Research on the coagulant aid effects of modified diatomite on coal microbial flocculation

Dong Jingshen and Liu Quanjun

ABSTRACT

Diatomite was modified by chitosan to prepare modified diatomite, and the modified diatomite in an optimized ratio was utilized in coal bio-flocculation. The interaction behavior and flocculation mechanism of modified diatomite on coal slurry water were investigated by single factor experiments, infrared spectroscopy, Brunauer-Emmett-Teller (BET) measurements, and zeta potential measurements. The single factor experiments showed that when the amount of microbial flocculant added was 1.5 ml, the temperature of coal slurry water was 39 °C, the pH was 5, and the amount of modified diatomite was 0.2 g, after 30 min of sedimentation, the flocculation transmittance of the coal slurry water reached 84.3%. The infrared spectra showed that the -NH_2 and -OH of the chitosan molecule had a polar interaction with the Si-OH bond in diatomite. The BET measurements showed that the specific surface area of diatomite was not a decisive factor affecting the flocculation effect. Zeta potential measurements indicated that the amino protonation of chitosan increased the isoelectric point (IEP) of modified diatomite. These results showed that modified diatomite has a good effect on coal bio-flocculation.

Key words | biological flocculation, chitosan, diatomite, light transmittance

Dong Jingshen
Liu Quanjun (corresponding author)
Faculty of Land Resources Engineering,
Kunming University of Science and Technology,
Kunming 650093,
China
and
State Key Laboratory of Complex Nonferrous Metal Resources Clean Utilization,
Kunming 650093,
China
E-mail: *kmliuqj@163.com*

INTRODUCTION

At present, most of the coal washing and dressing in China is carried out with water as the medium (Zhang *et al.* 2019). The process produces a lot of slime water, which has the properties of fine size distribution, high ash content on its particle surface. The flocculant sedimentation method is often used in the water treatment of coal slime (Yang *et al.* 2019). The most common flocculants are polyacrylamide and its derivatives, but these flocculants not only produce toxic monomeric acrylamide, but also pollute the environment (Ruden 2004). Microorganisms and their products can be excellent flocculants, and can even have the effect of selective flocculation in some cases (Zhang *et al.* 2007). Microbial flocculants can overcome the shortcomings of traditional flocculants; the main ingredients are proteins and sugars (Nwodo *et al.* 2014; Salehizadeh & Yan 2014). They are safe, reliable and biodegradable, and therefore pollution-free (Lian *et al.* 2008).

Coagulant aids are an auxiliary agent that can improve the flocculation effect (Abo-el-Enein *et al.* 2011). $CaCl_2$ is commonly used as a coagulant aid in the bio-flocculation of coal. Ca^{2+} is effective in promoting the flocculating activities of bio-flocculants (Yang *et al.* 2017). Diatomite is a widely used coagulant aid; its main component is SiO_2 (Al-Ghouti *et al.* 2003; Khraisheh *et al.* 2005). It has light texture, small porosity, large specific surface area and high chemical stability (Gao *et al.* 2005; Luan *et al.* 2019). Due to its excellent characteristics, it has received much attention in research (Lv *et al.* 2019). However, natural diatomite has poor coagulating properties, and chemical modification can be used to improve its coagulation efficiency. Chitosan is a chitin deacetylated product, insoluble in water, soluble in acid (Bonilla *et al.* 2019). It is a linear polymeric amino sugar substance. Chitosan has strong flocculation ability after hydrolysis, which can produce bridge and electric neutralization. Chitosan can also be biodegraded and not pollute the environment (Zimet *et al.* 2019). However, due to the high price of chitosan, it is generally not used alone. Currently, chitosan is usually modified or combined with other flocculants to improve water treatment.

At present, there are many studies on the use of chitosan-modified diatomite, but most of them are still used in the

doi: 10.2166/wst.2020.009

treatment of water, such as dyes and waste liquids (Song *et al.* 2019). However, there have been few reports on the use of modified diatomite for the flocculation treatment of coal slurry water. The research object of this paper is the light transmittance of coal slurry water after flocculation; and the diatomite modified by chitosan is used to explore the coagulation effect on microbial flocculation of coal slurry water. The flocculation mechanism of modified diatomite was investigated by infrared spectroscopy (IR) analysis, Brunauer-Emmett-Teller (BET) measurements, and zeta potential measurements.

MATERIALS AND METHODS

Materials

Characteristics of coal sample and coal slurry water

After the test coal sample was crushed and sieved, the sieve material having a particle diameter of less than 0.5 mm was taken as the test coal. The X-ray diffraction (XRD) pattern of coal slurry is shown in Figure 1. The size distribution of the coal particles were determined using a laser particle size analyzer (SALD-7101, Japan), as shown in Table 1.

Coal and water were mixed to make coal slurry water with a concentration of 35 g/L. 100 ml of coal slurry water was put into the measuring cylinder and left to settle. After 30 min, 5 ml of supernatant was taken out to measure its light transmittance with a UV-spectrophotometer. The results are shown in Table 2.

