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Article

An Adsorption Based Downstream Processing Approach for Penicillin V from a *Penicillium chrysogenum* BIONCL I22 Culture Filtrate

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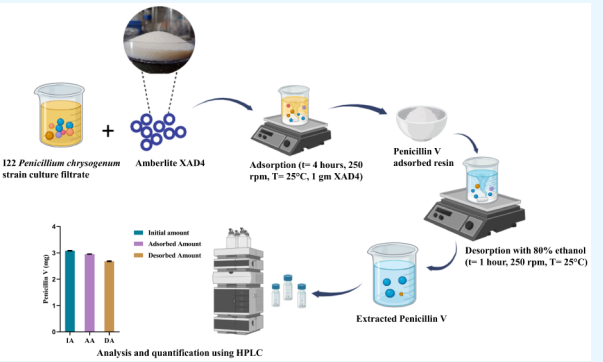
ABSTRACT: Penicillin V (phenoxy methyl penicillin) is highly sought after among natural penicillins because of its exceptional acid stability and effectiveness against common skin and respiratory infections. Given its wide-ranging therapeutic uses, there is a need to establish a greener method for its maximum recovery to reduce the carbon footprint. Here, we have identified and validated optimized operational conditions for resin-based penicillin V recovery. It was observed that Amberlite XAD4 had the highest penicillin V hydrophobic adsorption capacity among the other screened resins. Kinetic and isothermal studies using linear and nonlinear regression analysis showed that the adsorption process well fitted with pseudo-second-order kinetics (R^2 = 0.9816) and the Freundlich adsorption isotherm model (R^2 = 0.9871). Adsorption equilibrium was attained within 4 h, while maximum adsorption was observed at 3 mg/mL penicillin V concentration. Furthermore, the optimized extraction protocol was compared with the conventional butyl acetate-based downstream processing. Under optimum conditions resin-based penicillin V recovery was 2-fold higher as compared to the solvent extraction method and the resin could be reused for over six cycles without compromising the yield. These findings signify substantial progress toward the development of an environmentally sustainable approach for penicillin V recovery and a potentially viable method for extractive fermentation.



1. INTRODUCTION

Penicillins are an important class of beta-lactam antibiotics and can be classified as natural and semisynthetic therapeutic molecules. Natural penicillins are obtained by *Penicillium chrysogenum* or *Penicillium rubens* fermentation. Whereas the semisynthetic group includes penicillins prepared by modification of the 6-aminopenicillinic acid (6-APA) side chains providing a wide range of antibacterial properties to these antibiotics.[1−4] Among the natural penicillins most widely used are phenoxymethylpenicillin (penicillin V) and benzylpenicillin (penicillin G). Penicillins G and V show activity against a wide range of Gram-positive bacteria.[5] Penicillin V has an advantage over penicillin G and can be administered orally due to its acid stability. In contrast, penicillin G must be administered intravenously or intramuscularly. Penicillin V treats several mild to moderate bacterial infections, respiratory tract infections, pharyngitis, syphilis, skin infections, etc.[6]

As mentioned earlier, penicillin V is the first-choice medication in the treatment of community-acquired infections, such as pneumonia, pharyngitis, and progressive apical dental abscesses. Hence, in 2021 it was included in the World Health Organization's (WHO) model list of essential medicines.[7] According to the IQVIA MIDAS database, penicillins were considered the most commonly consumed antibiotics with a 36% increased antibiotic consumption rate between the years 2000 and 2015.[8] A 2021 study from Israel also reveals that penicillin was the most commonly prescribed antibiotic in certain community acquired infections.[9]

Microbial production of such antibiotics has been important since decades. Advancement in bioprocess and biochemical engineering has boosted the production and development of many bioactive compounds useful in the agriculture, food, and pharmaceutical industries.[10] Methods such as supercritical carbon dioxide based recovery of bioactive products using deep eutectic solvents[12] have been developed quite recently.[11] However, the efficient isolation and recovery of high purity products from the fermentation broth still remain a challenge. Apart from this, downstream processing accounts for almost 50

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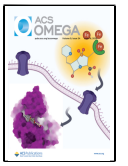

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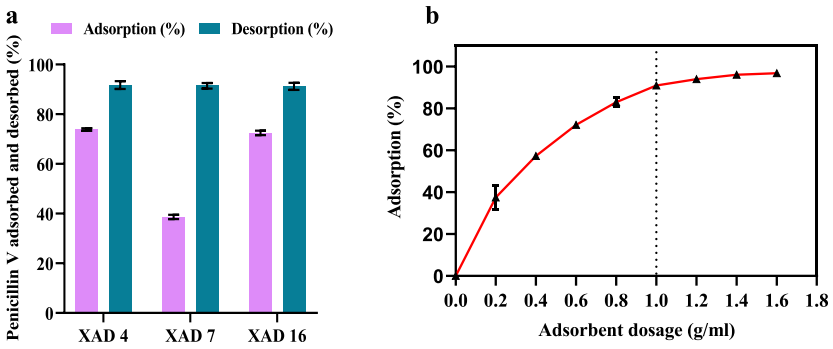

Figure 1. Resin screening and effect of adsorbent dosage. Selection and screening of Amberlite resins. (a) Three hydrophobic adsorbent resins Amberlite XAD4, XAD7, and XAD16 were screened for their penicillin V adsorption and desorption capacities. The adsorption and desorption experiments were carried out with 1 mg/mL standard penicillin V solution each for 1 h at 25 °C, 250 rpm without any changes in pH in triplicate (n = 3). The purple and blue bars represent the percentages of penicillin V adsorbed from the standard solution and the percentages desorbed from the adsorbed amount, respectively. (b) The effect of adsorbent dosage on penicillin V recovery was evaluated by performing triplicate (n = 3) assays. Experiments at various Amberlite XAD4 dosages (0.2 to 1.8 g/mL) were performed, keeping the initial penicillin V concentration fixed (1 mg/mL) and the volume of the solution (10 mL). Error bars depict the standard error of the mean (SEM).

