Action modes of surfactants on biodegradation of Wudong low-rank coal by *Pseudomonas aeruginosa*

Hao Wu (wuhao112116@163.com)
Xi'an University of Science and Technology https://orcid.org/0000-0003-0216-4781
Xiangrong Liu
Xi'an University of Science and Technology
Chen Shi
Xi'an University of Science and Technology
Jie Yang
Xi'an University of Science and Technology
Zaiwen Yang
Xi'an University of Science and Technology
Shunsheng Zhao
Xi'an University of Science and Technology

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Abstract

Adding surfactants is an effective method to improve the biodegradation rate of coal. In this study, the action modes of nonionic Triton X-100, anionic surfactant SDS and cationic surfactant DTAB on the biodegradation of Xinjiang Wudong low-rank coal by *Pseudomonas aeruginosa* were investigated. By measuring cell membrane permeability and cell surface hydrophobicity of *Pseudomonas aeruginosa*, it was found that the permeability of cell membrane was enhanced under the action of Triton X-100, and reached the highest at 4 CMC, while SDS and DTAB inhibited the permeability of cell membrane. Cell surface hydrophobicity increased under the influence of Triton X-100, SDS and DTAB and reached a maximum at CMC. According to the effects of surfactants on the degradation rates of coal, it was shown that the improvement of cell membrane permeability could promote the degradation of coal, but the increase of cell surface hydrophobicity could inhibit the degradation of coal. When Triton X-100, SDS and DTAB were all at their optimal concentrations for the growths of *Pseudomonas aeruginosa,* the coal biodegradation rates reached 78.63 %, 55.93 % and 20.93 %, respectively. The adsorption experiment of bacteria on the coal surface and the low-temperature nitrogen adsorption experiment of coal presented that the improvement of permeability of cell membrane was the main factor for Triton X-100 to improve the coal degradation rate, even if it can slightly increase cell surface hydrophobicity. For SDS and DTAB, the decrease of cell membrane permeability and the increase of cell surface hydrophobicity are all not conducive to the biodegradation of coal.

Statement Of Novelty

Surfactants can improve the biodegradation rate of coal sample. Due to the variety of coal samples and microorganisms, most studies focus on the matching test of surfactants in the biodegradation of coal, while there are few studies on the action modes of surfactants with microbial cells. Accordingly, we mainly explored the action modes of surfactants on biodegradation of low-rank coal through the interactions between surfactants and *Pseudomonas aeruginosa*. The results showed that surfactants could affect the cell membrane permeability, the cell surface hydrophobicity, and the adsorption of bacteria and its metabolites on coal surface, and then change the biodegradation rate of coal.

1 Introduction

Low-rank coal is a low metamorphic degree coal with a lignin structure [1], which is abundant in the world [2, 3]. Unfortunately, due to the high ash and volatile content of low-rank coal, the pollution caused by its direct combustion is serious [4–7]. Traditional low-rank coal treatment methods, such as gasification and pyrolysis, have the disadvantages of harsh process conditions and high energy consumption. Biodegradation of low-rank coal is carried out at normal temperature and pressure with simple equipment and convenient process, which is one of the effective ways for the green transformation of low-rank coal.

The biodegradation of low-rank coal originated in the 1980s, Cohen and Gabriele found that *Polyporus versicolor* and *Poria monticola* can degrade solid low-rank coals into black liquid products [8]. However,

the low biodegradation rate of coal is an obstacle to industrial application, so researchers have studied the biodegradation mechanism of coal [9–14], and tried various methods to improve the biodegradation rate of coal, among which adding surfactants is an effective method. At present, a variety of surfactants such as Triton X-100, Tween 80, LAS, SDS, and DTAB have obvious effects on the biodegradation of coal. Polman et al studied the effects of Triton X-100 and SDS on the degradation of bituminous coal and lignite by *Bacillus licheniformis*, and found that both surfactants can promote the organic components in the coal degraded by *Bacillus licheniformis* and improve the quality of coal [15]. Kang et al researched the effects of Triton X-100 and SDS on biodegradation of Shenmu lignite and found that Triton X-100 not only significantly improved the biodegradation rates of coal by *Ochrobactrum cytisi*, *Novospingobium naphthalenivorans*, *Alcaligenes faecalis* and *Pseudomonas fluorescens*, but also shortened their biodegradation time, while SDS only improved the degradation of coal by *Novospingobium naphthalenivorans* and *Pseudomonas fluorescens* [16].

The interaction between surfactants and microbial cells can change the properties of microorganisms. Surfactant molecules can insert, replace or dissolve lipids in microbial cell membranes, which leads to change in membrane area and membrane fluidity [17, 18]. Helenius reviewed the dissolution of surfactants on the membrane surface and considered that non-ionic surfactants such as Triton X-100 could dissolve lipopolysaccharides and phospholipids in bacterial cell walls [19]. Zhang et al studied the effects of five surfactants on *Klebsiella oxytoca* and found that Triton X-100 could destroy the integrity of cell membrane, improve the permeability of cell membrane, and then lead to the leakage of microbial metabolites [20]. Surfactants can also alter cell surface hydrophobicity (CSH) of bacteria, and then change the adsorption of cells on solid and mineral surfaces [21]. Chakraborty et al measured the adsorption capacity of *Burkholderia cepacia* ES1, *Burkholderia multifida* NG1 and *Burkholderia multifida* HN1 on the solid surface, and proved that the adhesion to the solid surface increased with the increase of CSH and the hydrophobicity of the solid surface [22]. Owsianiak et al investigated the effects of rhamnolipid and Triton X-100 on the hydrophobicity of 218 microbial consortia isolated from petroleum aminated soil and clarified that the effectiveness of surfactants on cell hydrophobicity depends on the specification of microorganisms rather than the type of surfactants [23].

Microbial cell metabolites are the main factor of coal biodegradation [24, 25], and *Pseudomonas aeruginosa* (*P. aeruginosa*) has a good degradation effect on coal sample, and its growth cycle is short, so it can produce more metabolites that can degrade coal in a short time [26], which can improve the economic benefits of production and promote the industrial application of coal biodegradation. As a result, *P. aeruginosa* is used as the experimental bacterium in this paper. Three kinds of surfactants, namely, nonionic surfactant Triton X-100, anionic surfactant SDS, and cationic surfactant DTAB, were selected to study the effects of *P. aeruginosa* on the degradation of Xinjiang Wudong low-rank coal. From the perspective of the interaction between surfactants and *P. aeruginosa* cells, the action modes of surfactants on biodegradation of coal were discussed with the goal to provide suggestions for the selection of surfactants to promote the industrial application of biodegradation of low-rank coal.

2 Materials And Methods

2.1 Chemicals

The three types of surfactants, non-ionic surfactant 2 (2 [4 (1,1,3,3 - tetramethyl butyl) phenoxy] ethoxy) ethanol (Triton X-100), anionic surfactant sodium dodecyl sulfate (SDS), and cationic surfactant dodecyl trimethyl ammonium bromide (DTAB) were purchased from Aladdin Reagent Co., Ltd, Kermel Reagent Co., Ltd., and Macklin Reagent Co., Ltd., respectively. Nitrocefin was purchased from Aladdin Reagent Co., Ltd. with a purity of 99%. N-hexane and ethyl acetate were purchased from Tianjin Kermel Chemical Reagent Co., Ltd. with a purity of 99%.

2.2 Coal samples

The coal sample was low-rank coal from Xinjiang Wudong Coal Mine (Xinjiang, China). The raw coal was crushed and sieved to a particle size range of 0.25 to 0.50 mm, and then pretreated with 8 mol/L nitric acid for 48 hours. The oxidized coal was washed with deionized water to pH 6.0 and dried in an oven at 80 ℃.

2.3 Microorganism and its culture conditions

2.3.1 Microorganism

Pseudomonas aeruginosa (*P. aeruginosa*) was purchased on the China Center of Industrial Culture Collection (CICC), and CICC number was 10204. The cells of *P. aeruginosa* are rod-shaped, arranged singly or in pairs, without spores, with polar flagella and strong motilities. The surface of the colony is moist and smooth, and the edges are neat, transparent, and white.

2.3.2 Culture conditions

Two kinds of culture media were used for the culture of bacteria, namely beef paste medium and mineral salt medium (MSM), which were composed as follows.

Beef extract medium: peptone 5.0 g, beef extract 3.0 g, NaCl 5.0 g, deionized water 1.0 L.

MSM: NH_4Cl 1.0 g, $KHPO_4$ 0.9 g, K_2HPO_4 1.7 g, NaH_2PO_4 0.5 g, $MnSO_4{\cdot}H_2O$ 3.6×10^{-4} g, $CaCl_2{\cdot}2H_2O$ 1.0×10^{-4} g, $CoCl_2{\cdot}6H_2O$ 1.0×10^{-4} g, $FeSO_4{\cdot}7H_2O$ 1.0×10^{-3} g, $MgSO_4{\cdot}7H_2O$ 3.0×10^{-3} g, $ZnSO_4{\cdot}7H_2O$ 3.0×10^{-4} g, deionized water 1.0 L.

Beef extract medium was used to culture bacteria and carry out coal biodegradation experiment, and MSM was used to wash and suspend bacteria. Two culture media must be sterilized at 121 ℃ and 0.11 MPa for 15 min before use.

P. aeruginosa was inoculated in a 150 mL flask with 50 mL beef extract medium and then transferred into a vibration incubator with a constant speed of 160 r/min at 30 ℃.

2.4 Determinations of biomass and hydrophobicity of P. aeruginosa

2.4.1 Biomass of P. aeruginosa

The biomass of *P. aeruginosa* was determined by the absorbance of its cell culture medium at the wavelength of 600 nm by Beijing Puxi TU-1950 ultraviolet-visible spectrophotometer (UV-Vis) [27].

