

The Alternating Growth of Bacteria within a Consortium During Desulfurization of Coal

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ABSTRACT

Efforts to reduce organic sulfur in coal are taken through biodesulfurization by using desulfurization bacteria to release covalently-bound sulfur from the coal matrix. Coal is a complex hydrocarbon material that requires collaboration from more than one type of bacteria in a consortium for desulfurization. The current study shows how the individual members of a bacterial consortium obtained directly from coal samples grew on the coal. Mineral medium containing sub-bituminous coal with a concentration of 10%, 15%, and 20% served as a carbon source and the only sulfur to support the consortium's growth. The examination included growth patterns, concentrations of dibenzothiophene as an organic sulfur representative, pH, and sulfate concentration as the sulfur product released into the medium. The growth of individual members of the consortium was observed for 336 h. The consortium grew in all three coal concentrations with slightly different cell growth patterns and the release of dibenzothiophene. Members of the consortium grew alternately and overlapped, which showed possible linkages or dependence on products and existence from the growth of other members. The existence of the primary strain *Moraxella osloensis* COK1 indicated that they played a role in the activities and growth of other members. The alternating growth is discussed to produce a hypothetical illustration of how several other members play in using sulfur in a well-known desulfurization pathway. In conclusion, this study provides a deeper insight into the value of consortium members individually but growing together while swarming coal as a complex resource to become low-sulfur coal.

1. INTRODUCTION

Coal is a complex natural material mainly composed of carbon interspersed with minerals. These minerals include sulfur-forming compounds such as; dibenzothiophene, thiophenol alkyl, aryl sulfide, and dialkyl sulfides. When coal is used as a fuel, sulfur compounds are emitted and lower the surrounding air quality. Therefore, various attempts should be made to address the sulfur content in coal. Biodesulfurization is an alternative to minimize the tightly associated sulfur content associated with coal which works at medium temperature, hence lower operational cost. Most studies on coal biodesulfurization employ

bacteria as the sulfur releasing agent, which is considered an economical and environmentally friendly technique (Jatoi et al., 2021; Kotelnikov et al., 2020).

As with any other natural materials, coal can be attacked and thus a harboring habitat for bacteria. Coal originally comes from plant material and is a target of carbon source for heterotrophic bacteria. Some studies have proved the presence of bacteria: the role of bacteria in coal formation (Burke and Wiley, 1937) and the isolation of bacteria from acid drainage of coal mines (Colmer et al., 1949). Other studies include recent publications about the presence of

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methanogenic bacterial communities on coal and water formations (Strapoć et al., 2008), the microscopic appearance of bacterial cells on coal particles (Yossifova et al., 2011), the dominance of hydrogen-producing bacteria and methanogens in coal seams (Su et al., 2018), and different taxa in the microbial community are responsible for the degradation of different coal components (Vick et al., 2019). A most recent study showed that the adherent microbial community preferred to form dense biofilms around cracks in the mineral-rich coal surface, which may provide shelter for the bacteria (McLeish et al., 2021). All the proof about the presence of bacteria that come from coal show the ability of the bacteria to adapt to the coal matrix environment. Accordingly, coal could be used as an object to explore desulfurization bacteria.

Apart from coal as a living space and its abiotic environment, the bacteria in coal cannot be separated from its biotic environment and other bacteria. The interactions have been established for a long time until coal formed, which caused the bacteria to coexist with the coal maturation. Although it is suspected that their growth occurs very slowly, or even quiescent or dormant in the coal (Burke and Wiley, 1937), due to limited growth factors, their coexistence in coal over millions of years of maturation has resulted in a consortium of bacteria that even helps restructure coal carbon. Some studies demonstrating the effectiveness of microbial consortia in reducing sulfur content in coal were oriented to the final results (Kotelnikov et al., 2020). Biodesulfurization of a mixed culture bacteria worked more efficiently than physico-chemical methods, lowering SO₂ emissions by 72.4% and reducing ash content by 33% (Makgato and Chirwa, 2020). Therefore, in the current study, we examined the activity of a consortium of bacteria from coal in batch culture with a medium containing minerals and coal as a sole source of sulfur. The consortium members were not isolated singly beforehand nor reconstructed, but mixed inoculum cultures were obtained directly from coal and enriched in stages. The results of this study provide an overview of how consortium members took turns in using coal as a sulfur source so that they overall worked to reduce sulfur in coal or carried out sequential biodesulfurization.

Examination of the bacterial consortium activity from coal samples was carried out in coal media of 10%, 15%, and 20% to determine the effect

of growth support, i.e., carbon and sulfur sources, on desulfurization activity and bacterial community. The concentration of dibenzothiophene is a parameter measured for desulfurization because it is the most significant organic sulfur in coal (Constanti et al., 1994; Jatoi et al., 2021) and has become an internal standard for assessing coal maturation (Li et al., 2012). In addition, the desulfurization of dibenzothiophene also means the attack of other organic compounds because the microorganisms that desulfurize the compound can use a wide range of sulfur compounds as substrates (Chen et al., 2019; Nassar et al., 2021).

