Green Conversion of Saponins to Diosgenin in an Alcoholysis System Catalyzed by Solid Acid Derived from Phosphorus Tailings

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1. INTRODUCTION
Dioscorea zingiberensis C.H. Wright (DZW) is a common vine grown widely in China. The rhizome of DZW contains diosgenin, an important raw material for the preparation of steroid hormone drugs. In recent years, scientists from various countries have found that diosgenin has very important and extensive biological activities, such as anti-inflammatory effects, antithrombotic effects, cardio-protective effects, anthelmintic properties, and anticancer effects. However, the method for industrial production of diosgenin is mainly through acid hydrolysis of glucoside bonds. A large amount of water with low pH and high chemical oxygen demand (COD) is discharged during the production, leading to serious environmental pollution. In addition, the chlorinated reaction taking place in the process of inorganic acid hydrolysis would reduce the yield of diosgenin.

In order to reduce pollution, protect the environment, and achieve the goal of sustainable development, many methods have been studied to replace direct acid hydrolysis to reduce COD discharge, such as microbial hydrolysis by Trichoderma reesei, enzymatic hydrolysis, catalytic hydrolysis with ion solution under microwave irradiation, supercritical CO2 methods, and pressurized biphasic acid hydrolysis. However, these methods are difficult to be applied in industrial production due to their respective problems. For example, although these methods significantly reduce the content of COD in wastewater, there is still a problem of potential environmental pollution, which is not a long-term solution. Enzymatic hydrolysis and natural fermentation hydrolysis in the biological method have the problems of a longer production period, difficult condition control, and high cost. The ion solution method has a high cost, complicated recovery process, and is easily contaminated by byproducts. The prestage investment of the supercritical CO2 method is large, the equipment requirements are harsh, and the high-pressure reaction operation is risky. Therefore, it is very important to explore an efficient, green, economical, and pollution-free catalytic way to obtain diosgenin. In recent years, solid acid as a cleaner production catalyst has achieved excellent performance in various research fields. Prior to this, we also successfully used solid acid to extract diosgenin from saponins of DZW. The studies showed that the alcoholysis method...
is a green approach to produce diosgenin from DZW, which does not have liquid waste discharging in the process of alcoholism; however, the cost of the solid acid greatly affects the production efficiency. Low cost for the preparation of the solid acid is the key factor to realize the potential applications.

Phosphate rocks, on the other hand, are the raw material for the preparation of fertilizers, detergents, animal food supplements, etc. The demand for phosphate rocks in China and even in the world is growing. With the consumption of the phosphate ore, about 30–40% of the quality ore is being discarded as tailings in processing operation of mining and the ore dressing process. More and more phosphorus tailings would stack as waste disposals if no effective utilization method is developed for them. However, a large number of tailing storage in tailing ponds is a great potential safety hazard, and accidents have been caused many times (Yang et al., 2017). Therefore, there is a need to explore a new way to reuse tailings.

In this study, a simple inorganic reaction was used to extract the main element Si from the phosphorus tailings for the synthesis of hydrophilic SiO2 microspheres and then the surface of SiO2 was sulfonated to prepare the silicon-based solid acid materials. The feasibility of using phosphorus tailings to synthesize silicic solid acid to catalyze the alcoholization of turmeric was studied. The optimum reaction conditions for extracting diosgenin from saponins in DZW were obtained by the response surface method (RSM) based on the results of the single factor experiment. The results showed that the application of the as-prepared solid acid can not only provide an approach to reduce both solid waste of phosphorus tailings and the potential safety hazards caused by accumulation but also propose a green method to overcome the environmental pollution caused by the hydrolisis of DZW at the present stage. The clean diosgenin production promoted by the utilization of waste material will have great significance for both reducing environmental pollution and sustainable development.

