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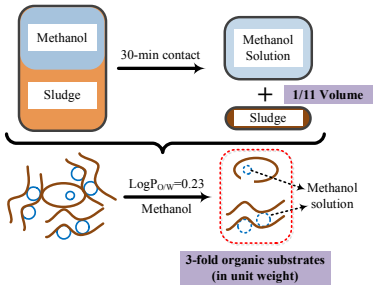
Graphical abstract



Highly effective in-depth dewatering of excess sludge using methanol

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Abstract

In-depth dewatering of excess sludge facilitates cost reduction of wastewater treatment plant, and make final disposal of sludge more economically feasible. In this study, contact with methanol was selected as an improved method to achieve in-depth sludge dewatering. After 30-min methanol contact and subsequent natural drying at ambient temperature for 6 h, the water content of the sludge was reduced to 13.4%. The thermogravimetric analysis depicted that the organic substance content (per unit weight) of the dewatered sludge was 3-fold higher than untreated sludge. The results suggested that methanol-aided dewatering did not influence the availability of sludge for subsequent utilization or energy recovery. The microorganisms that were retained in dewatered sludge were found to be Gram-negative bacteria, whose adaptive phospholipid composition possibly improved their tolerance to methanol. Hence, the methanol-aided dewatering mechanism can be concluded as (1) disruption of EPS structure and damage of cell membrane and (2) release and re-distribution of interstitial water. Furthermore, the optimal dewatering efficiency and operation parameters were predicted using response surface methodology, which could support as reference value for engineering application.

1. Introduction

Activated sludge system is the most prevalent technology in present municipal wastewater treatment plant (WWTPs). However, it has some serious drawbacks, e.g. production of huge amounts of excess sludge (ES), hazardous solid waste that could harm the environment.[1, 2] The associated capital and operating costs of treatment and disposal of ES may account for 25~50% of the total wastewater treatment costs.[3] Currently, thickening, stabilization, dewatering, incineration, and landfilling are widely applied for ES treatment and disposal.

The bottleneck of the sludge handling system is the dewatering operation.[4] Better dewatering performance could significantly reduce sludge volume, and benefit the subsequent processes by reducing the quantity of supporting agents in combustion or land use in landfill. At present, mechanical dewatering technologies are extensively used in WWTPs, including filter presses, belt presses, and centrifuges. However, the mechanical dewatering process could only reduce water content of ES to 70-80%, which might still be too high for direct combustion or landfill.[5] Thus, effective in-depth dewatering methods need further integrated investigation.

According to their respective bonding strength, the water contained in ES can be divided into four parts: free water, interstitial water, vicinal water, and water of hydration.[6] The free water could be removed by thickening or mechanical dewatering processes. The vicinal water and water of hydration are extremely difficult to remove, but they only account for small quantities in ES, therefore their removal doesn't significantly reduce sludge volume. Currently, the removal of interstitial water is deemed the crucial point for in-depth dewatering process, which determines the overall dewatering efficiency. The interstitial water is trapped in the shell layer by various metabolic products, i.e. protein, exopolysaccharides, and lipids, which in turn closely bound to internal polymers. Typically, conventional mechanical dewatering processes need certain pre-treatment procedures to remove the interstitial water. The techniques involved include ultrasonic aids, thawing aids, thermal aids, addition of chemical flocculent or surfactant, and electric aids.[7-10] With the assistance of these processes, mechanical dewatering could further reduce the water content to 55~80%, but water content less than 50% is seldom achieved.

Münter & Grén [11] used polar solvent contact as a pretreatment to achieve better dry peat solids. The most often used polar solvents are methanol, ethanol, and acetone, due to their

advantages of strong dehydration on the solids and low boiling point. As low boiling points entail easy separation after dehydration, these solvents could be a cost-effective solution for in-depth ES dewatering. Therefore in this study, methanol was selected to perform in-depth dewatering on excess sludge, as its price was around 1/3 of that of ethanol or acetone. Methanol concentration and contact time were tested as the two crucial parameters, for they affect not only the dewatering efficiency but also the overall cost. Thus, a response surface methodology (RSM) was adopted to evaluate the effects of the independent variables and their interaction on ES dewatering. In summary, this study (1) investigated the in-depth dewatering performance of methanol, (2) optimized the corresponding process parameters, and (3) investigated the transitions of microbial communities during the dewatering process for the first time, which aided in the understanding of the dewatering mechanism.