Table 1. Comparative Analysis of the Physical Properties, Adsorption Capacity, and Desorption Capacity of Amberlite Resins for Screening and Selection (n = 3)

properties	Amberlite XAD4	Amberlite XAD7	Amberlite XAD16
matrix	SDVB	acrylic	SDVB
surface area	750 m^2/g	500 m^2/g	800 m^2/g
pore volume (mL/g)	0.98	0.5	0.55
mean pore size (Å)	100	300	200
mesh size	20 to 60	20 to 60	20 to 60
amount adsorbed	0.80 mg/g	0.42 mg/g	0.81 mg/g
amount desorbed	0.73 mg/g	0.38 mg/g	0.73 mg/g
adsorption (%)	73.81 ± 0.92	38.64 ± 1.52	72.45 ± 1.56
desorption (%)	91.73 ± 2.77	91.41 ± 1.94	91.18 ± 2.49
application	small hydrophobic compounds adsorption, pharmaceutical manufacturing	adsorbent for insulin, fulvic, humic compounds, and antibiotic recovery	recovery of antibiotics (cephalosporin C), and proteins.

to 70% of the total production cost.[13] The downstream processing cost for penicillin G is about 50 to 55% when purified and formulated.[14] To overcome these challenges, an adsorption based recovery approach has been developed for compounds such as lactic acid,[15] antibiotics like cephalosporin, geldanamycin, tetracycline,[16−18] and many more as these methods are effective and relatively inexpensive.

Penicillin V being one such essential antibiotic, the development of a novel, effective, and environmentally friendly extraction process would be quite advantageous. Conventional downstream processing of penicillin V using solvent extraction is a multistep procedure, in which, it is first extracted into an organic solvent using n-butyl acetate or amyl acetate, followed by back extraction into phosphate buffer (PBS).[19,20] This method has various drawbacks, such as high solvent consumption, multiple extraction steps, less yield, and emulsification. Apart from butyl acetate-based extraction, numerous alternative approaches have been developed to extract penicillins. Edmundowicz et al.[21] proposed the method of nonextractive penicillin V recovery by acidification of the fermentation broth achieving an overall yield of about 56%. Penicillin G extraction using a liquid surfactant membrane prepared by water and oil emulsions and di-n-octylamine as a carrier was performed by Hano et al.[22] This provided a rapid recovery method but at the expense of low membrane stability and use of organic solvents. De Barros et al.,[23] investigated the use of hydrophobic resins for penicillin G recovery. Their approach provided useful insights into the adsorption based penicillin recovery process. However, the maintenance of low temperature and pH conditions is a quite challenging task. Other extraction approaches developed include carrier-supported liquid membranes,[24] microfiltration,[25] selective penicillin V recovery,[26] etc. A comparison for several such techniques developed is provided in Table S1. There are advantages and disadvantages to each approach, and further work still needs to be carried out in this area to come up with environment-friendly methods.

Here, we have attempted to develop and evaluate adsorption based penicillin V recovery to address shortcomings of the existing solvent extraction-based approaches. As penicillin V is a small organic molecule with phenyl groups, we used XAD resins for the hydrophobic adsorption of penicillin V. Most of the research reports on adsorption experiments at lower temperatures. We have screened multiple conditions and identified optimized parameters. Under the optimized conditions, penicillin V recovery was carried out from a *P. chrysogenum* BIONCL I22 culture filtrate. Quantification was carried out using high performance liquid chromatography (HPLC). We anticipate that this resin-based adsorption

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method will be a sustainable and efficient approach for penicillin V recovery.

2. RESULTS AND DISCUSSION

2.1. Higher Penicillin V Recovery by Amberlite XAD4 with Optimized Adsorbent Dosage. Three hydrophobic resins, viz. Amberlite XAD4, XAD7, and XAD16 were screened for their maximum penicillin V adsorption and desorption capacity. Amberlite XAD4 and Amberlite XAD16 both showed about 73% adsorption capacity whereas Amberlite XAD7 showed the least adsorption of about 38%. It was observed that 90 to 92% of the adsorbed penicillin V was desorbed and recovered from all three resins (Figure 1a and Table 1). Considering the slightly higher adsorption capacity and cost-effectiveness of Amberlite XAD4 as compared to Amberlite XAD16, the same was selected for penicillin V recovery and further optimization. As the amount of the adsorbent is one of the crucial factors in the recovery, the effect of the adsorbent dosage was evaluated. It can be clearly observed that penicillin V adsorption increased with the adsorbent dosage and was constant at dosages greater than 1 g of Amberlite XAD4. Over 91% penicillin V was recovered by the addition of 1 g of the pretreated XAD4 resin to 10 mL solution, after which equilibrium was reached (Figure 1b). Thus, 1 g of Amberlite XAD4 was optimized as the required adsorbent dosage. This may be explained by the fact that the adsorption sites are effectively utilized at lower adsorbent doses. A significant fraction of the accessible adsorption sites may remain exposed with an increase in adsorbent dosage, which could result in reduced adsorption per gram.[27] Researchers have used macroporous resins for the adsorption of several antibiotics.[28,29] Jain et al.[28] reviewed the use of XAD4 for the adsorption of penicillin, aspirin, ciprofloxacin, etc. In another study by De Barros et al.,[23] it was shown that XAD4 had the highest adsorption capacity for recovering penicillin G. Amberlite XAD4 is a nonionic, macroporous styrene-divinylbenzene copolymer. It possesses a relatively high Brunauer–Emmet–Teller (BET) surface area (750.2 m^2/g), mean pore size (100 Å), pore volume (0.98 mL/g), and a medium pore diameter of 5.58 nm. These physical properties and the aromatic nature of its surface enhance the adsorption performance of Amberlite XAD4 toward hydrophobic small organic molecules dispersed in aqueous media. Reports suggest Amberlite XAD4 has been used previously for the adsorption and recovery of many antibiotic compounds such as geldanamycin, penicillin G, and flavonoids like naringin.[30] The hydrophobic members of the beta-lactam antibiotic class, penicillin G and penicillin V, both contain nonpolar side chains. Therefore, it was believed that the presence of aromatic groups in penicillin V and XAD4 would give the best adsorption results. Accordingly, the maximum recovery of penicillin V using XAD4 was observed in the current investigation, as well.