2. METHODOLOGY

2.1 Materials and chemicals

The coal samples used are sub-bituminous coal from South Sumatra, Indonesia. The media used were mineral salts medium and nutrient agar. The mineral salt medium was sulfur-free and contained NaCl 0.075 g/L, NH₄Cl 2.0 g/L, Na₂HPO₄ 5.77 g/L, K₂HPO₄ 2.44 g/L, trace mineral solution 10 mL/L, and glucose 10.0 g/L (Gunam et al., 2006). All media was sterilized by autoclaving at 121°C for 15 min. Molecular identification of isolate used PeqGold Bacterial DNA kit (Peqlab, UK), DreamTaq polymerase PCR kit (Thermo Fisher Scientific), primers 27F/1492R, agarose, and buffer Tris-acetate EDTA pH 9.0.

2.2 Isolation of consortium inoculum

Isolation was carried out by using the stratified enrichment method in three stages. In the first stage, five grams of 100 mesh coal samples were used as a source of isolation and inserted into a 95 mL sterile mineral salts medium containing 10% (w/v) coal in a 250 mL Erlenmeyer flask. The coal concentration of 10% in the medium did not include coal added as a source of isolation. The cultures were incubated at room temperature for 24 h while being shaken at 120 rpm. In the second stage, following the duration of 24 h, 10 mL of culture solution from the first stage was transferred into 90 mL of new mineral salts medium and incubated under the same conditions for 24 h or achieved a minimum cell concentration of 10⁶ cells/mL. In the final stage, 15 mL of the second stage culture was transferred into 135 mL of new mineral salts medium and incubated under the same conditions for 24 h. This culture was used as an inoculum for examination. Bacteria in this culture were also isolated on a nutrient agar medium to obtain single colonies, and pure cultures were identified.

2.3 Examination of bacterial growth

The medium used for growth examination was a mineral salts medium treated with coal powder with concentrations (w/v) of 10%, 15%, and 20%. The examination was carried out by inoculating 10% (w/v) of the inoculum culture into the sterile mineral salts medium containing coal, i.e., 10%, 15%, or 20%. The cultures were incubated while shaking at 120 rpm at room temperature for 48 h. Sampling on culture solution was carried out at specific intervals to check the cell concentration, dibenzothiophene concentration, and culture pH. The cell concentration was measured using dilution and plating on a nutrient agar medium by the total plate count method. At the time of colony counting, colony morphology was observed to differentiate between bacteria in the consortium. Their morphology was compared to colony morphology in single isolates. Isolates were distinguished from their colony morphological characteristics: whole shape, margin, elevation, and color. Thus, each colony can be identified as a single species, and the individual species concentration can be calculated. For pH and dibenzothiophene measurements, the sample was filtered first to reduce the undissolved coal flakes. The concentration of dibenzothiophene was determined by spectrophotometric analysis using absorbance at a wavelength of 323.8 nm (Etemadifar et al., 2008). Sulfate measurements were carried out by the turbidimetric method at a wavelength of 420 nm after the addition of BaCl₂.

2.4 Identification of isolates

The pure bacteria were identified individually based on the 16S rRNA gene. The isolated DNA was PCR-amplified by using universal primers 27F/1492R according to the well-known general protocol. The following PCR conditions were carried out: initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 1 min, annealing at 51°C for 1 min, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The consensus sequences of forward and reverse PCR products were aligned with GenBank (NCBI) reference sequences retrieved by using BLAST (Altschul et al., 1990), and the phylogenetic tree was reconstructed with MEGA6 (Tamura et al., 2013) using the neighbor-joining method with bootstrap 1000. The identified sequences COK1 to COK5 were deposited in GenBank with accession numbers AB931117 to AB931121, respectively.

3. RESULTS AND DISCUSSION

Organic sulfur represented by dibenzothiophene has been detected in all coal media of 10%, 15%, and 20% since the beginning of incubation. The organic sulfur was released from the coal by previous autoclaving. It resulted in a low initial pH of the medium, with an average of 5.82, 5.42, and 4.78, respectively, in 10%, 15%, and 20% coal medium. The pH decreased because it was influenced by the sulfate content that increased simultaneously with the coal concentration, an average of 0.18, 0.61, and 1.19 mg/L, respectively, in 10%, 15%, and 20% coal medium. Sulfate is inorganic sulfur in coal and is easily leached from coal and is easily dissolved into the medium. Unlike sulfate, the initial concentration of dibenzothiophene decreased with an increase in coal concentration, i.e., an average of 0.56, 0.51, and 0.27 mM, respectively, in 10%, 15%, and 20% coal medium. Organic sulfur such as dibenzothiophene is covalently bound to form large complex structures in coal (Kotelnikov et al., 2020). If the coal is more concentrated, the heating effect using autoclaving-sterilization on coal degradation will decrease, resulting in less organic sulfur released into the medium.

