. Aiba, A. E. Humphrey, and N. F. Millis, Biochemical Engineering, Academic Press, Inc., New York (1973).

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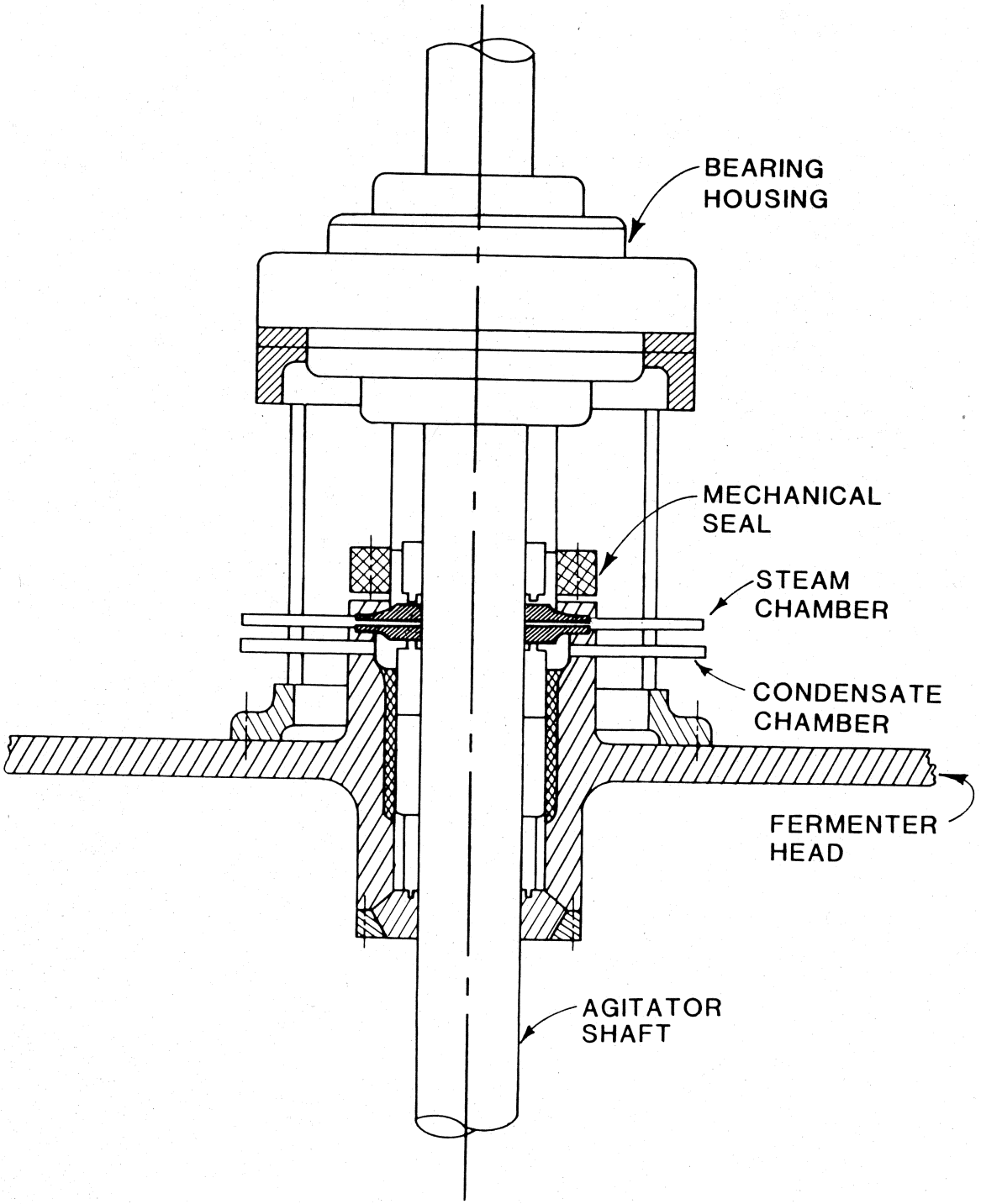