2.2. Adsorption of Penicillin V Shows the Maximum Recovery at pH 6 to 7. In adsorption experiments, the pH of the solution influences the interaction between adsorbent and adsorbate molecules. In the present study, batch adsorption experiments were performed at different pH values at room temperature (25 °C). It was observed that maximum penicillin V was recovered within the pH range of 6 to 7 at the given temperature (Figure 2). Studies by De Barros et al.[23] suggest that maximum penicillin G recovery could be achieved at pH 4 but requires maintenance of low temperature (4 °C).

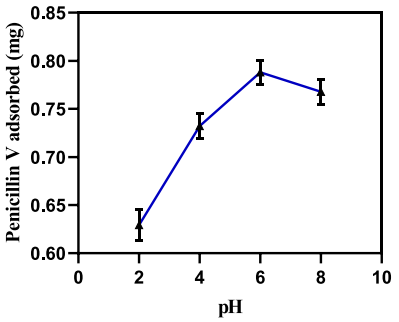

Figure 2. Effect of pH. The adsorption of penicillin V was analyzed for different pH values from 2, 4, 6, and 8. The effect of varying pH on the adsorption capacity of XAD4 for penicillin V was evaluated at room temperature 25 °C under stirring at 250 rpm. 1 g of XAD4 was added to 10 mL solution and samples were analyzed by HPLC. Average of the triplicate (n = 3) was considered. Error bars depict standard error of the mean (SEM).

According to the studies by Kheirolomoom et al.,[31] maximum penicillin G recovery could be achieved in the pH range of 5 to 8 at minimum temperatures to avoid loss of product. It can be observed that there is a considerable loss of the product at pH less than 4 at the given temperature. In the present study, the recovery could further be increased by decreasing the pH but requires maintenance of low temperatures, which further adds to the overall cost. Additionally, as the *P. chrysogenum* culture broth has a similar pH (6.5 to 7) no further changes in pH were made during the experiments to maintain the product integrity.

2.3. Adsorption Kinetics of Penicillin V on Amberlite XAD4 Shows the Best Fitting in the Pseudo-Second-Order Kinetic Model. Kinetic adsorption assays were performed by using XAD4 to confirm that the adsorption time was sufficient to reach equilibrium. Equilibrium was reached within 4 h and about 92 to 95% adsorption was achieved at room temperature (25 °C) and without pH changes (Figure 3a). Initially, there was a rapid increase in the amount of penicillin V adsorbed, which further slowed and eventually remained constant as the resin saturated. It was observed that most of the penicillin V was adsorbed within 160 to 200 min, leveling off and reaching a plateau stage by 240 min. The adsorption of penicillin V on Amberlite XAD4 was investigated using pseudo-first-order and pseudo-second-order kinetic models with linear and nonlinear fittings. The pseudo-second-order kinetic model represented the kinetic behavior more accurately for both the linear and nonlinear fittings. The plots for nonlinear fitting showed that, the data fitted best in the pseudo-second-order model with high R^2 (0.9816) as compared to the pseudo-first-order model with R^2 (0.9293) (Figure 3b–d). Coefficients of the adsorption kinetics for the adsorption of penicillin V onto Amberlite XAD4 are listed in Table 2. These kinetic models are commonly used for time-dependent experimental adsorption data fitting.[32] According to Lima et al. (2021), the correlation coefficient (R^2) values of most of the data sets reviewed in the study are better for the pseudo-second-order kinetic model.[33] Similar observations were made by William Kajjumba et al.[34] and suggest that the PSO better fits most of the experimental data at low solute concentrations, whereas at high concentrations the PFO model is preferred. This might be because of the exponentially increasing (q_e - q_t) values at low C_0 which is reversed at high C_0.

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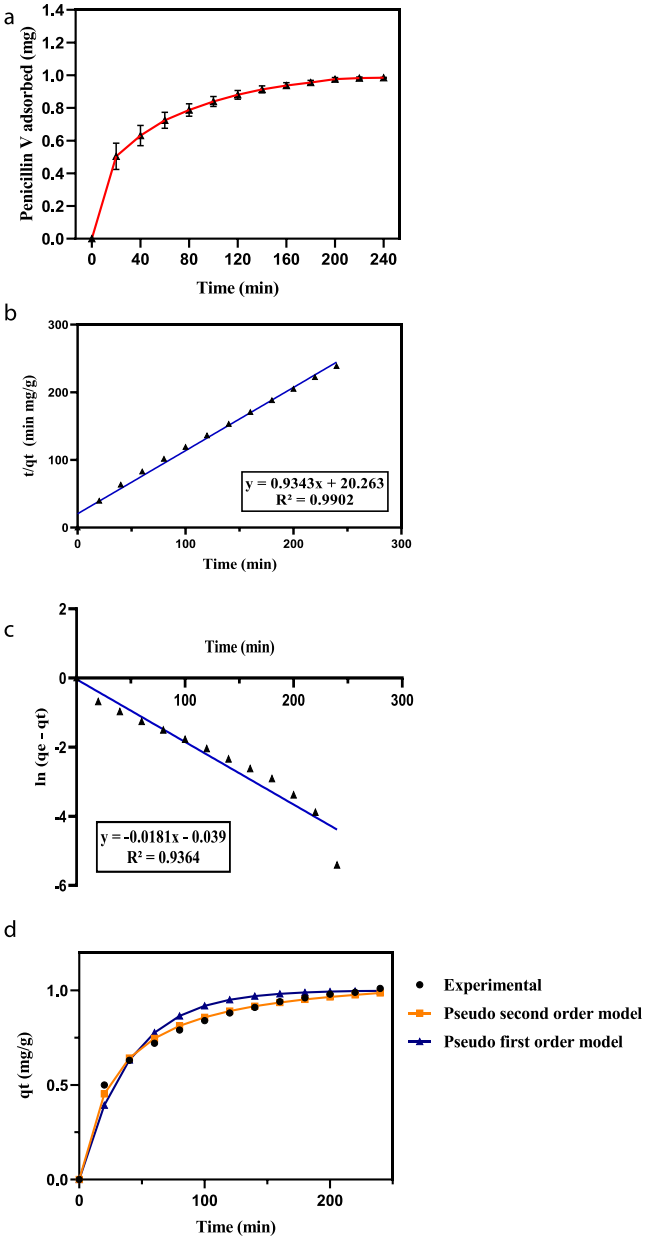