The X-ray diffraction pattern showed that the main mineral components of the coal included montmorillonite,

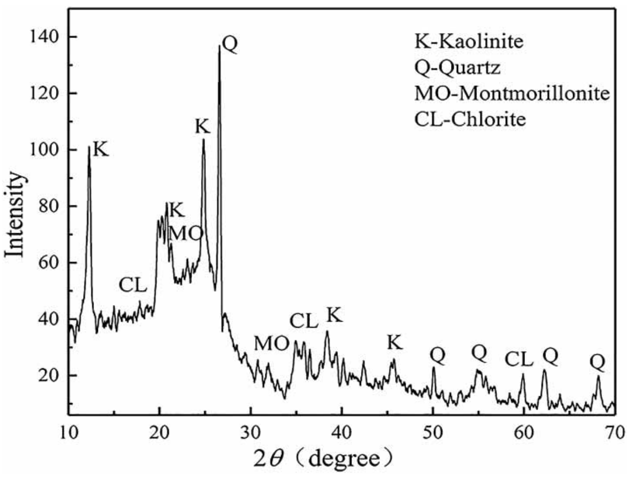

Figure 1 | XRD pattern of coal slurry.

Table 1 | The particle size distribution and ash content of coal

Size range/mm	Content/%	Ash/%
0.5–0.25	14.86	14.64
0.25–0.125	16.33	19.55
0.125–0.075	19.26	24.57
0.075–0.045	17.65	24.70
−0.045	31.90	36.01
Total	100	–

Table 2 | Characteristics of coal slurry water

Concentration	Water temperature	pH	Natural sedimentation transmittance
35 g/L	28 °C	6.6	7.29%

quartz, kaolinite, chlorite, etc. in which chlorite and montmorillonite were easily argillized (Chen *et al.* 2016a). At the same time, these minerals can increase the difficulty of coal slurry water treatment because they are all clay minerals (Ermolovich & Ermolovich 2016). It can be seen from the particle size composition and the ash content (Table 1) that the fine particles in the coal sample are high and the ash content is high. Combined with the results of the natural sedimentation transmittance in Table 2, it can be seen that the slime water prepared by the coal sample is difficult to settle.

Test reagents

The test diatomite (AR) was purchased from Chengdu Aikeda Chemical Reagent Co., Ltd, glacial acetic acid (AR) was purchased from Wuxi Yasheng Chemical Co., Ltd, medium-viscosity (200–400 mPa.s) chitosan was purchased from MACKLIN; the pH was adjusted to the predetermined value by 1 mol/L sodium hydroxide or hydrochloric acid.

Strain and strain culture medium

The strain used was *Bacillus subtilis* with good activity (Zhao *et al.* 2017), which was purchased from BeNa culture collection (number: BNCC109047).

Liquid medium: beef extract 4 g/L, peptone 10 g/L, NaCl 5 g/L, pH 7.0; the medium was sterilized at 121 °C for 15 min using a BOXUN vertical pressure steam sterilizer.

Experimental technical procedure

Culture of strains and determination of growth of strains

The liquid medium inoculated with the strain was placed in a constant temperature shaking incubator at 37 °C and 160 r/min. During the cultivation process, the rate of bacterial reproduction was measured with UV-spectrophotometer every 12 hours, and the growth curve of *Bacillus subtilis* was drawn.

Preparation of modified diatomite

Ten ml of different concentrations (10, 20, 30 g/L) of chitosan solution with 5% acetic acid solution were prepared. After adding 10 g diatomite, the mixture was stirred to fully mix the chitosan solution and diatomite. According to the mass ratio of diatomite and chitosan, the content of chitosan in diatomite was 10, 20, 30 mg/g, respectively. Finally, the modified diatomite was obtained after drying and grinding.

Flocculation tests

Flocculation test conditions included the amount of modified diatomite, the pH value, the amount of microbial flocculants, and the temperature of the water. Each condition was executed in turn as a single factor test, and the optimal conditions for each single factor test were used in the next set of tests.

Ninety ml of coal slurry water was added to a 250 ml beaker, plus a certain amount of coagulant. The beaker was placed on a magnetic stirrer and stirred for 1 min, then a certain amount of microbial flocculants was added, plus coal slurry water to 100 ml, and then it continued to be stirred slowly for 5 min. The stirred coal slurry water was poured into a 100 ml graduated cylinder to settle. After 30 min, 5 ml of the supernatant was taken to measure the transmittance under a UV-spectrophotometer.

In the flocculation experiment section, in order to determine the best content of chitosan in diatomite, the concentration of modified diatomite was studied: the test results show that the best flocculation effect cannot be achieved when the content of chitosan in diatomite is 10–30 mg/g, so the content of chitosan in diatomite was increased to 50–150 mg/g.

RESULTS AND DISCUSSION

Cultivation of *Bacillus subtilis*

As shown in Figure 2(a), the absorbance value of *Bacillus subtilis* reached the maximum at 60 hours, and the change was slow, so the microbial flocculants were cultured for 2.5 days. Figure 2(b) is an optical microscope image using Gram staining, as *Bacillus subtilis* is a Gram-positive bacteria. The *Bacillus subtilis* culture results are shown in Figure 2(c) and 2(d).