Fig. K-5. Mechanical shaft seal for fermenter agitator. Source: S. Aiba, A. E. Humphrey, and N. F. Millis, Biochemical Engineering, Academic Press, Inc., New York (1973).

temperature. There are many excellent oxygen probes on the market. Table K-1 gives a list of the manufactures. In antibiotic processes, it is desired to control the dissolved oxygen concentration at a set level. Control of the dissolved oxygen concentration at a given level can be achieved by altering the agitation rate, aeration rate, oxygen partial pressure in the influent gas or the fermenter head pressure, as indicated by the following equation:

$$N_A = K_g a(P_g - P_l)$$

where:

N_A = Rate of O_2 transfer by the fermenter.

K_g = Overall O_2 transfer coefficient.

a = Total interfacial area of gas bubbles in the fermenter.

P_g = Partial pressure of O_2 in the influent gas.

P_l = Dissolved O_2 concentration in the medium.

Temperature measurement and control are important and can be accomplished with conventional equipment plus the addition of chilling equipment for those locations which do not have cold (10°C) ground water for cooling. The importance of pH control has been well established in antibiotic fermentations. The microorganisms can secrete metabolites which will make drastic changes in the pH of the medium leading to nonproductive conditions. The control of pH is achieved by the automatic addition of acids (H_2SO_4) and/or bases (NaOH) with metering pumps. The pH-sensing electrodes that are inserted directly into a recirculating loop outside of the fermenter are used to measure the pH of the medium.

Table K-1. Suppliers of oxygen probes used in fermenters

Company	Model No.	Type	Steam Sterilizable	Oxygen Range
Cole-Parmer	J-5663-28	Galvanic	Yes	0-100%
	J-5663-00	Polarographic	Yes	0-100%
Ingold Electrodes, Inc.		Polarographic	Yes	0-100%
Beckman	777	Polarographic	Yes	0-100%
Lee Scientific	100	Polarographic	Yes	
Honeywell	55145-01,-02	Polarographic	No	
Technology Inc.	P0160L, B	Polarographic	No	0-116mm. Hg pO_2
Electronic Instr. Ltd.	A15A	Galvanic	No	0-14%
Union Carbide	1101	Polarographic	No	0-15ppm
E. H. Sargent & Co.	S-38640-10B	Galvanic	No	0-50 mg. O_2/liter
Delta Scientific	75	Galvanic	?	0-100%

APPENDIX L

Separation and Purification

Appendix L. SEPARATION AND PURIFICATION

This section will discuss two relatively new and potentially very powerful separation/purification methods for antibiotics, preparative chromatography and supercritical fluid extraction (SCF). If the size of the antibiotic manufacturing process is to be substantially reduced, the reduction will have to come in the separation and purification section of the plant which represents a large portion of a typical plant. Chromatography and SCF extraction are relatively new separation techniques being applied to the antibiotic process. Most of this work is still in the developmental stage, although some penicillins and cephalosporins are separated commercially using chromatography.

High Performance Liquid Chromatography

Figure L-1 illustrates the many different types of chromatography available. Figure L-2 presents a flow sheet for a commercial process for separating β-lactam antibiotics (i.e., penicillins and cephalosporins) using high performance liquid chromatography (HPLC). A sample chromatogram is given in Fig. L-3 for the purification of cephalosporin C (the most difficult separation for many antibiotics is the removal of derivatives and other antibiotics). Tables L-1 and L-2 summarize much of the work done on separation and purification of antibiotics using HPLC. These tables give the necessary information on the operation of the HPLC for many different antibiotics.