Figure 3. Effect of contact time. Penicillin V adsorption kinetics using Amberlite XAD4 and comparison between pseudo-second-order and first-order kinetic models. The adsorption kinetic data was fitted in pseudo-second-order and first-order kinetic equations using linear and nonlinear analyzes. The average of the triplicate data (n = 3) was considered for model fitting. (a) The effect of varying contact time on the adsorption capacity of XAD4 for penicillin V was evaluated for about 240 min (4 h) at fixed penicillin V concentration (1 mg/mL), temperature 25 °C, and stirring 250 rpm. 1 g of XAD4 was added to 10 mL solution, and samples were collected at 20 min intervals. (b) Linearized plot of pseudo-second-order kinetics for adsorption of penicillin V on Amberlite XAD4 with its equation and correlation coefficient value (R^2). (c) Linearized plot of pseudo-first-order kinetics for adsorption of penicillin V on Amberlite XAD4 with its equation and correlation coefficient value (R^2). (d) Nonlinearized plot of pseudo-second-order and first-order kinetic models compared with the experimental data. Error bars depict standard error of the mean (SEM).

2.4. Adsorption Isotherm of Penicillin V on Amberlite XAD4 Shows Consistency with the Freundlich Isotherm Model. Selecting the appropriate model that fits the adsorption data yields important insights into the adsorption process and the interaction between adsorbent and adsorbate molecules.[35] Adsorption isotherm for penicillin V revealed that the resin saturated at a concentration of 2.7 to 3 mg/mL. The equilibrium data fitted in the linearized as well as nonlinearized Langmuir and Freundlich isotherm models showed that penicillin V adsorption is more consistent with the Freundlich isotherm model. The nonlinear fitting for Freundlich model yielded a high R^2 (0.9871) in contrast to the R^2 (0.9664) for Langmuir model. The experimental data was compared with the data from both the models (Figure 4a–c). It was observed that the adsorption intensity ($1/n$) was less than 1 with the value of n above unity (n = 1.57 and n = 1.70 for linear and nonlinear models respectively) indicating favorable and physical adsorption. The maximum adsorption capacity (q_m) for penicillin V was reported using Amberlite XAD4 with physisorption mechanism of adsorption as indicated by the nonlinear Freundlich isotherm model. Similar observation was reported by Alnajrani et al.[35] for the polymer based adsorption of amoxicillin and penicillin G belonging to the same class of beta-lactam antibiotics as penicillin V. The separation factor (R_L) was found to be between 0 and 1; this showed a favorable adsorption process even though the Langmuir isotherm model represented the sorption process less accurately (Table 3). De Barros et. al., carried out the adsorption of penicillin G at 4 °C, pH 4 and showed data fitting in Langmuir adsorption isotherm but the data should not be directly compared due to a difference in the operating conditions.[23,36]

Table 2. Calculated Parameters for Linear and Nonlinear Adsorption Kinetic Models for Penicillin V/Amberlite XAD4 Adsorption Data Fitted in the Pseudo-First-Order and Pseudo-Second-Order Equations (n = 3)

	pseudo first order model			pseudo second order model		
parameter	q_e (mg/g)	k_1 (min^{-1})	R^2	q_e (mg/g)	k_2 (min^{-1})	R^2
linear	0.9617	0.0181	0.9364	1.07	0.0431	0.9902
nonlinear	1.01	0.025	0.9293	1.10	0.3375	0.9816

2.5. Desorption Yield of Penicillin V from Amberlite XAD4 Found Maximum in 80% Ethanol. Organic solvent ethanol is well-known for many advantages, ability to be recycled, low cost, low toxicity, and consequently safety for human usage.[37] Therefore, in the present study, it was chosen as a desorbent. The correlation between the concentration of ethanol and the desorption percentage of penicillin V showed that when the ethanol concentration was increased, the desorption percentage likewise increased (Figure 5a). It was observed that the penicillin V desorption percentage started decreasing after reaching the maximum peak at an 80% ethanol concentration. Based on the results of desorption experiments with various ethanol concentrations, it was found that maximum penicillin V was recovered in 80% ethanol solution, in the case of both standard penicillin V solution as well as the BIONCL I22 strain culture filtrate. Our findings are in agreement with the reports on penicillin G recovery by De Barros et al., (2020).[23] The amount of water present in ethanol had an impact on the polarity of the four concentrations employed in this study.[38] According to a report of Tiwari et al.,[39] as the water content in the aqueous ethanol solution increases, its polarity increases compared to absolute ethanol. Thus, at lower ethanol concentrations, i.e., higher water content (higher polarity), the extraction yield of penicillin V is

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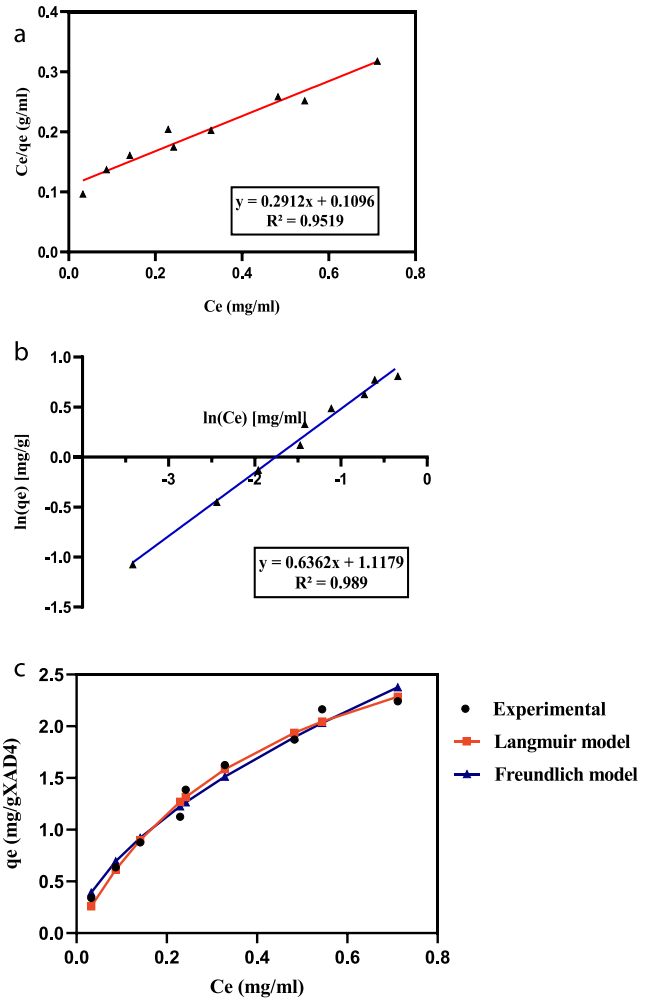

