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Enhanced Downstream Processing of 2,3-Butanediol through Salt Alcohol Aqueous Two-Phase Extraction

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Abstract

Separation of 2,3-butanediol (2,3-BDO) from broth is difficult because of the mixture's complexity and the presence of impurities. In this study, an integrated approach combining ATPE with distillation was developed to enhance recovery performance. Among several systems evaluated, the isobutanol (i-BuOH)/K_2HPO_4 (DKP) combination showed the best partitioning behavior. Process parameters, including salt concentration, temperature, and solvent fraction, were optimized using a CCD-RSM approach, leading to optimal conditions of 25% (w/v) DKP, 30% (v/v) i-BuOH and 40°C. Under these conditions, the model predicted an extraction yield of 98.23%, while experimental validation gave 97.81%, confirming good agreement between the predicted and experimental results. The optimized system achieved a high distribution coefficient of 60.47. The extract phase was then subjected to distillation for further concentration. Scale-up experiments demonstrated consistent performance, achieving 98.31% extraction and 98.02% overall recovery (purity > 99%) in a single run. Additionally, DKP was successfully reclaimed from the aqueous phase via methanol-assisted crystallization with an efficiency of 96.85%. Overall, this integrated and economically viable process significantly improves 2,3-BDO separation while enabling effective recycling of both solvent and salt, making it suitable for industrial-scale applications.

1. Introduction

2,3-Butanediol (2,3-BDO) is an important platform chemical with applications as a fuel additive, solvent, antifreeze agent, and intermediate for producing value-added chemicals such as butanone, 1,4-lactone, and buta-1,3-diene [1–3]. Microbial fermentation has emerged as a sustainable route for 2,3-BDO production because it can utilize renewable feedstocks and operates under milder conditions than conventional petrochemical processes [2,4–6]. However, downstream separation remains a major challenge for the commercial production of bio-based 2,3-BDO.

The recovery of 2,3-BDO from fermentation broth (FB) is difficult because of its high boiling point (184°C), strong affinity for water, relatively low broth concentration, and the presence of impurities such as proteins, residual sugars, salts, and organic acids [7–9]. These factors complicate the design of separation processes that are both cost-effective and energy-efficient. A range of recovery methods, including distillation [10], solvent extraction [11], sugaring-out [12], reactive extraction [13], aqueous two-phase extraction (ATPE) [14], salting-out [15], and membrane-based techniques [16], have been explored. However, these methods often suffer from high energy demand, poor selectivity, high solvent consumption, operational complexity, or limited scalability. For example, direct distillation can achieve high purity but requires high energy input, while conventional solvent extraction is limited by the hydrophilic nature of 2,3-BDO. Polymer-based ATPS also faces drawbacks such as low distribution coefficient, high polymer cost, and difficult product recovery [17]. Therefore, a scalable, cost-effective, and recyclable downstream process is still required.

Salt–alcohol-based ATPE has attracted attention as a promising alternative because it operates under mild conditions, provides rapid phase separation, uses relatively inexpensive phase-forming agents, and

can be integrated with solvent recovery steps [18–20]. In such systems, 2,3-BDO preferentially partitions into the alcohol-rich phase, while salts enhance phase formation through salting-out effects. Previous studies have reported several alcohol/salt systems for 2,3-BDO recovery, including IPA/AMS, EtOH/DKP, EtOH/AMS, and butanol/NaCl systems, with extraction efficiencies generally ranging from 91.7 to 98.13% [8,14,18,20,21]. Nevertheless, further improvement is needed in terms of distribution coefficient, material recyclability, salt recovery, and process validation at larger scale.

Compared with our previous studies on butanol/NaCl-based aqueous two-phase extraction and extraction-assisted distillation [14], the present work introduces a new i-BuOH/DKP salt–alcohol ATPE system with improved partitioning performance and better process recyclability. The selected i-BuOH/DKP system provides a higher distribution coefficient, efficient phase separation, and easier material recovery than several previously reported solvent/salt combinations, including IPA/AMS, EtOH/DKP, EtOH/AMS, and butanol/NaCl systems [8,14,18,20,21]. In addition to CCD-RSM-based optimization, this study demonstrates integrated solvent recovery by distillation, DKP regeneration through methanol-assisted crystallization, and validation at a 1 L scale. These features distinguish the present process from earlier reports and highlight its potential as a more practical, recyclable, and scalable approach for downstream recovery of bio-based 2,3-BDO from FB.

In this study, the distribution behavior of 2,3-BDO was systematically evaluated across different alcohol–salt ATPE systems, and the isobutanol (i-BuOH)/K_2HPO_4 (DKP) system was identified as the most effective combination. The effects of solvent fraction, salt concentration, and temperature were optimized using central composite design-response surface methodology (CCD-RSM). The optimized extract phase was further subjected to distillation for solvent recovery and product purification, while DKP was regenerated from the aqueous phase through methanol-assisted crystallization. The integrated process was also validated at a 1 L scale to assess its practical applicability for high-purity 2,3-BDO recovery.