2. RESULTS AND DISCUSSION

2.1. Catalyst Characterization. 2.1.1. FTIR Analysis. Fourier transform infrared (FTIR) analysis is one of the important tools for identifying functional groups in samples. Figure S1 shows FTIR spectra of SiO2 and SiO2,−SO3H, respectively. The FTIR spectra of SiO2 microspheres show two weak absorption bands at 3450 and 1634 cm−1, corresponding to the silica hydroxyl stretching and bending vibration on the silica surface, respectively. The wide strong band centered at 1095 cm−1 is assigned to Si−O−Si asymmetric stretching vibration. The bands at 798.81 and 469 cm−1 are symmetric stretching vibrations for the Si–O bond. Meanwhile, the existence of Si−OH symmetric stretching vibration is also confirmed by the absorption band at 965 cm−1. FTIR spectra of SiO2−SO3H microspheres show two characteristic adsorption bands at 3450.50 and 1634.91 cm−1 related to the O−H stretching and bending vibrations of the SO−H bond of the sulfonic acid groups (SO3H). The FTIR spectra of SiO2−SO3H microspheres at 3354 and 1609 cm−1 are significantly larger than those of SiO2; this is due to the enhancement of the characteristic peak due to the coincidence of the SO−H stretching and bending vibration with the O−H stretching peak and the sulfonic acid group.

2.1.2. Thermogravimetric Analysis. To further demonstrate the successful synthesis of SiO2−SO3H, the thermogravimetric analysis (TGA) studies of SiO2 and SiO2−SO3H and the relative analyses were conducted. Figure S2 shows the weight loss of SiO2−SO3H from room temperature to 800 °C. The weight loss (7%) from room temperature to 100 °C was caused by the removal of physically adsorbed water. The weight loss (3%) in 100–200 °C can be assigned to the physical adsorption −SO3H were lost in the weak acid region. The 5% weight loss in the temperature range of 200–800 °C is due to the −SO3H groups covalently bonded with SiO2 as well as the condensation of the hydroxyl groups on the solid acid.

2.1.3. XRD Analysis. Next, X ray diffraction (XRD) was used to study the crystalline structure of SiO2 sulfonated microspheres, as shown in Figure S3. There are broad peaks in the range of 2θ between 15 and 28°, and no other peaks, indicating the high purity of SiO2. However, the XRD of the sample did not show any significant diffraction peaks for −SO3H layers, indicating that the −SO3H amorphous shell on the SiO2 surface was too thin to be measured by XRD.

2.1.4. NH3-TPD Analysis. As shown in Figure 1, NH3 temperature programmed desorption (TPD) of the SiO2−SO3H desorption curve has a small peak in 100 °C (weak acid). The presence of weak acid sites on NH3-TPD may be due to the interaction between NH3 molecules and the −OH groups attached to the catalyst surface. The strong broad peak centered at 500 °C (strong acid >400 °C) belong to −SO3H bonded with SiO2. The calculated acidity of SiO2−SO3H was 0.93 mmol/g.

2.1.5. SEM, TEM, and TEM-Mapping Analysis. The morphologies of SiO2−SO3H particles were characterized by scanning electron microscopy (SEM), high resolution transmission electron microscopy (HRTEM), and transmission electron microscopy (TEM)-mapping. SEM and TEM revealed that the size of SiO2−SO3H was mostly in the range of 400–500 nm (Figure 3). In the preparation of SiO2 from the Na2SiO3 solution, a small amount of EtOH and PEG was added to avoid the aggregation of SiO2 and the addition of ethyl acetate was to decrease the pH value in the preparation of SiO2. Due to the fact that the preparation of SiO2 was performed by the precipitation reaction between Na2SiO3 and diluted H2SO4, the reaction speed was very fast, leading to the partial aggregation and the sizes of SiO2 were not completely consistent. Although there were no significant changes in the SEM and TEM images of the SiO2 samples before and after sulfonation, the existence of S can be determined by energy dispersive X-ray analysis (EDX) (Figure 2), and the TEM-mapping analysis, shown in Figure 3E−H, further confirms...
that O, Si, and S are uniformly distributed in the SiO$_2$−SO$_3$H particles.