2.4.2 Hydrophobicity of P. aeruginosa

The cell surface hydrophobicity (CSH) of *P. aeruginosa* was evaluated by the strain adhesion hydrocarbon method [20]. After the microorganisms were cultured to the logarithmic growth period, centrifugation was carried out under the condition of 4000 × g, and the bacterial precipitates were collected and washed 3 times with MSM, then resuspended in MSM, and the OD600 of cell suspension was adjusted to 0.6 (± 0.06) by UV-Vis. The concentrations of surfactants used in this experiment were as followed: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0 times of each critical micelle concentration (CMC). 5 mL cell suspension was mixed with a surfactant to the target concentration, left to stand for 10 minutes, then 1 mL of n-hexane was added, stirred well, allowed to stand for 30 minutes. The aqueous phase was separated, and its absorbance value at 600 nm wavelength was measured by UV-Vis [28]. The CSH was calculated according to formula (1).

$$\mathrm{CSH}(\%)=\left(\frac{\mathrm{OD}_1-\mathrm{OD}_2}{\mathrm{OD}_1}\right)\times 100\% \quad (1)$$

where, OD_1 is the initial biomass of *P. aeruginosa*, OD_2 the biomass of *P. aeruginosa* mixed with n-hexane.

2.5 Membrane permeability of P. aeruginosa

The permeability of cell membrane of *P. aeruginosa* was determined by hydrolysis of nitrocefin [29]. In the cell of *P. aeruginosa*, when β-lactamase was mainly periplasm, the hydrolysis rate of nitrocefin was limited by the rate of diffusion across the cell membrane and thus provided a measure of membrane permeability [30]. When nitrocefin was hydrolyzed by β-lactamase, the color will changed, and there was the maximum absorbance at 486 nm wavelength of UV-Vis. Therefore, A486 was used to evaluate the permeability of cell membrane of *P. aeruginosa*, and its value was proportional to the permeability of the cell membrane.

3 mL cell suspension of *P. aeruginosa* with OD600 = 1.0 (± 0.1) was evenly mixed with nitrocefin to the target concentration of 30 μg/mL, then cultured in a biochemical incubator with a constant temperature of 30 ℃ for 2 hours. Finally, it was centrifuged at 4000 × g for 5 minutes, and its absorbance was measured at 486 nm wavelength by UV-Vis.

2.6 Adsorption of P. aeruginosa on coal surface

The adsorption rate of bacteria on the coal sample surface adopts the method described by Guo [31]. After culturing at 30°C for 36 h, the *P. aeruginosa* that entered the stable stage were prepared into bacterial suspensions. 5 mL of bacterial suspension was mixed with 0.5 g of coal samples, shaken at 30°C for 120 min, which was sufficient to reach adsorption equilibrium, and then centrifuged at 390 × g for 5 min. The absorbance of the supernatant was measured at a wavelength of 600 nm by UV-Vis, and the adsorption percentage was calculated according to the formula (2).

$$\eta(\%)=\frac{OD_a\text{-}OD_b}{OD_a}\times 100\% \quad (2)$$

where, η is adsorption rate, OD_a the absorbance of bacteria before adsorption, OD_a the absorbance of bacteria after adsorption.

2.7 Biodegradation of coal samples

2.7.1 Biodegradation experiment of coal samples

P. aeruginosa was inoculated in a 150 mL conical flask with 50 mL beef extract medium, cultured in a vibrating incubator with a constant speed of 160 r/min at 30 ℃. Then, different concentrations of surfactant (0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0 times of each CMC) were added to the bacterial culture medium, and bacteria continue to be cultured. In addition, the permeability of cell membrane and CSH of bacteria were measured at different concentrations of surfactant. At the same time, 0.3 g sterilized coal was added to the culture medium containing *P. aeruginosa*, and the biodegradation experiment of coal was carried out. After biodegradation, the solid and liquid products were obtained by centrifugation and analyzed by low-temperature nitrogen adsorption and gas chromatography-mass spectrometry (GC-MS). The experimental flow chart of biodegradation of coal samples is shown in Fig. 1.

2.7.2 Evaluation of coal biodegradation effect

The evaluation method of the coal degradation effect used in this experiment was described by Shi [32]. After microbial degradation of coal, humic acids are released into the culture medium, and there is the maximum absorbance at 450 nm wavelength of UV-Vis, which is proportional to the degradation effect of coal. After biodegradation, the degraded products of coal samples were centrifuged at 5000 × g for 20 minutes, and supernatant (liquid product) was collected. The A450 value of liquid product diluted 30 times was used to evaluate the biodegradation effect of coal.

2.8 Analysis of biodegradation products

2.8.1 Analysis of solid products

The pore size distribution and cumulative area of solid products were measured by ASAP 2020 physical adsorption instrument (nitrogen) of American Micromeritics Company to explore the pore structure characteristics of coal samples.

2.8.2 Analysis of liquid products

The liquid product was extracted with ethyl acetate, and then the extract was analyzed by Agilent 8890-5977B gas chromatography-mass spectrometry (GC-MS).

3 Results And Discussion

3.1 Effects of surfactants on permeability of cell membrane of P. aeruginosa

The CMC of surfactant was determined by the surface tension method, and the CMC of Triton X-100, SDS, and DTAB were 1.93×10^{-1} g/L, 2.48 g/L, and 4.39 g/L, respectively.

The effects of surfactants on the absorbance of *P. aeruginosa* at 486 nm are shown in Fig. 2. The absorbance of *P. aeruginosa* is proportional to the permeability of cell membrane at 486 nm. It can be seen from Fig. 2 that Triton X-100 can improve the permeability of cell membrane, and with the increase of concentration, the permeability of cell membrane first increases and then decreases, and reaches the maximum at 4 CMC. SDS and DTAB inhibit the permeability of the cell membrane, and when their concentrations are 0.2 CMC, the permeability of cell membrane decrease to the lowest, and the permeability of cell membrane does not change significantly with the increase of their concentrations. Among them, the inhibitory effect of DTAB on the permeability of cell membrane is greater than that of SDS.

As can be seen from Fig. 2, a low concentration of Triton X-100 can slightly change the permeability of cell membrane. However, when the concentration of Triton X-100 exceeds CMC, the permeability of cell membrane changes greatly, indicating that the interaction between bacteria and surfactant depends on the ratios of surfactant concentration to membrane area [33]. When the concentration of Triton X-100 reaches CMC, Triton X-100 and lipid form mixed micelles, dissolve cell membrane, increase the permeability of cell membrane, and bacteria begin to leak cell metabolites [20]. Although part of the cell membrane is dissolved, this will not affect the normal life activities of *P. aeruginosa*, because Triton X-100 neither hinders DNA synthesis in bacterial cells nor changes the semi-conservative nature of the system [34].

In Fig. 2, adding SDS to *P. aeruginosa* can reduce the permeability of cell membrane, which may be that SDS interferes with proteins in the structure of the cell membrane and causes cell damage. In addition, the formation of cell aggregates may be responsible for the decreased permeability of the cell membrane, since the cells of *P. aeruginosa* will undergo division and proliferation in the SDS environment, and the cell division in this process will rearrange the surface structure of the membrane, which will make the sensitive part of the cell easier to be contacted by SDS, and their contact will lead to cell dissolution and the formation of cell aggregates [35]. Cell aggregates include damaged and uncultured cells and their formation may be an important reason for the decrease of membrane permeability.

It can be seen from Fig. 2 that DTAB inhibits the permeability of cell membrane most obviously. This may be the interaction between DTAB and cell surface proteins are ionic interaction. When the concentration of DTAB is low, it can affect the working environment of protein, and the hydrophobic and ionic interaction of higher concentrations of surfactant can destroy the secondary and tertiary structure of the partial protein [36], which may lead to the decrease of permeability of cell membrane.

3.2 Effects of surfactants on CSH of P. aeruginosa

The effects of Triton X-100, DTAB, and SDS on the CSH of *P. aeruginosa* are shown in Fig. 3. The CSH first increase and then decrease with the increase of the concentrations of three surfactants. When their concentrations are CMC, all three surfactants can make CSH reach the maximum. After treatment with Triton X-100, DTAB and SDS, CSH are 22.06%, 80.88%, and 79.90% respectively, which are 11.77%, 70.59% and 69.61% higher than that without surfactant.

It can be seen from Fig. 3 that DTAB and SDS can significantly improve the CSH of *P. aeruginosa*, because the surface of bacteria contains carboxyl, hydroxyl, and phosphate groups, and the interaction between cell and the surfactant is driven by polar interaction [37], which makes the hydrophobic tail of SDS and DTAB repel the hydrophobic part of the bacteria in the water environment [38]. *P. aeruginosa* belongs to Gram-negative bacteria, the inner membrane is composed of phospholipids, and its membrane contains lipopolysaccharide [39]. Moreover, Triton X-100 has strong emulsification, can destroy the cell membrane structure, and release lipopolysaccharide, and lipopolysaccharide is hydrophobic, which leads to the increase of CSH. This may be the reason why Triton X-100 improves CSH in a smaller range compared to SDS and DTAB.

It can be seen from Fig. 3 that when the surfactants concentrations are between 0 and 1 CMC, three surfactants can gradually increase CSH with the increase of concentrations. The reason may be that when the hydrophilic part of the cell surface of the bacteria is replaced by surfactant, the hydrophobic part is exposed outside the cell and shows hydrophobicity. When the surfactant concentration exceeds CMC, the CSH of bacteria decreases because the hydrophobic end of the surfactant molecule adsorbed on the surface of *P. aeruginosa* is replaced by the hydrophilic end of the surfactant molecule in the culture medium [40].