The molecular identification results indicated COK1 to COK5 belong to *Moraxella osloensis*, *Micrococcus endophyticus*, *Pseudomonas aeruginosa*, *Pseudomonas psychrotolerans*, and *Enhydrobacter aerosaccus*, respectively. However, COK6 was not identified because its viability could not be maintained during storage. Each isolate showed distinct morphological characteristics on the nutrient agar plate. Isolate 1 was circular, entire (smooth), raised, and translucent. Isolates 2, 3, and 6 were similar in shape, margin, and elevation but were light yellow, pink, and yellowish transparent, respectively. Isolate 4 was circular with a wrinkled surface, undulate margin, umbonate elevation, and yellow. Isolate 5 was circular, entire, flat, and was milky white or opaque in color. They are all heterotrophic bacteria that correspond to the predominant organic sulfur in the coal sample. The organic sulfur content in the coal sample was 57% of the total sulfur, meaning that the high organic sulfur environment selected the organic sulfur users. The species name followed by the isolate codes are used in the following discussions.

In the 10% coal medium, *Moraxella osloensis* COK1 was the only bacteria detected at the beginning of incubation; cell concentration decreased until hour 6, accompanied by an increase in dibenzothiophene

and a slow increase in sulfate concentration (Figures 1 and 2). The decrease in the concentration of *M. osloensis* COK1 indicates the adaptation of bacteria once they enter the coal medium. Growth of *M.*

osloensis COK1 continued to be detected until the end of incubation, indicating that its growth dominates the consortium.