Single factor experiment

Study on the concentration of modified diatomite

It can be seen from the analysis in Figure 3(a) that the direct use of diatomite as a coagulant has little effect on the transmittance of coal slurry water. It was found that the transmittance of the supernatant increased with the increase of chitosan content in diatomite, with the best flocculation effect at the dosage of 0.2 g. With the other conditions unchanged, the content of chitosan in diatomite was increased to 50, 75, 100, 125, 150 mg/g and the controlled addition amount was kept at 0.2 g. The test results are shown in Figure 3(b).

It can be seen from Figure 3(b) that the results of the flocculation experiment using the diatomite with different chitosan contents under the same factors show a trend of increasing and then decreasing. With the content of chitosan in diatomite at 100 mg/g, the transmittance value reached the maximum. Adding chitosan above this level caused excessive adsorption of chitosan on the surface of diatomite, resulting in a decrease in light transmittance. If the chitosan is added in excess, it will be suspended in the coal slurry water and will have a certain adverse effect on the flocculation effect.

Effect of the addition amount of modified diatomite

The modified diatomite with a chitosan concentration of 100 mg/g was used, and the amount was varied; the microbial flocculants were 1 mL, and the test results are shown in Figure 4(a). The experimental data show that the modified diatomite was added in the range of 0.05–0.20 g and the light transmittance gradually increased. When the amount added was 0.2 g, the light transmittance reached a maximum value of 62.3%; as the amount of modified diatomite continued to increase, the light transmittance began to decrease. This result indicated that a large amount of modified diatomite had an adverse effect on flocculation, because the chitosan in the modified diatomite is viscous, and the excessive amount will be suspended in the coal slurry water. Therefore, the dosage of the modified

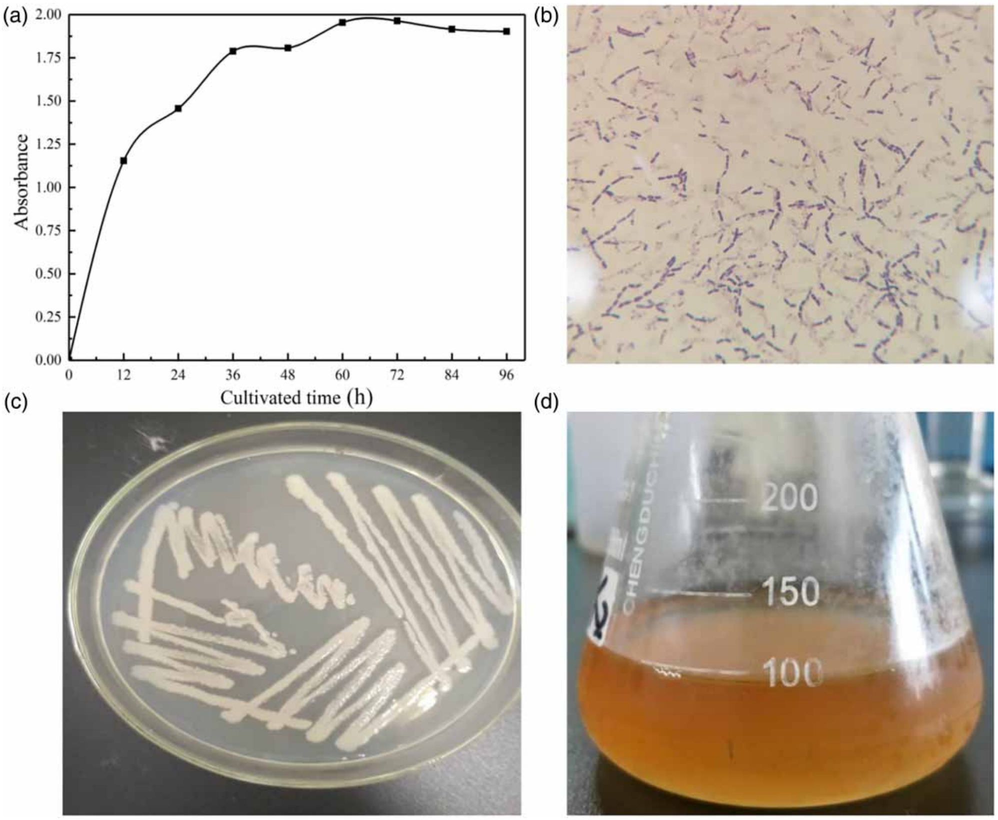

Figure 2 | *Bacillus subtilis* growth curve (a); image under light microscope (b); *Bacillus subtilis* plate growth colony morphology (c); *Bacillus subtilis* liquid colony morphology (d).