2. Materials and Methods

2.1 Excess sludge and thickened sludge

The excess sludge was collected from the sludge recycling tank of a municipal wastewater treatment plant in Shanghai, China. The excess sludge was then left to settle for 1 h in the lab to obtain a primary concentrated sludge, which was further centrifuged (3,000 ×g, 10 min) to produce a secondary thickened sludge. [12] The water content of the thickened sludge was determined to be 92.1±1.84%.

2.2 In-depth dewatering test with the aid of methanol

An Erlenmeyer flask was loaded with 15 mL methanol and 8 g thicken sludge. The methanol contact was performed in an air-bath shaker at 20 ºC, 150 rpm for 30 min. The untreated sludge was set as control. The mixture was then centrifuged at 3,000 ×g for 10 min at 4 ºC, and the residual sludge was spread in a 5-cm plate, and exposed to air at room temperature (22.1±3 ºC) for 600 min. The water content was measured at intervals. All experiments were conducted in triplicate with average and standard deviation reported.

2.3 Experimental design of response surface methodology

The response surface methodology (RSM) was used to evaluate the effect of methanol concentration (X_1) and contact time (X_2) on the dewatering effect. Three different coded levels (-1,

0 and +1) with low, medium and high values (Table 1) were adopted for each variable using central composite design analysis. The specific experimental design is shown in Table 2.To predict the optimal point, a second order polynomial function (Eq. (1)) was fitted to correlate the relationship between independent variables and response.

$$Y = \beta_0 + \sum \beta_n X_n + \sum \beta_{nn} X_n^2 + \sum \beta_{nm}^2 X_n X_m \qquad (1)$$

where, Y is predicted response (water content), β_0: offset term, β_n: liner coefficient, β_{nn}: squared coefficient, β_{nm}: interaction coefficient, X_n: nth independent variable, X_n^2 and $X_n X_m$ are squared effect and interaction effects. For the two variable systems, the equation is as Eq.(2):

$$Y = \beta_0 + \beta_1 X_2 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_1^2 X_1 X_2 \qquad (2)$$

Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). The equation was then used to predict the results by three dimensional response surfaces and contour plot.

Table 1 Actual values and levels values of the variables employed in RSM.

Table 2 Experimental arrangement of RSM

2.4 Analytical methods

The water content was measured after105 ºC oven drying for 6 h.[13] The dewatering rate was calculated as Eq(3):

dewatering rate (g/min) = $(W_1\text{-}W_2)/t$ (3)

where, the W_1 (g) and W_2 (g) was the initial and terminal weight of the dewatered ES respectively, during t min.

In order to analyze the water distribution in dewatered sludge, certain amount of sludge sample was used for thermogravimetric (TG) analysis by Perkin Elmer Pyris 1 TGA (PerkinEimer co., Ltd. USA). The dewatered sludge and untreated sludge were heated from room temperature to 900 ºC with a rate of 10 ºC/min, but were kept at 105 ºC and 550 ºC for 10 min respectively.[14]

2.5 Analysis for microorganism community

The microbial communities of the sludge samples from RSM were analyzed using PCR-DGGE.[15] Before the extraction of DNA, the sludge samples were pre-treated to remove methanol. The dewatered sludge was left still for 10 min, and re-suspended in 10 mL ddH_2O. The suspension was then centrifuged at 10,000×g for 10 min, and the pellets were used for DNA

extraction. The genomic DNA (100 μL) was extracted according to manual of PowerSoil DNA isolation kit (Mobio Inc., USA). The procedures of DNA extraction, polymerase chain reaction amplification (PCR), and denaturing gradient gel electrophoresis (DGGE) analysis were all according to Wan *et al.*.[16]