2.2. Single Factor Experiment. 2.2.1. Effect of Different Amount of SiO$_2$−SO$_3$H. Alcoholysis activity of SiO$_2$−SO$_3$H depended mainly on the acidic groups −SO$_3$H in the solid acid, so the amount of SiO$_2$−SO$_3$H was the key factor for alcoholysis. To find out the optimal mass of SiO$_2$−SO$_3$H in the alcoholysis reaction, the effect of the SiO$_2$−SO$_3$H dosage (0.2−0.6 g) on the yield was explored and shown in Figure 4. With increase of the dosage of SiO$_2$−SO$_3$H from 0.2 to 0.5 g, the yield of diosgenin increased gradually, indicating that SiO$_2$−SO$_3$H plays an irreplaceable role in the alcoholysis of saponins and directly affects the yield of diosgenin. When the amount of SiO$_2$−SO$_3$H reached 0.5 g, the yield of diosgenin was the highest. However, as more SiO$_2$−SO$_3$H was used for alcoholysis, the diosgenin yield decreased slightly, indicating that the high amount of SiO$_2$−SO$_3$H might destroy the generated diosgenin and result in the formation of a side reaction. It was found that diosgenin was easily dehydrated to Δ3,5-deoxytigogenin. 30,31

2.2.2. Effect of Solvent Volume. Anhydrous ethanol has a great influence on the yield of diosgenin in the hydrolysis of total saponins in traditional processes. Therefore, the effect of different solvent volumes (4−8 mL) on the alcoholysis reaction was studied. The results are shown in Figure 5. It showed that the yield rises rapidly when the volume of ethanol is increased from 4 to 6 mL. After the solvent reached 6 mL, the yield of diosgenin decreased with further increase in the ethanol volume, which can be ascribed to the decrease in the contact probability between SiO$_2$−SO$_3$H and total saponins. 

![Figure 2. EDX of SiO$_2$−SO$_3$H.](image)

![Figure 4. Effect of different quantities of SiO$_2$−SO$_3$H.](image)

![Figure 5. Effect of solvent volume.](image)

![Figure 3. (A and B) SEM of SiO$_2$−SO$_3$H; (C and D) HRTEM of SiO$_2$−SO$_3$H; (E–H) TEM-mapping of SiO$_2$−SO$_3$H.](image)
2.2.3. Effect of Reaction Time. In the conventional hydrolysis, the hydrolysis reaction time was an important factor. Therefore, the influence of reaction time was investigated. As shown in Figure 6, the yield increased linearly when the reaction time increased from 4 to 6 h, indicating that the total saponins were more fully transferred into diosgenin and sugars with the increase in reaction time. The highest yield was obtained when the reaction time was 6 h. However, the yield of diosgenin decreased with further increase in the reaction time, which can be contributed to the decomposition of diosgenin in the longer reaction time.12

2.2.4. Effect of Reaction Temperature. The reaction temperature is a very important factor that directly affects all chemical reactions. Therefore, the yield in the process was investigated from 70 to 110 °C, shown in Figure 7. The optimum reaction temperature was 100 °C. When the temperature increased from 70 to 100 °C, the yield of diosgenin was significantly increased. The rise in the temperature accelerated the alcoholysis reaction and enhanced the SiO2−SO3H catalytic efficiency. However, the yield of diosgenin showed little change when the reaction temperature exceeded 100 °C.

2.3. Analysis of Response Surface Results. The results of the above single factor tests showed that the yield of diosgenin in the 100 °C temperature (yield 2.30%) was almost the same with that in 110 °C (yield 2.33%). For the consideration of reducing energy consumption, we set the reaction temperature at 100 °C. The independent variables and relative levels are listed in Table 1, the amount of SiO2−SO3H (X1) was 0.4−0.6 g, reaction time (X2) was 5~7 h, and ethanol solvent volume (X3) was 5~7 mL.

| independent variables | symbols | coded levels |
|-----------------------|---------|--------------|
| SiO2−SO3H/g | X1 | 0.4 0.5 0.6 |
| reaction time/h | X2 | 5 6 7 |
| ethanol/mL | X3 | 5 6 7 |

Box−Behnken design (BBD) software was used to optimize the variables and levels, and 17 experimental schemes were designed. After the % values obtained from the experiment for each scheme design were entered, the response regression model was established (Table 2).