2. Experimental section

2.1. Chemicals and microorganism

Analytical-grade glucose (99.5%), DKP (99%), $MgSO_4 \cdot 7H_2O$ (99–102%), and AMS (99.5%) were purchased from Merck (India). Deuterium oxide (D_2O, 99%) and 2,3-BDO (99%) were obtained from Sigma-Aldrich (USA). $MgCl_2 \cdot 6H_2O$ (99–102%) and $CaCl_2 \cdot 6H_2O$ (97%) were sourced from Fisher Scientific (USA). Organic solvents (OS), including ethyl ethanoate (EE), MeOH, octyl alcohol, butyl ethanoate (BE), hexamethylene, i-BuOH, and (≥ 99% purity) were supplied by SRL (India). The *Bacillus licheniformis (BS)* culture was procured from the Microbial Type Culture Collection, Chandigarh, India. All experimental procedures were carried out using distilled H2O purified through a Millipore system.

2.2. Fermentation procedure

Production of 2,3-BDO was performed using *BS* in FB formulated with glucose and a basal salt medium following the method reported in [22]. The cultivation was performed at 37°C with the pH maintained at 6 and agitation set to 250 rpm. An inoculum size of 5% (v/v), corresponding to an optical density of 1, was used, and the process was continued for 48 h. Following fermentation, the broth was centrifuged to separate the biomass, and the resulting supernatant was found to contain 76.5 g/L of 2,3-BDO. The chromatographic profile of the product is provided in **Fig. S1**.

2.3. Analytical methods

The composition of the FB was determined using gas chromatography (Bruker 450GC, USA) equipped with a CP Wax 57CB column and a flame ionization detector (FID). The oven T program began at 60°C (held for 1 min) and was then increased to 220°C at a heating rate of 20°C/min. Nitrogen served as the carrier gas at a flow rate of 30 mL/min, while the injector and detector were maintained at 220 and 250°C, respectively. Quantitative analysis was performed using a calibration curve for 2,3-BDO in the range of 0.2–1 g/L, which exhibited strong linearity (**Fig. S2**).

GC and NMR spectroscopy (Bruker ASCEND 600 MHz) were employed to confirm the purity of the recovered product. For NMR measurements, 250 µL of sample was diluted with an equal volume of D_2O. ^{1}H spectrum was recorded with 16 scans and a 1s relaxation delay, while ^{13}C spectrum was collected using 1000 scans and a 2 s delay to achieve dependable structural identification.

2.4. ATPE experiments

In a typical experiment, a predetermined quantity of salt was dissolved directly into the FB to obtain a homogeneous aqueous solution. Subsequently, an equal volume of OS was introduced, and the mixture was agitated for 10 min to ensure proper phase interaction. The mixture was then poured into a separatory funnel and left undisturbed until distinct phase separation was observed. The upper, solvent-rich phase was separated and processed by distillation or evaporation to recover the solvent and concentrate 2,3-BDO. At each step, GC analysis was performed to monitor the concentration and purity of the product. For comparative partitioning studies, the same protocol was applied using a range of OS, including BA, octyl alcohol, n-butyl alcohol, EA, isopentanol, MeOH, i-BuOH, and hexamethylene. In addition, the fermentation broth was preconditioned with 20% (w/v) of different salts such as DKP, AMS, $MgSO_4{\cdot}7H_2O$, $MgCl_2{\cdot}6H_2O$, and $CaCl_2{\cdot}6H_2O$. The efficiency of extraction was assessed in terms of extraction yield (Y) and D, calculated using Eqs. (1) and (2).

$$Y\ (\%) = \frac{C_O \times\ V_O}{C_{FB} \times\ V_{FB}} \times\ 100$$

1

$$D = \frac{C_O \times\ V_O}{C_W \times\ V_W}$$

2

Here, C_O, C_W, and C_{FB} represent the concentrations of 2,3-BDO in the organic phase (OP), AP, and initial FB, respectively, while V_{FB}, V_W, and V_O correspond to their respective volumes.

2.5. Process optimization and statistical evaluation

To enhance the extraction efficiency of 2,3-BDO, a CCD integrated with RSM was applied, considering three variables at three levels. The selected factors included salt concentration (10–40% w/v), T (20–60°C), and solvent proportion (10–50% v/v), with Y taken as the response. A total of 20 experimental trials, covering different parameter combinations, were generated using Design-Expert 13 software. The influence of individual variables and their interactions was statistically examined using one-way ANOVA at a confidence level corresponding to $p \leq 0.05$. The performance of the developed model was assessed using the R^2 and adjusted R^2 values. A second-order polynomial equation (Eq. 3) was further employed to correlate the independent variables with extraction yield, aiding in the optimization of operating parameters.

$$Y = \alpha_0 + \sum_{i=1}^{n} \alpha_i X_i + \sum_{i=1}^{n} \alpha_{ij} X_i X_j + \sum_{i=1}^{n} \alpha_{ii} X^2$$

3

In the polynomial model, α_0 represents the constant term, while α_i, α_{ii}, and α_{ij} describe the contributions from linear, quadratic, and interaction effects, respectively. The dependent variable Y corresponds to the extraction yield of 2,3-BDO, and X_i and X_j denote the coded process variables, where $i \neq j$. The optimal operating conditions were determined using a desirability function (d), which varies between 0 and 1. Here, 0 denotes non-compliance with the criteria, whereas 1 corresponds to the optimal conditions that maximize 2,3-BDO recovery while fulfilling all specified constraints [14,23–25].