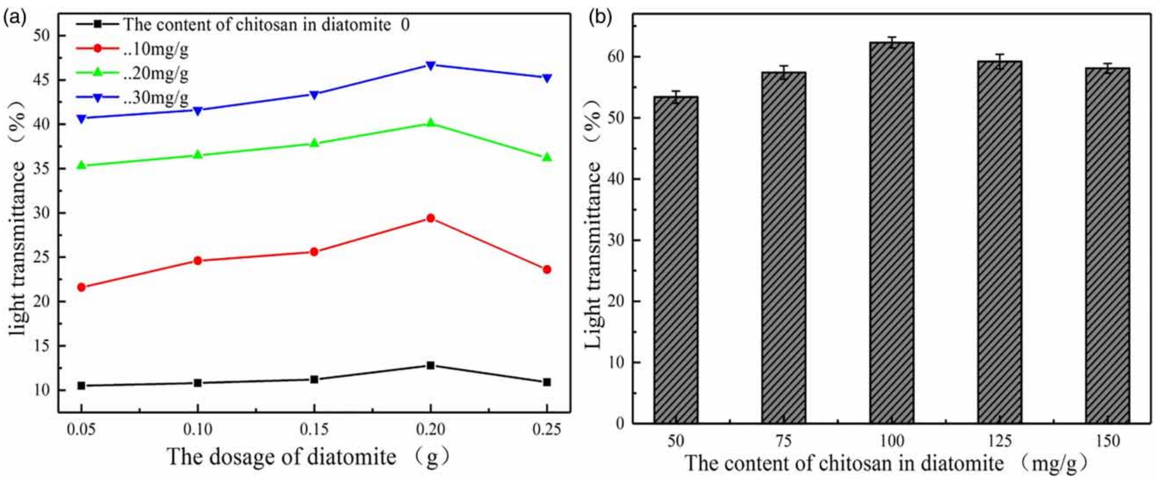

Figure 3 | Effect of diatomite with different chitosan content (a) (b).

diatomite was maintained at 0.2 g in subsequent flocculation tests.

Effect of pH

The pH value of coal slurry water is a vital factor affecting microbial activity. The amount of the modified diatomite added was 0.2 g, the amount of the microbial flocculants added was 1 ml, and the pH value was changed. The test results are shown in Figure 4(b). When the pH value was 5, the transmittance value reached 68.3%; as the pH value increased the light transmittance gradually decreased. The reason for this is that the change in pH affected the activity of *Bacillus subtilis*, there was less

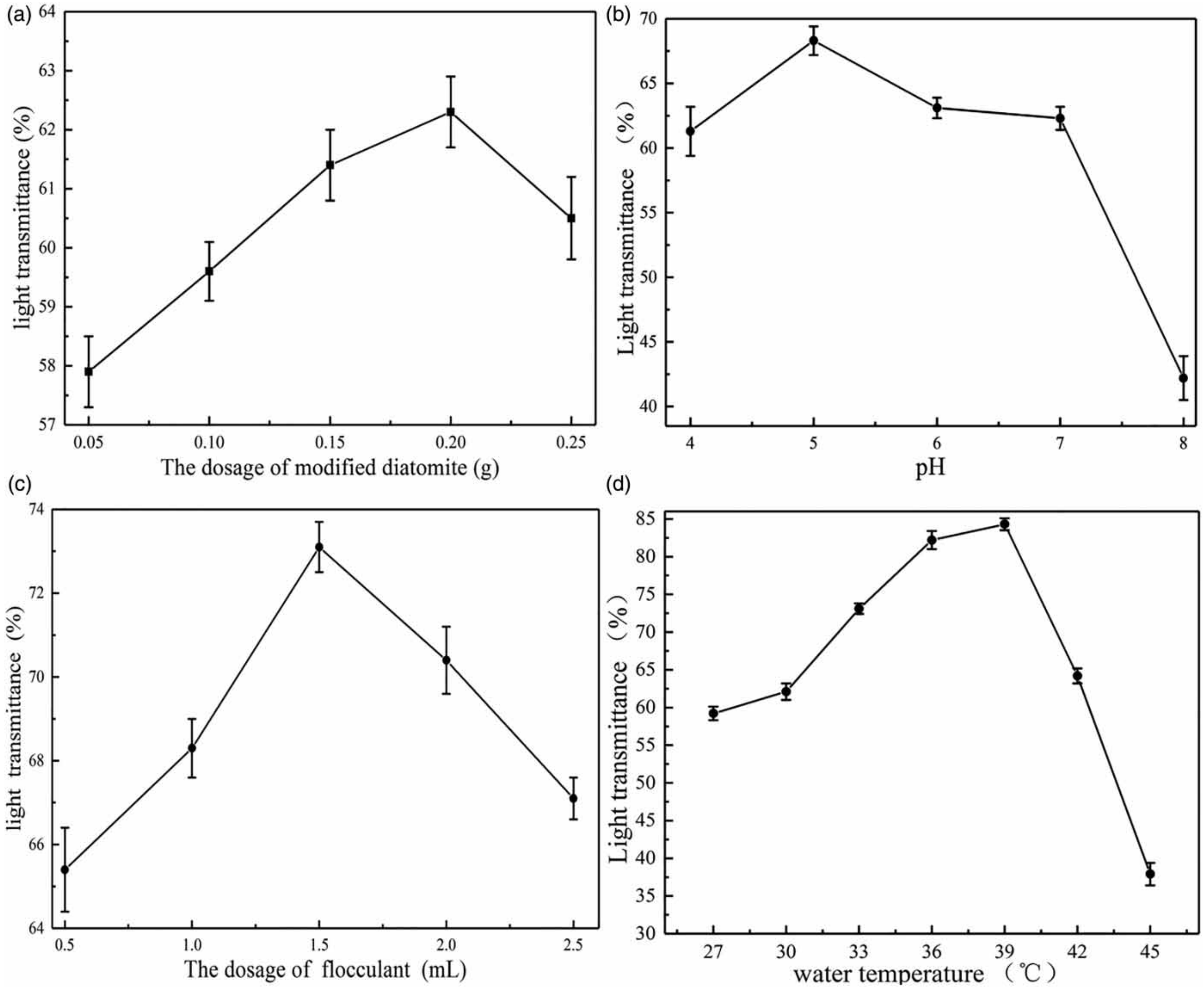

Figure 4 | Effect of the amount of modified diatomite added (a); effect of pH (b); effect of the amount of flocculant added (c); effect of coal slurry water temperature (d).

flocculation and the light transmittance of the coal slurry water after sedimentation decreased. Therefore, the amount of the modified diatomite was kept at 0.2 g, and the pH kept at 5 for subsequent tests.