3.3 Effects of surfactants on the growth of P. aeruginosa

The effects of surfactants on the absorbance of *P. aeruginosa* at 600 nm are shown in Fig. 4. When *P. aeruginosa* grew for 24 hours, Triton X-100, SDS, and DTAB were added to make the final concentrations of three surfactants in the culture medium reach 4 CMC, CMC, and CMC respectively, and bacteria without surfactants were used as the control.

It can be seen from Fig. 4 that the biomass of *P. aeruginosa* with Triton X-100 and SDS and without surfactant increase first, then stabilize, and finally decrease, while the biomass of bacteria with DTAB continue to decrease. Among them, Triton X-100 can promote the growth of *P. aeruginosa*, increase the

biomass of bacteria, and reach a stable period within 36–48 h. In addition, in the later stages of growth, the decay speed of bacteria also slows down. The reason may be that the permeability of the cell membrane increases, the metabolic rate increases, and the division of bacteria is promoted. The addition of SDS significantly increases the bacterial biomass and reaches a stable period within 108–120 h. The reason may be that SDS can make bacterial cells form cell aggregates, which leads to a significant increase in the absorbance of bacteria at 600 nm [35]. However, DTAB seriously reduces the biomass of the bacteria, which may be that DTAB is adsorbed on the cell surface and can cause cell lysis [41].

3.4 Influences of surfactants on biodegradation of coal samples

3.4.1 Effects of surfactants concentrations on biodegradation of coal samples

The effects of surfactants on the absorbance of liquid products at 450 nm are shown in Fig. 5. The absorbance of liquid products at 450 nm is proportional to the degradation rate of coal, which can be used to measure the degradation effect of coal samples. It can be seen from Fig. 5 that the non-ionic surfactant Triton X-100 can promote the degradation effects of coal samples, and with the increase of its concentration, the degradation effects of coal samples first increase and then decrease, and reach the best at 4 CMC. According to Fig. 2, it can be seen that the permeability of cell membrane also reaches the maximum at this concentration, and the trend of the degradation effects of coal samples in the presence of Triton X-100 is the same as the trend of the permeability of cell membrane in Fig. 2. Therefore, the increase of cell membrane permeability is the main factor for Triton X-100 to improve the degradation effect of coal sample, and the change of cell membrane permeability is proportional to the degradation effect of coal sample, that is, the increase of cell membrane permeability improves the degradation effect of coal sample. The reason may be that the increase of permeability of cell membrane promotes bacteria to secrete more metabolites that can degrade coal.

Both SDS and DTAB can inhibit the degradation effects of coal samples, and with the increase of concentrations of surfactants, the degradation effects of coal samples first decrease and then increase. When the concentrations of SDS and DTAB are CMC, the degradation effects of coal samples are the worst. According to Fig. 2, it can be seen that after adding SDS and DTAB, the permeability of cell membrane hardly changes with the increase of surfactant concentration. Combined with Fig. 5, it can be found that the change of CSH is inversely proportional to the degradation effect of the coal sample, that is, the degradation effect of the coal sample decreases with the increase of CSH. Moreover, the coal surface is hydrophobic, and the increase of CSH may increase the adsorption capacity of the coal surface, and the pore sizes of the micropores in the coal samples are smaller than that of the microorganisms. Therefore, the increase of bacterial adsorption on the coal surface may lead to the blockage of pores in the coal, which makes it difficult for microbial metabolites to degrade the interior of the coal sample [42, 43].

According to Fig. 2 and Fig. 3, DTAB and SDS reduce the permeability of cell membrane and significantly improve CSH of *P. aeruginosa*, which may increase the adsorption of the bacteria on the coal surface, decrease the secretion of metabolites, and reduce the contact area between metabolites and the coal surface, so the degradation effect of coal become worse. According to the enzyme mechanism of microbial degradation of coal, DTAB can denature proteins and thus reduce the coal degradation effect [10, 44, 45].

3.4.2 Effects of degradation time on the absorbance of liquid products at 450 nm

The effects of degradation time on the absorbance of liquid products at 450 nm are shown in Fig. 6, and the corresponding degradation rates of coal samples are shown in Table 1. The control group is the absorbance of liquid product degraded by *P. aeruginosa* without surfactant at 450 nm. The concentrations of Triton X-100, SDS and DTAB are 4 CMC, CMC and CMC, respectively.

It can be seen from Fig. 6 that the degradation effect of *P. aeruginosa* on coal samples increases and then tends to be stable after adding surfactants, and the degradation speed is the fastest in 0-2 days. Triton X-100 not only improves the degradation effect of coal sample but also reduces the time for the degradation effect of coal sample to reach degradation stability, and the degradation effect reaches stability on the 8th day, which is two days shorter than that of the control group. The degradation rate of the coal sample by *P. aeruginosa* added Triton X-100 is 78.63 % at 14 d, 7.43 % higher than that of the control group. SDS inhibits the degradation effect of coal, and the degradation effect is stable on the 10th day, and the degradation rate is 55.93 % at 14 d, 15.27 % lower than that of the control group. DTAB has the strongest inhibitory effect on the degradation of coal samples, and the degradation rate is only 20.93 % at 14 d, 50.27 % lower than that of the control group.

3.5 Adsorption rates of P. aeruginosa on coal samples surface

The adsorption rates of *P. aeruginosa* on coal samples surface under the action of different surfactants are shown in Fig. 7. The control group is the adsorption rate of *P. aeruginosa* on the coal surface without adding surfactant, and the concentrations of Triton X-100, SDS, and DTAB are 4 CMC, CMC, and CMC, respectively.

It can be seen from Fig. 7 that the three surfactants promote the adsorption of *P. aeruginosa* on the coal surface. According to Fig. 3, it can be found that the CSH of bacteria is related to the adsorption rate of bacteria on the surface of coal sample. SDS and DTAB have similar effects on CSH of *P. aeruginosa*, but under the action of DTAB, the adsorption rate of bacteria on the surface of coal sample reaches 44.85%, which is 13.21% higher than that of SDS. This may be because there is a negative charge on the coal surface [16], and DTAB is a cationic surfactant with a positive charge in the culture medium, which may increase the adsorption capacity of bacteria on the coal surface.

3.6 Analysis results of biodegradation products

3.6.1 Ultimate and proximate analysis

The ultimate and proximate analyses of coal samples are given in Table 2. The biodegradation products of coal samples by *P. aeruginosa* added Triton X-100, SDS, DTAB and without adding surfactant are solid product (Triton X-100), solid product (SDS), and solid product (DTAB), and solid product (Control). It can be seen from Table 2 that after nitric acid treatment, the content of C, H and S decreases, the content of O and N increases, the volatile matter increases, and the ash content decreases, which may be due to the reaction of nitric acid with some functional groups in the molecular structure of coal, leading to aromatic carboxylation, side chain alkyl oxidation and nitration, and nitric acid dissolves inorganic substances in the raw coal [46]. After biodegradation, the contents of C, H, N, and S decrease, the volatile contents decrease and the ash contents increase of three solid products. The reason may be that bacterial metabolites can degrade the oxygen-containing functional groups and aromatic rings in oxidized coal [47]. By comparing the three solid products, it can be seen that the solid product (Triton X-100) with the highest degradation rate has lower volatile content and higher ash content. In addition, coal samples with higher degradation rates have lower volatile content and higher ash content, which may be that the metabolites of *P. aeruginosa* can degrade organic components in coal samples.

3.6.2 Low-temperature nitrogen adsorption analysis of coal samples

The cumulative pore area distribution of the coal sample is shown in Fig. 8. It can be seen from Fig. 8 that the cumulative pore area distribution curves of the five coal samples are similar, and four solid products increase the cumulative pore area. Among them, the cumulative pore area of coal samples from large to small is solid product (Triton X-100) > solid product (Control) > solid product (SDS) > solid product (DTAB) > oxidized coal, that is, the degradation rates of the coal samples are proportional to the cumulative pore areas in the coal samples.

As can be seen from Fig. 8, the pore sizes of coal samples are mainly concentrated between 50–500 nm, while the sizes of *P. aeruginosa* are 1.5-5.0 μm × 0.5-1.0 μm, larger than the maximum pore diameters of coal samples. Therefore, the bacterial cells adsorbed on the coal surface may block the pores in coal samples, prevent the bacterial metabolites from entering the pores of the coal to degrade the coal, decrease the contact area between the metabolites and the coal sample, and then reduce the degradation effect of the coal. The change of CSH can affect the adsorption of bacteria on the coal surface and the contact between bacterial metabolites and coal samples, and then change the biodegradation effect of the coal sample.

3.6.3 GC-MS analysis

The total ion chromatograms of liquid products are shown in Fig. 9, and the compositions of the liquid products are shown in Table 3–6. During the degradation of coal samples by *P. aeruginosa*, the liquid products obtained by adding Triton X-100, SDS, DTAB, and without surfactant are liquid product (Triton X-100), liquid product (SDS), liquid product (DTAB), and liquid product (Control), respectively. As can be

seen from Fig. 9, the chromatograms of the four liquid products are similar. It can be seen from Table 3–6 that the compositions of the four liquid products are also similar, including alkanes, esters, alcohols, and other substances, which shows that surfactants only affect the degradation effects and speed of coal samples, and do not affect the compositions of biodegradation products.

3.7 Action modes of surfactants on biodegradation of coal samples

The action mode diagram of Triton X-100 in the biodegradation process of coal sample is shown in Fig. 10. The hydrophobic end of Triton X-100 is adsorbed on the cell surface to dissolve the cell membrane, which improves the permeability of cell membrane and leads to the leakage of metabolites in the cell. That is, cellular metabolites can either be secreted by carrier proteins and channel proteins, or leaked from lysed cell membranes, which increases the metabolite content in the medium. In addition, Triton X-100 can slightly increase CSH, and will not make a large number of cells adsorb on the coal sample, which increases the contact area between cell metabolites and the surface of the coal sample, thereby improving the degradation effect of the coal sample.