The disadvantages of HPLC are that it is a batch process, it causes dilution of the product, and it is difficult to scale up due to the flow dynamics in the narrow bore columns which are required. These problems were solved with the introduction of the annular chromatograph depicted in Fig. L-4.[51] This device uses the same exact resins which are used in HPLC columns except that now the material is packed into the narrow rotating annulus. The rotation is timed to be in phase with the feed rate so that each component inthe feed exits the adsorption bed at a specific point, thus making the process continuous and of much greater utility from a processing

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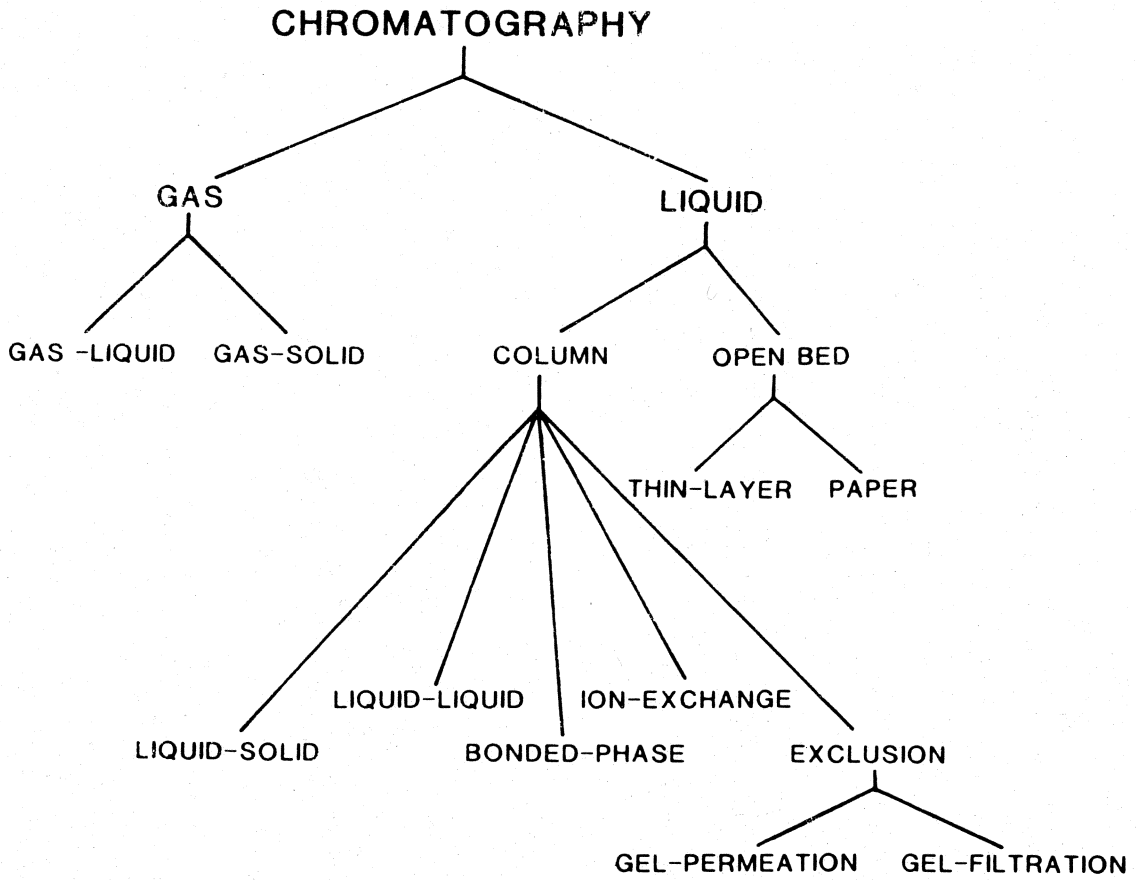

Fig. L-1. Schematic diagram showing the many types of chromatography that are in use.