Effect of the addition amount of bio-flocculants

The amount of modified diatomite added was 0.2 g, the pH value was adjusted to 5, and the amount of the microbial flocculants was varied; the test results are shown in Figure 4(c). When the amount of flocculants added was between 0.5 and 1.5 mL, the light transmittance gradually increased. When the amount of flocculant added was 1.5 mL, the light transmittance reached 73.1%; as the flocculants addition amount continued to increase, the light transmittance gradually decreased. The cause of this was that adding an excessive amount of microbial flocculants caused a part of the microbial flocculants to be suspended in the solution, affecting the light transmittance. Therefore, the amount of the modified diatomite was set at 0.2 g, the pH was adjusted to 5, and the amount of flocculant added was 1.5 mL for subsequent tests.

Effect of coal slurry water temperature

The temperature of coal slurry water also has an important influence on the activity of microbial flocculants. The amount of modified diatomite added was 0.2 g, the pH value was adjusted to 5, the amount of flocculant added was 1.5 mL, and the temperature of the water was varied. The test results are shown in Figure 4(d). When the temperature of the coal slurry water was controlled at 27 to 39 °C, the light transmittance gradually increased. When the temperature was 39 °C, the light transmittance reached 84.3%; as the water temperature gradually increased, the light transmittance decreased. The reason for this was that the change in water temperature affected the activity of *Bacillus subtilis*, there was less flocculation and the light transmittance of the coal slurry water after sedimentation decreased.

According to the single factor test, at a temperature of 39 °C, modified diatomite addition of 0.2 g, pH of 5, and microbial flocculant addition of 1.5 mL, the light transmittance of coal slurry water with 35 g/L reached 84.3% after 30 min of settling.

The modified diatomite was replaced by the coagulant $CaCl_2$, which commonly used in microbial flocculation. Under the same test conditions, the supernatant transmittance of the coal slurry water flocculation test was 71.1%. It can be concluded that the effect of modified diatomite is better than that of the conventional coagulant.

The sedimentation effect under different conditions is shown in Figure 5.

Results of the characterization

Infrared spectroscopy analysis

As can be seen in Figure 6(a), in the diatomite spectra, the typical wide peak region from 3,103 cm^{-1} to 3,671 cm^{-1} was caused by the stretching vibration of hydroxyl (-OH) and amine (-NH_2) (Pandi *et al.* 2017; Mu *et al.* 2018). The peaks at 1,627, 1,069, 792 and 462 cm^{-1} showed four important characteristic peaks of diatomite, that is to say, asymmetric vibration of Si-O, symmetric vibration of Si-O, and Si-O-Si bridge vibration (Mu *et al.* 2018). In the modified diatomite spectra, three characteristic peaks of diatomite at 1,069, 786, 462 cm^{-1} were preserved, the characteristic peak at 1,627 cm^{-1} was strengthened, and the peaks from

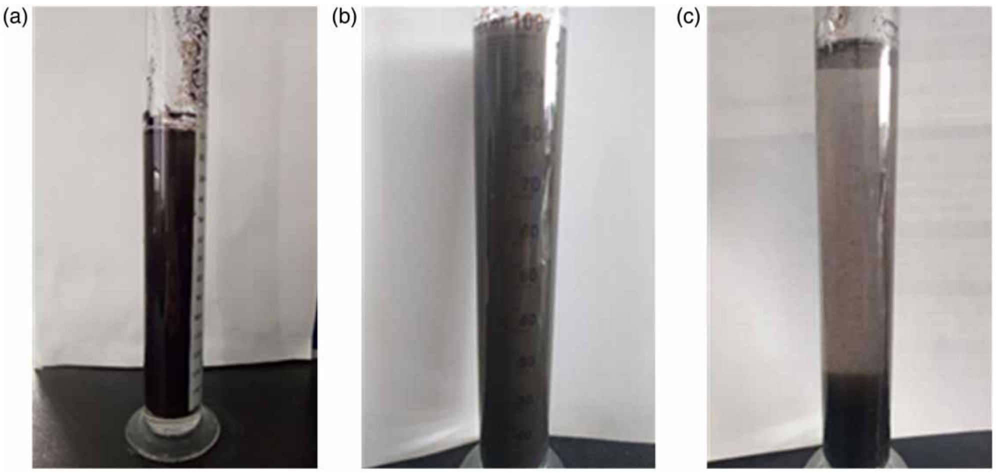

Figure 5 | Settlement effect of different coagulation conditions: natural settling (a); $CaCl_2$ as a coagulant (b); modified diatomite as coagulant (c).