The PCR was conducted using extracted DNA with primer 8F-GC (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAGAGTTTGATCCTGGC TCAG) and primer 518R (ATTACCGCGGCTGCTGG). The 50 μL PCR mixture consisted of 5 μL 10 × Ex Taq buffer, 4 μL dNTP (2.5 mmol), 1.5μL primer 8F-GC, 1.5 μL primer 518R, 1.25U Ex Taq and 1 ng DNA template. The PCR temperature program was as follows: 94 ºC for 10 min, 30 cycles consisting of 94 ºC for 1 min, 55 ºC for 1 min, 72 ºC for 1 min 30 s and final extension at 72 ºC for 10 min.

The denaturing gel was 8% polyacrylamide gel that contained 30–60% denaturant (100% denaturing solution contained 7 mol/L urea and 40% formamide). The DGGE was performed at 140 V for 600 min. The gel was stained with 0.1% $AgNO_3$ and 2.5% $NaCO_3$ and then it was scanned, and the principal component analysis (PCA) was conducted by SPSS 12.0.

The subjective bands were recovered by reamplification using primers mentioned in Section 2.3.1. The purified amplicons were ligated into pMD-18T and transformed into *Escherichia coli* DH5α cells. Then, the positive colonies were randomly picked for sequencing by Sangon Co., Ltd. (Shanghai, China). The nucleotide sequences were compared with those available from the GenBank to identify the closest genes using the BLAST alignment tool.

3. Results and Discussion

3.1 In-depth sludge dewatering performance by methanol

After contact with methanol for 30 min, the change of sludge weight during 600 min air drying was monitored and the results shown in Fig. 1. During the whole process, the weight of the untreated sludge gradually reduced from 7.54 g to 3.34 g, accompanied by water reduction from 92.1% to 81.7%. The dewatered sludge, on the other hand, showed a much more pronounced loss of weight from 7.69 g to 0.69 g within 300 min, and the maximum dewatering rate of 0.069 g/min was detected at 30 min. The final water content of the dewatered sludge was 13.4%, which was only 16.4% of that of the untreated sludge. More importantly, the price of methanol was 1/3 of

ethanol or acetone, suggesting an economical advantage in engineering application. In this test, the water content decreased from 92.1% to 13.4%, achieving an 11-fold volume reduction. Thus, comparing with the common mechanical dewatering methods, [7-9] in-depth dewatering with the aid of methanol could be deemed as a promising alternative method.

Fig.1 In-depth dewatering performances

3.2 Thermogravimetric behavior of dewatered sludge

Thermogravimetric (TG) analysis was used to measure the weight loss in a controlled atmosphere, evaluating the variances of water and organic substrates with or without methanol treatment.[14,17] The temperature of TG analysis was divided into three stages: lower than 105 ºC, 105 ºC ~ 550 ºC, higher than 550 ºC. The weight losses of untreated and dewatered sludge were measured and the results are shown in Fig. 2. Both weight loss curves were analyzed through these three stages. In the first stage (room temperature to 105 ºC), the weight loss of untreated sludge was 73.74% and the biggest change was detected around 100 ºC. However for dewatered sludge, only 3.61% of weight loss was detected at 70 ºC, and no obvious peak was observed around 100 ºC. The boiling point of methanol is 64.7 ºC, thus the weight loss of dewatered sludge in the first stage was speculated to be caused by a slight methanol residue. In Fig.1, the final water content of the dewatered sludge was shown to be 13.4%. This discrepancy was probably caused by different retention times used in these two tests. For measurement with oven drying, six hours were adopted in order to observe the changes of all the four parts of water in ES. For the TG analysis, only 10 min was used, which mostly affected relative weakly bounded water, i.e. free water and partial interstitial water.[14] Taken together, the methanol-aided dewatering process could remove the free water and interstitial water entirely, achieving effective in-depth dewatering.

