Biosolubilization of Indonesia’s Subbituminous Coal Using *Neurospora intermedia*

Elvi Restiawaty1,3*, Dwiwahju Sasongko2, Ahmad Furqon Hala2, Ryan Fitrian Sofwan Fauzan2, Nendry Nurramdani Solihah2, & Ardiyan Harimawan2

1Bioenergi Engineering and Chemurgy Study Program, Faculty of Industrial Technology, Institut Teknologi Bandung. Jl. Let. Jend. Purn. Dr. (HC) Mashudi No.1, Kec. Jatinangor, Kab. Sumedang, West Java 45363, Indonesia.
2Chemical Engineering Study Program, Faculty of Industrial Technology, Institut Teknologi Bandung. Jalan Ganesa 10 Bandung 40132, Indonesia.
3Biosciences and Biotechnology Research Center, Institut Teknologi Bandung. Jalan Ganesa 10 Bandung 40132, Indonesia
*Email: erestiawaty@che.itb.ac.id

Abstract. Recognizing its high volatile matter content, liquefaction to convert subbituminous coal with relatively low calorific value into liquid fuel by thermal or biological process has been subjected to extensive research. The former has been implemented commercially whereas the latter challenges for further studies. This paper looks into low-rank coal upgrading through subbituminous biosolubilization using *Neurospora intermedia* as the bioconverting agent. *N. intermedia* was initially cultured on agar medium containing a carbon source (glucose, sucrose, or cellulose) in a petri dish. After four days cultivation, sterilized coal particles with diameter of 1.5-2.5 mm were spread on *N. intermedia* culture. It was observed that *N. intermedia* could grow on the solid culture containing glucose, sucrose, and cellulose. However, the biosolubilization product was only observed from the solid culture with glucose and sucrose as carbon source. The highest biosolubilization rate was $1.07 \times 10^{-3}$ abs/min and about 83% of coal could be solubilized when the media contained 15 g/L of glucose. The FTIR analysis showed that the spectrum of biosolubilization product was similar to the initial coal spectrum, but it contained small amount of aliphatic C-H group compounds and high amount of carbonyl, hydroxyl, and amine groups.

Keywords: Biosolubilization, Carbon sources, Neurospora intermedia, Subbituminous coal.

1 Introduction
Nowadays, coal utilization for industrial sector in Indonesia has been increasing, while the utilization of petroleum has been decreasing. In 2010, approximately 86% of Indonesia’s coal reserve are low-rank coal, i.e. 20% lignite and 66% subbituminous, with the rest are high-rank coal, i.e. 13% bituminous and 1% anthracite [1]. The use of the low-rank coal as fuel can potentially threaten the environment due to its combustion products such as sulfur oxide, nitrogen oxide, fly ash, etc. In
addition, low-rank coal has low calorific value and high moisture content [2]. Problems arising from utilization of low-rank coal resulted in the need for the development of low-rank coal utilization technology. Some green technologies can be potentially developed such as co-gasification technology [3], co-liquefaction technology [4] and biosolubilization [5]. Biosolubilization is preferable as it can be carried out at atmospheric pressure and mild temperature [6]. Coal biosolubilization is a process of solubilizing coal using microorganism as solubilizing agents [7], such as Trichoderma asperellum [5], and Neurospora crassa [8]. Neurospora is a genus of mostly edible fungi that can be easily isolated from a traditional Indonesia’s fermented food, known as oncom [9]. Coal biosolubilization products can be used as suitable substrate for other biological process such as biogas production by methanogenic bacteria [10]. This study focused on the process of biosolubilization of Indonesian subbituminous coal using Neurospora intermedia. Glucose, sucrose, and cellulose were used to examine the effect of the type of carbon source and its concentration on the process and products of the subbituminous coal biosolubilization.

2 Materials and Methods

2.1 Biological Agent and Indonesian Subbituminous Coal

N. intermedia was cultivated in Vogel medium [11] and incubated at 30 °C for four days. Subbituminous coal used in this study was received from Pusat Penelitian dan Pengembangan Teknologi Mineral dan Batubara (PUSLITBANG tekMIRA) in Bandung – Indonesia. The coal was ground and soaked by 8 M nitric acid for two days. It was then washed until the washing water reached pH 5.5 and dried in an oven at 50 °C. The next step was sieving to get the particle size between 1.5 and 2.5 mm. The coal particle was then sterilized using autoclave at 121 °C and 2 atm for 15 minutes.