The action mode diagram of SDS in the biodegradation process of coal samples is shown in Fig. 11. The binding of SDS to proteins disrupts the function of some carrier proteins and channel proteins and hinders the secretion of metabolites. Moreover, SDS significantly increases the CSH of bacteria and causes a large number of bacterial cells to adsorb on the surface of the coal sample, which reduces the contact area of microbial metabolites with the surface of the coal sample, and leads to a decrease in the degradation effect of the coal sample.

The action mode of DTAB in the biodegradation process of coal samples is shown in Fig. 12. DTAB adsorbs on bacterial surfaces and metabolite molecules, which lyses a large number of cells and denatures some cellular metabolites. Moreover, the increase of CSH makes a large number of live bacteria and dead bacteria adsorb on the surface of coal samples, which reduces the contact area between metabolites and coal samples. In addition, DTAB also reduces the permeability of live bacterial cell membranes, decreases the secretion of metabolites, and some denatured metabolites can not degrade coal sample. These factors together lead to poor degradation of coal sample.

4 Conclusion

(1) Triton X-100 increases the cell membrane permeability of *P. aeruginosa*, and the permeability of cell membrane reaches the highest at 4 CMC, while SDS and DTAB decrease the permeability of cell membrane. Triton X-100, SDS, and DTAB can improve the CSH of *P. aeruginosa*. When the concentrations of the three surfactants are CMC, the maximum CSH of bacterial cells are 22.06%, 80.88%, and 79.90% respectively, which are 11.77%, 70.59%, and 69.61% higher than that of the control group.

(2) Triton X-100 can improve the biodegradation rate of coal sample by *P. aeruginosa*. When the concentration is 4 CMC, the degradation rate of coal sample is the highest of 78.63%, which is 7.43%

higher than that of the control group. Both SDS and DTAB can inhibit the degradation of coal, and when their concentrations are both CMC, the lowest degradation rates of coal samples are 55.93% and 20.93%, which are 15.27% and 50.27% lower than the control group, respectively.

(3) Triton X-100 SDS and DTAB can affect the degradation rate of coal sample, but do not change the liquid products compositions of coal samples degraded by *P. aeruginosa*. The compositions of the liquid products are similar, mainly containing alkanes, esters, alcohols, etc. Surfactants also affect the cumulative pore areas of coal samples, and coal samples with a high degradation rate have a larger cumulative pore area. Among them, the cumulative area from large to small is solid product (Triton X-100) > solid product (Control) > solid product (SDS) > solid product (DTAB) > oxidized coal.

(4) The permeability of cell membrane is proportional to the degradation rate of coal, and CSH is inversely proportional to the degradation rate of coal. Among them, the main factor for TritonX-100 to improve the degradation rate of coal is to improve the permeability of cell membrane and increase the release of cell metabolites, even if it can slightly increase CSH; The main reasons for the reduction of coal degradation rate by SDS and DTAB are the decrease of cell membrane permeability and the significant increase of CSH, which lead to the adsorption of a large number of bacteria on the surface of coal samples and reduce the contact area of coal samples with bacterial metabolites.

Declarations

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There is no conflict of interest in the submission of this paper, and the paper can be published only after all authors have approved it. I would like to declare on behalf of my co-authors that the work described is original research, which has not been published before and has not been considered for publication elsewhere, in whole or in part.

Author Contributions

All authors contributed to the concept and design of this study. Material preparation, data collection and analysis, and the first draft of the manuscript were carried out by Hao Wu. Conceptualization, formal analysis, writing comments and editing were carried out by Xiangrong Liu, supervised and verified by Chen Shi, Jie Yang, Zaiwen Yang, and Shunsheng Zhao, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Tables

Table 1
Degradation rates of coal samples

Groups	Degradation rates (%)						
	2nd day	4th day	6th day	8th day	10th day	12th day	14th day
Control	63.07	64.73	66.80	68.80	71.10	71.17	71.20
Triton X-100	70.43	73.10	76.23	78.50	78.57	78.60	78.63
SDS	47.77	50.07	52.33	54.60	55.77	55.83	55.93
DTAB	20.70	20.77	20.83	20.83	20.90	20.90	20.93

Table 2
Ultimate and proximate analysis of coal samples

Coal samples	Ultimate analysis (%)					Proximate analysis (%)			
	C	H	O	N	S	M_{ad}	A_{ad}	V_{daf}	FC_{daf}
Row coal	69.41	3.60	25.10	0.97	0.92	11.65	6.31	55.24	45.76
Oxidized coal	60.00	3.06	32.73	4.12	0.08	6.23	3.01	81.21	18.79
Solid product (Control)	59.01	2.37	35.21	3.41	0.02	6.33	7.54	67.54	32.46
Solid product (Triton X-100)	58.75	2.30	35.57	3.36	0.02	6.35	7.71	66.83	33.17
Solid product (SDS)	59.38	2.59	34.18	3.83	0.02	6.33	7.35	72.21	27.79
Solid product (DTAB)	60.07	2.91	32.98	4.01	0.03	6.41	6.28	79.03	20.97

Tables 3-6 are available in the Supplementary Files section.

