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TECHNICAL NOTE

Technical Aspects of Extractive Purification of Penicillin Fermentation Broth by Aqueous Two-Phase Partitioning

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ABSTRACT

A new attempt was made to reform the original penicillin recovery technology, and fundamental data were provided for further scale-up. The volume of fermentation broth processed was 1000 mL, the mass of penicillin crystal obtained was 7.228 g with an average purity of 84.15%, the overall recovery ratio of aqueous two-phase partitioning (ATPP) technology was 76.56%, and the entire technical process is described. Compared to the original organic solvent extraction, ATPP technology can extract penicillin from whole fermentation broth directly. The pH should be adjusted only once and the activity of penicillin can be improved. The extraction steps are decreased from three times to one, and the extraction technology is integrated. A new field is opened up by this powerful ATPP technology.

Key Words. Aqueous two-phase partitioning; Penicillin; Liquid–liquid equilibrium; Extractive fermentation

INTRODUCTION

The antibiotics recovery technology commonly used in industry employs organic solvent extraction, ion-exchange and precipitation, etc. The

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flow sheet (1) for recovery of penicillin from fermentation broth is given in Fig. 1. Penicillin G has a half-life of 15 minutes at pH 2.0 at 20°C. The harvested broth is therefore initially cooled to 0–3°C. Finally, penicillin is crystallized out of aqueous solution at a concentration of approximately 1.5×10^6 units·cm^{-3}. At each stage, the spent liquids should be checked for residual penicillin and solvent usage carefully monitored. Since the solvents are relatively expensive, they are recovered for recirculation through the extraction process. As shown in Fig. 1, the penicillin recovery technology of organic solvent extraction has characteristics of complex high energy consumption, high solvent expense, and the biomolecule liable to denature. It is necessary in both theory and practice to develop a new recovery technology for antibiotics to reduce the process.

Since being introduced by Albertsson (2), aqueous polymer–polymer or polymer–salt two-phase systems (ATPS) have been shown to be useful for the extractive separation of biomolecules such as enzymes and other

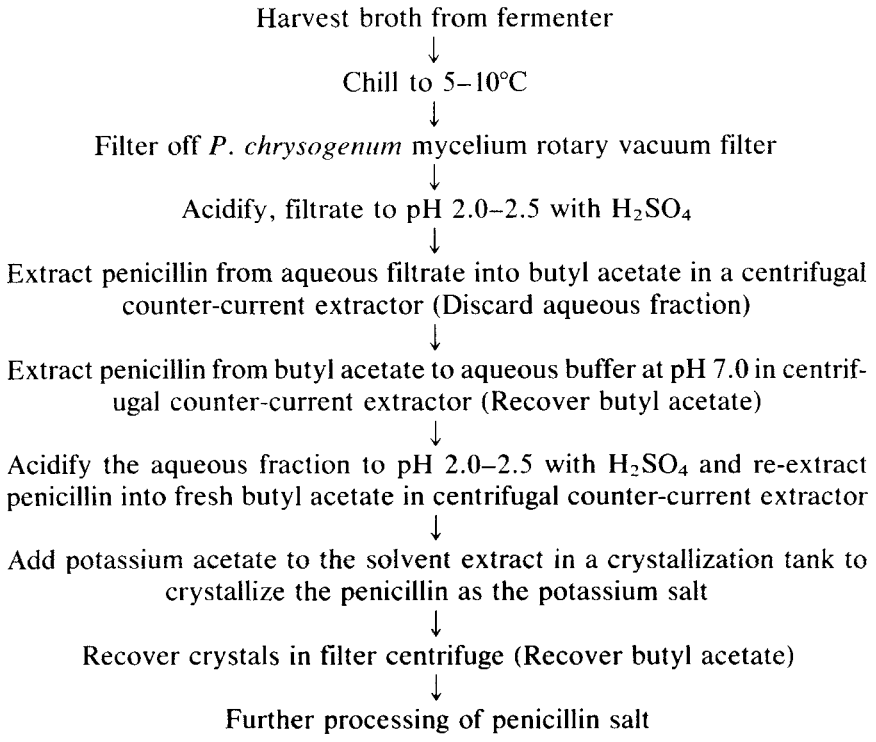

FIG. 1 Recovery and partial purification of penicillin G.

biologically active proteins, nucleic acids and cells, etc. In recent years they have also found application in various areas such as analytical measures, extractive bioconversion, and extractive fermentation. According to the present theory, large biological molecules are expected to have an uneven distribution in ATPS. Small molecules, e.g., amino acids and antibiotics, are expected to have a nearly equal distribution in the system. Recent reports indicate that small molecules such as lysine, phenylalanine, glutamic acid (3), penicillin G (4), vancomycin (5), cephalosporin C (6), pristinamycin (7), and acetylspiramycin (8, 9) can unequally partition in ATPS. The results referred to indicate that partitioning of small molecules needs to be reassessed.