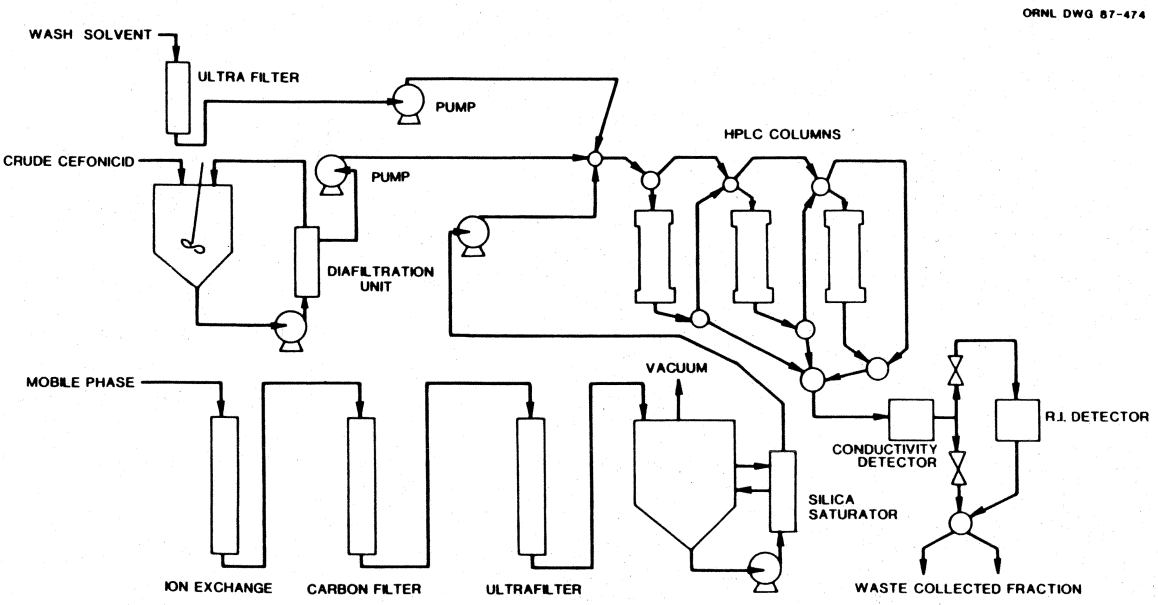

Fig. L-2. Process chromatograph flow diagram.
Source: A. M. Cantwell, R. Calderone, and M. Sienko, "Process Scale-Up of a β-Lactam Antibiotic Purification by High-Performance Liquid Chromatography," Journal of Chromatography. 316 133 (1984).

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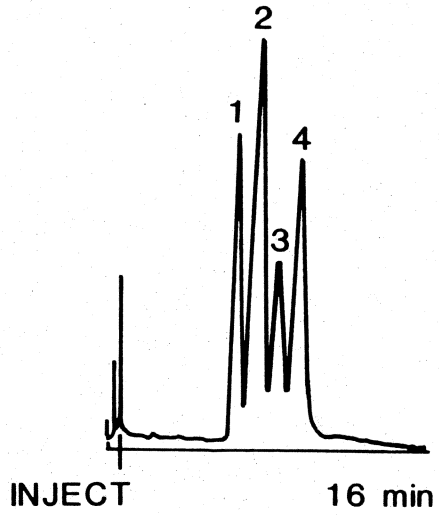
U.V. RANGE: 254 nm, 0.08 AUFS
CHART SPEED: 5 mm/min
FLOW RATE: 1.5 ml/min
COLUMN SIZE: 2x250 mm
PACKING: μ NH_2 (VARIAN AEROGRAPH)
SOLVENT: HOAc–CH_3OH–CH_3CN–H_2O(2:4:7.5:86.5)
P.S.I.: 3400



1. DEACETOXYCEPHALOSPORIN C, 1.5 μg
2. DEACETYLCEPHALOSPORIN C, 1.5 μg
3. 5-METHYCEPHALOSPORIN C, 1.5 μg
4. CEPHALOSPORIN C, 2.0 μg

Fig. L-3. Sample chromatogram for the separation of cephalosporin C.