Seventeen runs designed by the BBD of the RSM were used to optimize the conditions for alcoholysis of total saponins to diosgenin with solid acid. The predicted response of Y for the yield of diosgenin extracts can be expressed by the following second-order polynomial equation:

\[
Y = 2.43 + 0.079X_1 + 0.18X_2 + 0.023X_3 + 0.029X_1X_2 + 0.028X_1X_3 + 0.030X_2X_3 - 0.13X_1^2 - 0.27X_2^2 - 0.074X_3^2
\]

The model of the RSM was verified by analysis of variance (ANOVA) for the yield of diosgenin (Table 3). These results showed that the P-value of the regression model was less than 0.0001 and the ANOVA lack-of-fit was 0.1394, demonstrating that the model is “highly significant” and the lack-of-fit is “not significant”.52 Moreover, the coefficient of multiple determination \( R^2 \) for the yield of diosgenin was 99.59% and the coefficient of adjustment (Adj-R²) was 99.06%, C.V. = 0.96%, indicating that the resultant second-order equation fits well. In the variables, the highly significant effect (<0.0001) factors were the SiO2−SO3H quantity and reaction time, and the quadratic terms of the SiO2−SO3H quantity and reaction time.
also had highly significant (<0.001) effects. All other reaction conditions ($X_3, X_1X_2, X_1X_3, X_2X_3,$ and $X_3^2$) were significant (<0.05) on the yield of diosgenin.33

The three-dimensional response surface was drawn according to the BBD experimental data (Figures 8−10), and the interaction between various factors was studied to determine the optimal value to achieve the maximum yield.

Based on the aforementioned RSM model, the optimal extraction conditions for alcoholysis of total saponins was estimated to be 0.54 g (amount of $\text{SiO}_2−\text{SO}_3\text{H}$), 6.38 h (reaction time), 6.30 mL (ethanol), and 100 °C, the optimum yield of diosgenin was 2.48%. According to the optimal reaction conditions calculated by the RSM, we carried out three independent experiments to verify that the average yield was 2.45%, which was very close to the calculated data of the RSM.

2.4. Recycling Experiments. Although solid acid catalysts were recyclable and had high-level catalytic activity in the alcoholysis process, 0.03−0.05 g solid acid will be lost in the process of centrifugal separation, drying, regeneration, and recycling of the recovered catalyst.34 In order to ensure the activity of the catalyst and avoid the influence of operational loss on the catalytic activity, the corresponding loss amount of the solid acid was mixed with the recovered solid acid as the catalyst for the next run.

The results for the recycling experiments are shown in Figure 11, the activity of the catalyst decreases a little after each run, which may be ascribed to the decrease in active sites. The sugars produced by alcoholysis has a low solubility in ethanol and can deposit on the surface of solid acid in the process of centrifugal separation, resulting in a part of the active sites of solid acid being covered. However, it only decreased by 0.57% after five runs, indicating its good stability and reusability. The solid acid collected after five cycles can be easily reactivated by mixing it with diluted sulfuric acid at room temperature for 3 h, and its activity was tested after washing and drying, which can restore the yield of diosgenin to 2.34%. The reactivated process is easy to do, and the used sulfuric acid in the reactivated process can be reused in the next recycle.

The stability of the solid acid was also verified by XRD and EDX results, shown in Figures 12 and 13. It showed that the XRD curve of $\text{SiO}_2−\text{SO}_3\text{H}$ after five times of recycling use is almost the same with that of fresh $\text{SiO}_2−\text{SO}_3\text{H}$. The EDX of $\text{SiO}_2−\text{SO}_3\text{H}$ after five runs is basically the same as that of fresh solid acid (Figure 2). It indicating that the solid acid is very stable in the alcoholysis process.