2.2 Biosolubilization of Subbituminous Coal on Solid Culture of N. intermedia

The subbituminous coal biosolubilization process was carried out in a solid agar culture of N. intermedia placed on a petri dish with a diameter of 9 cm. The medium of solid culture used was Vogel medium containing a determined concentration of carbon source: cellulose, sucrose, or glucose. Inoculum of N. intermedia spores was cultured by a spread plate method. The cultures were incubated at 30 °C for four days. On the fourth day, the mushroom fungal hyphae filled the surface of the solid medium. About 0.5 g of pretreated coal particles were spread on surface and then the petri dish was incubated at 30 °C for more six days. The black liquid sample of biosolubilized products was collected by using sterile syringes and then it was stored in microtube for analysis.
2.3 Analysis

The absorbance analysis of the product was carried out by the UV-Vis spectrophotometer Thermo Genesys 20. Absorbance was measured to determine the level of liquidity of coal during the biosolubilization process [12]. Measurement of product absorbance was performed at wavelengths of 450 nm and 280 nm to measure the absorbance of humic acid and fulvic acid, respectively. In addition, analysis by infrared spectroscopy method was conducted using a FTIR spectrometer to determine the organic chemical groups contained in the product sample [13]. The analysis was done with a wavelength of 4000 cm$^{-1}$ to 400 cm$^{-1}$. In addition, the proximate and ultimate analysis of the remaining particles of coal was done to determine its contents. The results were compared with the coal content before the biosolubilization process.

3 Results and Discussion

3.1 *N. intermedia* Growth on Solid Medium with Different Carbon Sources

It was observed that the higher the concentration of carbon sources, the more *N. intermedia* grew on the surface of the solid medium. On the fourth day of cultivation, the pretreated and sterilized coal particles were spread on the surface of the solid medium which has been covered with mold mycelia and it continued to incubate in the same condition for six more days. After six days, some coal particles were solubilized which was marked by the formation of black droplet over the surface of a solid medium containing carbon sources of glucose and sucrose at various concentrations (see Figures 1 and 2). However, biosolubilization products were not observed in the petri dishes containing cellulose as shown in Figure 3. Moreover, the growth of *N. intermedia* on solid state culture containing cellulose was slower than that of *N. intermedia* on solid state cultures containing glucose and sucrose. The fact that cellulose is a polysaccharide so that *N. intermedia* needs a longer time to break down the complex sugar was likely to be the reason. On the other hand, the monosaccharide of glucose and the disaccharide of sucrose can be consumed easily by *N. intermedia*. The coal in the medium containing cellulose did not solubilize until the end of observation (tenth day). It is suspected that the growth of *N. intermedia* has not reached the stationary phase. A possible explanation is that tyrosinase and laccase enzymes that play a major role in biosolubilization had not been produced at that time [14]. Faison [15] reported that the coal biosolubilization process is categorized as a secondary metabolic function or it occurs when the growth of the microorganism enters the stationary phase. Visual observations strengthened the fact that the color of mold grown on a cellulose carbon source was bright yellow, while molds grown in the medium contain glucose and sucrose had a dark yellow or orange color. The *N. intermedia* cultures with glucose and sucrose as the carbon source had reached the stationary phase and excreted the tyrosinase and laccase enzymes after the fourth day of cultivation.
The black liquid products of coal biosolubilized after five days cultivation with *N. intermedia* on medium containing glucose with concentration of (a) 5 g/L; (b) 15 g/L; (c) 30 g/L.

**Figure 1**

The black liquid products of coal biosolubilized after five days cultivation with *N. intermedia* on medium containing sucrose with concentration of (a) 5 g/L; (b) 15 g/L; (c) 30 g/L.

**Figure 2**

The coal had not been solubilized yet for ten days cultivation in medium containing cellulose with concentration of (a) 5 g/L; (b) 15 g/L; (c) 30 g/L.

**Figure 3**

3.2 Production of Humic and Fulvic Acid

Humic and fulvic acids can be used as markers for coal solubilization [16]. Both of these compounds are contained in coal and released when the biosolubilization takes place. The biosolubilization product obtained from the *N. intermedia* cultivation in solid medium containing glucose or sucrose were analyzed further in this study. The absorbance of both humic and fulvic acids compounds has similar trends and values.