Table L-1. Chromatographic parameters for tetracyclines

Chromatographic system and column	Support	Mobile phase	Comments
Ion Exchange	Zipax SCX	0.4M $K_3B_4O_7$, 0.01M EDTA 1% isopropyl alcohol (pH 9.8)	Poor column efficiency
		0.1M citric acid, 0.001M EDTA pH 5.2	Very poor efficiency
Adsorption Chromatography	Partisil (18μ)	Aqueous 0.08M $HClO_4$	Broad overlapping peaks
	Sil-X (13μ)	0.1M $HClO_4$, 1.9M $NaClO_4$, 0.005M citric acid-acetonitrile (2:1)	Good separation
Liquid-liquid Partition Chromatography	Corasil Coated with Polyethylene glycol 400	Dioxane-pentane (1.5:1)	Poor column efficiency Poor reproducibility
Reversed-Phase Partition Chromatography	Octadecyl silane Bonded on Partisil (18μ)	0.1M $HClO_4$, 0.4M $NaClO_4$ 0.0025M citric acid-acetonitrile (85:15 v/v)	Poor partition efficiency

Table L-2. HPLC assays of antibiotics

Drug	Extraction	Recovery (%)	Mobil phase	[a]Stationary Phase	Mode of Separation	Detection	Sensitivity (mg/liter)	Time (min)
Cefamandole	methanol-sodium acetate	---	Methanol-Sodium acetate	C-18	Partition/ Reverse phase	UV absorption	0.3	
Cefazolin	Trichloroacetic	85+	Methanol-Acetic acid	Phenylsilane	Partition/ Reverse phase	UV absorption	1.0	30
Cefuroxime	Dimethylformamide	95-100	Acetic acid-methanol-water	C-18	Partition/ Reverse phase	UV absorption	1.0	
Cephalexin	Methanol	≈100%	Methanol-water	C-18	Partition/ Reverse phase	UV absorption	0.5	
Cephaloridine	Trichloroacetic acid	98	Methanol-ammonium acetate	Phenylsilane	Partition/ Reverse phase	UV absorption	2.0	30
Cephalothin	Trichloroacetic acid	---	Methanol-ammonium acetate	Phenylsilane	Partition/ Reverse phase	UV absorption	10.0	
Cephalothin	Dimethylformamide	97+	Methanol-acetic acid	C-18	Partition/ Reverse phase	UV absorption	1.0	30
Cephradine	None	92-100	Ammonium carbonate	C-18	Partition/ Reverse phase	UV absorption	0.5	
Ampicillin/ Amoxicillin	Perchloric acid	---	Methanol-Phosphate buffer	C-8	Partition/ Reverse phase	UV absorption	0.5	15
Penicillins		---	0.05M phosphate Acetonitrile	C-18	Partition/ Reverse phase	UV absorption		16
Tetracyclines		---	55% acetonitrile/ 45% 0.05M phosphoric acid	C-18	Partition/ Reverse phase	UV absorption		5
Erythromycin	Diethyl ether	96+	Acetronitrile-Ammonium acetate-water	C-18	Partition/ Reverse phase	U Fluorometry	0.1	