Figure 4. Effect of penicillin V concentration and isotherm fitting. Adsorption isotherm fitting in Langmuir and Freundlich isotherm models for penicillin V adsorption on Amberlite XAD4. Adsorption experiments were conducted using different penicillin V concentration solutions (0.3 to 3 mg/mL) keeping adsorbent dosage and contact time fixed. Experiments were performed in triplicate (n = 3) and the average of the triplicate data was considered for model fitting. Correlation coefficients (R^2) of both the models were compared: (a) linearized Langmuir adsorption isotherm of penicillin V/Amberlite XAD4 adsorption, (b) linearized Freundlich Langmuir adsorption isotherm of Penicillin V/Amberlite XAD4 adsorption, and (c) nonlinear isotherm model fittings for penicillin V adsorption on Amberlite XAD4 and comparison with the experimental adsorption isotherm data. Error bars depict standard error of the mean (SEM).

lower probably because of its nonpolar nature. With increasing ethanol concentration (80%) the penicillin V yield also increases. Furthermore, an increase in the ethanol concentration might have allowed the extraction of a few other adsorbed impurities along with Penicillin V, in turn decreasing the overall penicillin V yield (Figure S1).

2.6. Penicillin V Recovery from the *P. chrysogenum* BIONCL I22 Culture Filtrate. Under all the optimized conditions, temperature 25 °C, pH 6.5 to 7, 4 h adsorption time, 250 rpm, 80% ethanol for desorption, 1 h desorption time, recovery was carried out from the typical BIONCL I22 strain culture broth spiked with 3 mg/mL standard penicillin V. Spiking was carried out as the penicillin V concentration in the culture broth very low. The experimental adsorption yield was about 90 to 95%, whereas about 80 to 85% of adsorbed Penicillin V was desorbed and recovered in ethanol. The overall penicillin V recovery achieved by using Amberlite XAD4 was 80 to 85% (Figure 5b). The chromatograms represent the selective recovery of penicillin V from the culture filtrate (Figure 5c–f). According to De Barros et al., about 91% penicillin G was recovered by adsorption on Amberlite XAD4 at 4 °C and pH 4 with an initial penicillin G concentration of 50 g/L in the fermentation broth, which is comparable to the recovery in the present work.[23] However, recovery at 25 °C and without a pH change would be more favorable and sustainable than recovery at 4 °C and pH 4.

2.7. Amberlite XAD4 Resin is Reusable Over Six Cycles. Estimation of the resin reuse capacity is one of the important factors of the adsorption studies.[40] Following this, the reusability of the resin was evaluated by conducting batch adsorption experiments after optimization of all the parameters. The percentage of penicillin V adsorbed after each regeneration cycle was estimated using HPLC (Figure 6). It was observed that an overall yield of about 80 to 85% was achieved in each cycle. According to the results, the resin can be reused for about six cycles or even more without compromising the yield, making the method more sustainable and environment friendly.

2.8. Comparative Analysis of Solvent Extraction and Resin-Based Recovery. To compare the extraction of penicillin V from the culture filtrate using the traditional solvent extraction method and the resin-based recovery method, various operating parameters and extraction efficiency were taken into account. According to the findings of the present study, adsorption-based recovery can be carried out at room temperature (25 °C) without any pH adjustment, whereas solvent-based recovery requires low temperature (chilled solvents) and acidic pH for extraction. Along with fewer processing steps, the problem of emulsification was also eliminated in the resin-based recovery method. Additionally, the resin showed better reusability and could be used for six or more cycles without compromising the yield. It can be observed that the time required for adsorption based recovery was slightly more compared to solvent extraction, which was further compensated by 2-fold more penicillin V recovery by the former method.

3. CONCLUSION

The present work aimed to develop a feasible extraction approach that eliminated drawbacks of solvent extraction yet

Table 3. Coefficients of Langmuir and Freundlich Adsorption Isotherm Models for Penicillin V/Amberlite XAD4 Adsorption Data Fitted in Linear and Nonlinear Equations along with Their Correlation Coefficient (R^2) (n = 3)

	Langmuir				Freundlich		
parameter	q_m (mg/g)	K_L	R_L	R^2	n	K_F	R^2
linear	3.4335	2.6572	0.1223	0.9519	1.57	3.06	0.989
nonlinear	3.6883	2.29	0.1392	0.9664	1.70	2.90	0.9871