Total absorbances of humic and fulvic acids formed from biosolubilization process with *N. intermedia* in various concentration of glucose or sucrose are shown in Figures 4a and b, respectively. The highest total content of humic and fulvic acids was produced from the medium with an initial concentration of 15 g/L, for both glucose and sucrose. The concentration of 5 g/L from the carbon source of glucose or sucrose
showed the lowest total humic and fulvic acids. Total humic and fulvic acids on medium with 30 g/L carbon sources was lower than that of 15 g/L. As previously mentioned, laccase and tyrosinase enzymes play an important role in the process of coal biosolubilization. The production of these two enzymes can be enhanced by limiting the presence of carbon sources [17]. Based on these observations, the concentration of 15 g/L glucose or sucrose was likely to be the optimum concentration to obtain high total absorbance of humic and fulvic acids.

If glucose or sucrose in the medium have been depleted, then *N. intermedia* will use humic and fulvic acids as the carbon sources for its metabolism. At the concentrations of 5 g/L and 15 g/L of glucose or sucrose, both carbon sources were depleted on the fourth day of biosolubilization. On the other hand, the carbon source with a concentration of 30 g/L depleted on the fifth day. It was characterized by a decrease in the concentration of humic and fulvic acids (see Figures 4a and b), where both of these compounds were consumed as a substitute carbon source by *N. intermedia*.

Figure 5 depicts that the carbon source of glucose has higher total absorbance than that of sucrose. Sucrose decomposes into glucose and fructose before it can be consumed by *N. intermedia*. This decomposition process causes a longer metabolism pathway. *N. intermedia* growth in the sucrose medium became slower than the one with glucose as a carbon source. This affected the biosolubilization process, in which the higher production of humic and fulvic acids occurred in the use of glucose.

**Figure 4** The total absorbance of humic and fulvic acids from coal biosolubilization with *N. intermedia* in medium containing (a) glucose and (b) sucrose in various concentrations.
The biosolubilization rate is equivalent to the production rate of humic and fulvic acids. The humic and fulvic acids can be optimally produced from coal biosolubilization on certain days. The initial rate approach was used to determine the biosolubilization rate. The total production rate of humic and fulvic acids was based on the initial production which were $1.23 \times 10^{-4}$ abs/min, $1.07 \times 10^{-3}$ abs/min, and $4.92 \times 10^{-4}$ abs/min for medium containing glucose as carbon source of 5 g/L, 15 g/L, 30 g/L, respectively. While the total production rate of humic and fulvic acid of sucrose as carbon source of 5 g/L, 15 g/L, and 30 g/L were $1.31 \times 10^{-4}$ abs/min, $6.37 \times 10^{-4}$ abs/min, and $1.83 \times 10^{-4}$ abs/min, respectively.

### 3.3 Proximate and Ultimate Analysis

Table 1 and 2 presents the proximate and ultimate analyses, and also percentage of biosolubilized coal by *N. intermedia* that was cultivated in medium with various carbon sources. The percentage of biosolubilization was calculated by differences volatile matter and fixed carbon (VM + FC) residual coal and initial coal. Moreover, assuming that the amount of ash content in coal was constant during the biosolubilization process and only the volatile matter and fixed carbon components were solubilized. The percentage of solubilized coal from cultivation medium containing glucose is higher than that of medium containing sucrose. The metabolic pathway of *N. intermedia* in utilizing glucose is shorter than in the use of sucrose. Consequently, the growth of *N. intermedia* and the process of coal biosolubilization in a medium containing glucose becomes faster than in a medium containing sucrose. Unlike the use of glucose and sucrose as a carbon source, cellulose must be hydrolyzed before it is used for growth and metabolite formation by *N. intermedia*. The highest percent of biosolubilization was achieved when 15 g/L glucose was applied. The ultimate analysis showed the content of C, H, O, and S in coal residues were lower than in the initial coal. The initial coal was degraded into liquid products so that the mass of the components of the coal residue is reduced.
3.4 Characterization of Coal Biosolubilization Product

FTIR analysis was performed on the initial solid coal fed and on the liquid coal product resulting from biosolubilization process using medium containing glucose and sucrose. Generally, the results provided by FTIR analysis for initial coal and the products were be similar (see Figure 6). A few difference between solid coal and solubilized coal were at wavelengths of 3000 - 2800 cm⁻¹, in which the absorbance of solubilized coal was reduced and almost disappears. This indicated that the amount of aliphatic C-H bonds such as CH, CH₂ and CH₃ in the biosolubilization products was reduced by decomposition during biosolubilization. Results reported by Romanowska et al. [19] on lignite biosolubilization using *Gordonian alkanivorans* S7 and *Bacillus mycoides* NS1020 and a study by Cohen [20] on leonardite and lignite biosolubilizations using *Polyporus versicolor* gave the similar results with this study.