[a]C-18 is Octadecyl silane bonded phase, C-8 is Octyl silane bonded phase

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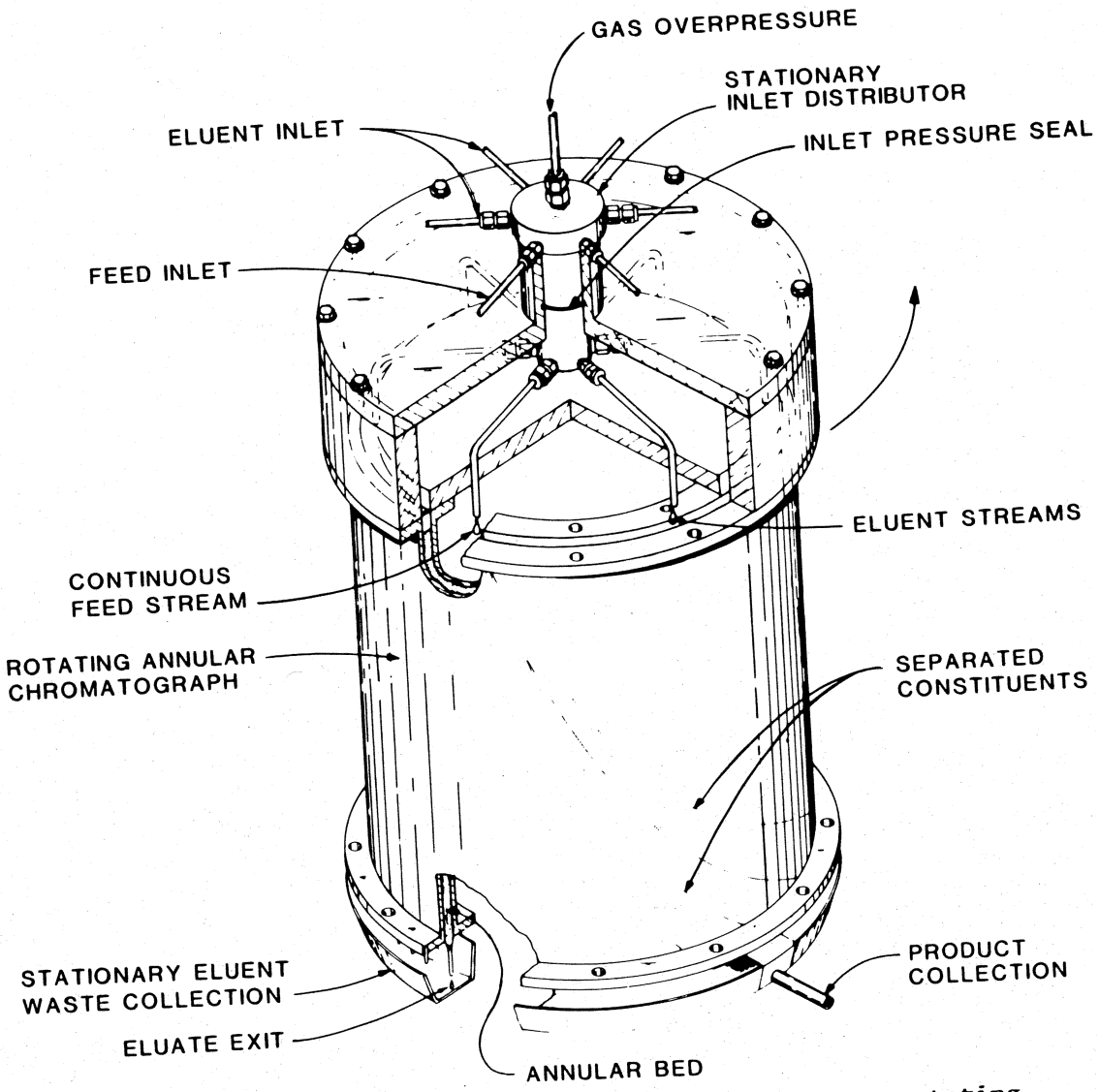

Fig. L-4. Schematic diagram of continuous rotating annular chromatograph.

standpoint. The use of an annulus for the resin instead of a packed bed eliminates the scale-up problems with bed compression as the column diameter is increased.[35] Recently the Oak Ridge National Laboratory (ORNL) has demonstrated that annular chromatography can concentrate products in addition to separating them. There is a wealth of experimental data in the literature on the selectivity of resins for antibiotic separation which has been evaluated in HPLC columns. These same data can be used with the aid of mathematical models developed at ORNL for evaluating the performance of other annular chromatographs with these same resins.[52] Annular chromatography has the potential to effect a vast decrease in the size and complexity of the separation and purification of antibiotics on a scale comparable with the great advances made in the 1940s by the submerged culture process for the fermentation of antibiotics. Several large-scale pilot units of the annular chromatograph are available at ORNL. Because these units have a very high capacity, it would be possible during a national emergency to skid-mount an annular chromatography system on a truck and simply drive it to any emergency production facility.