Figures

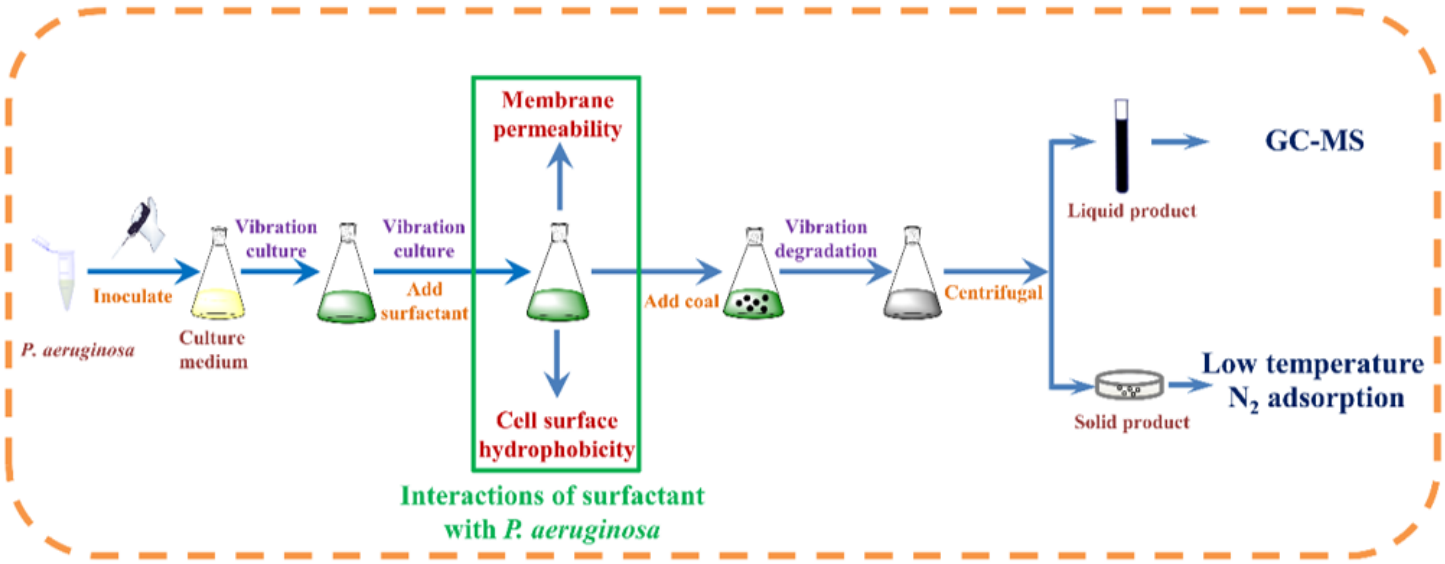

Figure 1

Experimental flow chart of biodegradation of coal samples

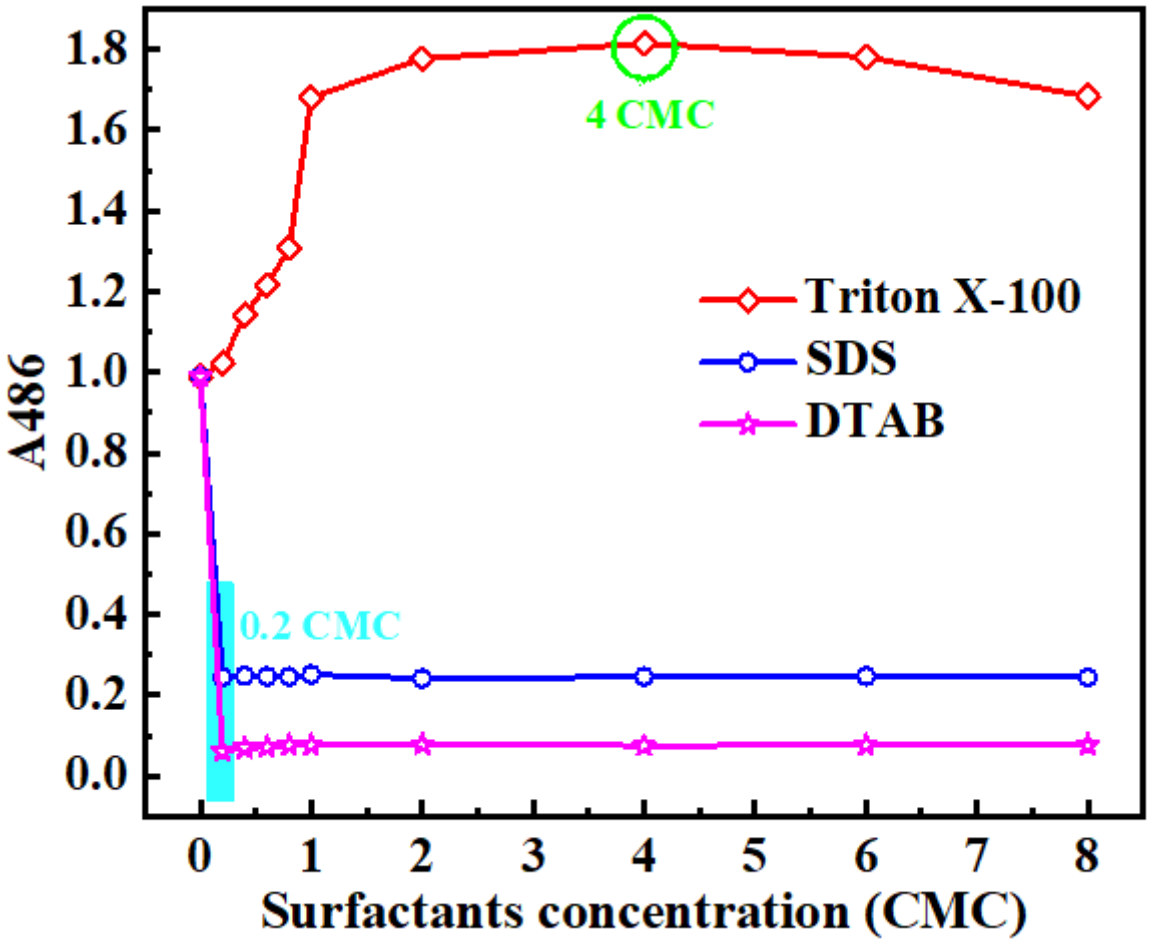

Figure 2

Effects of surfactants on absorbance of *P. aeruginosa* at 486 nm

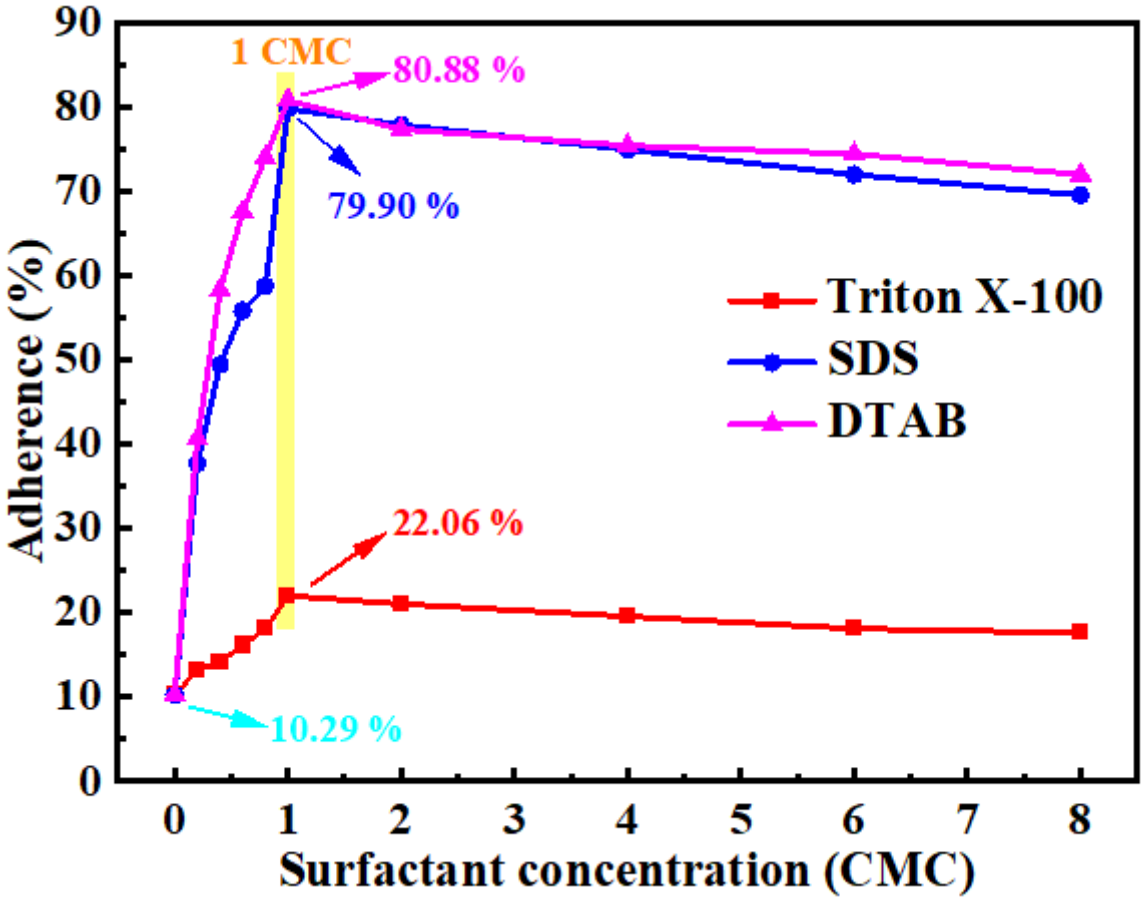

Figure 3

Effects of surfactants on CSH of *P. aeruginosa*

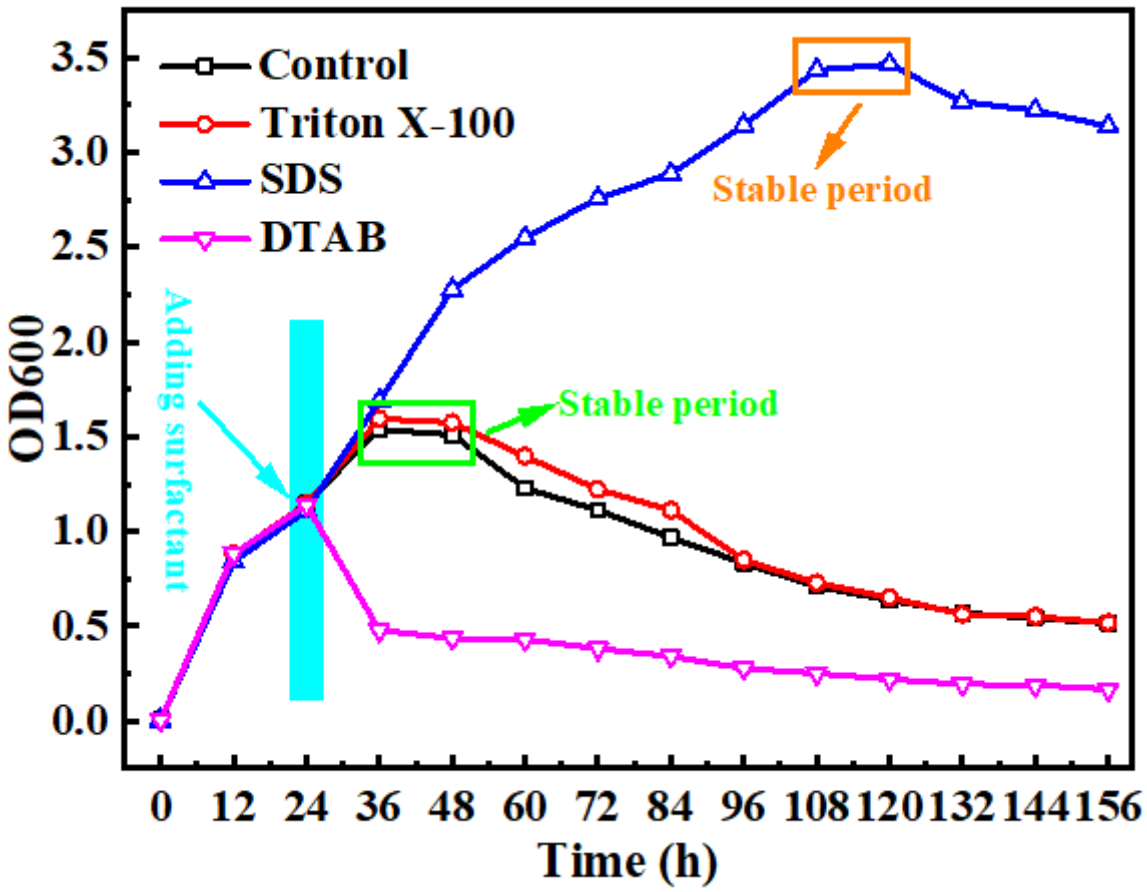

