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Extraction of Penicillin V from Simulated Fermentation Broth by Liquid-Liquid Membrane Technique

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Abstract

Liquid-liquid membrane extraction technique, pertraction, using three types of solvents (methyl isobutyl ketone, n-butyl acetate, and n-amyl acetate) was used for recovery of penicillin V from simulated fermentation broth under various operating conditions of pH value (4-6) for feed and (6-8) for receiver phase, time (0-40 min), and agitation speed (300-500 rpm) in a batch laboratory unit system. The optimum conditions for extraction were at pH of 4 for feed, and 8 for receiver phase, rotation speed of 500 rpm, time of 40 min, and solvent of MIBK as membrane, where more than 98% of penicillin was extracted.

***Keywords**: Liquid-liquid membrane, Penicillin V, pertraction, extraction.*

1. Introduction

Liquid-liquid membranes extraction technique combines extraction and stripping into one step, rather than the two separate steps required in conventional processes such as solvent extractions. A one-step liquid membrane process provides the maximum driving force for the separation of a targeted species, leading to the best clean-up and recovery of the species[1]. Liquid membrane process, called also pertraction process, have gained increased attention due to its ambient temperature operation, relatively low capital cost, high separation efficiencies and modular construction. The process is inherently low-energy, continuous, and can be made highly-automated. The amount of organic solvent required are generally very small, and thus the technology is environmentally benign[2, 3]. Two aqueous solutions, feed solution F, and receiver solution R, are separated by a third, organic liquid M, representing the "liquid membrane" which is insoluble in the other two liquids. The solute is transferred from the feed to the acceptor solution under the effect of appropriately chosen equilibrium conditions at the two interface F/M and M/R. In liquid membranes, facilitated transport is the mass transfer mechanism for the target species to go from the feed solution to the receiver solution.[4, 5]. Extraction using liquid membranes has been studied since the 1980s and is one of the most advantageous techniques of separation at the present. This separation method consists in the transfer of a solute between two aqueous phases of different pH which are separated by a solvent and carrier layer. The claimed advantages are as follows: the quantity of solvent used is small because of its continuous regeneration, the loss of solvent is small during extraction process provided the pH gradient between the two aqueous phases is maintained, there is a possibility of solute transport through liquid membranes that have been used for the separation of some biosynthetic products, namely carboxylic acids, amino acids and antibiotics[6, 7].The membrane interposed between two miscible aqueous solution, at one side (feed phase) in which the solute to be transport is extracted, while at the other side (strip phase), re-extraction occurs. Since in each of the aqueous phase some specific, and different for each of them, thermodynamic conditions exist, the extraction and re-extraction occur simultaneously[8, 9].

The steps of transport of solute in the pertraction system are described as: diffusion through the boundary layer in the feed solution, sorption on the feed solution/liquid membrane interface, diffusion through boundary layer on the feed side, transport in the membrane, diffusion through boundary layer on the receiving side, desorption on the membrane/receiving solution interface and diffusion through the boundary layer in the receiving solution [10].

The incessant stripping of solute of the liquid membrane keeps low concentration of solute in this phase and therefore provides its complete recovery from the feed solution. One of the principal advantages of pertraction process is the practically complete removal of the valuable component from the source material using, in most cases, not sophisticated, friendly solvents, in particular-water. As far as the membrane liquid is considered, it is noteworthy to mention that the requirements to the liquid membrane are not the same as to the conventional solvents used in a solvent extraction process, because in pertraction, priority is given to the membrane selectivity, rather than to the capacity and the solute distribution coefficient [11].

Penicillin V is a secondary metabolite produced at low growth rates and its syntheses have been described extensively in the literature. Penicillin formation starts from three activated amino acids, and involves several enzymes and isopenicillin N as a major intermediate. Penicillin V (phenoxymethylpenicillin) is the commercially most important penicillin. It is mainly converted to 6-aminopenicillanic acid (6-APA), which in turn is used to make amoxicillin and ampicillin. Penicillin V is a weak acid and it is extracted with n-butyl acetate at pH 2-3. In this pH range penicillin V is unstable and decomposes, therefore the aqueous medium in fermentation broth is cooled to 0^{o}C and extracted in centrifugal extractor to keep contact time as short as possible[12].

In the present work the fermentation broth was simulated by dissolving penicillin V sodium salt in distilled water. Liquid-liquid pertraction technique was conducted for the recovery of penicillin V in a batch pertraction laboratory unit. Methyl isobutyl ketone (MIBK), n-amyl acetate, and n-butyl acetate were proposed as membranes for penicillin V pertaction at 25^{o}C. The effect of speed of agitation, time, and pH were studied.