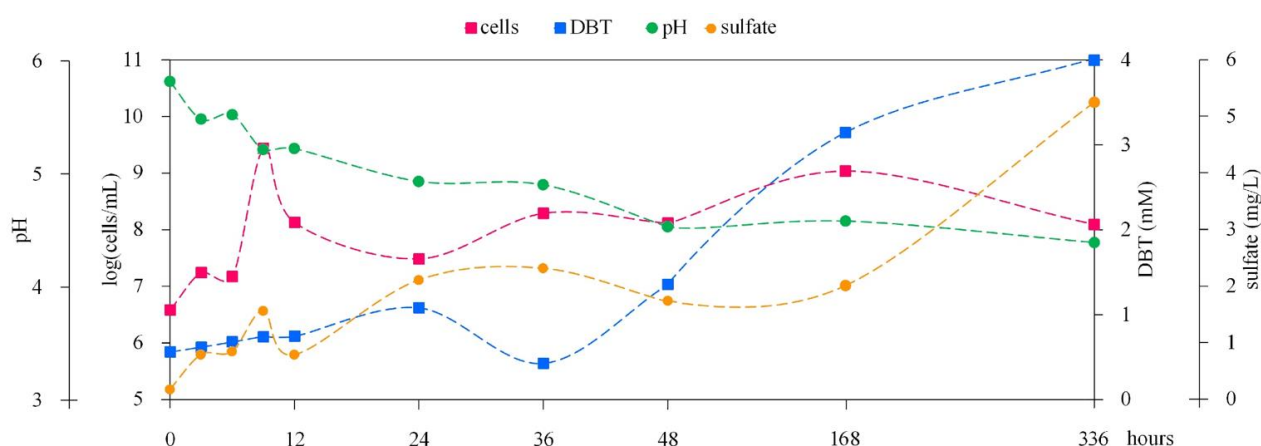


Figure 1. The activity of coal-origin bacteria consortium in 10% coal medium

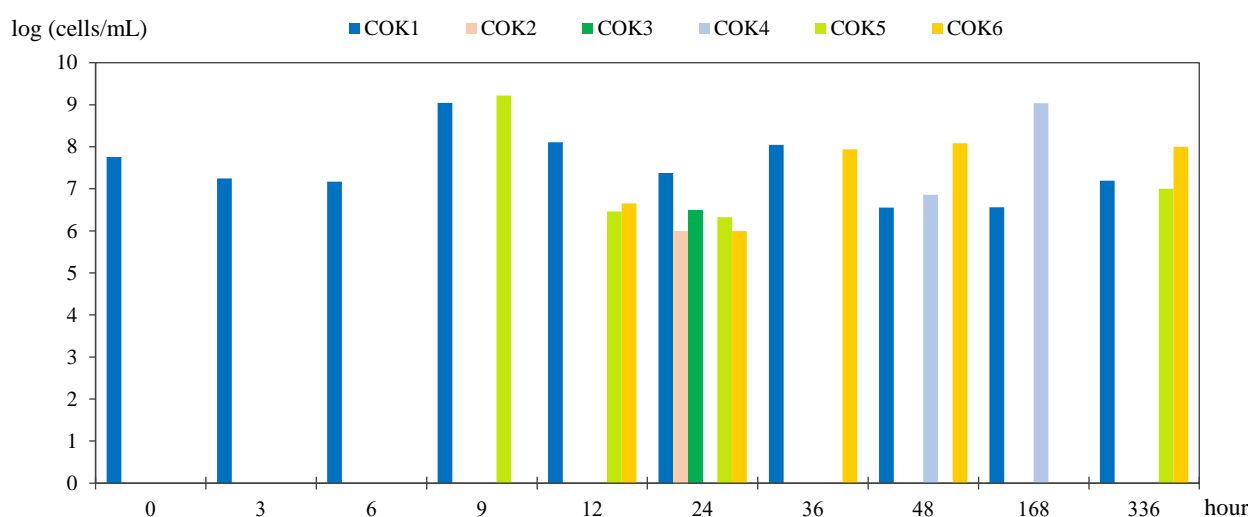


Figure 2. The concentrations of individual consortium members in 10% coal medium

At the beginning of its growth in a coal medium containing glucose, *M. osloensis* COK1 got carbon from glucose and sulfur released from coal due to autoclaving-sterilization to be assimilated into cells. Due to this action, the cells obtained energy to produce enzymes used to remove organic sulfur from coal which was further used and can be seen from an increase in the concentration of dibenzothiophene and sulfate. Degradation of black carbon in nature, including coal, is through the mechanism of cometabolism, which increases with the addition of glucose, namely through increased growth of biomass and the enzymes it produces (Hamer et al., 2004; Jatoti et al., 2021). Meanwhile, culture pH tended to decrease from the beginning to the end of incubation,

although it appears that there was a fluctuation. pH changes were affected by sulfates and the release of organic acids resulting from coal degradation, such as fulvic acid and humic acid found in coal. These acids are products from extracellular laccase activities while using coal as a carbon source (Taiwo et al., 2020).

During hours 6-9, the cell concentration increased rapidly, accompanied by decreased pH, and increased sulfate and dibenzothiophene concentrations. The growth of *M. osloensis* COK1 cells, which is heterotrophic bacteria (use organic matter), and an increase in sulfate concentrations, indicated that degradation of organic compounds, including the organic sulfur, was taking place. The concentration of dibenzothiophene that also increased revealed that not

all of the released organic sulfur was used by bacteria. This is possible because other organic sulfur compounds can be released when coal degradation occurs and become an alternative sulfur source. Degradation of dibenzothiophene released sulfur in the sulfate form, allowing some sulfur to be assimilated for cell growth. At this stage, the desulfurization by *M. osloensis* COK1 was assisted by *E. aerosaccus* COK5. The emergence of *E. aerosaccus* COK5 led to an assumption that they were the user of degradation products by *M. osloensis* COK1. This assumption was supported by the fact that *E. aerosaccus* COK5 could not grow well as a single culture (data not shown).

During hours 9-24, the cell concentration decreased significantly, the dibenzothiophene concentration increased, and the sulfate concentration decreased then increased. At this stage, desulfurization was not only played by *M. osloensis* COK1 and *E. aerosaccus* COK5 because *Micrococcus endophyticus* COK2, *Pseudomonas aeruginosa* COK3, and COK6 also appeared. *Micrococcus endophyticus* is a gram-positive bacterium relatively recently discovered and characterized from the roots of *Aquilaria sinensis* (Hamer et al., 2004). Meanwhile, *Pseudomonas aeruginosa* has been widely known for its ability to degrade hydrocarbon compounds, including dibenzothiophene. *P. aeruginosa* isolated from oil sludge was able to degrade a mixture of carbazole, dibenzothiophene, and fluorene in petroleum refinery wastewater (Ghosh and Mukherji, 2020). In the current stage, the cell concentrations were lower than before. However, the consortium members were more diverse, so the release of organic sulfur compounds and their desulfurization continued. Desulfurization can be triggered by a decrease in sulfate during hours 9-12. Under limited sulfate conditions, some bacteria synthesize an extra set of proteins required to metabolize an alternative sulfur source, called sulfate-starvation-induced proteins, which are synthesized only in the absence of a sulfur source, including sulfate. The proteins can be enzymes and transport systems involved in the metabolism of alternative sulfur sources from the environment, including Dsz desulfurization enzymes in the 4S pathway (Mohebbi and Ball, 2016). The sulfate-starvation reactions include restricting sulfur assimilation, enforcing sulfur-sparing, and maintaining redox homeostasis. However, it is currently unknown how desulfurizing bacteria respond to this stressor (Hirschler et al., 2021).

At hour 24, five strains of bacteria were detected, and *M. osloensis* COK1 still predominated. However, at hour 36, only *M. osloensis* COK1 and COK6 dominated, approximately in the same proportion. COK6 was challenging to grow as a single culture, either in general bacterial media (nutrient agar) or minimal media containing dibenzothiophene. The decrease in dibenzothiophene concentration accompanied by an increase in sulfate concentration at this stage indicated that COK6 was the user of organic sulfur released by *M. osloensis* COK1 from coal.