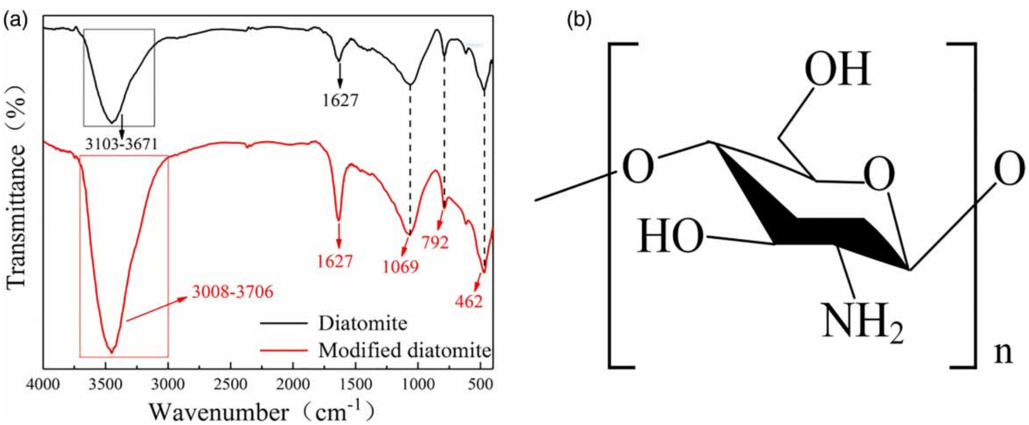

Figure 6 | Infrared spectra of diatomite and modified diatomite (a); molecular structure of chitosan (b).

3,038 cm^{-1} to 3,696 cm^{-1} of -OH and $-NH_2$ were enhanced. It can be seen from the molecular structure diagram of chitosan in Figure 6(b) that there are a large amounts of -OH and free $-NH_2$ on the chitosan molecule. This is due to the fact that chitosan has abundant polar groups. Specifically, $-NH_2$ and -OH had a polar interaction with the Si–O bond in diatomite, which promotes the binding of polar groups. In summary, diatomite successfully completed the modification.

BET measurements

The specific surface area of diatomite before and after modification was measured. The specific surface area, pore volume and average pore diameter of diatomite before and after modification are shown in Table 3. The results showed that the specific surface area of the modified diatomite decreased slightly compared with diatomite. This is also the result of chitosan loading on the surface of diatomite. It can be seen that the specific surface area of diatomite was not a decisive factor affecting the flocculation effect.

Zeta potential analysis

As shown in Figure 7, the zeta potential of diatomite and modified diatomite decreased with the increase of the pH value. At pH <4.39, the silanol (-Si-OH) of diatomite was protonated and attained a positive charge to become $-Si-OH_2^+$. In comparison, at pH >4.39, $-Si-OH^{2+}$ started to deprotonate and became negatively charged, in the form of -Si-O-. The isoelectric point (IEP) of diatomite was 4.39. The IEP of modified diatomite was 7.41. As a result, the amino protonation of chitosan increased the IEP of modified diatomite (Chen *et al.* 2016b). When the pH was between 4.39 and 7.41, the potential of the diatomite was negative, and the potential of the modified diatomite was positive. Because of this, the positive charge on the surface of the modified diatomite was easily electrostatically attracted and combined with the anionic groups in the coal slurry water.

Table 3 | Comparison of BET surface area, pore volume and BJH average pore diameter of diatomite and modified diatomite

Sample	BET surface area (m^2/g)	Pore volume (cm^3/g)	BJH average pore diameter (nm)
Diatomite	20.3	0.06	8.4
Modified diatomite	18.1	0.05	7.3

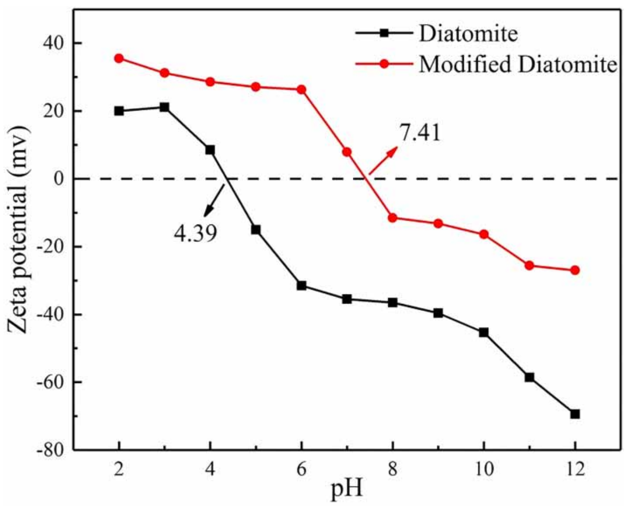

Figure 7 | Zeta potential of diatomite and modified diatomite as a function of pH.