2.6. Scale-Up of the integrated ATPE-distillation operation

For larger-scale operation, a 10 L extraction unit was integrated with a batch distillation system. The separation column had an internal diameter of 21 mm and a height of 60 cm, and it was designed with 50 theoretical stages. A Teflon spinning band was incorporated to improve separation efficiency and aid in effective solvent recovery. The setup accommodated boiler volumes ranging from 1 to 50 L and offered flexible reflux ratio control from 0:1 up to 999:1. It could be operated either manually or through automated control, providing the precision and adaptability required for scaling up 2,3-BDO recovery.

In the scale-up study, 1 L of FB was first treated with 25% (w/v) DKP and mixed thoroughly until the salt was completely dissolved. Subsequently, 30% (v/v) i-BuOH was added, and the system was agitated for 10 min at 40°C to promote phase formation and solute transfer. Once a clear separation occurred, the upper organic phase enriched with 2,3-BDO was carefully withdrawn. The collected extract was then processed by atmospheric distillation column. During operation, the condenser T was maintained at 25°C, while heating was operated at 50% of the system's capacity to selectively vaporize i-BuOH. This enabled effective solvent recovery along with concentration and purification of 2,3-BDO.

3. Results and Discussion

3.1. Screening of solvent/salt system

The distribution of 2,3-BDO in different solvent–salt systems is summarized in **Table 1**. Systems based on more polar (hydrophilic) solvents exhibited noticeably higher D = 0.38–7.3 and Y = 25.3–87.5% than those using nonpolar solvents. Within ATPS, 2,3-BDO preferentially accumulates in the solvent-rich phase. By contrast, when hydrophobic solvents were employed, most of the compound remained in the salt-rich AP, indicating poor separation performance.

Salt composition plays a critical role in determining partitioning behavior. Anions such as phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}) exert a stronger influence than cations like Mg^{2+} and Ca^{2+} [14,26,27]. These highly charged anions promote phase formation and enhance the selective transfer of 2,3-BDO into the organic layer. Although $CaCl_2$ and $MgCl_2$ were capable of forming two-phase systems with all tested solvents, the most effective extraction was achieved using hydrophilic solvents and, in some cases, moderately nonpolar ones such as octyl alcohol, hexamethylene, and isoamyl alcohol. The highest extraction performance was observed with i-BuOH paired with DKP or AMS. Compared to IBA alone, the presence of salts substantially improved both D and Y, reaching D = 7.3 and Y = 87.5% for DKP, and D = 5.8 and Y = 80.5% for AMS. This enhancement is attributed to the salting-out effect, where increased ionic strength reduces the affinity of 2,3-BDO for H_2O and promotes its transfer to the solvent phase.

The presence of salts also facilitates phase disengagement by disrupting hydrogen bonding between H_2O and 2,3-BDO, thereby lowering its solubility in the AP. Highly charged ions reduce the dielectric constant of H_2O and strengthen hydration interactions, intensifying this effect; the extent depends on ion charge, concentration, and overall ionic strength [18,20]. In practice, systems containing AMS occasionally formed cloudy interfaces due to precipitation of proteins and amino acids, whereas DKP produced a sharp and rapidly settling boundary, indicating cleaner separation. Unlike solvent-only systems such as i-BuOH, which may form azeotropes requiring additional separation units, salt-assisted systems enable straightforward phase splitting without such complications, reducing both operational complexity and cost [28].

Based on these observations, the i-BuOH/DKP system was selected for further study. Its rapid phase separation, combined with low material recovery costs, makes it particularly suitable for scale-up and industrial application in 2,3-BDO extraction.

3.2. RSM optimization of 2,3-BDO extraction

The experimental and model-predicted Y of 2,3-BDO under different conditions are presented in **Table 2**. The observed yields varied widely, from 43.09% to 98.23%, with an average value of 75.4%. The highest recovery (98.23%) was achieved at 30% (v/v) solvent, 25% (w/v) salt and 40°C, whereas the lowest yield (43.09%) occurred at 10% solvent, 10% salt and 25°C. The regression model demonstrated strong

predictive performance, with R^2 = 0.9997, adjusted R^2 = 0.9994, and predicted R^2 = 0.9985, confirming its high accuracy.

ANOVA further verified the statistical significance of the model, as indicated by a large F-value (3673.64) and a p-value below 0.0001 (**Table 3**). Among the studied variables, solvent fraction had the most pronounced influence on extraction efficiency (F = 4948.57), followed by salt concentration (F = 946.17), while T showed a comparatively minor effect (F = 3.42), though still statistically significant. The lack-of-fit test was found to be insignificant (F = 1.6), indicating that the model sufficiently describes the experimental data. From these findings, **Eq. 4** was derived from Eq. (3) to estimate the extraction yield as a function of the key parameters.

$$Y = 98.06 + 10.13A + 4.42B - 0.2637C - 0.51AB + 0.35AC - 0.5729BC - 12.19A^2 - 13.42B^2 - 14.14C^2 \quad (4)$$

The reliability of the model is supported by the close agreement between the predicted R^2 values and coefficient of variation (CV%) (**Table S1**). As illustrated in **Fig. 1a**, the predicted and experimental yields exhibit excellent correlation, with a very small average deviation (~ 0.61%). The normal probability plot (**Fig. 1b**) demonstrates that the residuals conform to a normal distribution, thereby validating the assumptions of the regression model. Additionally, residuals plotted against predicted values (**Fig. 1c**) show random dispersion, confirming homoscedasticity, while their distribution versus run order (**Fig. 1d**) reveals no systematic pattern, indicating the absence of experimental bias. Collectively, these statistical diagnostics confirm that the model is robust and suitable for process optimization.