The current investigation deals with extractive purification of penicillin whole broth based upon the partitioning of pure penicillin solution in PEG and ammonium sulfate. A new attempt was made for the reformation of the original recovery technology of penicillin.

MATERIALS AND METHODS

Materials

The filtered fermentation broth (15892 U/mL) was kindly supplied by Dongfeng Pharmaceutical Factory, Jiangxi, People's Republic of China.

Polyethylene glycol (PEG) of different standard molecular weights, ammonium sulfate, and all other chemicals were of analytical reagent grade.

Water was redistilled by our own laboratory.

Analyses

The iodine value (10) was adopted for the determination of penicillin concentration in solution. The purity of penicillin crystal was assayed by HPLC with a Lichrosorb RP-8(10 μm) Nucleosil C_{18} or Nucleosil CN column. The mobile phase was composed of 1:5 v/v of methanol:0.05 mol/L pH 7 phosphate salt buffer with a flow rate of 0.6 to 0.8 mL/min. A Waters 990 photodiode array detector at 220 nm was used. The concentration of saccharide was measured by Fehling's method (11), and the Coomassie Brilliant Blue method (12) was employed for the determination of protein content in fermentation broth.

Formula

If penicillin concentration in the whole fermentation broth is C_0, and C_t and C_b are the concentrations of the partitioned substance in milligram

per milliliter of the top and bottom phases, respectively, then

$$\text{Partition coefficient of biomolecule:} \quad K = C_t/C_b \tag{1}$$

$$\text{Enrichment coefficient of penicillin:} \quad \alpha = C_t/C_0 \tag{2}$$

$$\text{Selectivity of penicillin to saccharide:} \quad \beta_1 = K/K_1 \tag{3}$$

$$\text{Selectivity of penicillin to protein:} \quad \beta_2 = K/K_2 \tag{4}$$

$$\text{Recovery ratio:} \quad Y_t = RK/(1 + RK) \tag{5}$$

where K represents the partition coefficient of penicillin, K_1 is the partition coefficient of saccharide, K_2 is the partition coefficient of protein, and R is the phase volume ratio of top to bottom in ATPS.

RESULTS AND DISCUSSION

Enrichment of Penicillin from Whole Fermentation Broth by ATPS

For the enrichment of filtered broth, solid ammonium sulfate and concentrated PEG solution were added directly to the whole broth. The solution was centrifuged at 2500 rpm for 5 minutes after it was vigorously mixed. All the operations were conducted at room temperature (25°C). A sample was carefully withdrawn from each phase, and the penicillin concentrations were determined by the above method. The effects of PEG molecular weight, PEG concentration, and salt concentration on penicillin enrichment were investigated for the filtered broth. The relationship between penicillin partition coefficients and tieline length is illustrated in Fig. 2. Penicillin partitions unevenly in ATPS, and the concentrations of penicillin are much higher in the PEG-rich top phase. Increasing the polymer molecular weight in one phase decreases the partition of the biomolecules to that phase because of the repulsion of the polymer to the biomolecule. The penicillin partition coefficient was significantly improved when the concentrations of polymer and salt were increased; in other words, the system tieline length was increased. A linear relationship between ln K and tieline was obtained.

The experimental results of extractive purification of penicillin for the filtered broth are all listed in Table 1. The enrich coefficients are larger than 1, and penicillin was enriched in the PEG-rich top phase. The recovery ratio of penicillin in ATPS is bigger than that in organic solvent extraction when the concentrations of PEG 2000 is 8%, $(NH_4)_2SO_4$ is 20%, the partition coefficient of penicillin is 58.39, the enrich coefficient is 3.53, and the recovery ratio can be 93.67%. It is practical to process the filtered broth directly by ATPP.