In the second stage, weight loss of the untreated and dewatered sludge was 12.84% and 51.74%, respectively, and figures were only 8.18% and 19.89%, respectively in the third stage. The weight loss in the last two stages was respectively noted as caused by biodegradable organic and refractory organic.[14, 17] The dewatered sludge preserved 3.4-fold organic substrates (per unit weight) than the untreated sludge, suggesting that methanol targeted specifically on water than other matters. It is noted that methanol-aided dewatering did not influence the availability of sludge for subsequent utilization or energy recovery.

Fig.2 Themogravimetric and differential thermal behaviors of treated and dewatered sludge

3.3 Optimization of methanol amended in-depth dewatering

Methanol concentration and contact time were chosen as the two parameters to be evaluated. The optimum levels of these two operational parameters and the effect of their interactions on dewatering performances were determined by RSM. Based on water content of the dewatered sludge, a quadratic model was utilized to fit the experimental data. Since the units of the two variables are different in this study, coefficients for coded factors were adopted to represent water content as a response surface, as shown in Eq. (4).

$$Y\,(\text{water content}) = 17.53-2.91X_1-0.36X_2+6.92X_1X_2+3.69X_1^2-0.07X_2^2 \qquad (4)$$

Analysis of variance (ANOVA) for the response surface quadratic model of water content was used to justify the adequacy of the models, [18] and the results are summarized in Table 3. The model F-value of 8.39 implies the model is significant. The '*Lack of Fit F-value*' of 7.73 implies the *Lack of Fit* is significant, and there is only a 3.86% chance that a 'Model *F-Value*' this large could occur due to noise. The values of 'Prob>F' less than 0.05 indicate model terms are significant, but values greater than 0.1 designate as insignificant terms. In this case, the higher *F-value*s of X_1 and X_1^2 suggested that methanol concentration had much more significant effect than the contact time on the dewatering process. However, X_1X_2 shows the highest *F-value*, whereupon the interaction of methanol concentration and contact time was the epistatic to simplex term.

The value of adjusted R^2 (94.59%) for Eq. (3) suggests that the total variation of 94% is attributed to the independent variables and only about 6% of the total variation cannot be explained by the model.[19] The difference of predicated R^2 and adjusted R^2 was approximate 7%, which was within a reasonable agreement of 20%.[18] Therefore, this quadratic equation can be used for predicting response to any combination of the two variables in the experimental range.

Table 3 ANOVA for response surface reduced quadratic model.

The fitted 3D response surface plot (Fig.3A) was generated by the above statistical model to understand the interaction between parameters on water content. The water content was decreased with an increase of methanol concentration and also the contact time. However, if methanol concentration and contact time simultaneously reached the maximum value, the water content was not the lowest. It is suggested that the interaction of two parameters was more predominant. The

predicted contour lines of water content could be observed in the contour diagrams (Fig.3B). Accordingly, if the water content of 20% is desired, methanol concentrations of 50%, 60%, 70% would require contact time of 100 min, 84 min, and 30 min, respectively. It is noted that relative small increase of methanol concentration could largely shorten the contact time, which is highly relevant to engineering application. Based on the data collected in this study, the model predicted a minimum water content of 11.7%, under the conditions of methanol concentration 100% and contact time 30 min.

Fig.3 The response surface (A) and contour diagrams (B) of water content as a function of methanol concentration (X_1) and contact time (X_2).