Mechanisms analysis

The flocculation mechanism is shown in Figure 8. The main effect of microbial flocculants on coal slime water is selective adsorption and bridging (Salehizadeh & Shojaosadati 2003; Yang *et al.* 2017), while the function of modified diatomite is mainly electrical neutralization. The presence of these effects destroys the stability of the coal particles and mineral suspended particles, prompting rapid settling. Hydroxyl and carboxyl groups on the molecular chain of bio-flocculants determine the bridging mechanism. The hydroxyl groups of the bio-flocculants can form hydrogen bonds with the OH groups of the clay mineral particles (Falade *et al.* 2019). In addition, when carboxyl groups are present there are chemical bonds between the bio-flocculants and the clay minerals. Furthermore, there is a hydrophobic force between the coal surface and the bio-flocculants polysaccharide, which may also be the cause of the bridging mechanism. In short, the modified diatomite produces an electric neutralization effect on the basis of the action of the microbial flocculants adsorption and bridge, hydrogen bonds, chemical bonds, etc., thereby achieving faster sedimentation of the coal slurry water.

CONCLUSIONS

The optimum conditions for the combination of modified diatomite (the content of chitosan in diatomite was 100 mg/g) and *Bacillus subtilis* were as follows: the temperature of the slime water was 39 °C, the pH value of the

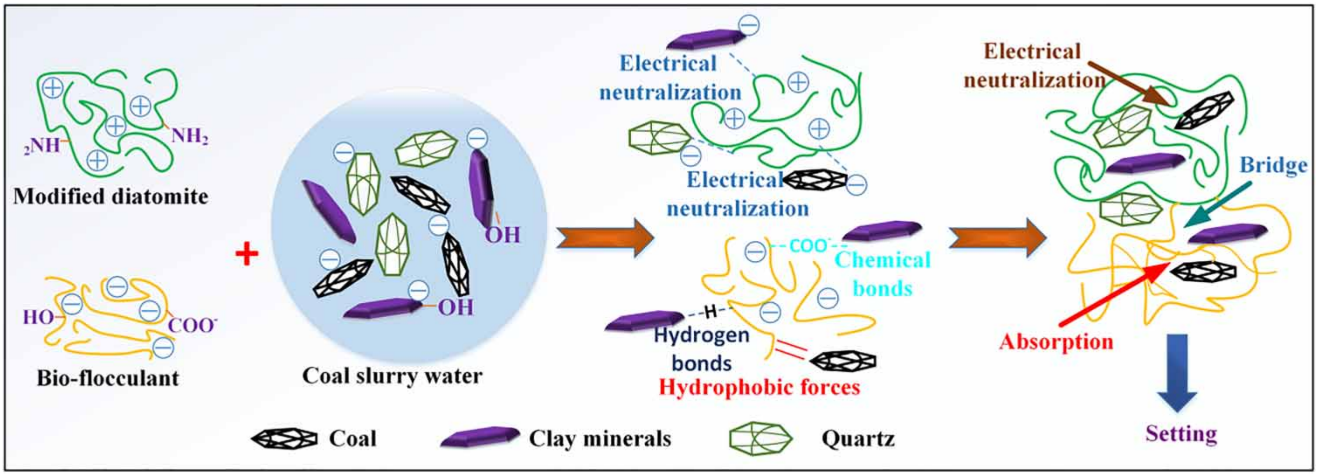

Figure 8 | The schematic diagram of flocculation mechanism of modified diatomite and bio-flocculants in coal slurry water.

coal slurry water was 5, and the amount of modified diatomite was 0.2 g. The amount of the microbial flocculants added was 1.5 ml, and under these conditions, the flocculation of the slime water for 30 min allowed the light transmittance to reach 84.3%. When the modified diatomite was replaced by $CaCl_2$, and the flocculation test of the slime water was carried out under the same test conditions, the transmittance of the supernatant was 71.1%.

Due to the protonation of the amino group on the chitosan making the diatomite positively charged, the modified diatomite easily results in electrical neutralization by the anion group of the coal slurry water, and with the adsorption and bridging action of the microbial flocculants, the coal slurry water settles faster.

ACKNOWLEDGEMENT

The authors wish to express to financial support of the research from the National Natural Science Foundation of China (project no. 51764021).

REFERENCES

Abo-el-Enein, S. A., Eissa, M. A., Diafullah, A. A., Rizk, M. A. & Mohamed, F. M. 2011 Utilization of a low cost agro-residue for production of coagulant aids and their applications. *Journal of Hazardous Materials* **186** (2–3), 1200–1205.

Al-Ghouti, M. A., Khraisheh, M. A. M., Allen, S. J. & Ahmad, M. N. 2003 The removal of dyes from textile wastewater: a study of the physical characteristics and adsorption mechanisms of diatomaceous earth. *Journal of Environmental Management* **69** (3), 229–238.

Bonilla, F., Alexander Chouljenko, Andy Lin, Britt M. Young, Tanishq Sai Goribidanur, James C. Blake, Peter J. Bechtel & Subramaniam Sathivel 2019 Chitosan and water-soluble chitosan effects on refrigerated catfish fillet quality. *Food Bioscience* **31**, 100426.

Chen, J., Fanfei Min, Lingyun Liu, Chenliang Peng & Fangqin Lu 2016a Hydrophobic aggregation of fine particles in high muddied coal slurry water. *Water Science and Technology* **73** (3), 501–510.