Three-dimensional response surface and contour plots (**Fig. 2**) provide insight into the interaction effects between variables. The combined influence of solvent and salt concentrations (**Figs. 2a and 2d**) shows that optimal performance occurs near 30% solvent and 25% salt, indicating a synergistic effect that enhances partitioning. The interaction between salt concentration and T (**Figs. 2b and 2e**) highlights improved extraction at around 40°C, emphasizing the role of T in conjunction with salting-out. Similarly, the relationship between solvent fraction and T (**Figs. 2c and 2f**) suggests that higher solvent levels combined with moderate T favor 2,3-BDO recovery. These visualizations clearly demonstrate how parameter interactions influence extraction efficiency and help identify optimal conditions.

Optimization using Design-Expert software identified 30% (v/v) solvent, 40°C and 25% (w/v) salt as the most favorable conditions. Under these conditions, the model predicted a Y of 98.23%, slightly exceeding the theoretical estimate by 0.42%. The experimentally obtained yield of 97.81% was in close agreement with the predicted value, demonstrating the model's reliability. These results establish a strong basis for scaling up the process, improving both efficiency and feasibility for industrial recovery of 2,3-BDO from FB.

3.3. Integrated recovery, solvent recycling, and salt regeneration

Following the optimization of the ATPE system, the selected conditions were applied to maximize the recovery of 2,3-BDO from FB. After phase separation, the solvent-rich layer containing the target compound was subjected to distillation for purification (**Fig. 3**). Mass balance analysis (**Table S2**) indicated that the IATPED achieved an extraction efficiency of 98.31% and an overall recovery of 98.02% (purity > 99%) in a single operation. In addition, the process showed strong solvent recyclability, with approximately 97% of the solvent being recovered at a purity greater than 97%. The chromatographic profile of the purified product (**Fig. S3**) further confirms the efficacy of the separation process.

While solvent recovery through distillation is relatively simple, regeneration of the salt phase presents additional challenges. In this system, DKP accumulates in the AP, and there is a possibility of salt crystallization during processing, which could affect separation efficiency. To overcome this issue, a MeOH-assisted crystallization approach was employed (**Fig. 4**). By adding methanol to the salt-rich AP, selective precipitation of DKP was induced. Increasing the MeOH-to-AP volume ratio from 0.5 to 2 significantly improved salt recovery, rising from 74.75% to 96.85%. However, further increases beyond a ratio of 2.5 reduced recovery, likely due to enhanced solubility and co-dissolution effects [29]. Therefore, a ratio of 2 was identified as optimal for achieving high recovery while maintaining process practicality.

Reported efficiencies for ATPE-based recovery of 2,3-BDO generally fall within the range of 94.5–98.18%. For example, Li et al. reported a yield of 91.7% using 16% AMS and 32% EtOH [18], while Narisetty et al. achieved 97% recovery with a D of 45 using 50% IPA and 30% AMS [21]. Similarly, Sun et al. obtained 93.7% recovery with 20% AMS and 34% 2-propanol [8]. Compared to these systems, the i-BuOH/DKP-based ATPS developed in this study provides improved extraction efficiency, recovery, and product purity (**Table S3**), highlighting its advantages for large-scale implementation.

Overall, the i-BuOH/DKP system demonstrates strong potential for efficient separation of 2,3-BDO from FB. The process enables effective reuse of i-BuOH via distillation and recovery of DKP through methanol-induced crystallization, thereby reducing material consumption. Its operational simplicity and scalability make it a promising candidate for industrial production of bio-based 2,3-BDO.

3.4. Structural verification of recovered 2,3-BDO

The identity and purity of the isolated 2,3-BDO were confirmed using NMR, with the corresponding spectra provided in **Fig. S4**. Both ^{13}C and ^{1}H NMR analyses were carried out, and the results were compared with those of a commercially available standard to ensure accurate structural validation. In the ^{13}C NMR spectrum, two clear signals were observed at 17.3 ppm and 71.2 ppm, corresponding to the methyl ($-CH_3$) and methine (–CH) carbon atoms, respectively, which are characteristic of 2,3-BDO. The ^{1}H NMR spectrum further supported this assignment, showing the methine proton as a quartet at 2.52 ppm and the methyl protons as a doublet at 1.01 ppm. A signal near 4.79 ppm was also detected, which is attributed to the residual solvent peak from D_2O used during analysis. The consistency of these spectral features with those of the reference standard confirms that the recovered product possesses the expected molecular structure and a high level of purity.