3.4 Destruction and preservation of microbial communities

The microbial population in the untreated and dewatered sludge was compared using PCR-DGGE technology. With methanol addition, microbial diversity was severely decreased (Fig.4A). The PCA analysis (Fig.4B) revealed that two RSM variables (methanol concentration and contact time) both affected the transitions of microbial communities, but the methanol concentration had more influences (70.646%) than contact time (12.279%). The DGGE profile suggests that S1 (with the lowest methanol concentration) had less bands than the initial sludge sample S0, but showed higher abundance than the other samples. Although 50% and 100% methanol was added in S7 and S13, respectively, the microbial communities in these two samples showed 99.6% similarity. In this regard, the microbial communities showed negligible differences when the methanol concentration was higher than 50%. In addition, the S4 and S10 received equivalent methanol of 75% but different contact time of 11.36 min and 138.64 min, but the S4 and S10 also shared 99.9% of similarity. Taken together, the methanol concentration was epistatic to contact time on the microbial structure, but once the methanol dosage exceeded 50%, it's significant on microbial communities was no longer concentration dependent.

The predominant bands were numbered and extracted for cloning and sequencing, and the results are shown in Fig.4C. The bands 1-8 were clustered as *Sphingomonas rhizogenes* strain, *Zoogloea* sp., *Ornithinibacter aureus* strain, *Rhodobacter* sp., *Amaricoccus veronensis* strain, *Plasticicumulans lactativorans* strain, *Cytophagaceae* bacterium, *Hydrogenophaga* sp., respectively. They were distributed in phyla Proteobacteria, Acinobacteria, and Bacteroidetes, And the bacteria of phylum Proteobacteria accounted for 75% of the detected population.

Microbial population changes from S0 to S1 suggested that the majority of microorganisms were generally killed during dewatering, possibly due to their poor resistance to the solvent methanol. In the preserved bacteria, *Rhodobacter* sp. (band 4) could utilize organic solvent and survive in benzene solution.[21] *Ornithinibacter aureus* strain (band 3) originates in actinomycetes, and some members of actinomycetes have also been reported to transform organic solvents.[21] Ramos *et al.* [22] noted that intrinsic characters of bacterial species decided the microbial resistance to such toxicants. Studies have pointed out that *Sphingomonas rhizogenes* strain, *Zoogloea* sp., *Rhodobacter* sp., *Amaricoccus veronensis* strain, *Plasticicumulans lactativorans* strain, *Cytophagaceae* bacterium, and *Hydrogenophaga* sp. are all gram-negative bacteria. And gram-negative bacteria in general process adaptive phospholipid composition in their cell membranes that might assist in the microorganisms' resistance and adaptation to hostile environments.[22] Taken together, the eight surviving bacteria detected showed decisive tolerance to methanol, and their cell membrane structure might be the primary reason.

Fig.4 Microbial communities of sludge in RSM test. A: DGGE profile, S0 represents the initial thickened sludge sample, and S1-S13 corresponded to the RSM test sample list in Table 2; B: PCA results; C: phylogenetic tree.

3.5 Mechanisms of the in-depth sludge dewatering process by methanol

The mechanisms of the in-depth sludge dewatering were inferred and concluded as the following: (1) contact and mixing between the sludge and methanol; (2) disruption of EPS structure and damage on cell membrane; (3) release and re-distribution of the interstitial water; (4) separation of solid and liquid.

Organic solvents are toxic for the majority of microorganisms, by attacking cell membrane and destroying cellular integrity.[23, 24] Cell membrane encloses intracellular components by lipid bilayer, and embedds enzymes and channel proteins to regulate entrance and exit the cell, thus facilitating the transport of materials needed for survival.[25] The toxic effects on cells probably started from the contact with and accumulation of solvent molecules in the membrane. The long-chain alkane components of cell membrane are hydrophobic, providing an external barrier to charged or toxic particles. However organic solvent contact could largely enhance the polarity of long-chain alkanes, and weakening their protection on cell membrane.[26] Due to the loss of cell

integrity, the intracellular components could be leaked, which would finally lead to the death of a microbial cell.[27] Generally, if the dead cells kept their structural integrity, in which the DNA molecules were still preserved, the species could also be detected using PCR-DGGE. However in this case (as shown in Fig. 4A), only very few bacteria were observed in the dewatered sludge, supporting that methanol contact actually destroyed the cell membrane and cause leakage of intracellular components.