| source | sum of squares | Df | mean square | F     | P-value |
|--------|----------------|----|-------------|-------|---------|
| model  | 0.76           | 9  | 0.085       | 188.53| <0.0001 |
| $X_1$  | 0.050          | 1  | 0.050       | 111.06| <0.0001 |
| $X_2$  | 0.27           | 1  | 0.27        | 599.34| <0.0001 |
| $X_3$  | $4.325 \times 10^{-3}$ | 1  | $4.325 \times 10^{-3}$ | 9.62  | 0.0173  |
| $X_1X_2$ | $3.422 \times 10^{-3}$ | 1  | $3.422 \times 10^{-3}$ | 7.61  | 0.0281  |
| $X_1X_3$ | $3.080 \times 10^{-3}$ | 1  | $3.080 \times 10^{-3}$ | 6.85  | 0.0345  |
| $X_2X_3$ | $3.660 \times 10^{-3}$ | 1  | $3.660 \times 10^{-3}$ | 8.14  | 0.0246  |
| $X_1^2$ | 0.075          | 1  | 0.075       | 167.77| <0.0001 |
| $X_2^2$ | 0.30           | 1  | 0.30        | 661.97| <0.0001 |
| $X_3^2$ | 0.023          | 1  | 0.023       | 51.06 | 0.0002  |
| residual | $3.14 \times 10^{-3}$ | 7  | $4.495 \times 10^{-4}$ | |
| lack of fit | $2.24 \times 10^{-3}$ | 3  | $7.470 \times 10^{-4}$ | 3.30  | 0.1394  |
| pure error | $9.052 \times 10^{-4}$ | 4  | $2.263 \times 10^{-4}$ | |
| cor. Total | 0.77          | 16 |             |       |         |

$R^2 = 0.9959$ Adj-$R^2 = 0.9906$ C.V.% = 0.96.

Figure 8. Effect of interaction of two parameters reaction time ($X_2$) and solid acid dosage ($X_1$): (A) response surface 3D plot and (B) contour plot.
2.5. Comparison with the Different Extraction Methods. In order to evaluate the solid acid-catalyzed alcoholysis efficiency of total saponins to diosgenin, the conventional method to extract diosgenin from DZW was also performed according to the general procedure. The contents of diosgenin in the samples were detected by high pressure liquid chromatography (HPLC) and UV−vis after hydrolysis. The results are listed in Table 5. It shows that the alcoholysis method catalyzed by SiO$_2$−SO$_3$H had a higher diosgenin yield than other methods. The alcoholysis yield of solid acid was 12.90% higher than that of the H$_2$SO$_4$ hydrothermal reaction. In order to exclude the influence of experimental operation on the results, the same method was used for the blank experiment except for no addition of solid acid. As a result, the yield of diosgenin in blank extraction was much lower than that of other experiments, indicating that the solid acid plays a crucial role in increasing the yield of diosgenin extracted from diosgenin of DZW. At the same time, we also compared the reaction conditions and yield of other different extraction methods of diosgenin. It can be seen from Table 4 that the solid acid in this work has a comparable catalytic activity to those with better catalysts reported in the literature.

2.6. LC–MS Analysis. In order to better understand the alcoholysis process of saponins to diosgenin by solid acid and prove that diosgenin existed in the alcoholysis products, liquid chromatography-mass spectrometry (LC–MS) detection was carried out to analyze standard diosgenin, total saponins, and the alcoholysis samples before and after purification, shown in Figure 14. The peak of the standard diosgenin appeared in a
The retention time of 7.80 min (Figure 14A), and the peak of the alcoholysis products before and after purification also appeared in about 7.80 min (Figure 14C,D), but the peak at about 7.80 min was not observed in LC−MS of the total saponins (Figure 14B). The MS spectra showed that the peak at about 7.80 min has an m/z value of 415 (Figure 15), which is the aggregation peak of the alcoholysis products.

Table 4. Comparison Experiment Results

| number | catalyst    | solvents | method          | yield of diosgenin (%) |
|--------|-------------|----------|-----------------|------------------------|
| 1      | H₂SO₄       | H₂O      | oil bath        | 2.17 ± 0.15            |
| 2      | H₂SO₄       | H₂O      | hydrothermal reaction | 2.05 ± 0.16            |
| 3      | SiO₂        | ethanol  | solvothermal reaction | 0.29 ± 0.11            |
| 4      | SiO₂−SO₃H   | ethanol  | solvothermal reaction | 2.45 ± 0.17            |
| 5      | Fe₃O₄@SiO₂−SO₃H | ethanol | solvothermal reaction | 2.64 ± 0.12¹⁶         |
| 6      | Si−SO₃H     | ethanol  | solvothermal reaction | 0.70¹⁸                 |
| 7      | Fe₃O₄@ETMS-TETA-SO₃H | ethanol | solvothermal reaction | 1.62¹⁷                 |
| 8      | [BHSO₄MIm]  | HSO₄     | ionic liquid microwave irradiation | 1.02¹²             |
| 9      | Aspergillus | H₂O      | microbial hydrolysis | 2.58¹⁵                 |
| 10     | none        | ethanol  | solvothermal reaction | 0.36 ± 0.12            |

Figure 11. Recycling experiments of SiO₂−SO₃H in the alcoholysis.