During hours 36-48, the cell concentration decreased until it was relatively constant, the dibenzothiophene concentration increased again, and the sulfate concentration decreased, but the pH continued to decrease. *Pseudomonas psychrotolerans* COK4 appeared, in addition to *M. osloensis* COK1 and COK6, which remained the members who played a role. The increase in dibenzothiophene concentration indicated that *P. psychrotolerans* COK4 enhanced the release of organic sulfur from coal. The growth of *P. psychrotolerans* COK4 was still detected at hour 168, causing high cell concentrations and dibenzothiophene concentrations. The genus *Pseudomonas* is known to degrade various aromatic hydrocarbons. They were widely used as a bioremediation agent of environmental pollutants (Wasi et al., 2013) and a member of consortia in coal desulfurization (Kotelnikov et al., 2020; Makgato and Chirwa, 2020).

The decrease in sulfate concentration and the increase in the concentration of dibenzothiophene indicated that the release of organic sulfur compounds from coal did not increase their use. This result could be due to the high concentration of sulfate from the previous step (before hour 36), resulting in repression of the desulfurization activity. The byproduct of desulfurization, including sulfate in a particular concentration, has an inhibitory effect on the activity of desulfurization enzymes and repression on the expression of *dsz* genes, although not on microbial growth (Martín-Cabello et al., 2020; Sousa et al., 2020).

At hour 336, bacterial growth still occurred; the concentrations of dibenzothiophene and sulfate showed the highest values compared to previous times. The detected bacteria were *M. osloensis* COK1, *E. aerosaccus* COK5, and COK6, so it is assumed that these bacteria are the most adapted in the coal environment than other bacteria in this coal consortium. In another study, the activity of a bacterial consortium on coal continued for up to 18 days

(432 h), which significantly reduced almost 70% of the sulfur (Makgato and Chirwa, 2020).

In the 15% coal medium, the presence of bacteria was not detected at the beginning of growth; it could be due to an adaptation to a higher coal concentration (Figure 3). Then the cell concentration increased from hour 3 to 6, and the growth was kept relatively constant until hour 168. Meanwhile, the pH tended to decrease steadily until the end of incubation. Fluctuations occurred in the concentrations of dibenzothiophene and sulfate with a tendency: the DBT concentration decreased, the sulfate concentration increased, and vice versa. The consortium remained dominated by *M. osloensis* COK1, then *E. aerossacus* COK5 (Figure 4). *M. osloensis* COK1 just appeared at hour 6, followed by the emergence of other bacteria. The appearance of other bacteria after

M. osloensis COK1 indicated the interdependence of the consortium members. When the consortium reached the highest diversity (hours 36-48), the dibenzothiophene concentration increased, and the sulfate concentration decreased. It indicated that there was cooperation between bacteria in releasing sulfur compounds from coal and using them. The consortium members generally appeared to maintain the desulfurization function in the 15% coal medium, a relatively constant cell concentration since hour 6, with organic sulfur available and used. Although at hour 360, the release of dibenzothiophene from coal and sulfate uptake still occurred (Figure 3), the cultured bacteria COK1-COK6 were no longer detected (Figure 4), possibly due to the activity of unculturable microorganisms, and this requires further study.