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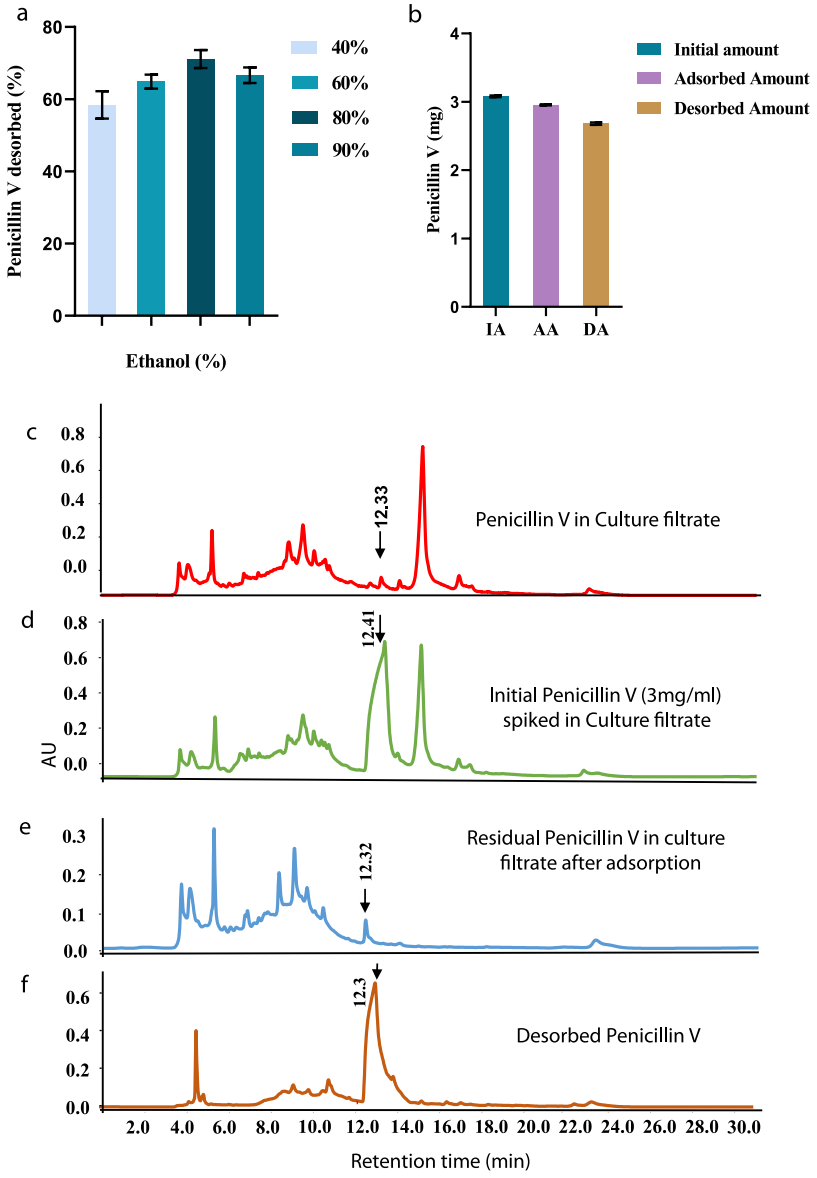

Figure 5. Optimization of ethanol concentration for desorption and penicillin V recovery from culture filtrate. Final penicillin V recovery compared in different ethanol concentrations. (a) Desorption of penicillin V adsorbed on the resin from the BIONCL I22 strain culture filtrate was carried out using an ethanol:water binary mixture. penicillin V percent recovery was examined at various ethanol concentrations, including 40, 60, 80, and 90%. Desorption studies were carried out in triplicates (n= 3) for each ethanol concentration. (b) Recovery of penicillin V was performed from the BIONCL I22 strain culture filtrate under all the conditions optimized initially with standard penicillin V. The experiments were performed in triplicates (n= 3). The graphs indicate the amount of penicillin V at different stages of resin-based recovery process where IA is the initial amount of penicillin V spiked in the culture filtrate, AA is the amount adsorbed on the resin and DA is the amount desorbed in 80% ethanol. The chromatograms represent (c) initial penicillin V in the culture broth, (d) penicillin V concentration in the culture broth after spiking with 3 mg/mL standard penicillin V, (e) residual penicillin V concentration after adsorption, and (f) final penicillin V concentration in desorption liquid. Error bars depict standard error of the mean (SEM).

achieved maximum penicillin V recovery. The optimized operational conditions for Amberlite XAD4 resin-based recovery and its reuse were found to be superior over the conventional butyl acetate-based solvent extraction. Importantly, Amberlite XAD4 had the best adsorption capacity for hydrophobic adsorption of penicillin V and equilibrium could be attained within 3 to 4 h with 3 mg/mL penicillin V. A maximum recovery of about 80 to 85% penicillin V from the *P. chrysogenum* strain BIONCL I22 culture filtrate is one of the key findings of this study. The results indicate that 2-fold higher penicillin V was recovered by the Amberlite XAD4 resin-based recovery method compared with the conventional *n*-butyl acetate-based extraction method. It could be hypothesized that high productivity and efficiency would certainly come from scaling up the process further to column and continuous mode. Further optimization of the process parameters might enhance the penicillin V recovery. Collectively, the findings show progress toward creating a more environmentally friendly alternative approach for recovering penicillin V and toward a feasible extractive fermentation method.

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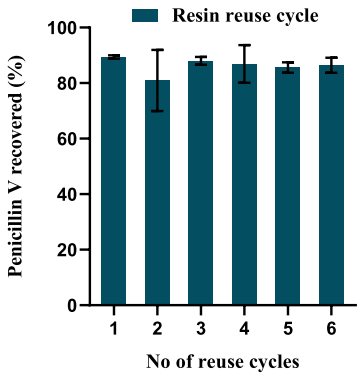

Figure 6. Determination of resin regeneration capacity. Evaluation of reusability of the resin for different resin regeneration cycles. The adsorption and desorption capacities of the resin were determined with intermediate washes between each cycle by 100% ethanol for about six cycles. The overall percentage of penicillin V recovered during each cycle was plotted. Adsorption and desorption for each cycle was performed in triplicates (n = 3). Error bars depict standard error of the mean (SEM).

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents. Penicillin V potassium salt (98% pure) was procured from Cayman Chemical (Michigan, USA) and used to make a 1 mg/mL stock solution for initial adsorption tests, without pH adjustment. The *Penicillium chrysogenum* BIONCL I22 strain (in-house collection) was used to obtain culture filtrate. All of the media ingredients were purchased from HiMedia (Mumbai, India). Ammonium lactate and beef extract were obtained from Sigma-Aldrich (St. Louis, USA). Phenoxy acetic acid (PoA) was procured from Loba Chemicals (Mumbai, India). Tween 80 was purchased from Merck Specialties Pvt. Ltd. (Mumbai, India). Three nonionic, polymeric adsorbent resins with the trade names Amberlite XAD4, Amberlite XAD7, and Amberlite XAD16 were obtained from Sigma-Aldrich (St. Louis, USA). Absolute ethanol (99.9%) was obtained from Changshu Hongsheng Fine Chemicals (Changshu, China). *n*-butyl acetate of analytical grade was purchased from Sisco Research Laboratories (Mumbai, India). Acetonitrile for the HPLC mobile phase was obtained from Avantor Performance Materials (Radnor, USA).