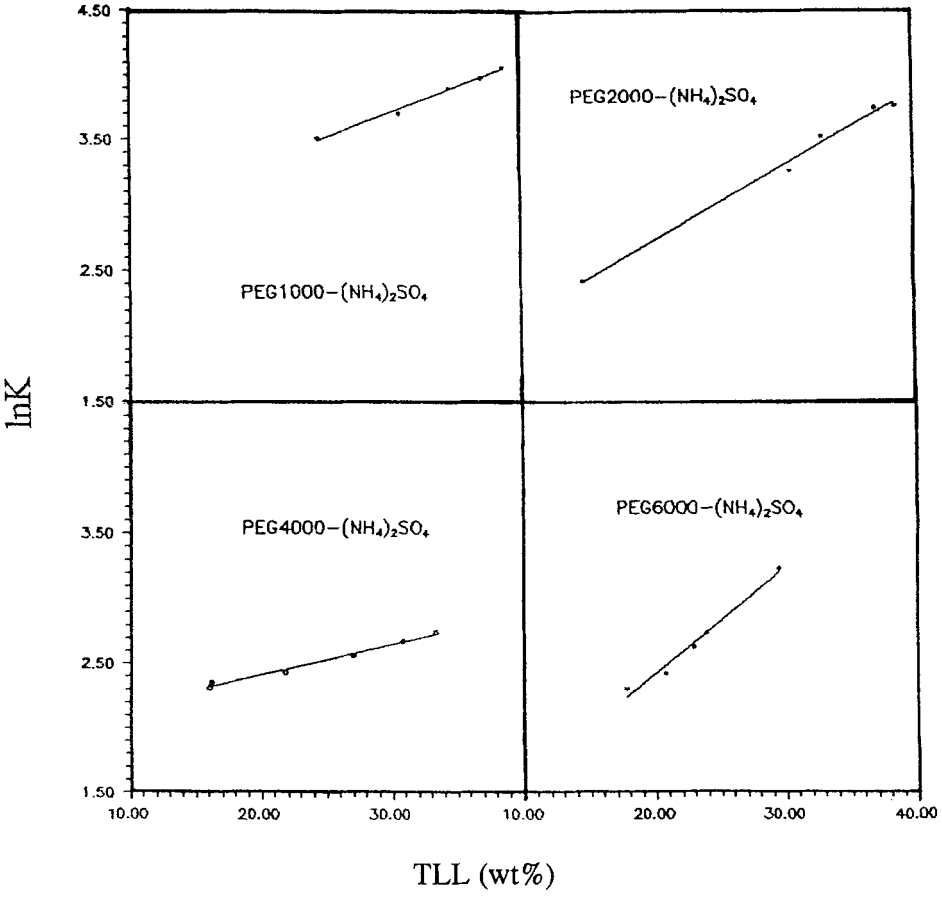

FIG. 2 The relationship between penicillin partition coefficients and tieline length (TLL).

Extraction of Penicillin by Butyl Acetate (BA)

After penicillin was enriched from filtered broth by ATPP, the bottom phase of ATPS was discarded and butyl acetate was added to the PEG-rich top phase to reextract penicillin. The solution was mixed vigorously and left undisturbed for 20 minutes in a 10°C water bath. The penicillin concentrations from the PEG-rich phase and the organic phase were measured by the above method. The effect of pH on extraction was investigated. The experimental results are represented in Table 2. As expected, when the pH value was reduced, the partition coefficients of penicillin

TABLE 1
Enrichment of Penicillin from Whole Fermentation Broth by ATPS

	No.	Total composition			Phase volume ratio R	Partition coefficient K	Enrichment coefficient α	Recovery ratio $Yr\%$
		PEG (wt%)	$(NH_4)_2SO_4$ (wt%)	Broth (ml)				
PEG 1000	1	7.0	17.0	7.10	0.26	33.30	3.04	89.54
	2	7.0	18.0	7.00	0.25	40.35	3.01	99.51
	3	7.0	19.0	6.90	0.25	49.19	2.78	92.67
	4	7.0	20.0	6.80	0.26	77.98	3.12	95.25
	5	7.0	21.0	6.70	0.24	86.12	3.29	95.44
	6	5.0	19.0	7.10	0.20	46.11	3.90	90.34
	7	6.0	19.0	7.00	0.22	66.70	3.43	93.68
	8	8.0	19.0	6.75	0.29	53.59	2.81	93.89
	9	9.0	19.0	6.70	0.33	39.63	2.51	92.96
PEG 2000	1	8.0	12.0	7.40	0.39	11.41	2.09	81.80
	2	8.0	14.0	7.30	0.33	26.11	2.57	89.69
	3	8.0	16.0	7.10	0.30	42.13	2.91	92.33
	4	8.0	18.0	6.90	0.27	68.74	2.90	94.84
	5	8.0	20.0	6.70	0.24	58.39	3.53	93.67
	6	6.0	16.0	7.50	0.21	33.87	3.21	88.61
	7	9.0	16.0	6.90	0.34	31.69	2.51	91.58
	8	12.0	16.0	6.35	0.50	53.71	1.78	96.11
	9	15.0	16.0	5.97	0.61	36.64	1.56	95.70
	10	18.0	16.0	5.20	0.80	46.66	1.23	97.38
PEG 4000	1	8.0	10.0	7.60	0.48	10.09	1.94	83.00
	2	8.0	11.0	7.50	0.41	11.33	2.11	82.15
	3	8.0	12.0	7.40	0.36	12.94	2.29	82.47
	4	8.0	13.0	7.20	0.34	9.66	2.67	85.35
	5	8.0	14.0	7.00	0.33	17.09	2.57	90.55
	6	4.0	12.0	8.00	0.17	10.53	2.70	92.55
	7	6.0	12.0	7.80	0.29	12.84	1.73	67.85
	8	10.0	12.0	7.20	0.49	8.70	2.56	99.99
	9	12.0	12.0	6.60	0.58	12.86	2.11	99.98
PEG 6000	1	5.0	11.0	7.80	0.28	10.00	2.80	73.53
	2	5.0	12.0	7.70	0.25	9.95	2.94	71.46
	3	5.0	13.0	7.70	0.24	15.44	3.25	78.62
	4	5.0	14.0	7.60	0.23	25.31	4.25	90.58
	5	5.0	15.0	7.50	0.22	25.54	3.63	84.67
	6	7.0	12.0	7.40	0.34	21.75	2.56	88.03
	7	6.0	12.0	7.50	0.27	13.84	2.73	78.74
	8	4.0	12.0	7.90	0.20	11.29	3.61	69.02
	9	3.0	12.0	8.05	0.11	5.11	2.19	36.20