$LogP_{O/W}$ is the octanol/water partition coefficient, depicting the hydrophobicity of an organic solvent. When the solvent has a lower $LogP_{O/W}$, it is considered to have higher polarity. Thereby, the solvent with a lower $LogP_{O/W}$ is likely to be more soluble in aqueous phase, and has more opportunity to attack the lipid bilayer of cell membrane.[27, 28] The $LogP_{O/W}$ value of methanol is 0.23, thus the strong polarity of methanol could be the major reason behind its advanced dewatering performances.

The interstitial water was bound to the EPS, which was a primary hindrance to in-depth sludge dewatering.[29] If EPS was disorganized, the interstitial water would be re-distributed in sludge flocs or removed into the bulk liquid. Furthermore, the EPS is constructed by various organic substrates, including: protein, polysaccharides, humic acids, and lipids. As methanol has a lower $LogP_{O/W}$ value than EPS molecules, it would favor the attachment to interstitial water rather than the generally hydrophobic EPS molecules. Thus, methanol can help to overcome the obstacle of EPS-bound water.

Whether the fundamental mechanism lied in the loss of cell integrity or the separation of interstitial water from EPS, methanol did achieve the release and re-distribution of water in ES. The external mechanical process could then separate the bacterial cells and the bulk liquid, and the supernatant would be mainly composed of methanol solution. Due to the low boiling point of methanol (64.7 ºC), the separation could also be easily achieved by naturally evaporation or low temperature distillation, thus greatly saves energy. Comparing to the untreated thickened sludge (Fig.2), the dewatered sludge possessed only 16.4% water but 3.4-fold higher organic substrates (in unit weight), which is beneficial to sludge incineration as post treatment. As methanol is also the common additional carbon source to enhance denitrification in WWTPs, [30, 31] the supernatant of in-depth dewatering process could also be recycled into anoxic tank.

4. Conclusion

The water content of dewatered ES can be reduced to 13.4% with the aid of methanol contact. More importantly, the dewatered sludge (per unit weight) preserved 3-fold organic substrates, compared to untreated sludge. Due to the low-cost of methanol, the in-depth dewatering method has promising prospective for engineering application. Additionally, the optimal water content was estimated to be 11.7% under 30-min contact with 100% methanol. The core mechanism for methanol aided in-depth dewatering was hypothesized to be the destruction of cell membrane and re-distribution of trapped water in cells and EPS. The in-depth dewatering process was concluded as follows: (1) contact of ES and methanol, (2) destruction of cell membrane and bacterial death; (3) release and re-distribution of interstitial water; (4) separation of solid and liquid.

Acknowledge

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Figure captions

Fig.1 In-depth dewatering performances

Fig.2 Themogravimetric and differential thermal behaviors of treated and dewatered sluge

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Table 1 Designed levels and values of the variables employed in RSM.