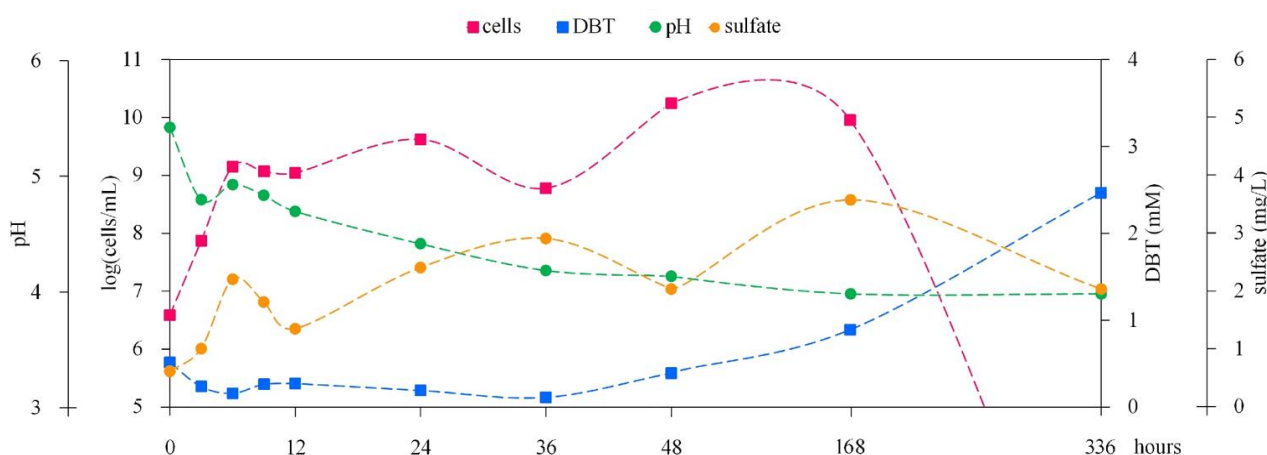


Figure 3. The activity of coal-origin bacteria consortium in 15% coal medium

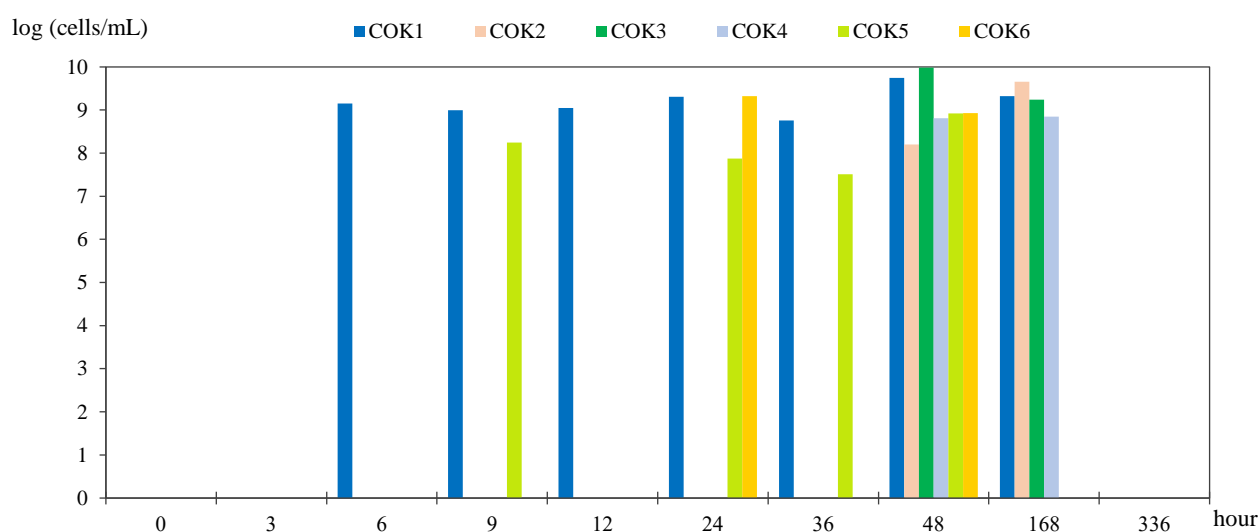


Figure 4. The concentrations of individual consortium members in 15% coal medium

In the 10% and 15% coal medium, *M. endophyticus* COK2 and *P. aeruginosa* COK3 always appeared concurrently and occurred after the appearance of *M. osloensis* COK1, *E. aerosaccus* COK5, and COK6. They were thought to be closely associated with each other and depended on the metabolic products of *M. osloensis* COK1, *E. aerosaccus* COK5, and COK6. *Pseudomonas* has been confirmed in degrading coal and dibenzothiophene (Li et al., 2019; Taiwo et al., 2020). Thus, *Pseudomonas aeruginosa* COK3 was assumed of using organic acids released from coal by the activities of the other members. In addition, *P. aeruginosa* COK3 was thought to facilitate the growth of *M. endophyticus* COK2. The rhamnolipid biosurfactant produced by *Pseudomonas* bacteria (Li et al., 2019) can facilitate the growth of other bacteria using hydrocarbon compounds in a consortium. The members that produce surfactants among coal-using bacteria can effectively lower the water surface pressure, indirectly assisting the absorption of nutrients into other cells. In an environment with complex hydrocarbons such as coal, microorganisms live in groups and complement

each other through substrate exchanges to produce degradation products (Su et al., 2018).

Similar to the 15% coal medium, the growth in the 20% coal medium did not start immediately (Figure 5). The consortium members were adapting to a higher coal environment, and then the cell concentrations were relatively constant until the end of incubation. Dibenzothiophene and sulfate concentrations also fluctuated, showing a dynamic between the released and the used, as it did in the 10% and 15% coal medium (Figure 6). *M. endophyticus* COK2 and *P. aeruginosa* COK3, which appeared concurrently in the 10% and 15% coal medium, were not detected at the sampling points applied in the coal medium 20%. Presumably, these bacteria would appear slower, like the delay in their appearance in the 15% coal medium compared to the 10% coal medium. Higher coal concentrations mean a larger share of coal that microorganisms have to elaborate. They are limited in the face of challenges in the coal's porosity, cracks, and surface areas to further oxidation of sulfur forms, and intermediate products of the sulfur pathway can inhibit their growth (Makgato and Chirwa, 2020).