Figure 4

Effects of surfactants on absorbance of *P. aeruginosa* at 600 nm

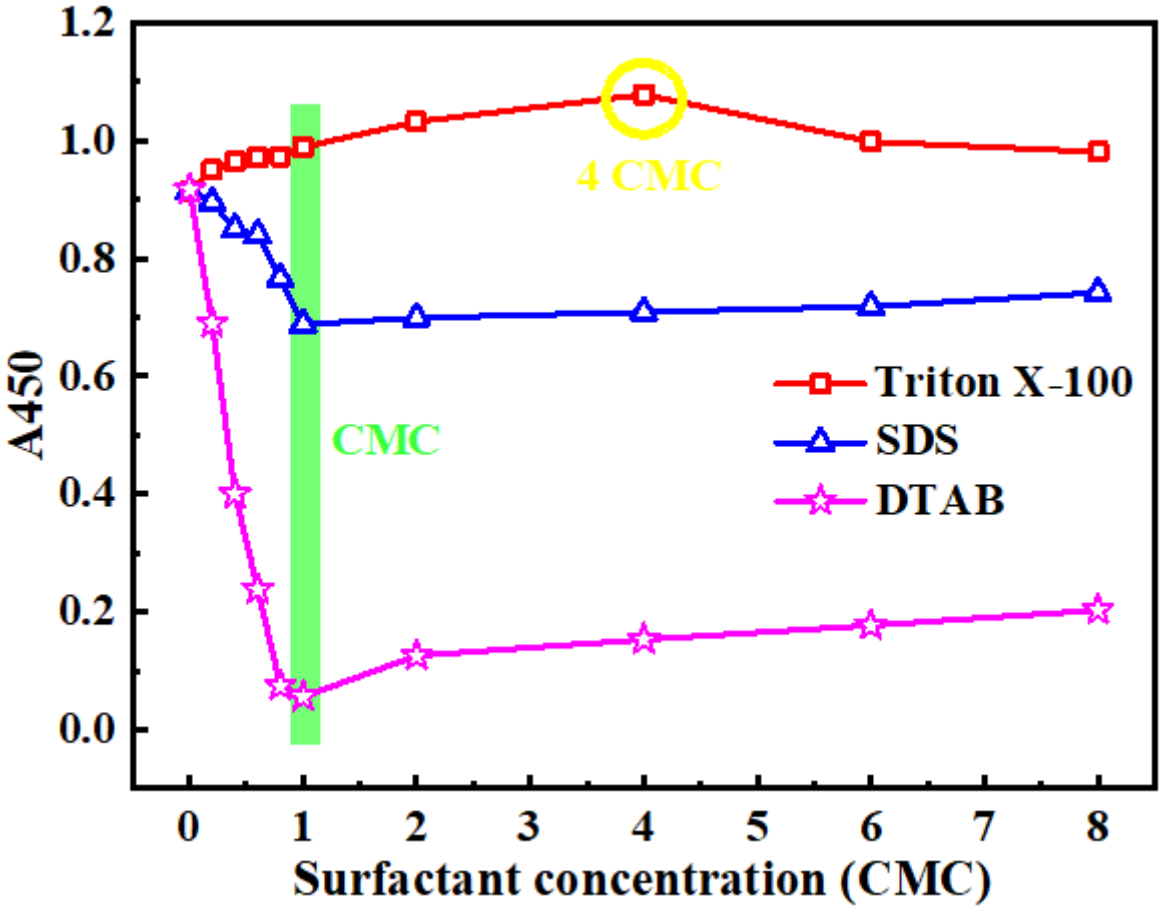

Figure 5

Effects of surfactants on absorbance of liquid products at 450nm

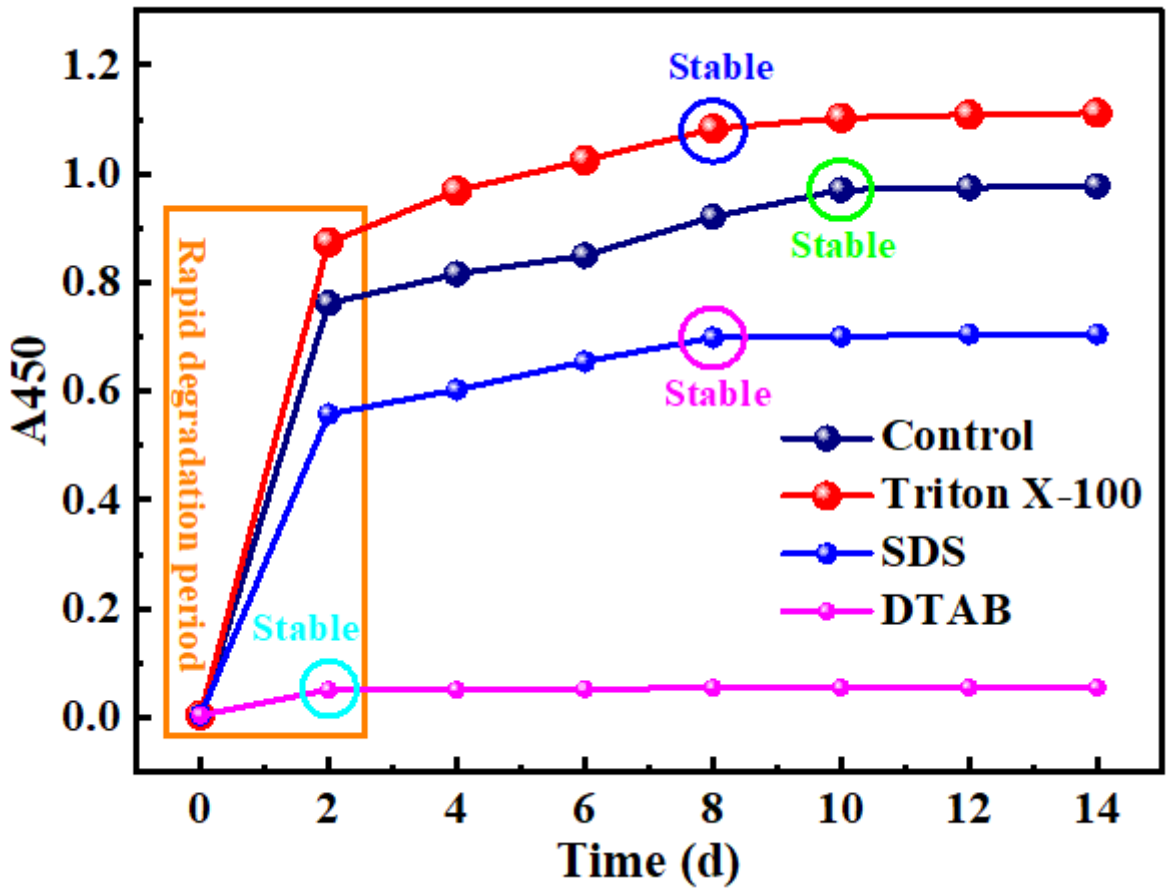

Figure 6

Effects of degradation time on absorbance of liquid products at 450 nm

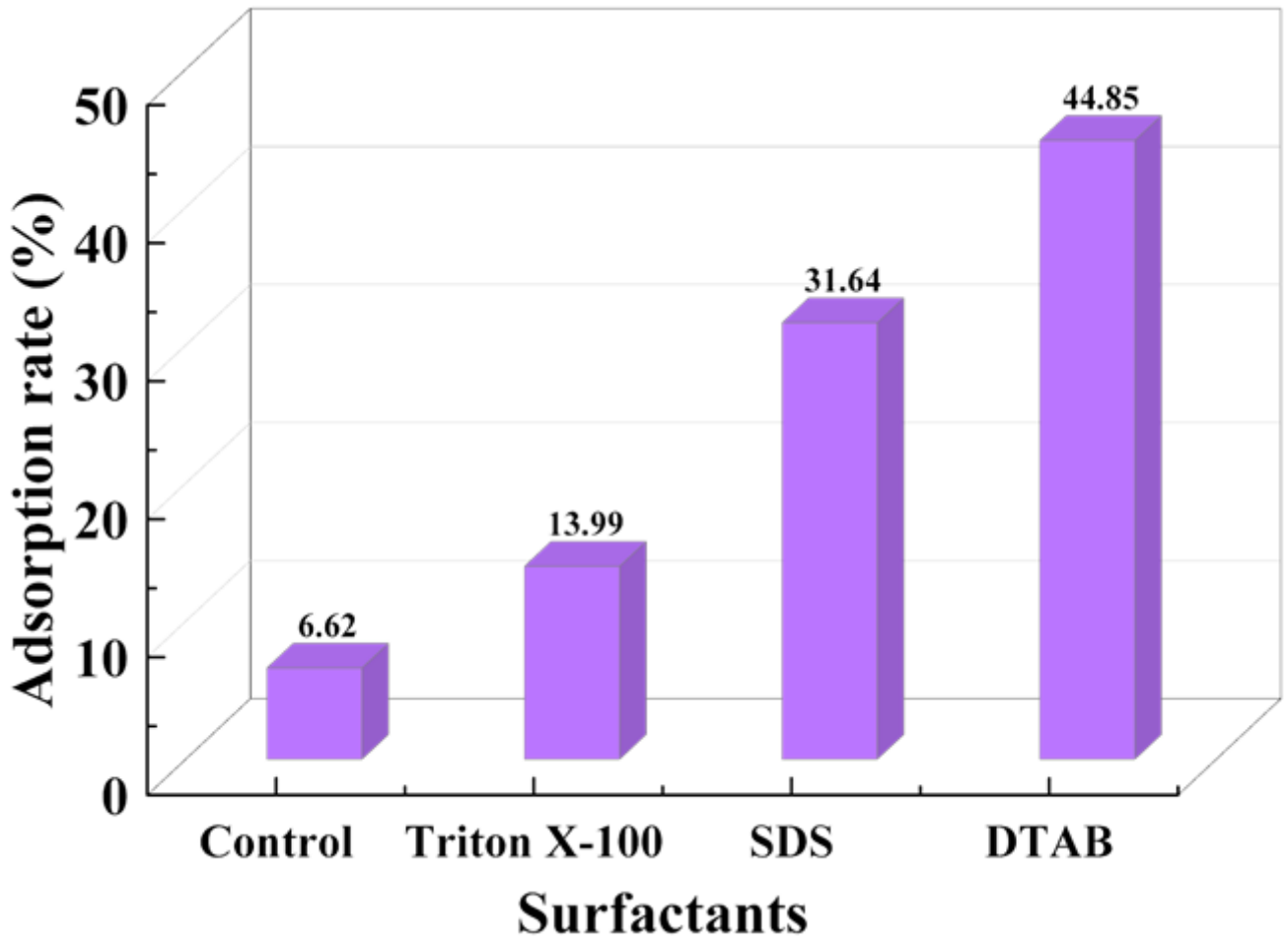

Figure 7