2. Experimental Work

2.1. Material

Feed Phase (Donor Phase):

The feed phase was prepared by dissolving 1 g of penicillin V sodium salt in 1 L of distilled water, the pH of solution is adjusted by [4% H_2SO_4 (BDH)] and [5% Na_2CO_3 (BDH)][6].

Receiver Phase (Stripping Phase):

A sodium carbonate solution was used as a receiver phase.

Membrane Phase:

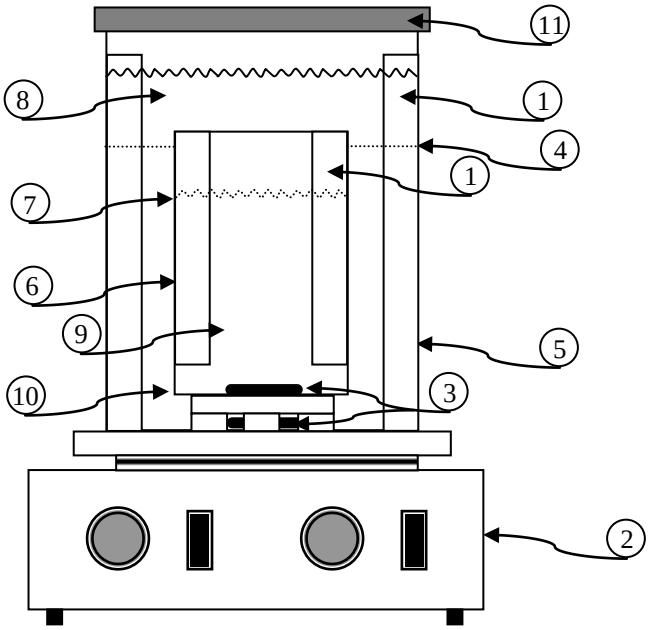
In the present study, methyl isobutyl ketone (BDH), n-amyl acetate (BDH), and n-butyl acetate (BDH) were used as liquid membrane.

2.2. Pertraction Lab Unit

Pertraction experiments were carried out in 1 liter laboratory pertractor as shown in Fig. 1. The pertractor consists of two coaxial Pyrex beakers and baffles where placed in each beaker as shown in Fig. 1. The outer beaker is 1 liter and the inner is 250 ml. The two beakers were arranged as shown in Fig. 1 and placed on a magnetic stirrer with heater in order to control the temperature and the speed. The membrane, feed, and receiver phases were stirred by using two Teflon-coated magnetic bars.

2.3. Experimental Setup

500 ml of feed phase was placed in the annular space between the two beakers, and 200 ml of receiver phase was placed in the inner beaker. After that 300 ml of membrane phase was added to cover the other two phases as shown in Fig.1. The outer beaker was covered with a thin plastic layer to prevent evaporation of membrane phase. In the present study the effect of speed of agitation using the three proposed membranes was studied in the range of 300-500 rpm. The speed of agitation and temperature were adjusted and controlled by using hotplate and magnetic stirrer. The pertraction time was continuing up to 40 min and during this period of time samples were taken at a specified time interval from the feed and receiver phases for penicillin V analysis by HPLC. HPLC type Shimadzu model LC20AD was used in this analysis using column 100 RP-18 (5 μm). The penicillin V in the membrane organic phase was evaluated by material balance.



1	Baffles	7	Membrane-Receiver interface
2	Magnetic Stirrer	8	Membrane phase
3	Magnetic bars	9	Receiver phase
4	Membrane-Feed interface	10	Feed Phase
5	Feed-baker	11	Plastic cover
6	Receiver Baker		

Fig. 1.Schematic Diagram of Pertraction Laboratory Unit.

3. Results and Discussion

In the present work, the batch pertraction of penicillin V using the three proposed liquid membranes was studied, the agitation speed, and liquid membrane type was conducted in this work. The efficiency of penicillin V extracted, *E*, was calculated as follows:

$$E = \frac{C_r V_r}{C_{fo} V_f} \times 100\% \quad \ldots(1)$$

Where C_r is the penicillin V concentration in the receiver phase, C_{fo} is the initial concentration in the feed phase, V_r is the volume of receiver phase, and V_f is the volume of feed phase.

3.1. Effect of pH

Figures 2 and 3 show the relationship between the penicillin transport from feed and to the receiver phases respectively with pH at different liquids membrane and at agitation speed of 400 rpm and time of extraction of 40 min. The range of pH for feed phase is taken between (4-6) and for receiver phase between (6-8) because the penicillin V is unstable and decomposes for pH < 4 and pH > 8[13]. From Figure 2, it can be seen that the extraction of penicillin V from feed is increasing with the decrease of pH value and also from Figure 3 the extraction of penicillin V by the receiver is increasing by increasing of pH value because the over all mass transfer coefficient increases when the difference in the pH value between two phases is high as possible[14]. In the Figure 2, it seems that the best value of pH for extraction of penicillin V from feed is 4 where about 98% of penicillin V is extracted by MIBK, while from Figure 3, the best value of pH for extraction of penicillin V by the receiver phase is 8 although the pH value of 7.5 has little effect on the extraction.