Figure 12. XRD (A) fresh Fe₃O₄@SiO₂−SO₃H and (B) Fe₃O₄@SiO₂−SO₃H after five runs.

Figure 13. EDX of SiO₂−SO₃H after five runs.

Figure 14. LC−MS of (A) standard of diosgenin, (B) total saponins, (C) before purification of diosgenin, and (D) after purification of diosgenin.

Figure 15. MS spectra of the peak at about 7.80 min.
of one diosgenin molecule combining with one $\text{H}^+$, proving that diosgenin is involved in alcoholsysis products.\cite{36,37}

The other components in the alcoholsysis products were further investigated via LC−MS, and the results are exhibited in Figures S4 and S5. On the basis of all the experimental results and the previous studies, we believe that there are two possible pathways for the transfer of total saponins to diosgenin, shown in Figures 16 and 17, where various fragment
peaks with different m/z values can be found in Figures S4 and S5. The structures and formulas for each of the fragments are their relative assignments, which match very well with the practical m/z values. It can be seen that there are some substances containing ethyl glucosides in the alcoholysis, such as the substances with m/z = 443.29, 207.98, and 605.46, giving a strong support for the release of diosgenin from saponins by alcoholysis.

3. CONCLUSIONS
This work introduces a method for preparing solid acid of SiO$_2$−SO$_3$H using phosphorus tailings (PT) as a raw material. The successful synthesis of SiO$_2$ and the modification of SiO$_2$ with sulfonic acid groups were verified by IR, TGA, XRD, NH$_3$-TPD, SEM, TEM, and TEM-mapping. The total saponins in DZW were successfully converted into diosgenin by SiO$_2$−SO$_3$H catalytic alcoholysis under the condition of solvothermal reaction. Through the single factor test and RSM, the optimal conditions were determined as follows: SiO$_2$−SO$_3$H 0.54 g, reaction time 6.30 h, ethanol solution 6.38 mL, and reaction temperature 100 °C. The highest yield of saponin was 2.45%. Moreover, the alcoholysis reaction mechanism was studied through LC−MS analysis. It was found that the solid acid catalyst is not only environmentally friendly and pollution-free but also provides a solution to the environmental pollution of phosphorus tailings. This method has a broad industrial prospect in the production of diosgenin and the comprehensive utilization of phosphorus tailings.

4. EXPERIMENTAL SECTION
4.1. Materials. High silicon phosphate tailings (PT) were obtained from Hubei Ezhong Ecological Engineering Co. Ltd.
and the composition of the PT is shown in Table 5 identified by XFR analysis. \( \text{Na}_2\text{CO}_3 \) (AR), \( \text{H}_2\text{SO}_4 \) (AR), sodium dodecyl sulfate (SDS, AR), anhydrous ethanol (AR), polyethylene glycol (PEG-1000), ethyl acetate (AR), dichloromethane (CH\(_2\)Cl\(_2\)), and chlorosulfonic acid (ClSO\(_3\)H) were purchased from Sinopharm Chemical Reagent Co., Ltd. Dioscorea zingiberensis C. H. Wright (DZW) was provided by Danao Pharmaceutical Co., Ltd., Hubei, China. Chloroform (AR) and acetonitrile (chromatographically pure) were used in the HPLC analysis, and perchloric acid (AR) and diosgenin (analytical standard) were purchased from Aladdin. The reagents and chemicals were used without further purification.