At wavelengths of about 1600 and 1700 cm⁻¹, the absorbance of solubilized coal was increased (see figures 6). This indicated that the number of C=C bonds and the carbonyl group (C=O) in the solubilized coal was increased. Laborda et al. 1999 [6] conducted biosolubilization of three types of coal (lignite, subbituminous, and bituminous) using *Aspergillus* S10H and *Doratomyces* S3X-and it yielded similar results with this study. the research gave similar results with this study. In addition, at the wavelength of 3500-3200 cm⁻¹, the absorbance of the biosolubilization product also increased (see Figure 6). This indicated that the number of hydroxyl (O-H) and N-H bonds increases in the solubilized product. Romanowska et al. [19] and Cohen [20] found similar FTIR analysis results with this study.

Table 1 Proximate analysis and percent of biosolubilization (PB) of coal residue after biosolubilization using *N. intermedia* in medium containing various initial concentration of glucose or sucrose compared to initial coal.

| Samples          | Coal Mass (g) | Moisture (g) | Ash (g) | Volatile matter (g) | Fixed carbon (g) | PB (%) |
|------------------|---------------|--------------|---------|---------------------|------------------|--------|
| Initial Coal     | 2.306         | 0.216        | 0.01    | 1.346               | 0.733            | -      |
| 5 g/L glucose    | 0.415         | 0.036        | 0.01    | 0.232               | 0.136            | 82.26  |
| 15 g/L glucose   | 0.407         | 0.035        | 0.01    | 0.219               | 0.141            | 82.67  |
| 30 g/L glucose   | 0.669         | 0.063        | 0.01    | 0.379               | 0.217            | 71.34  |
| 5 g/L sucrose    | 0.519         | 0.045        | 0.01    | 0.303               | 0.160            | 77.72  |
| 15 g/L sucrose   | 0.546         | 0.052        | 0.01    | 0.316               | 0.168            | 76.73  |
| 30 g/L sucrose   | 0.532         | 0.048        | 0.01    | 0.302               | 0.172            | 77.22  |
Table 2 Ultimate analysis of coal residue after biosolubilization using N. intermedia in medium containing various initial concentration of glucose or sucrose compared to initial coal.

| Samples          | Coal Mass (g) | C (%) | H (%) | N (%) | S (%) | O (%) |
|------------------|---------------|-------|-------|-------|-------|-------|
| Initial Coal     | 2.306         | 53.89 | 5.45  | 3.75  | 0.38  | 36.08 |
| 5 g/L glucose    | 0.415         | 52.45 | 5.17  | 3.86  | 0.37  | 35.65 |
| 15 g/L glucose   | 0.407         | 52.29 | 5.15  | 3.97  | 0.37  | 35.67 |
| 30 g/L glucose   | 0.669         | 52.65 | 5.21  | 4.10  | 0.37  | 36.12 |
| 5 g/L sucrose    | 0.519         | 53.70 | 5.42  | 3.97  | 0.36  | 34.55 |
| 15 g/L sucrose   | 0.546         | 53.19 | 5.77  | 3.82  | 0.36  | 34.96 |
| 30 g/L sucrose   | 0.532         | 52.93 | 5.61  | 3.80  | 0.36  | 35.35 |

Figure 6 FTIR analysis of initial coal fed (a), solubilized coal on glucose (b), and sucrose media (c).

4 Conclusions

N. intermedia could grow on different carbon sources, such as glucose, sucrose, or cellulose. However, it could solubilized coal easily on surface culture media containing glucose or sucrose. The optimum condition was obtained on medium containing 15 g/L glucose with 82.67% biosolubilization. The FTIR analysis showed that the biosolubilization product's characteristics were similar to the solid coal’s, but there was a reduction in aliphatic C-H group and addition of carbonyl, hydroxyl and amine groups.

Acknowledgement

This research was supported by P3MI 2018 research program, Institut Teknologi Bandung.

References
[1] KESDM, Bahan Direktur Jenderal Mineral dan Batubara dalam Rapat Kerja tahun 2012 dengan Kementerian Perindustrian: Akselerasi Industrialisasi dalam Rangka Mendukung Percepatan dan Pembangunan Ekonomi, Jakarta, 2012.