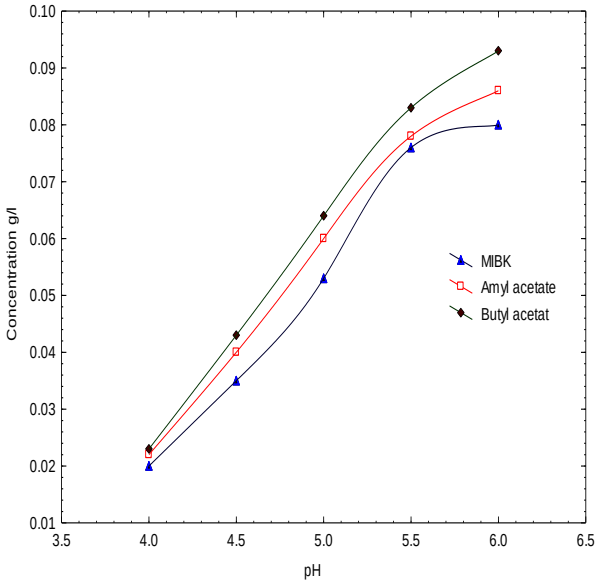


Fig. 2. pH Effect on Penicillin Extraction from Feed Phase after 40 min and Agitation Speed of 400 rpm.

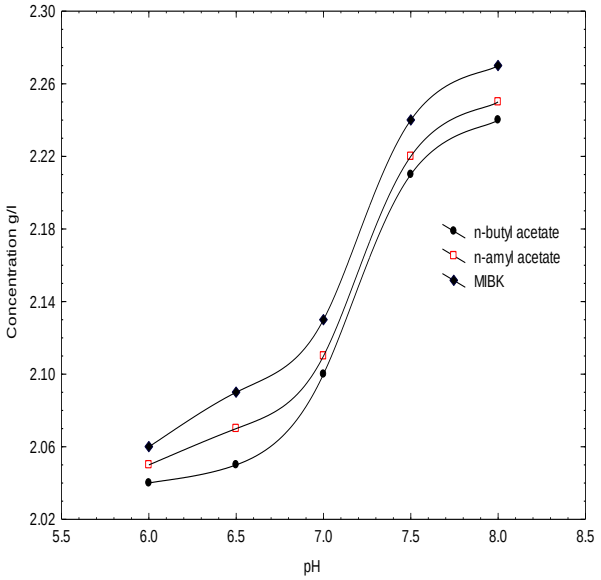


Fig. 3. pH Effect on Penicillin Extraction by Stripping Phase after 40 min and Agitation Speed of 400 rpm.

3.2. Effect of Membrane type

From figures 2 and 3, it can be seen that is a better solvent as a membrane and it can give better extraction efficiency methyl isobutyl ketone (MIBK) with respect to other solvents (n-amyl acetate and n-butyl acetate). The extraction efficiency of penicillin V is evaluated by using equation 1 for three types of membranes at temperature of 25°C, rotation speed of 400 rpm and pH for feed 4 and for receiver phase 8 as shown in Table 1, where the difference in extraction efficiency for three types of membrane are very little; i.e., the effect of membrane type on extraction is little.

Table 1,
Extraction Efficiency, *E*, of Penicillin V for 3 Types of Membrane at 25°C, 400 rpm and pH of Feed 4 and of Receiver 8.

Membrane type	***E***
MIBK	90.8
n-amyl acetate	90
n-butyl acetate	89.6

Figure 4 shows the penicillin V content in the feed ($R_f = \frac{C_f}{C_{fo}}$), membrane, MIBK, ($R_m = \frac{C_m}{C_{fo}}$) and receiver phases ($R_r = \frac{C_r}{C_{fo}}$) during the extraction at temperature of 25°C, agitation speed of 400 rpm and pH of 4 for feed and 8 for receiver phases. It seems that about 80% of penicillin V is extracted during 15 min; i.e., the extraction process during this time is fast, while after 30 min the extraction is stable and there is no effect of time on extraction.