4.2. Conventional *n*-Butyl Acetate-Based Penicillin V Recovery. As mentioned earlier, several techniques have been developed for the extraction of penicillins, among which butyl acetate-based extraction is one of the conventionally used techniques. To compare solvent extraction with the resin-based recovery method, butyl acetate-based extraction of penicillin V was initially demonstrated using a 1 mg/mL solution of standard penicillin V followed by extraction from the culture filtrate. It was first extracted into *n*-butyl acetate (pH 2.5), followed by back extraction into phosphate buffer (pH 7.5)[19,41,42] (Figure S2). The effect of varying volume ratios of penicillin V solution to the *n*-butyl acetate organic phase was studied (Figure S3 and Table S2).

4.3. Initial Optimization of Adsorption Based Recovery Using Standard Penicillin V: Pretreatment of Adsorbent Resins and Batch Studies. The adsorbent resins were pretreated before use to remove preservative salts and impurities if any. Resin was washed thoroughly with methanol at 30 min intervals between subsequent cycles, followed by distilled water wash.[43] The resin was then dried for 24 h at 50 °C. Hydration of the resins was carried out with absolute ethanol owing to their hydrophobic character making permeation easier.[23] Furthermore, the resins were abundantly washed with distilled water to remove any storage solution. The adsorption and desorption yield of the resins with and without ethanol treatment was evaluated to determine the impact of ethanol hydration (Figure S4).

For initial batch studies, 1 g of pretreated resin was added to 10 mL of standard penicillin V solution and stirred at 25 °C with 250 rpm for 1 h. At the end of the incubation period, 1 mL samples were collected and quantified using HPLC. The adsorption yield was calculated using eq 1:[40]

$$\text{Percentage penicillin V adsorbed} = \frac{(C_0 - C_a)}{C_0} \times 100 \quad (1)$$

where C_0 is the initial zeroth min penicillin V concentration and C_a is the residual adsorption supernatant concentration after 1 h.

Desorption was carried out initially by adding 10 mL of 82.5% ethanol to the penicillin V adsorbed resin under stirring at 250 rpm for 1 h, as maximum recovery was reported in the same work.[23] About 1 mL samples were collected at the end of desorption for HPLC analysis. The following eq 2 was used to calculate the desorption yield:[44]

$$\text{Percentage penicillin V desorbed} = \frac{(C_d \times V_d)}{[(C_0 - C_a) \times V_a]} \times 100 \quad (2)$$

where C_0 is the initial concentration at zeroth min, C_a is the residual concentration in the adsorption solution, C_d is the concentration in the desorption solution, and V_a and V_d are the volume of the adsorption and desorption solutions, respectively.

4.4. Resin Screening and Estimation of Adsorbent Dosage. Three different resins, namely Amberlite XAD4, Amberlite XAD7, and Amberlite XAD16 were screened for their maximum penicillin V adsorption and desorption capacity.[23] The adsorption and desorption capacities were evaluated by performing batch experiments in triplicate for each resin using a standard penicillin V solution. To determine the effect of adsorbent dosage on penicillin V adsorption, batch experiments were performed at different dosages (0.2 to 1.8 g) of the selected pretreated resin, fixed initial concentration of penicillin V, and 10 mL volume of the solution. The amount of penicillin V adsorbed was further quantified by using HPLC.

4.5. Effect of pH. Batch assays were performed in triplicates to determine the effect of pH on adsorption of penicillin V. The pH of unbuffered aqueous penicillin V solutions was adjusted to the range 2 to 8. 1 g of selected pretreated adsorbent was added to 10 mL of each of these solutions, stirring at 250 rpm at room temperature (25 °C). Further quantification of the samples collected initially and at the end of the experiment was carried out using HPLC.

4.6. Effect of Contact Time (Adsorption Kinetics). Kinetic adsorption assays were performed to determine the equilibrium adsorption time. This was carried out by adding 1 g of the selected pretreated adsorbent to 10 mL of 1 mg/mL standard penicillin V solution, stirring at 250 rpm, room temperature (25 °C). Samples were collected at 20 min intervals for about 240 min (4 h) to determine the amount of penicillin V adsorbed. The data obtained were fitted in the

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pseudo-first-order and pseudo-second-order equations to determine the best-fit model.[45] The models were analyzed by linear as well as nonlinear fittings. Pseudo-first-order and pseudo-second-order kinetic models are expressed by eqs 3 and 4 respectively.[34,46]

$$\ln(q_e - q_t) = -(K_1) \times t + \ln(q_e) \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{q_e}t + \frac{1}{K_2 q_e^2} \quad (4)$$

where q_e (mg/g) is the amount adsorbed at equilibrium, q_t (mg/g) is the amount adsorbed at time t (min), K_1 (min^{-1}) is the rate constant of the pseudo-first-order equation, and K_2 (g $mg^{-1}{\cdot}min^{-1}$) is the rate constant of the pseudo-second-order equation.

4.7. Effect of Penicillin V Concentration (Adsorption Isotherm). To find out the maximum adsorption capacity of the resin, adsorption isotherm studies were conducted. 10 mL of standard penicillin V solutions in concentrations ranging from 0.3 to 3.0 mg/mL were equilibrated with 1 g of resin for 4 h at 250 rpm without changing the pH. HPLC was used to measure the residual penicillin V concentration in the solution, and the data were then fitted into linear and nonlinear models for the Langmuir and Freundlich adsorption isotherms. Some studies suggest that the nonlinear data fitting provides more accurate results compared to the linear data fitting.[45,47] In the present study, the adsorption models were analyzed by both linear as well as nonlinear fittings. The Langmuir adsorption isotherm assumes a fixed number of adsorption sites, monolayer adsorption, and constant adsorption energy.[48] The Freundlich adsorption isotherm describes not only monolayer adsorption but also multilayer adsorption. It assumes adsorption on a heterogeneous surface. The Langmuir and Freundlich isotherms are represented by eqs 5 and 6 below:[49,50]

$$\frac{1}{q_e} = \frac{1}{(q_m \times K_L \times C_e)} + \frac{1}{q_m} \quad (5)$$

$$\ln(q_e) = \frac{1}{n} \times \ln(C_e) + \ln(K_F) \quad (6)$$

where q_e is the amount adsorbed per g resin (mg/g), q_m is the maximum amount adsorbed, C_e is the equilibrium concentration in the liquid phase (mg/mL), K_L and K_F are the Langmuir and Freundlich constants, respectively.[51] In the Langmuir isotherm, the following values of separation factor R_L indicate favorability of adsorption: unfavorable for $R_L > 1$, linear for $R_L = 1$, favorable for $0 < R_L < 1$, and irreversible for $R_L = 0$.[52−55] The important feature of Freundlich isotherm can be represented by $1/n$ which indicates favorability of the process. If the value of n is above unity ($n > 1$), it indicates that adsorption is favorable and physical.[56]