4. Conclusions

This study investigated the recovery of 2,3-BDO using different combinations of OS and inorganic salts in ATPS, with the i-BuOH/DKP system emerging as the most effective due to its economic feasibility and ease of recycling both solvent and salt. Process optimization using CCD and RSM highlighted the significant roles of salt concentration, solvent fraction, and T in influencing extraction performance. The developed quadratic model accurately described the relationship between these variables and the Y. At optimized conditions 30% (v/v) i-BuOH, 25% (w/v) DKP, and 40 °C, the system delivered a high D of 60.47 along with a Y of 98.31%. The solvent-rich phase was further processed by distillation to obtain a concentrated product. Scale-up experiments demonstrated consistent and reliable performance, achieving approximately 98.31% extraction and 98.04% recovery (purity> 99%) in a single step. In addition, recovery of DKP from the AP reached 96.85% through MeOH-assisted crystallization, supporting material reuse and lowering operational costs. Overall, the proposed approach offers a practical, scalable, and cost-efficient strategy for 2,3-BDO recovery, with reduced waste generation and improved suitability for industrial implementation.

Declarations

CRediT authorship contribution statement

PMG: Conceptualization, Investigation, Formal analysis, Data curation, software, Writing- original draft, review & editing. **SL**: Conceptualization, Investigation, Formal analysis, Software.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

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Tables

Table 1. Distribution of 2,3-BDO across different solvent/salt systems.

Organic solvent/Inorganic salt		$MgSO_4$	DKP	AMS	$CaCl_2$	$MgCl_2$
MeOH	**D**	1.01	3.23	3.8	1.1	1.03
	Y(%)	44.23	72.53	77.5	40.5	39.5
i-BuOH	**D**	1.74	7.68	5.8	1	1.16
	Y(%)	66.14	87.5	80.5	42.33	42.25
BE	**D**	1.02	2.18	2.21	0.97	1.06
	Y(%)	53.25	66.36	56.3	44.23	45.6
EE	**D**	1.08	3.21	3.28	1.12	1.16
	Y(%)	46.31	76.6	62.3	49.3	48.2
Butanol	**D**	1.1	4.37	3.64	1.08	1.27
	Y(%)	51.58	78.85	72.36	44.8	51.05
2-ethylhexanol	**D**	1.12	5.25	2.3	1.15	1.13
	Y(%)	54.12	79.23	69.5	48.5	42.3
Octyl alcohol	**D**	0.56	1.72	1.65	0.58	0.51
	Y(%)	35.21	46.53	38.3	31.25	30.2
Hexamethylene	**D**	0.5	0.52	0.51	0.42	0.38
	Y(%)	34.58	37.56	35.6	35.63	33.56
Isopentanol	**D**	0.46	0.56	0.51	0.49	0.43
	Y(%)	25.3	45.2	38.52	39.5	32.25

Volume of FB: 5 mL; Salts concentration (% w/v):20; Volume of solvent: 5 mL; Concentration of 2,3-BDO in FB: 76.5 g/L; Mixing duration: 15 min

Table 2. CCD-RSM-design matrix and response data for 2,3-BDO extraction from FB.

Run	A:Solvent(% v/v)	B: Salt (%w/v)	C:Temperature(°C)	Actual yield(%)	Predicted yield (%)
1	50	40	60	72.08	71.87
2	50	10	60	65.2	65.21
3	50	10	25	63.58	63.89
4	10	40	60	52.22	51.93
5	30	25	40	98.23	97.81
6	30	5	40	68.23	67.96
7	10	40	25	54.39	54.31
8	30	25	40	98.23	97.81
9	50	25	40	95.89	95.70
10	10	10	60	43.31	43.21
11	50	40	25	72.79	72.84
12	30	25	40	98.23	97.81
13	5	25	40	65.98	66.16
14	30	25	60	83.03	83.66
15	30	25	40	98.23	97.81
16	30	25	40	98.23	97.81
17	10	10	25	43.09	43.30
18	30	25	20	75.35	75.03
19	30	40	40	88.3	88.89
20	30	25	40	98.23	97.81

Table 3. ANOVA summary for best-fit RSM model adequacy and 2,3-BDO extraction from FB.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	7108.3	9	789.81	3673.64	< 0.0001	Significant
A-Solvent	1063.91	1	1063.91	4948.57	< 0.0001	
B-Salt	203.42	1	203.42	946.17	< 0.0001	
C-Temp	0.7358	1	0.7358	3.42	0.094	
AB	2.12	1	2.12	9.87	0.0105	
AC	0.985	1	0.985	4.58	0.058	
BC	2.64	1	2.64	12.27	0.0057	
A^2	661.84	1	661.84	3078.4	< 0.0001	
B^2	885.82	1	885.82	4120.21	< 0.0001	
C^2	912.48	1	912.48	4244.22	< 0.0001	
Residual	2.15	10	0.215			
Lack of Fit	1.32	5	0.2647	1.6	0.309	Not insignificant
Pure Error	0.8265	5	0.1653			
Cor Total	7110.45	19				