Adsorption rates of *P. aeruginosa* on coal samples surface

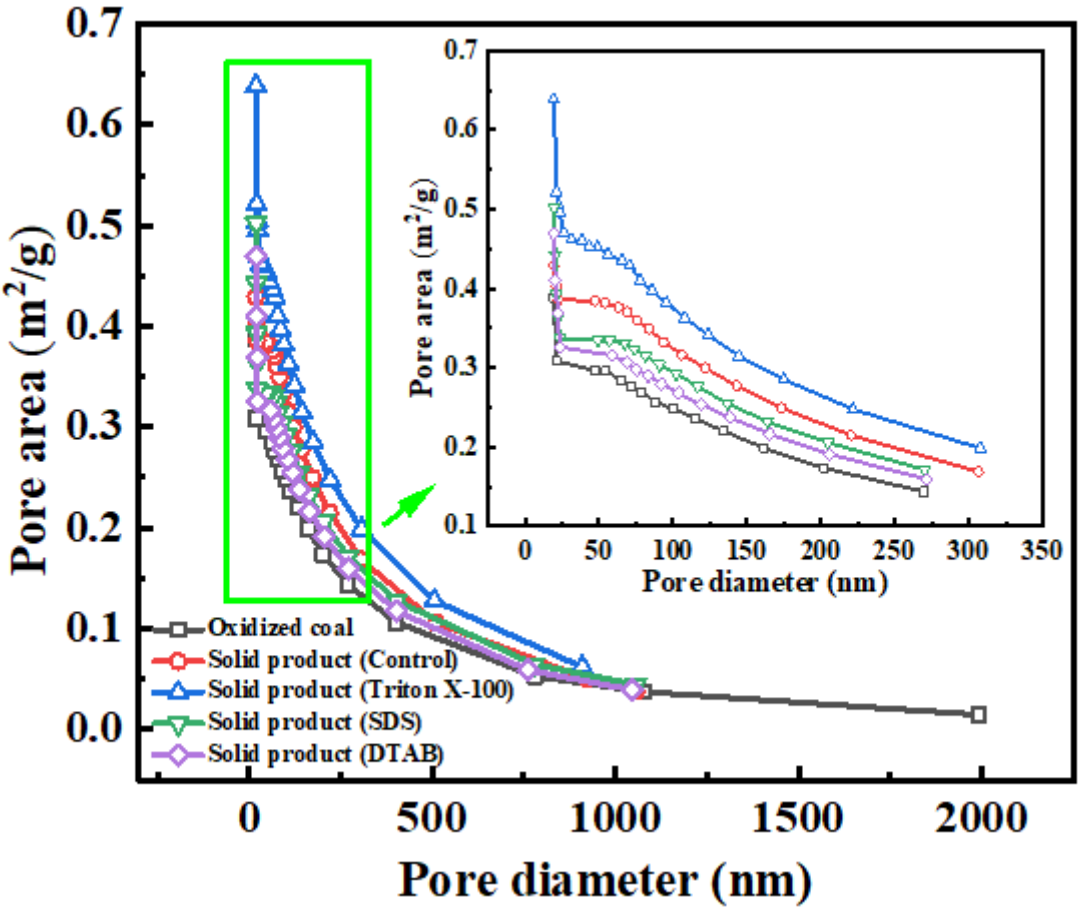

Figure 8

Cumulative pore area distribution of coal sample

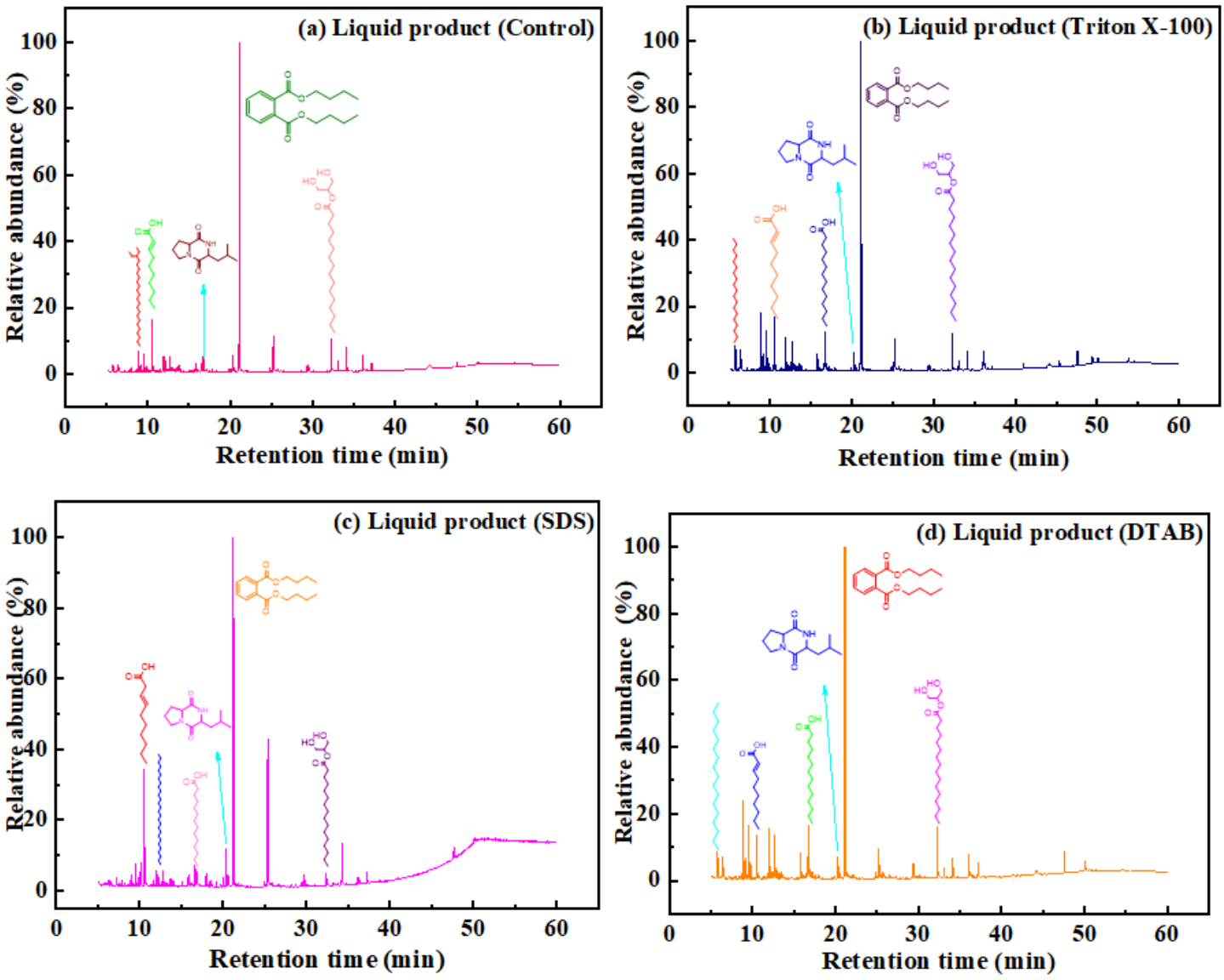

Figure 9

Total ion chromatograms of liquid products

(a) liquid product (Control) (b) liquid product (Triton X-100)

(c) liquid producst (SDS) (d) liquid product (DTAB)