Supercritical Fluid Extraction

The solubility of solids in SCF was first discovered over 100 years ago. Recently SCF extraction was applied commercially to the decaffination of coffee and has received widespread interest in the pharmaceutical industry because this separation technique can be performed at low temperatures (20 to 60°C). The advantages are obvious. SCF extraction operates like distillation or liquid/liquid extraction but at lower temperatures and with milder less toxic solvents. Figure L-5 presents a crude flow sheet for the process. The separation and purification of antibiotics with SCF extraction has been patented in Germany.[41] Carbon dioxide is the solvent of choice because of its availability, nontoxicity, and proper thermodynamic properties. The solubility of most antibiotics in SCF CO_2 is low; however, if 5 to 10% of a polar second solvent is added, these solubilities can increase by one or two orders of magnitude.[53]

ORNL DWG 88-254

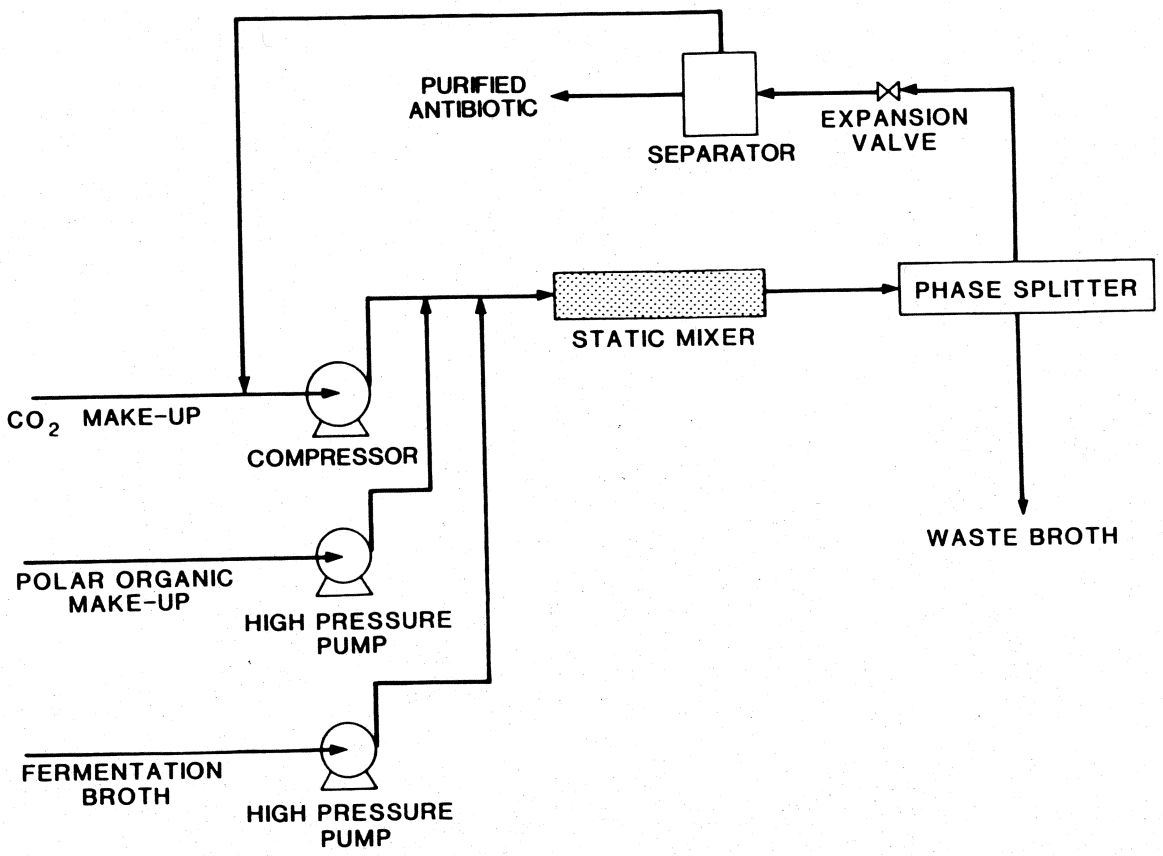

Fig. L-5. Schematic diagram for supercritical fluid extraction using CO_2.