Figures

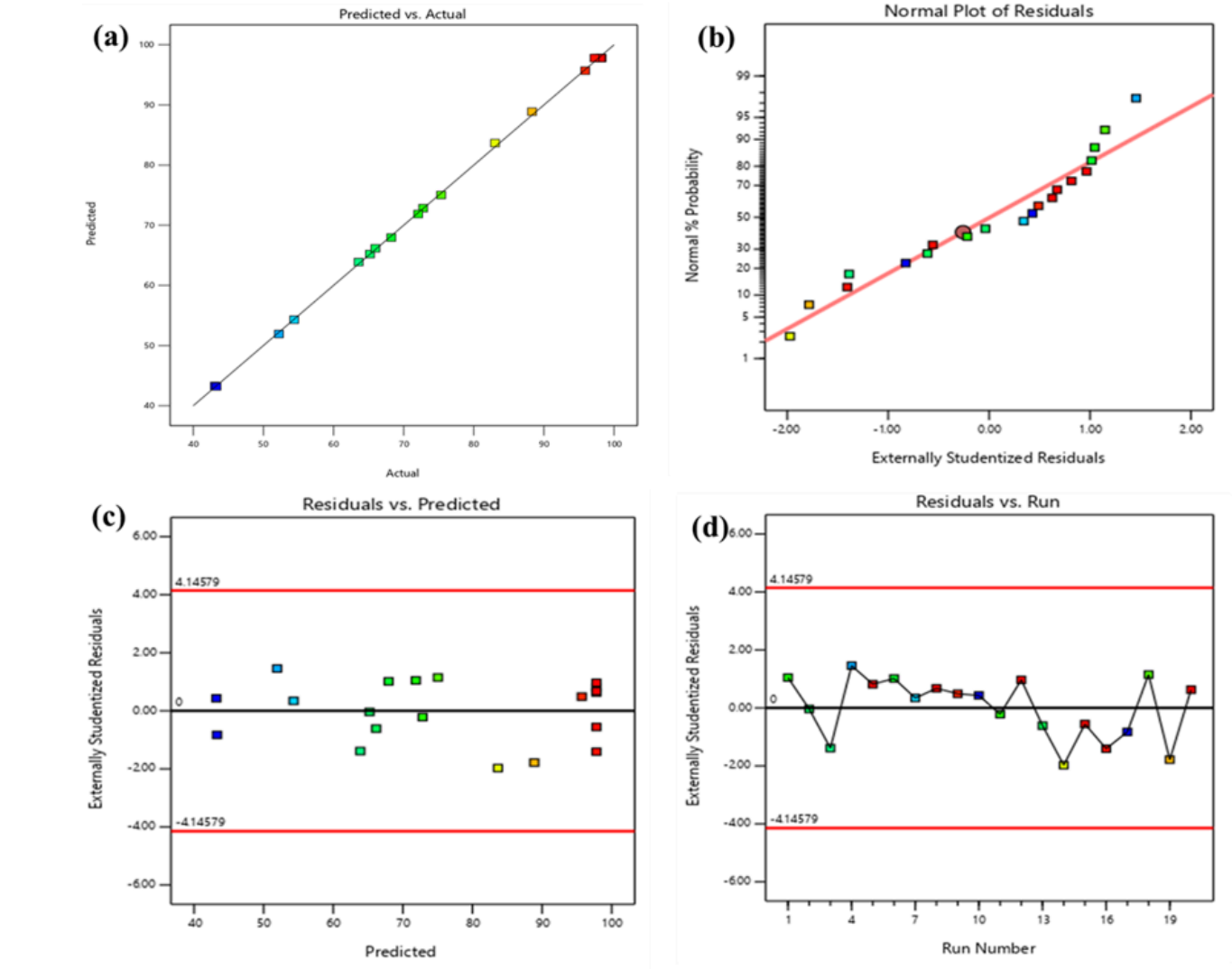


Figure 1

Diagnostic plots for the RSM model of 2,3-BDO extraction yield: (a) actual Vs predicted yield, (b) normal probability Vs externally studentized residuals, (c) externally studentized residuals Vs predicted yield, and (d) externally studentized residuals Vs run number.