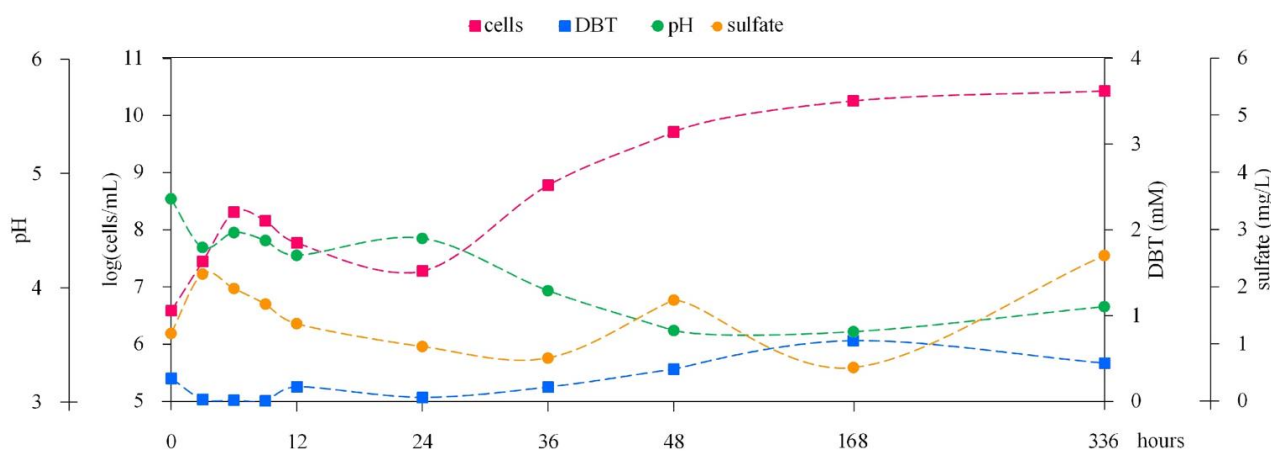


Figure 5. The activity of coal-origin bacteria consortium in 20% coal medium

In the three coal media (10%, 15%, and 20%), *M. osloensis* COK1 was the dominant bacteria in the coal consortium. Although the starter inoculum was made from a medium containing dibenzothiophene only, the bacteria increased their growth in the coal medium, which contained various organic sulfur. Likewise, *E. aerosaccus* COK5 has a role in providing sulfur in coal. These results add information about the bacterial species able to grow in coal as well as the desulfurization of organic compounds. Those bacteria that consume various organic sulfurs such as benzo- and dibenzothiophenes for their growth and

metabolism are called sulfur-dependent bacteria, such as *Rhodococcus erythropolis*, *Lysinibacillus sphaericus*, and *Sphingomonas subarctica*, and were used to conduct biodesulfurization (Sousa et al., 2020).

The sequential appearance of consortium members in the coal media indicated a syntrophism interaction. For example, the growth of *P. psychrotolerans* COK4 depended on COK6, while the appearance of COK6 depended on *E. aerosaccus* COK5. Naturally, the bioconversion of complex materials often involves syntrophic interactions

among many microorganisms. The growth also occurred in bacterial communities during coal bioconversion in a coalfield, where the growth of *Methanosarcina* increased after consuming acetate produced by acetogenic *Propionibacterium* (Wang et

al., 2019). This interaction caused partial degradation of the substrate while reducing the coal's complexity to be used by other bacteria that do not have the same ability.