### Table 5. Main Oxide Composition of Phosphorus Tailings

| sample | \( \text{SiO}_2 \) | CaO | \( \text{Al}_2\text{O}_3 \) | \( \text{Fe}_2\text{O}_3 \) | \( \text{P}_2\text{O}_5 \) | MgO | K\(_2\)O |
|--------|----------------|-----|----------------|----------------|----------------|-----|--------|
| PT (%) | 42.08          | 19.73 | 8.19          | 5.49             | 4.80             | 2.13 | 6.08   |

4.2. Synthesis of Sulfonated Silica Microspheres.

#### 4.2.1. Preparation of Silica Microspheres from Phosphorus Tailings.

The process for preparing solid acid from PT was performed in a similar way as reported in literature studies. Typically, 25 g of PT were ball-milled to fine powder and 99% of it was passed through 300 mesh and then the screen underflow was transferred to a ceramic crucible. Anhydrous sodium carbonate (50 g) was added and mixed evenly. Then, it was put into a muffle furnace and heated to 850 °C for 4 h. After natural cooling to room temperature, the solid was transferred to a beaker and 250 mL of deionized water was added. After stirring at 50 °C for 4 h, the mixture was filtered while hot.

The silicon-containing leaching solution obtained above was transferred to a 500 mL beaker, and 0.5 g PEG-1000, 2 g EtOH, and 3.7 g ethyl acetate were added into the above solution in turn and mixed evenly; then, the pH value of the leaching solution was adjusted to 1 with 10% \( \text{H}_2\text{SO}_4 \); a white precipitate was formed at 60 °C for 3 h. The obtained \( \text{SiO}_2 \)
composites were washed with water until the filtrate was neutral, dried, and calcined at 400 °C.

4.2.2. Synthesis of Sulfonated Silica Microspheres Materials. The sulfonated silica microspheres materials were prepared in a similar method for silica-supported sulfonic acids. First, the prepared SiO2 (2.5 g) was transferred to a 50 mL two-neck flask, which was connected to a gas absorption device to absorb the escaped HCl reaction gas from the sulfonation reaction. Dry dichloromethane (20 mL) was added and the resulting mixture was dispersed by ultrasound. Then, chlorosulfonic acid (2 mL) was added dropwise and stirred for 3 h at 0–5 °C. After the reaction, the catalyst was filtered, washed with neutral with CH2Cl2 and ethanol, and dried overnight in vacuum.

4.3. Characterization. X-ray fluorescence (XRF, ARL PERFORM'X) was used to determine the composition of the phosphorus tailings. FTIR spectra were recorded in a spectrometer (IACST, Guwahati) in the range of 500–4000 cm−1. XRD data were recorded by BRUKER D6 with radiation (λ = 1.54 Å) at 40 kV 44 mA. TGA was tested by Mettler-Toledo TG2 series under the conditions of heating rate 10 °C/min from 25 to 800 °C and a nitrogen atmosphere at flow rate 100 mL/min. The morphologies of the catalyst were recorded by a transmission electron microscope (JEM2100F, 200 kV, JEOL) and a scanning electron microscope. The acid value was determined by TPD-NH3 (AutoChem II2920). UPLC-MS (Agilent 1100, thermos TSQ quantum Ultra AM) was used for qualitative and quantitative analyses.

4.4. Alcoholysis of Total Saponin by the SiO2−SO3H Catalyst. The alcoholysis schematic diagram is shown in Scheme 1. DZW was washed, dried, and crushed; the fine powder screened through 200 mesh was collected. To extract steroid saponins, the powder (60 g) was mixed with ETOH (600 mL) overnight, then heated at 85 °C, and refluxed for 12 h. The hot mixture was filtered and washed with ethanol. The combined filtrate was concentrated to dry using a rotary evaporator, and the obtained products were used as crude saponins, and dried in vacuum at 40 °C. Finally, 6.65 g of total saponins were obtained with a yield of 11.08%.