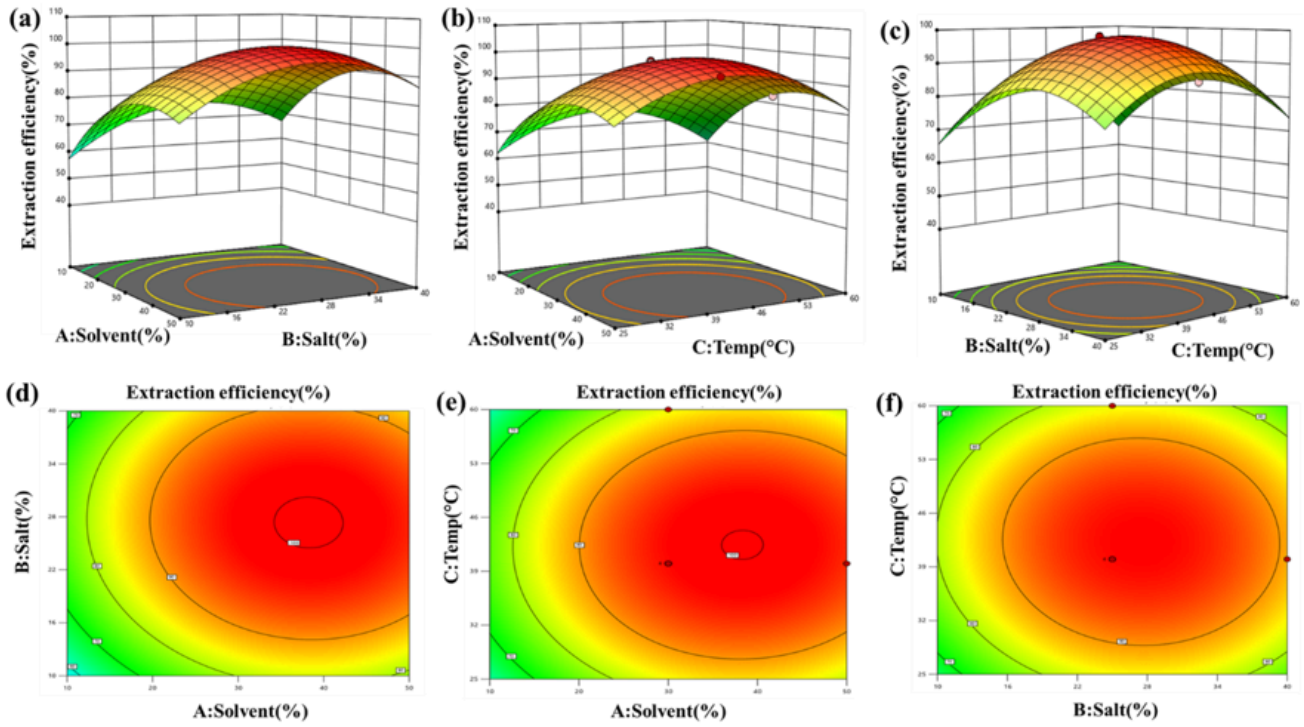


Figure 2

Response surface and contour plot showing the effect of process variable on of 2,3-BDO extraction yield: (a & d) AB, (b & e) AC, and (c & f) BC [A: Salt (%); B: Solvent (%); C: Temperature (°C)].