TABLE 2
Results of Butyl Acetate (BA) Extraction

No.	pH	K	α	$Yt\%$
1	2.5	2.54	2.17	33.72
2	2.0	30.95	5.91	86.09
3	1.7	60.99	6.30	92.42

rose because in low pH the content of acidic penicillin in larger than of penicillin. It is much easier for acidic penicillin to enter the butyl acetate phase, and increases of the partition coefficient, the enrichment coefficient, and the recovery ratio are obtained.

Crystallization

The fermentation broth extracted with BA was pipetted out of the penicillin-rich BA phase. It was added to potassium acetate in ethanol to crystallize the penicillin in the potassium salt from the BA phase. The molar ratio of potassium acetate and penicillin in the BA phase is 1.45:1. The crystals were filtered, washed with fresh alcohol; dried on a vacuum desiccator, and frozen for storage. The purity of the penicillin crystals was assayed by HPLC. The purities of three independent samples were 88.48, 80.39, and 83.59%, respectively, and the average purity was 84.15%. The purity could be increased by recrystallization.

Flow Sheet of Extractive Fermentation of Penicillin by ATPP

A schematic presentation of the extractive purification of penicillin from filtered fermentation broth by ATPS is shown in Fig. 3, where the operation conditions and experimental results in three successive steps are as follows.

(1). Enrichment by ATPP:
PEG2000 8% (wt%), $(NH_4)_2SO_4$ 20% (wt%), pH 5.0, T = 293 K
K = 58.39, α = 3.53, Yt = 93.67%
K_1 = 2.68, K_2 = 4.37, β_1 = 21.79, β_2 = 13.36
(2). Extraction by BA:
pH 1.7, T = 293 K, R = 0.2
K = 60.99, α = 6.30, Yt = 92.42%

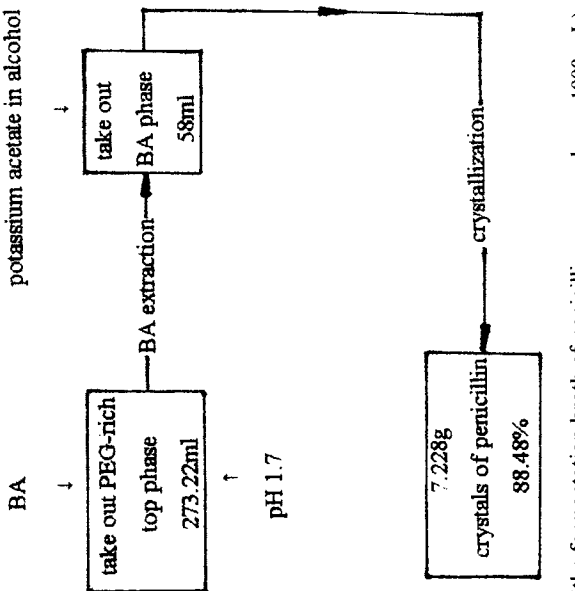

FIG. 3 Simplified flow diagram of extractive purification by ATPP (the fermentation broth of penicillin processed was 1000 mL).

(3). Crystallization:
The mass of crystal is 7.228 g; the purity is 88.48%

The total recovery ratio of ATPP is 76.56%.

The new technology of extraction purification of penicillin from filtered broth by ATPP can extract penicillin from the fermentation broth directly, free from the pretreatment of filtration and acidification; the activity of penicillin can be improved; the amount of solvent needed is reduced considerably; and the extraction technology is integrated. A new attempt has been made to reform the original antibiotics recovery technology, and fundamental data were provided for a further scale-up. This first application of ATPP into the recovery of small molecular biomaterials opens up a new field for this powerful technology.

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