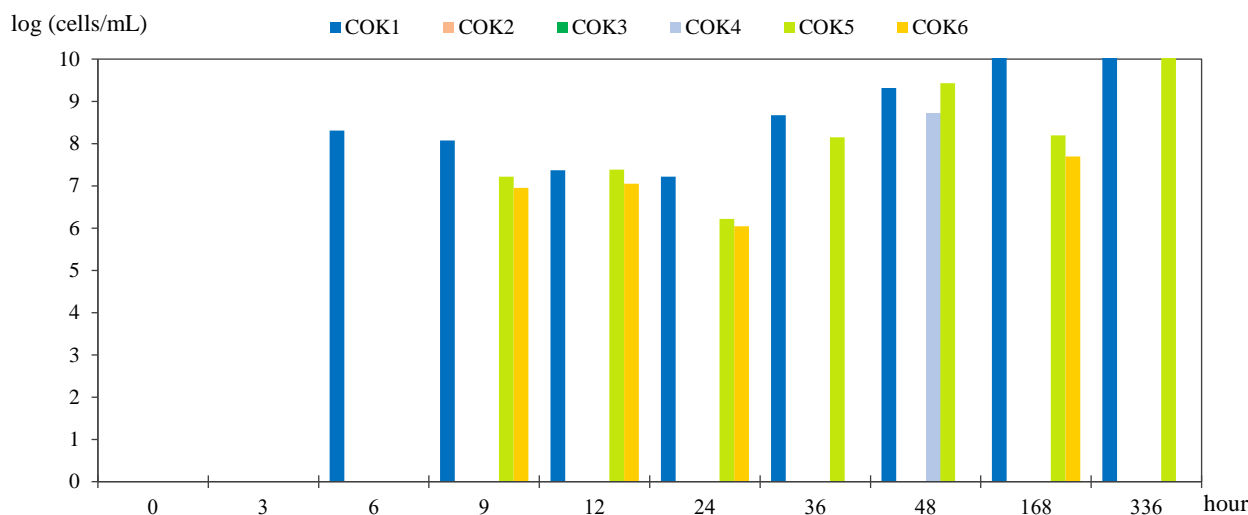


Figure 6. The concentrations of individual consortium members in 20% coal medium

By considering the occurrence and activity of consortium members, and their proximity to the published desulfurization bacteria, the potential role of each bacterium in the consortium can be predicted. The illustration in Figure 7 placed the bacteria members in the 4S pathway based on their activities and growth turnover in coal medium (Figures 1-6) and some references. The 4S is a well-known biodegradation pathway of dibenzothiophene (Jatoi et al., 2021; Li et al., 2019; Martín-Cabello et al., 2020; Mishra et al., 2016; Sousa et al., 2020). The most dominant role member in the coal consortium is *M. osloensis* COK1. Its activities at all stages and emergences in the three concentrations of coal medium made it thought to have a complete gene or enzyme system in processing organic sulfur in coal. In addition, it did not depend on the other members, especially in consuming the dibenzothiophene. Therefore, this bacterium plays in all steps in dibenzothiophene transformations until the release of sulfate in the 4S pathway.

Furthermore, according to several references, *P. aeruginosa* COK3 is an efficient user of hydrocarbon compounds related to its ability to produce biosurfactants (Ghosh and Mukherji, 2020; Li et al.,

2019), but in this consortium, it seems to compete with *M. osloensis* COK1. Therefore, it is thought to play a secondary role in releasing dibenzothiophene from coal and some transformation steps, such as the further degradation of hydroxy biphenyl (HBP) *P. aeruginosa* is known to have an enzyme system to utilize biphenyl, a form of toxic persistent organic pollutants in the environment (Chakraborty and Das, 2016). Meanwhile, those that occasionally appear, such as *P. psychrotolerans* COK4, *E. enhydrobacter* COK5, and *M. endophyticus* COK2, required products of the primary members before playing the next steps of dibenzothiophene transformations; moreover, *M. endophyticus* COK2 played at a more downstream stage. Other consortium members who are challenging to detect in this experiment do not mean any role. The non-desulfurizing members make essential contributions to the desulfurization activity, including creating sulfur-deficient conditions by removing sulfur (sulphite/sulfate), thereby increasing the sulfur requirement for the desulfurized cells (Kilbane, 2016). In any case, the presumptions require further study of the role of each consortium member, including the participation of unculturable members.

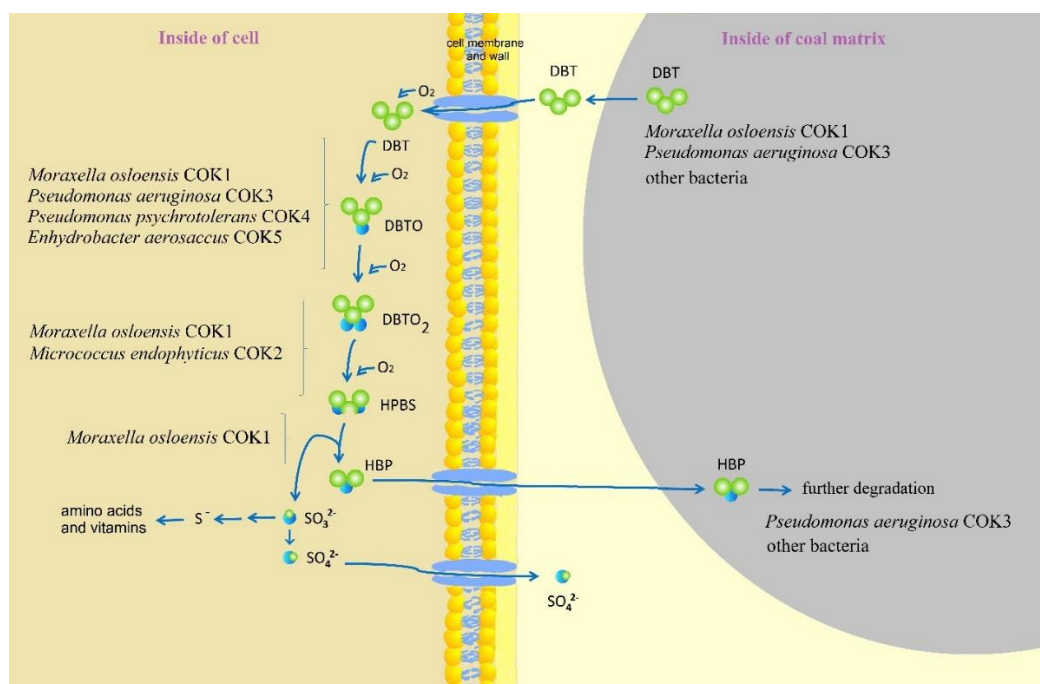


Figure 7. Hypothetical workflow regarding the potential role of consortium bacteria in the 4S pathway

4. CONCLUSION

This study revealed that bacteria work concurrently or sequentially in processing organic sulfur sources available in coal. They should be applied as a consortium culture because coal is a complex hydrocarbon interspersed by organic sulfur compounds that the degradation needs to be swarmed. Degradation and desulfurization of coal will be more extensive when a microbial consortium is applied because various bacteria cooperate to release the sulfur from the coal. The goal of producing clean coal is expected to be easily achieved by providing a diverse mixture of bacteria.

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