the first 40 h of fermentation rapid cell growth is achieved with a doubling time of nearly 6 h. After a high cell density has been obtained, nutrients (glucose and CSL) and precursors are added slowly or intermittently to reduce cell growth to 0.02 h^{-1} and maximize penicillin production. Oxygen, carbon, nitrogen, and phosphate concentrations should be low. A simplified diagram of the process scheme is presented in Fig. 1.3. Culture preparation starts with lyophilized spores and agar slant cultures. Vegetative cells are cultivated in shake flasks and then are transferred to seed fermenters (10–100 l). Production fermenters are agitated tanks 200–500 m^3 in volume made of stainless steel. Mechanical agitation is provided at a rate of 100 to 300 rpm. Temperature is controlled around 25–28°C (26°C optimum) by using cooling coils. Antifoam is added to reduce foam formation. Dissolved oxygen is controlled at > 2 mg/l and pH at 6.5. Vigorous aeration is supplied from the bottom of fermenters by ring or tube spargers. Due to the high viscosity of the broth, oxygen transfer is a major problem in penicillin fermentations. In some cases, strains from pellets are preferred because the medium is less viscous and oxygen supply is improved. The fermentation is stopped when the oxygen uptake rate of the culture exceeds the oxygen transfer rate of the reactor, or when 80% of the fermenter is full.

The original process for the recovery of penicillin from fermentation broth was based on adsorption on activated carbon. After washing with water, the activated carbon was eluted with 80% acetone. The penicillin was concentrated by evaporation under vacuum at 20 to 30°C. The remaining aqueous solution was cooled to 2°C, acidified to pH = 2–3, and the penicillin extracted with amyl acetate. Penicillin was crystallized from amyl acetate with excess mineral salts at pH of 7 under vacuum. This process is uneconomical because of the high cost of activated carbon.

The current recovery process includes filtration, extraction, adsorption, crystallization, and drying. Filtration is usually achieved by using high-capacity, rotary vacuum drum filters for separation of the mycelia. The mycelia are washed on the filter and disposed. The penicillin-rich filtrate is cooled to 2–4ºC to avoid chemical or enzymatic degradation of the penicillin. In some early processes, the filtrate was further clarified by a second filtration with the addition of alum. In recent years, macroporous filters have been used in some plants for separation of the mycelia.

Solvent extraction is accomplished at low pH such as 2.5–3, using amyl acetate or butyl acetate as solvent. Continuous, countercurrent, multistage centrifugal extractors (Podbielniak D-36 or Alfa–Laval ABE 216) are used for this purpose. The distribution coefficient of penicillin G or V between organic and aqueous phase depends strongly on the pH of the medium. At a pH of 3 the distribution coefficient is about 20. Penicillins G and V degrade under acidic conditions with a first-order kinetics. To avoid degradation of penicillin during solvent extraction at low pH, temperature is kept around 2–4°C and filtration time is kept very short (1–2 min). Two extractors used in series result in nearly 99% penicillin recovery. Whole broth extraction (without filtration) is possible by using Podbielniak extractors in series. Due to operational difficulties, however, this approach is not used in practice. Carbon adsorption is used to remove impurities and pigments from penicillin-rich solvent after extraction. Several activated carbon columns in series can be used for this purpose.

Penicillin may be back extracted into water by addition of alkali (KOH or NaOH) or buffer at pH of 5 to 7.5. The water-to-solvent ratio in this extraction is usually between 0.1 and 0.2. A continuous, multistage, countercurrent extractor may be used for this purpose. This step is usually omitted in the new separation schemes.