4.8. Production and Final Recovery of Penicillin V from the *P. chrysogenum* BIONCL I22 Strain Culture Filtrate. *P. chrysogenum* BIONCL I22 was maintained on potato dextrose agar (PDA). The spore suspension was prepared from a 7-day-old culture by gently scraping the spores from the *P. chrysogenum* culture with a solution of sterile NaCl (0.08%) and Tween 80 (0.01%). The Neubauer chamber (Marienfeld, Germany) was used to count the spores, which were preserved in 50% glycerol at −80 °C for future use.

A 10% seed medium containing (g/L) glucose (40), ammonium lactate (21), sodium sulfate (0.74), calcium carbonate (13), $ZnCl_2{\cdot}2H_2O$ (0.25), $MgCl_2{\cdot}2H_2O$ (0.2), $FeCl_3$ (0.2), $CuCl_2{\cdot}2H_2O$ (0.2), and $CaCl_2{\cdot}2H_2O$ (0.5) was inoculated with a 7-day-old *P. chrysogenum* spore suspension (1×10^8 spores/mL) and incubated for 48 h at 25 °C with 180 rpm in a rotary incubator shaker (Steelmate Industries, India). The acquired seed media were then inoculated in freshly prepared production medium (PM8) that contained (g/L) lactose (40), beef extract (20), and phenoxy acetic acid (PoA) (0.01), and the pH of the medium was adjusted to 6.5. Furthermore, it was cultured for 8 days at 25 °C and 180 rpm in a rotary incubator shaker. Following incubation, the culture was harvested and initially filtered with a muslin cloth, followed by filtering with a 0.22 μm filter.[19,57,58]

4.9. Batch Adsorption Experiments from the *P. chrysogenum* BIONCL I22 Culture Filtrate. The initial penicillin V concentration in the filtered broth was determined using HPLC and found to be 0.8 mg/mL. As the penicillin V concentration in the culture broth was low, it was spiked with standard penicillin V equal to its equilibrium concentration. From the initial optimization experiments, the contact time, adsorbent dosage, and penicillin V concentration for the screened resin were estimated at 25 °C. Furthermore, the adsorption of penicillin V from *P. chrysogenum* BIONCL I22 culture broth was carried out under these optimized operational conditions. The adsorption experiments were performed in triplicates with a 10 mL culture volume for each set. Once adsorption equilibrium was reached, 1 mL aliquots were withdrawn for HPLC analysis.

4.10. Desorption Studies and Evaluation of Resin Reusability. The adsorption experiments were performed under all optimized conditions. After reaching adsorption equilibrium, the residual solutions were removed from each beaker and 10 mL aqueous ethanol solutions of various concentrations (40, 60, 80, and 90%) were added for the desorption process. Desorption was performed at 250 rpm and 25 °C for 1 h. At the end of 1 h, 1 mL aliquots were withdrawn for HPLC analysis. The desorption capacity was calculated, and percentage penicillin V desorbed vs solvent concentration plots were plotted to determine the maximum penicillin V recovered. Desorption studies were carried out in triplicate for each ethanol concentration. Initially, the maximum penicillin V recovery was determined by using a standard penicillin V solution. The experiment was then duplicated in a culture filtrate. Furthermore, the reusability of the resin was evaluated by regenerating the resin with 100% ethanol wash after each cycle.[59,60]

4.11. Estimation and Analysis Using HPLC. The wavelength was confirmed initially by performing a spectral scan for penicillin V using a LabIndia UV 3000+ UV/vis spectrophotometer. A standard calibration curve was plotted for the HPLC analyses. Samples were prefiltered through 0.22 μm sterile syringe filters and analysis was performed using an HPLC instrument (Waters, Milford, USA) assembled with a C18 X-bridge column (4.6 × 260 mm, 5 μm, Waters, Milford, USA). 50 μL of the sample was injected and analyzed at 25 °C, 263 nm wavelength using 100% acetonitrile (ACN) as the mobile phase and 25 mM KH_2PO_4 buffer (pH 4.8) in gradient, maintaining a flow rate of 0.7 mL/min. The limit of detection (LOD) for penicillin V was 0.07 mg/mL for the HPLC below which penicillin V was not detectable. The lower limit of

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quantification (LLOQ) was 0.1 mg/mL, and the upper limit of quantification was about 3.4 mg/mL.

4.12. Statistical Analysis. Statistical analysis, adsorption kinetics, and adsorption isotherm model fitting were performed by using Microsoft Excel 2016 and GraphPad Prism 8 software. All of the experiments were performed at least in triplicate (n = 3). The findings are presented as the mean ± SEM of triplicate determinations. Kinetic and isotherm plots were plotted by considering the average of the triplicate data. Linear and nonlinear regression analysis was performed to calculate R^2 values and model fitting. Details are provided in Section 4 as well as in the main and supplementary figure legends wherever necessary.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00251.

Chromatograms for penicillin V recovered in different ethanol concentrations (Figure S1); separation of organic and aqueous phases in butyl acetate-based extraction (Figure S2); impact of different butyl acetate volumes on the percentage of penicillin V recovered and butyl acetate based penicillin V extraction methodology and results (Figure S3 and Table S2); percentage penicillin V adsorbed and desorbed on the resin treated with and without ethanol (Figure S4); summary of existing penicillin recovery approaches (Table S1) (PDF)

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Notes

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