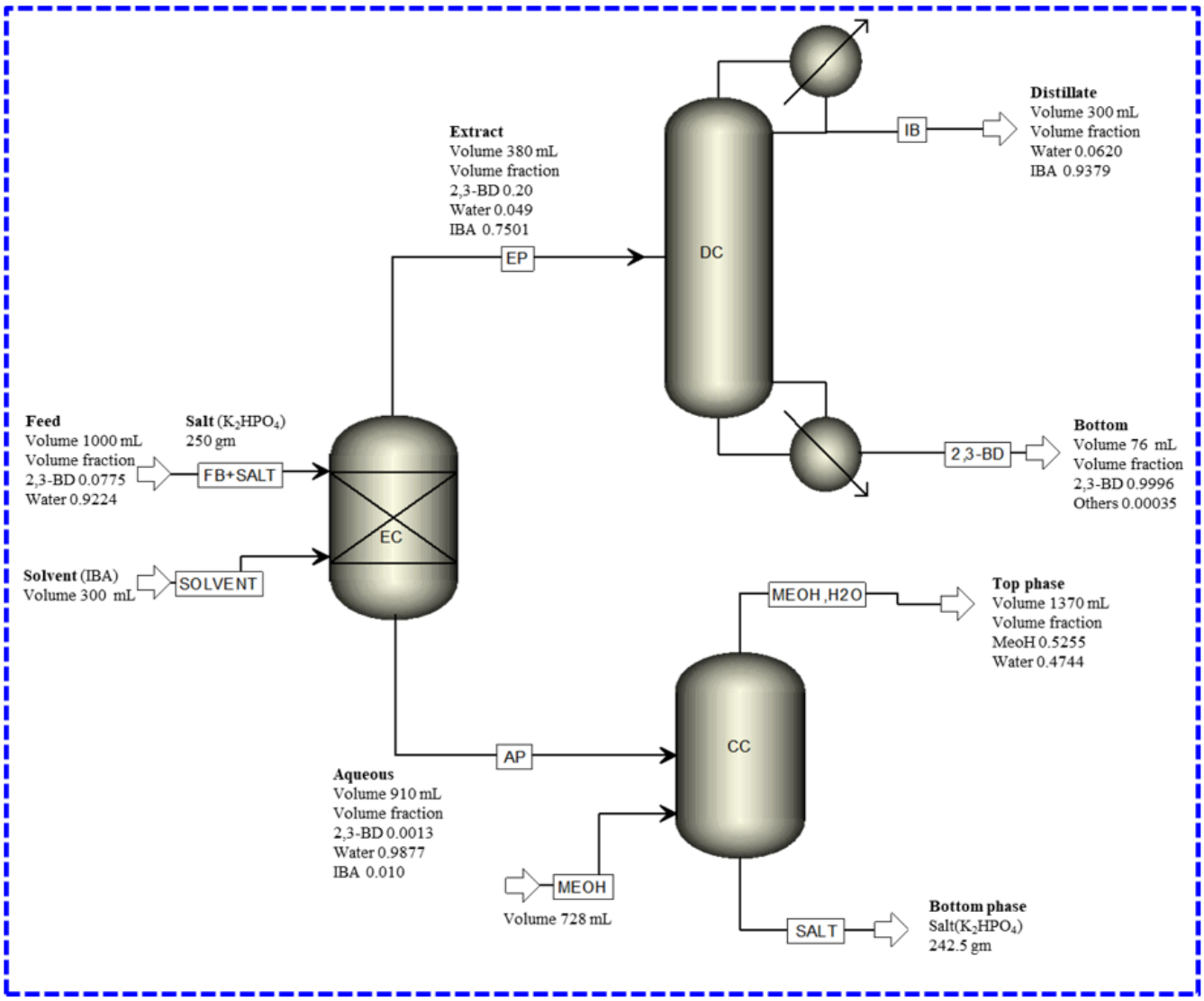


Figure 3

Schematic of the integrated ATPE-distillation using an i-BuOH/DKP system for 2,3-BDO recovery from FB[CC: crystallization column; DC: distillation column; EC: extraction column; EP: extract phase; AP: aqueous phase; FB: fermentation broth; IB: isobutanol; MeOH: methanol; 2,3-BDO: 2,3-butanediol].

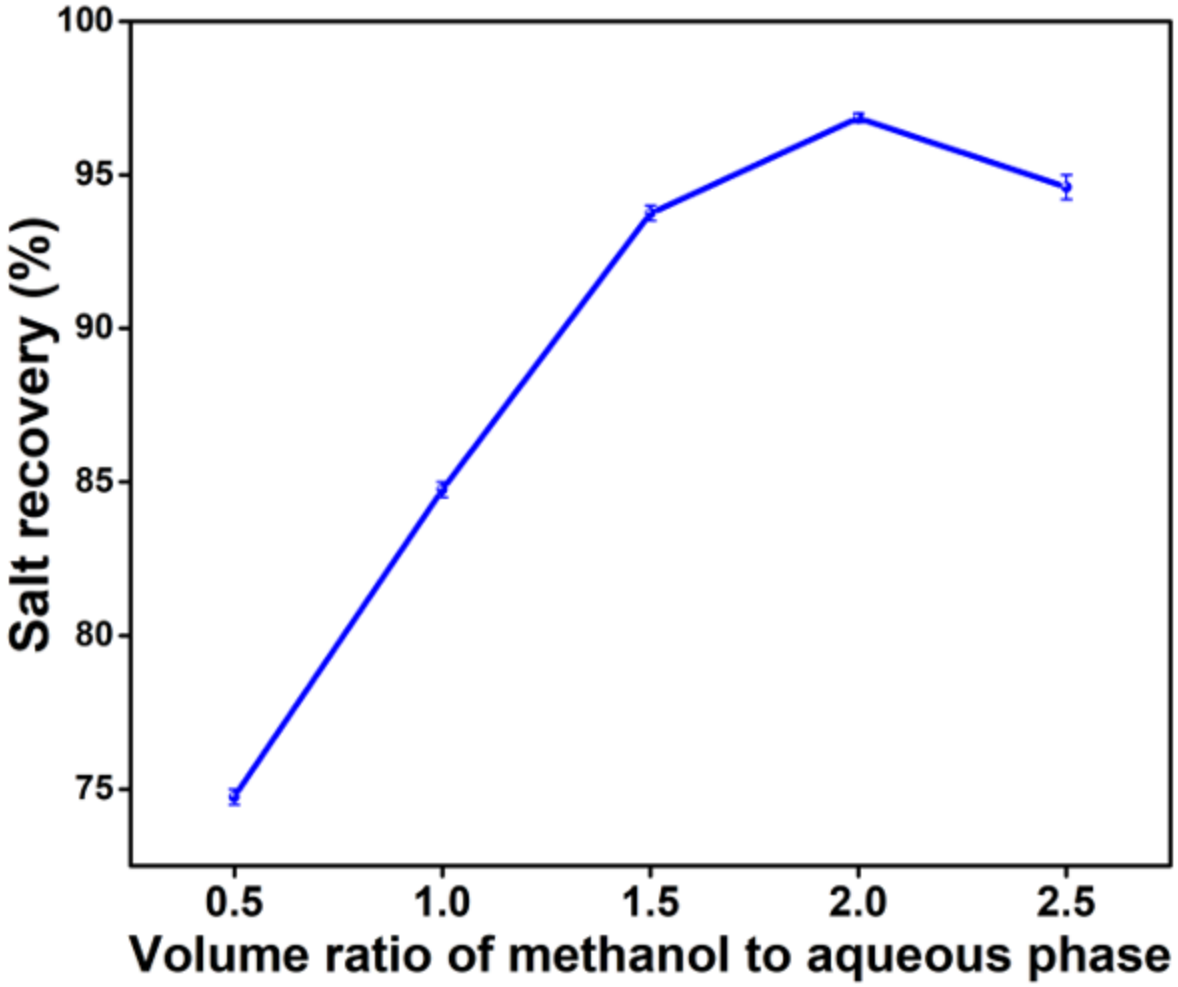


Figure 4

Effect of methanol-to-aqueous-phase volume ratio on DKP recovery by methanol-assisted crystallization.

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