A unique property of this process is that the dissolved substance may be separated by decreasing the fluid density by either decreasing the pressure or increasing the temperature. The key point is that a small change in temperature or pressure will result in a large change in solvent density and thus a large change in solubility. The process can be easily staged so as to provide enhanced recovery with a small expenditure of energy.[35,43] The pressures required are in the same range as the high pressure steam which is found at most industrial manufacturing facilities.

Conventional liquid-liquid-extraction, which is quite common in the antibiotic industry, often requires a difficult crystallization, a distillation and/or use of a second solvent. A solvent residue may be left on the product thus requiring further purification. With SCF extraction, the extract is separated by reducing the solvent density to the gaseous state, thereby leaving very little solvent on the product. In addition, the transport properties of SCF's are closer to those of the gaseous state than the liquid state, giving rise to enhanced diffusivities and viscosities which will minimize any mass transfer problems in the extraction. Recently, a German patent has been issued for the separation and purification of antibiotics using supercritical fluids. The major advantages would be a reduction in the number of processing steps and the elimination of the large and expensive solvent recycle system.

Many different SCF solvents are possible as extraction agents. The best solvents can often be easily identified from thermodynamic data which is rapidly becoming available in the open literature. For example, from Table L-3, the solubility of penicillin V in ethanol is over twice what it is in water (the major constituent of the fermentation broth). In the supercritical region ethylene and water are immiscible, with ethanol distributing between the two phases. It is reasonable to assume that if a SCF mixture of ethylene and ethanol were used for fermentation broth extraction, penicillin V would be concentrated in the SCF phase. An exact prediction of the final result can be made if the thermodynamic data are used to tune a mathematical model known as an equation of state.

Table L-3. SCF related physical properties of selected antibiotics

Antibiotic	Molecular Weight	Melting Point (°C)	Solubility in		
			Water	Ethyl Acetate	Ethanol
PENICILLIN V (Phenoxymethyl penicillinic acid)	350.0	124.0	9.0	>20.0	20.0
K salt	388.0	263.0	>20.0	0.65	1.34
PENICILLIN G (Benzyl-penicillin)	334.0				
Na salt	356.0	215.0	>20.0	0.40	9.97
K salt	372.0	215.0	>20.0	0.42	10.4
TETRACYCLINE	444.0	172.5	1.7	17.3	>20.0
OXYTETRACYCLINE	460.0	182.0	0.60	0.85	8.1
CEPHALOSPORIN C	415.4				
CEPHALOTHIN	382.4	160.5			
Na salt	404.4		>20.0	0.02	17.7
CEPHALORIDINE	399.0		>20.0	0.035	1.95
BACITRACIN	1421.0		>20.0	0.047	9.1

[a]Solubility in g/l at 294-301 °C

EXPEDIENT ANTIBIOTICS PRODUCTION -- 1988

Unclassified
April 1988
200 pages

by P. R. Bienkowski, C. H. Byers, and D. D. Lee
Oak Ridge National Laboratory, Oak Ridge, TN 37831
Interagency Agreement: FEMA No. EMW-84-E-1737; DOE No. 1457-1457-A1

The literature on the manufacture, separation and purification, and clinical uses of antibiotics was reviewed, and a bibliography of the pertinent material was compiled. Five antimicrobial drugs, penicillin V and G, cephalosporin, tetracycline, Bacitracin, and sulfonamide, were identified for emergency production. Antibiotics plants in the United States, Mexico, and Puerto Rico and potential alternate sites such as single cell protein, enzyme, and fermentation ethanol plants were identified.

Antibiotic-manufacturing plants contain a generic fermentation unit with specialized sterile equipment and an antibiotic-specific separation and purification unit. Feasible emergency production will require a substantial reduction in the complexity and degree of separation and purity normally required. Detailed instructions and recommendations were developed to assist State and Federal officials in directing the resumption of antibiotic production. Alternate sites require a team of skilled personnel to convert to antibiotic production in the austere environment.

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