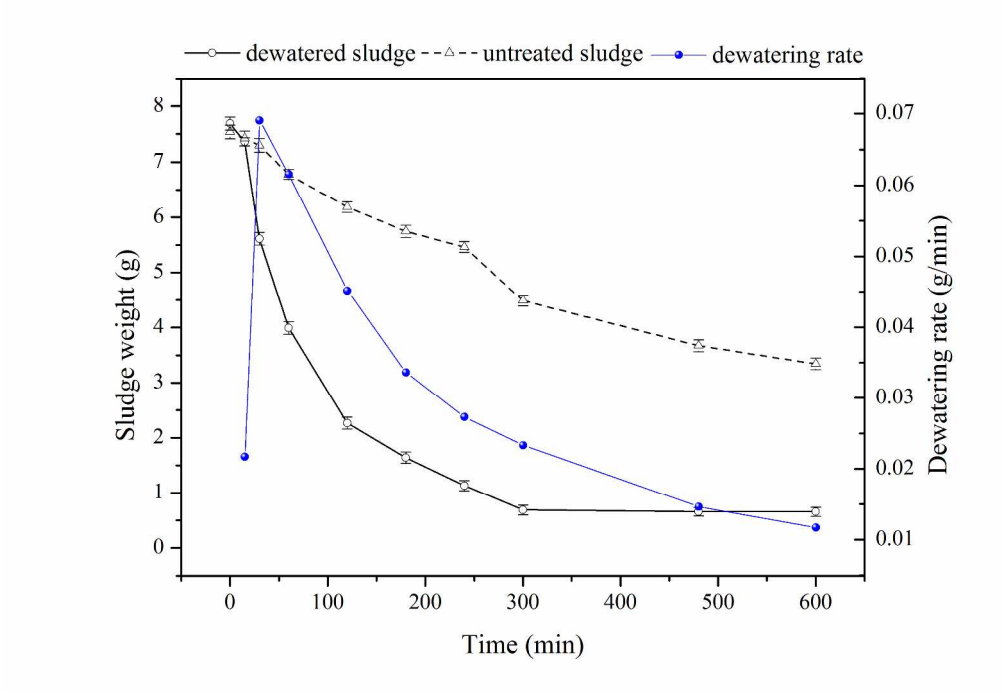
Variables	Range and level		
	-1	0	+1
X_1/ Methanol concentration (%)	50	75	100
X_2/ Contact time (min)	30	75	120

Table 2 Actual experimental design of RSM.

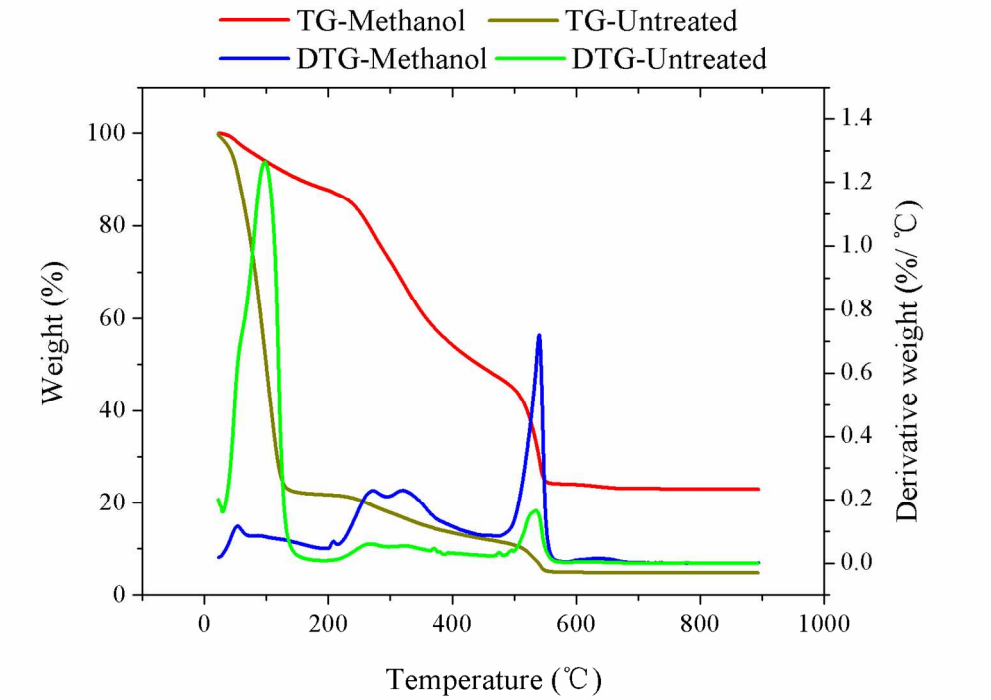
Test	Methanol concentration (%)	Contact time (min)
S1	39.64	75
S2	100	120
S3	75	75
S4	75	11.36
S5	110.35	75
S6	75	75
S7	50	30
S8	50	120
S9	75	75
S10	75	138.64
S11	75	75
S12	75	75
S13	100	30

Table 3 ANOVA for the response surface reduced quadratic model.