Chen, J., Liang-guo Yan, Hai-qin Yu, Shuang Li, Li-lu Qin, Guo-qiang Liu, Yun-fei Li & Bin Du 2016b Efficient removal of phosphate by facile prepared magnetic diatomite and illite clay from aqueous solution. *Chemical Engineering Journal* **287**, 162–172.

Ermolovich, E. A. & Ermolovich, O. V. 2016 Effects of mechanical activation on the structural changes and microstructural characteristics of the components of ferruginous quartzite beneficiation tailings. *International Journal of Mining Science and Technology* **26** (6), 1043–1049.

Falade, A. O., Leonard V. Mabinya, Anthony I. Okoh & Uchechukwu U. Nwodo 2019 Biochemical and molecular characterization of a novel dye-decolourizing peroxidase from *Raoultella ornithinolytica* OKOH-1. *International Journal of Biological Macromolecules* **121**, 454–462.

Gao, B., Pengfei Jiang, Fuqiang An, Shuying Zhao & Zhen Ge 2005 Studies on the surface modification of diatomite with polyethyleneimine and trapping effect of the modified diatomite for phenol. *Applied Surface Science* **250** (1–4), 273–279.

Khraisheh, M. A., Al-Ghouti, M. A., Allen, S. J. & Ahmad, M. N. 2005 Effect of OH and silanol groups in the removal of dyes from aqueous solution using diatomite. *Water Research* **39** (5), 922–932.

Lian, B., Ye Chen, Jin Zhao, H. Henry Teng, Lijun Zhu & Sheng Yuan 2008 Microbial flocculation by *Bacillus mucilaginosus*:

applications and mechanisms. *Bioresource Technology* **99** (11), 4825–4831.

Luan, X., Jinhong Li, Lijie Liu & Zhiwei Yang 2019 Preparation and characteristics of porous magnesium phosphate cement modified by diatomite. *Materials Chemistry and Physics* **235**, 121742.

Lv, J., Bin Sun, Jing Jin & Wei Jiang 2019 Mechanical and slow-released property of poly(acrylamide) hydrogel reinforced by diatomite. *Materials Science and Engineering: C Materials for Biological Applications* **99**, 315–321.

Mu, Y., Miao Cui, Shaoqing Zhang, Jing Zhao & Changgong Meng, QiSun 2018 Comparison study between a series of new type functional diatomite on methane adsorption performance. *Microporous and Mesoporous Materials* **267**, 203–211.

Nwodo, U. U., Ezekiel Green, Leonard V. Mabinya, Kunle Okaiyeto, Karl Rumbold, Lawrence C. Obi & Anthony I. Okoh 2014 Bioflocculant production by a consortium of *Streptomyces* and *Cellulomonas* species and media optimization via surface response model. *Colloids and Surfaces B: Biointerfaces* **116**, 257–264.

Pandi, K., Periyasamy, S. & Viswanathan, N. 2017 Remediation of fluoride from drinking water using magnetic iron oxide coated hydrotalcite/chitosan composite. *International Journal of Biological Macromolecules* **104**, 1569–1577.

Ruden, C. 2004 Acrylamide and cancer risk–expert risk assessments and the public debate. *Food and Chemical Toxicology* **42** (3), 335–349.

Salehizadeh, H. & Shojaosadati, S. A. 2003 Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*. *Water Research* **37** (17), 4231–4235.

Salehizadeh, H. & Yan, N. 2014 Recent advances in extracellular biopolymer flocculants. *Biotechnology Advances* **32** (8), 1506–1522.

Song, X., Zhihui Chai, Yuan Zhu, Chenglin Li & Xinquan Liang 2019 Preparation and characterization of magnetic chitosan-modified diatomite for the removal of gallic acid and caffeic acid from sugar solution. *Carbohydrate Polymers* **219**, 316–327.

Yang, Z., Wang, W. & Liu, S. 2017 Flocculation of coal waste slurry using bioflocculant produced by *Azotobacter chroococcum*. *Energy & Fuels* **31** (2), 1460–1467.

Yang, Z., Shengyu Liu, Wenbing Zhang, Quanbao Wen & Yongjie Guo 2019 Enhancement of coal waste slurry flocculation by CTAB combined with bioflocculant produced by *Azotobacter chroococcum*. *Separation and Purification Technology* **211**, 587–593.

Zhang, Z.-q., Lin, B., Xia, S.-q., Wang, X.-j. & Yang, A.-m. 2007 Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. *Journal of Environmental Sciences* **19** (6), 667–673.

Zhang, N., Xumeng Chen, Timothy Nicholson & Yongjun Peng 2019 The effect of saline water on the settling of coal slurry and coal froth. *Powder Technology* **344**, 161–168.

Zhao, C. Q., Yang, Q. H. & Zhang, H. 2017 Optimization of microbial flocculant-producing medium for *Bacillus subtilis*. *Indian Journal of Microbiology* **57** (1), 83–91.

Zimet, P., Álvaro W. Mombrú, Dominique Mombrú, Analía Castro, Juan Pablo Villanueva, Helena Pardo & Caterina Rufo 2019 Physico-chemical and antilisterial properties of nisin-incorporated chitosan/carboxymethyl chitosan films. *Carbohydrate Polymers* **219**, 334–343.

First received 4 September 2019; accepted in revised form 7 January 2020. Available online 14 January 2020