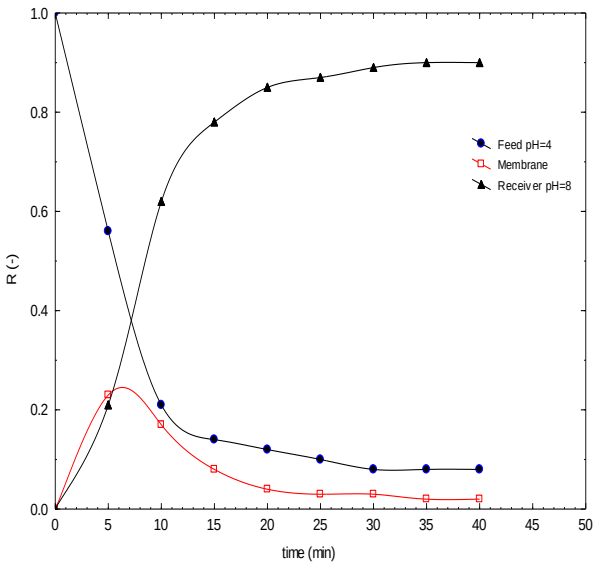


Fig. 4. Penicillin V Content in Feed, Membrane (MIBK), and Receiver Phases with Time at 25°C and 400 rpm .

3.2. Effect of Agitation Speed

Figure 5 shows the effect of speed of agitation on the extraction efficiency, *E*. In order to explore the effects of stirring rate, the extraction experiment were carried out at three different stirring rates, 300, 400, and 500 rpm. *E* value increases with increasing speed of agitation, which means that the extraction efficiency of penicillin V from feed phase to the receiver phase through liquid membrane improved with increasing the speed of agitation; this is because the higher stirring rate leads to much severer mixing between the aqueous solution and organic phase, which could accelerate the transport of penicillin V and enhance the mass transfer area between the aqueous solution and liquid membrane solution and reduce the mass transfer resistances of penicillin V from feed to liquid membrane in the extraction process, and from liquid membrane to the receiver phase in the stripping process. This variation in the efficiency indicates a diffusion control of the extraction process. According to previously published literature, although the mass transfer improved with higher speed of agitation, it was not applied because of increased risk of droplet formation which causes phase intermixing and deterioration of the process [10].

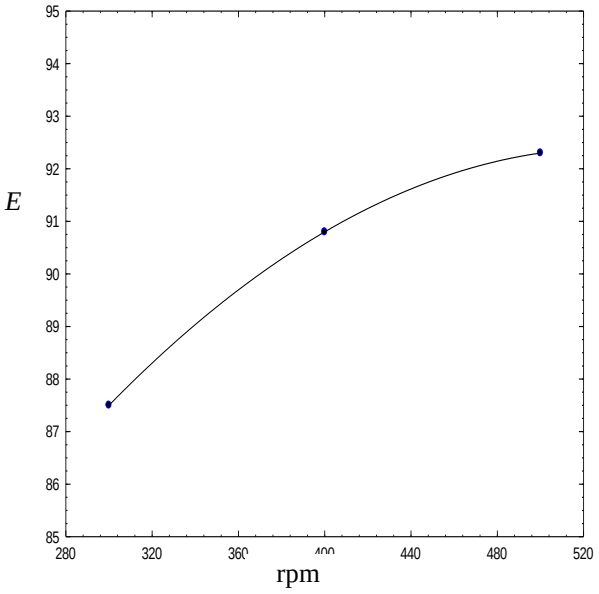


Fig. 5. Effect of Rotation Speed on the Extraction Efficiency Using MIBK as Membrane, at 25°C, and pH of 4 for Feed and 8 for Receiver Phases.

4. Conclusion

The liquid-liquid membrane extraction, pertraction, of biosynthetic products constitutes advantageous alternatives to conventional separation methods because it reduces the number of stages required for an efficient separation and, therefore, for the corresponding energy and material consumption. It can be concluded that the separation of Penicillin V from simulated broth could be enhanced by decreasing the pH value for feed up to 4 and increasing pH value up to 8 for receiver phase and increasing rotation speed up to 500 rpm. The type of solvent as a membrane has little effect on the extraction efficiency.

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استخلاص البنسلين V من ناتج التخمير المصطنع بأستخدام تقنية الغشاء السائل

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الخلاصة

تم استخدام تقنية الأستخلاص بطريقة الغشاء السائل لثلاث انواع من المذيبات العضوية (ايزوبيوتايل مثل كيتون، اسيتات البيوتايل الأعتيادي، اسيتات الأمايل الأعتيادي) والتي أستخدمت لغرض استخلاص البنسيلينV من ناتج التخمير المصطنع تحت ظروف تشغيل متباينة من اس هيدروجيني بمدى (4-6) للقيم و (6-8) للطور المستلم، زمن استخلاص بمدى (0-40 دقيقة)، وسرعة خلط بمدى (300-500 دورة/دقيقة) في منظومة مختبرية ذات النظام الدفعي. الظروف المثلى للأستخلاص كانت عند اس هيدروجيني 4 للقيم و 8 للطور المستلم، سرعة خلط 500 دورة/دقيقة، زمن استخلاص 40 دقيقة وللمذيب العضوي ايزوبيوتايل مثل كيتون، حيث اكثر من98% من البنسلين تم استخلاصه ضمن هذه الظروف.