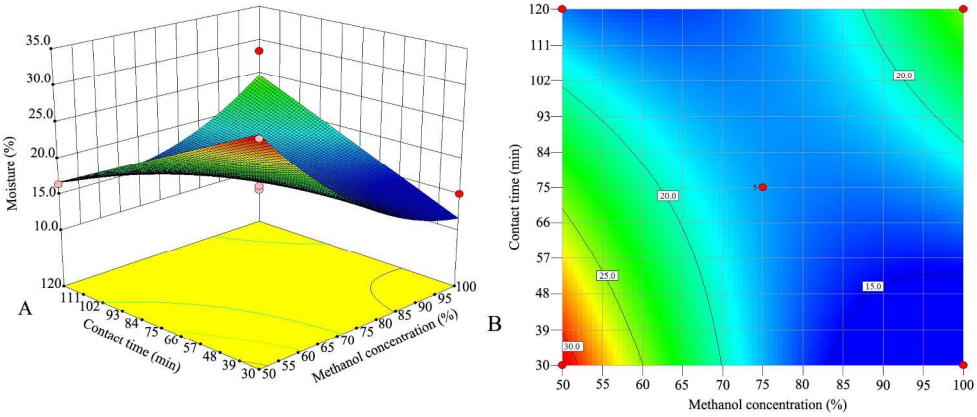
Source	Sum of squares	df	Mean square	F-value	p-Value Prob>F	
Model	357.05	5	71.41	8.39	0.0072	Significant
X_1-methanol	67.76	1	67.76	7.96	0.0257	
X_2-contact time	1.02	1	1.02	0.12	0.7397	
$X_1 X_2$	191.47	1	191.47	22.50	0.0021	
X_1^2	94.65	1	94.65	11.12	0.0125	
X_2^2	0.034	1	0.034	3.982E-003	0.9514	
Residual	59.57	7	8.51			
Lack of Fit	50.80	3	16.93	7.73	0.0386	Significant
Pure Error	8.77	4	2.19			
Cor Total	416.62	12				
R^2=95.70%, R^2(adjusted)=94.59%, R^2(predicted)=87.97%						



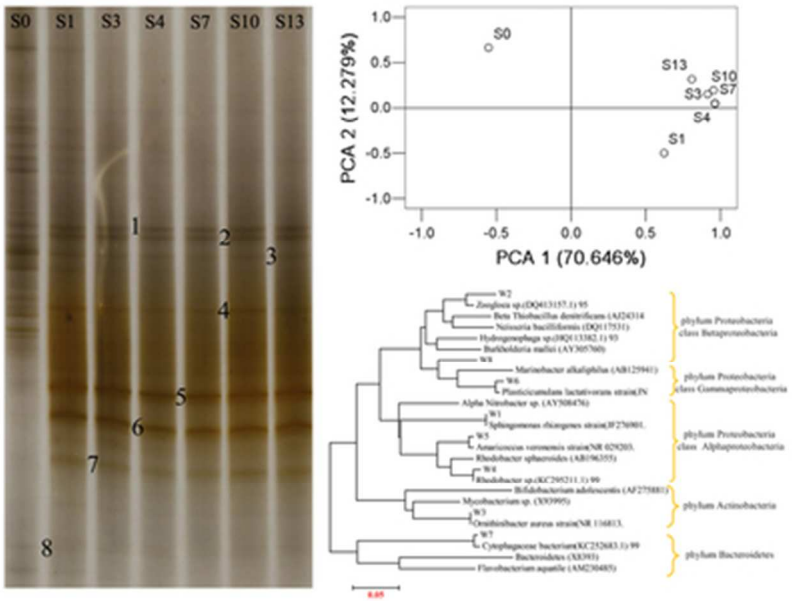
288x200mm (300 x 300 DPI)



287x202mm (150 x 150 DPI)



627x275mm (96 x 96 DPI)



33x25mm (300 x 300 DPI)