[2] Oborien, B.O., Burton, S.G., Cowan, D., & Harrison, S.T.L., The Effect of the Particulate Phase on Coal Biosolubilisation Mediated by Trichoderma atroviride in a Slurry Bioreactor, Fuel Processing Technology, 89, 123-130, 2008.

[3] Ali, D. A., Gadalla, M.A., Abdelaziz, O.Y., & Ashour, F.H., Modeling of Coal-Biomass Blends Gasification and Power Plant Revamp Alternatives in Egypt’s Natural Gas Sector, Chemical Engineering Transactions, 52, 49-54, 2016.

[4] Pinto, F., Paradela, F., Costa, P., Andre, R., Rodrigues, T., Snape, C., Herrador J.M.H., & Fratzczak, J., The Role of Solvent and Catalysts on Co-Liquefaction of Coal and Waste, Chemical Engineering Transactions, 70, 1735-1740, 2018.

[5] Sasongko, D., Restiawaty, E., Rochman, F., & Gamal, M., Biosolubilization of Indonesian Lignite Coal using Trichoderma asperellum. Proceeding of International Seminar on Chemical Engineering in Conjunction with Seminar Teknik Kimia Soehadi Reksowardojo (STKSR 2016), October 27th - 28th 2016, Bandung Indonesia, ISSN: 2353-5917, E34.

[6] Laborda, F., Monistrol, I. F., Luna, N., & Fernandez, M., Processes of liquefaction/solubilization of Spanish coals by microorganisms, Applied microbiology and biotechnology, 52, pp. 49-56, 1999.

[7] Faison, B. D., & Lewis, S.N., Microbial Coal Solubilization in Defined Culture Systems: Biochemical and Physiological Studies, Resources, Conservation and Recycling, 3, 59-67, 1990.

[8] Odom, B., Cooley, M., & Mishra, N.C., Genetics of coal solubilisation by Neurospora crassa, Resources, Conservatio and Recycling, 5, 297-301, 1991.

[9] Restiawaty, E., & Dewi, A., Comparison of Pretreatment Methods on Vertiver Leaves for Efficient Processes of Simultaneous Saccharification and Fermentation by Neurospora sp., Journal of Physics: Conference Series, 877, 2017.

[10] Faison, B. D., Scott, C. D., & Davison, B. H, Biosolubilization of coal in aqueous and non-aqueous media, Oak Ridge National Laboratory, 1987.

[11] Vogel, H.J., A Convenient Growth Medium for Neurospora crassa. Microbial Genetics Bulletin, 13, 42-47, 1956.

[12] Tao, X., Pan, L., Shi, K., Chen, H., Yin, S., Luo, Z., Bio-solubilization of Chinese Lignite I: Extracellular Protein Analysis, Mining Science and Technology, 19, 358-362, 2009.

[13] Shi, K., Tao, X., Yin, S., Du, Y., & Lv, Z., Bio-liquefaction of Fushun Lignite: Characterization of Newly Isolated Lignite Liquefying Fungus and Liquefaction Products, Procedia Earth and Planetary Science 1, 627-633, 2009.

[14] Mishra, N. C., Genetics and molecular biology of Neurospora crassa, In Advances in genetics, 29, 1-62, 1991.

[15] Faison, B. D., Biological Coal Conversions, Critical Reviews in Biotechnology 11, 347-366, 1991.

[16] Sekhohola, L.M., Igbinejie, E.E., & Cowan, A.K., Biological Degradation and Solubilization of Coal”, Biodegradation, 24, pp. 305-318, 2013.

[17] Horowitz, N. H., Feldman, H. M., & Pall, M. L., Derepression of Tyrosinase Synthesis in Neurospora by Cycloheximide, Actinomycin D, and Puromycin, Journal of Biological Chemistry, 245, pp. 2784-2788, 1970.

[18] Sugoro, I., Sasongko, Astuti, D.I., & Aditiawati, P., Comparison of Gamma Irradiated and Raw Lignite in Bioliquefaction Process by Fungus T5, Atom Indonesia, 38, 2, pp. 51-56, 2012.

[19] Romanowska, I., Strzelecki, B., & Bielecki, S., Biosolubilization of Polish Brown Coal by Gordonia alkanivorans S7 and Bacillus mycoides NS1020, Fuel Processing Technology, 131, pp. 430-436, 2015.

[20] Cohen, M. S., Enzymatic Solubilization of Coal, Chapter In Wise, D. L. (Ed): Bioprocessing and Biotreatment of Coal, Marcel Dekker, Inc., New York, 1990.