The total saponins (0.1 g) extracted in the above experiments were fully dissolved in EtOH (4–8 mL) in Penicillin bottles using ultrasound for 15 min. Then, SiO2−SO3H (0.2−0.6 g) was added and ultrasonically dispersed. The mixture was transferred to a polytetrafluoroethylene-lined stainless-steel autoclave, after sealing. A small amount of solvothermal reaction was programmed at different temperatures (70–110 °C) and reaction times (4–8 h). After each run, SiO2−SO3H was recovered by centrifugation and the centrifugate was evaporated to dry by a rotary evaporator; meanwhile, the ethanol liquid collected from the evaporator was reserved for further use. The obtained solid products were extracted by a Soxhlet extractor using chloroform as solvent for 3 h. The content of diosgenin in the sample was analyzed with HPLC and UV−vis. In order to obtain the optimal alcoholysis conditions, four experimental factors were studied and optimized by the single variable analysis and RSM. The optimal reaction conditions were obtained by studying the effect of SiO2−SO3H, dosage of ethanol, reaction time, and reaction temperature on the yield of diosgenin. Then, the best reaction conditions for the preparation of diosgenin from DZW were obtained by the RSM. Finally, the path of total saponins to diosgenin with solid acid alcoholysis was investigated by LC−MS.

4.5. Response Surface Method. The RSM was used to obtain the optimal conditions for extraction of diosgenin. According to the previous results of the single factor test, the three-level-three-factor BBD was designed to calculate the optimal conditions. All RSM experiments were performed with random to minimize the influence of uncertainty of the external unknown factors. A second-order polynomial equation was postulated to express the yield of diosgenin response Y, as eq 2.40

$$Y = \beta_0 + \sum_{i=1}^k \beta_iX_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^k \beta_{ij}X_iX_j + \sum_{i=1}^k \beta_i^2X_i^2$$

(2)

where Y is the response (the diosgenin yield); $\beta_0$ is the regression coefficient for the intercept (constant); and $\beta_i, \beta_{ij}$ and $\beta_i^2$ are the coefficients for the linear and interaction terms of variables i and j, respectively. $\beta_i$ is the quadratic coefficient and k is the number of variables. The three independent

![Scheme 1. Flow Chart of the Alcoholysis of Saponins for the Production of Diosgenin with SiO2−SO3H Solid Acid from Phosphorus Tailings](image-url)
parameters were assigned as: \( X_1 (\text{SiO}_2-\text{SO}_3\text{H}, \text{g}) \), \( X_2 (\text{reaction time, h}) \), and \( X_3 (\text{ethanol dosage, mL}) \). Therefore, the second-order polynomial models of the three independent variables can be expressed as eq 3:

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2
\]  

(3)

4.6. Regeneration and Recycling Experiments. The \( \text{SiO}_2-\text{SO}_3\text{H} \) solid acid is a recyclable catalyst. Therefore, we also need to research on its recovery and reuse. After each reaction, the solid acid was separated from the reaction mixture by centrifugation, the recovered \( \text{SiO}_2-\text{SO}_3\text{H} \) was washed with water and ethanol, and dried at 40 °C under vacuum. The recovered \( \text{SiO}_2-\text{SO}_3\text{H} \) will be reused in five cycles under the same reaction conditions. After five cycles, \( \text{SiO}_2-\text{SO}_3\text{H} \) was soaked in dilute sulfuric acid for 3 h to regenerate, and its activity was restored to the original one.

4.7. Conventional Sulfuric Acid Hydrolysis Method. For evaluating the novel method, the conventional sulfuric acid hydrolysis method described in the literature was carried out for comparison. A sample of 0.10 g total saponin powder was hydrolyzed in 6 mL 2 mol/L sulfuric acid for 5 h at 100 °C. After the reaction, the mixture was filtered and the solid residues were washed with hot water and alcohol three times and dried at 40 °C in vacuum. Then, the diosgenin in the residues was extracted by a Soxhlet extractor for 3 h with chloroform. The diosgenin content in the sample of sulfuric acid hydrolysis was analyzed under the same conditions as the solid acid alcoholysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c05627. FTIR and TGA of \( \text{SiO}_2 \) and \( \text{SiO}_2-\text{SO}_3\text{H} \), XRD of \( \text{SiO}_2-\text{SO}_3\text{H} \), total ion chromatogram (TIC) of alcoholysis products and total saponins, the mass spectrogram of total saponins, and alcoholysis products of the pathway (PDF).

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Notes

The authors declare no competing financial interest.

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