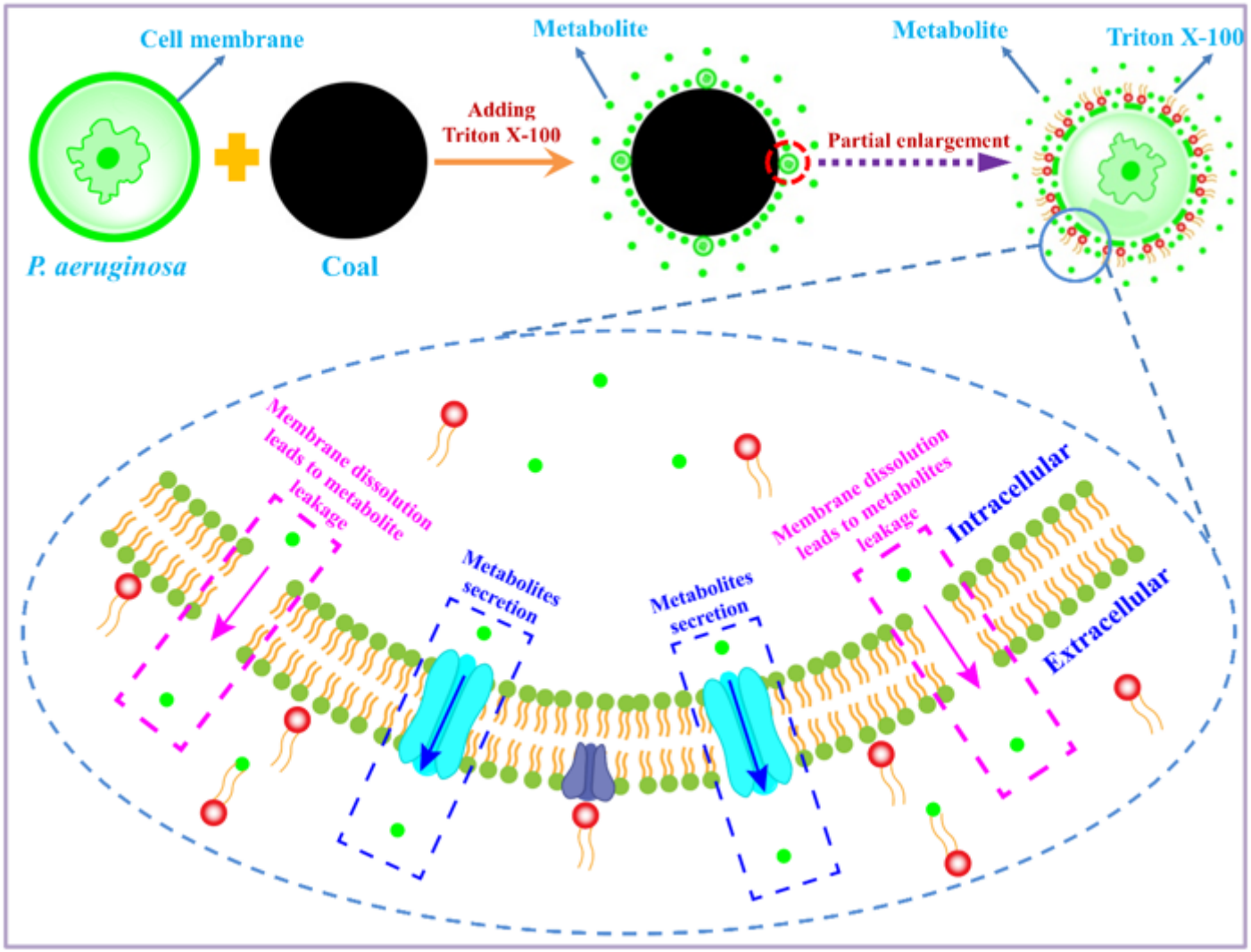

Figure 10

Action mode diagram of Triton X-100 in biodegradation process of coal samples

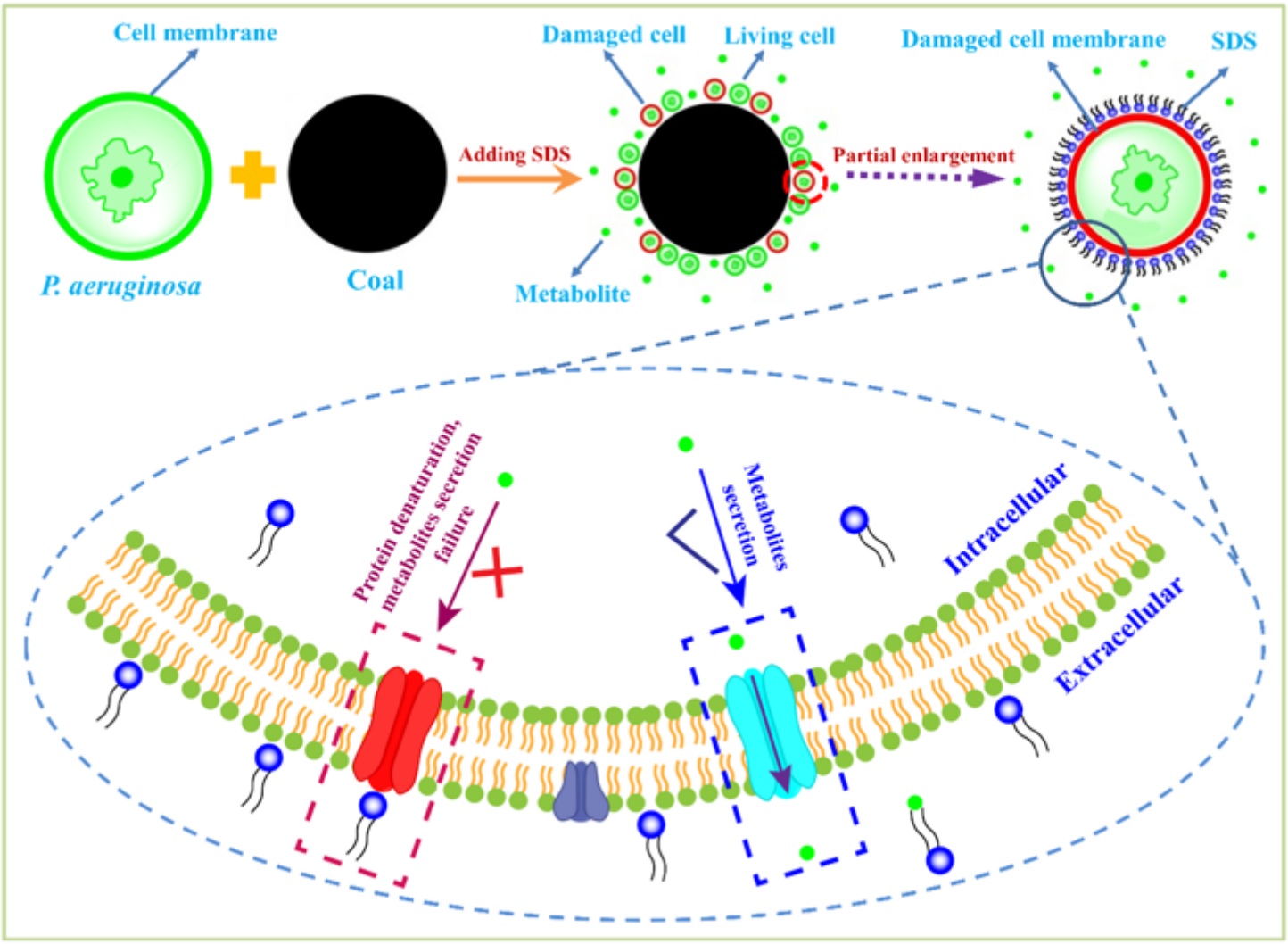

Figure 11

Action mode diagram of SDS in biodegradation process of coal samples

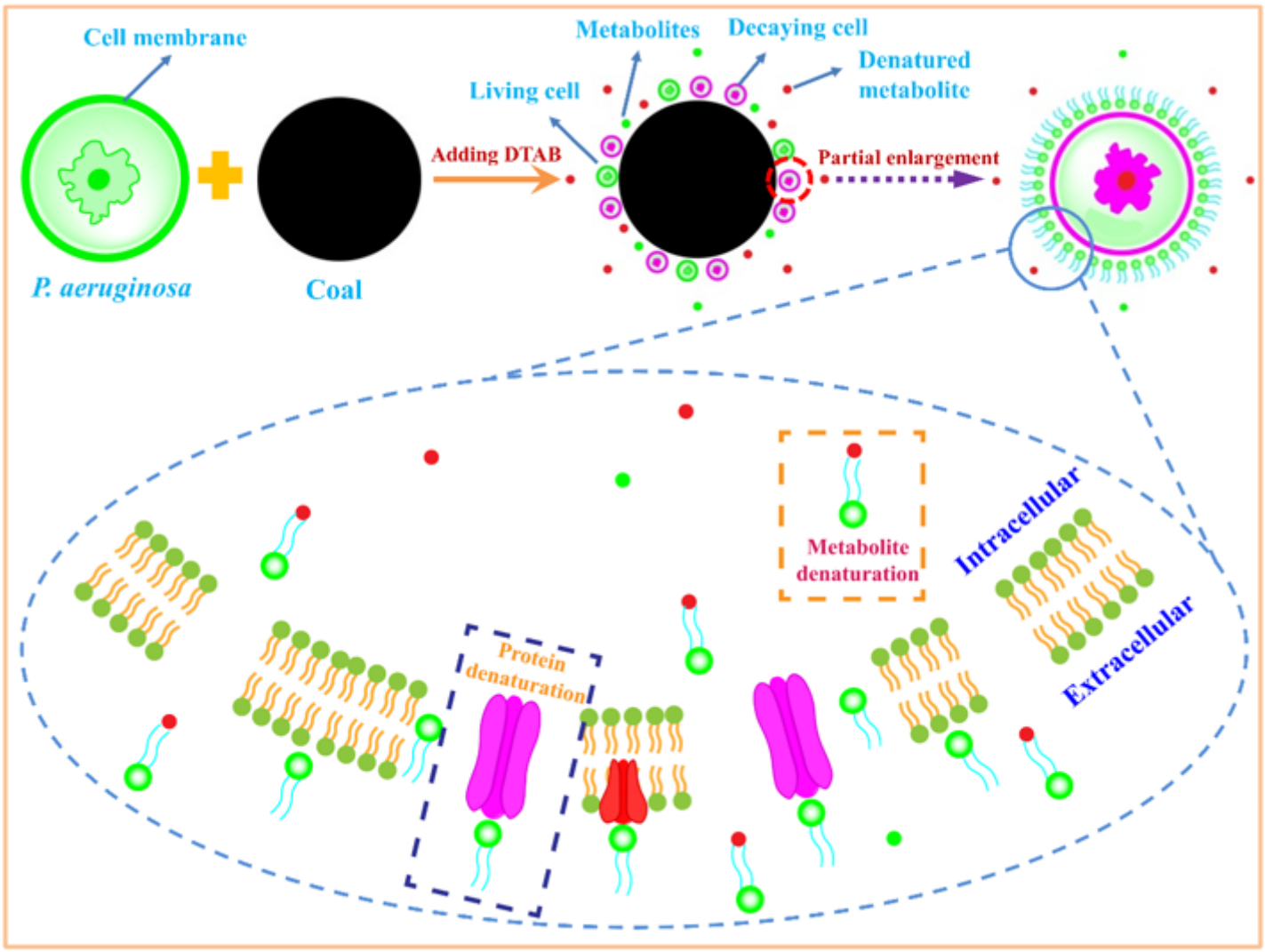

Figure 12

Action mode diagram of DTAB in biodegradation process of coal samples

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table3to6.docx
- GraphicalAbstract.png