Fertilizer is one of the vital ingredients in plant growth and nitrogen is the main nutrient in the agroecosystems (Abalos et al., 2019; Gogoi et al., 2018; Ichihashi et al., 2020; Umar et al., 2020). Wastewater produced by urea fertilizer factories has characteristics, namely the presence of high urea and ammonia content (Machdar et al., 2018). According to Swaminathan (Swaminathan et al., 1984), for every ton of urea produced, about 12 m$^3$ of water is needed and 2.3 m$^3$ of wastewater is produced. In the wastewater stream, the concentration of urea is between 1500–10 000 ppm and ammonia is between 400–3000 ppm (Darmadi, 2014; Prasetyo et al., 2019). This wastewater containing high concentrations of urea and ammonia will trigger eutrophication which is very detrimental to aquatic organisms (Afifah et al., 2020; Suryawan et al., 2021; Suryawan I.W.K. et al., 2021). Ammonia content usually increases when contaminated with domestic and agricultural waste because ammonia is found in urea fertilizers and detergents. However, the ammonia content can decrease when a body of water contains a high content of dissolved oxygen (DO).

Black liquor is a residual cooking solution from a pulp mill with a chemical process. In the sulfite process, the chemical commonly used is sodium bisulfite (NaHSO$_3$) (Apriani et al., 2020; Singh & Chandra, 2019). In pulp mills with alkaline processes (soda process...
and kraft process), the black liquor content is not lignosulfonates but lignin insoluble in water and other compounds (Reyes et al., 2020). Black liquor has the main component of water, inorganic compounds derived from the chemical residue of wood chip cooking, the reaction products during the cooking process, and organic compounds derived from the wood chips. In the pulp and paper industry, lignin must be separated from cellulose to obtain whiter fibers because lignin turns paper yellow (Reddy et al., 2007). The content of inorganic components in black liquor reaches ~ 45% (Magdeldin & Järvinen, 2020). Besides cellulose, many important polymer organic substances in the plant world are lignin. Lignin is found in the cell wall and partly in the middle lamella (in the intercellular area). The structure of lignin is very diverse depending on the type of plant. In general, lignin polymers are composed of phenyl propane units, namely p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which are the parent compounds (pre-substance) of lignin (Davin & Lewis, 2005). The purpose of this study was to determine the effect of using isolated lignin from black liquor as urease inhibitor.

**MATERIAL AND METHOD**

**Black liquor-based inhibitor**

Two solutions were prepared to study the inhibition of urease by black liquor. To hydrolyse the urea, solution of urease enzyme was added to the mixture of urea, ethylenediaminetetraacetic acid (EDTA) and buffer solution. To study the inhibition, urease was mixed with isolated lignin from black liquor as inhibitor and the mixture was then added to the mixture of urea, EDTA and buffer solution.

**FTIR Analysis**

FTIR Thermo NicoletTM iSTM 5 was performed to determine functional group of the samples. Dried isolated lignin from black liquor as inhibitor was mixed with solid potassium bromide (KBr) (0.2–1%), then ground and pressed to form pellet. Each FTIR spectrum was recorded with a blank KBr pellet as background. The samples were then run under transmittance mode in the wavelength range of 500 cm⁻¹ to 4000 cm⁻¹.

**RESULT AND DISCUSSION**

**Characterization of black liquor**

Infrared radiation, which is essential in determining the structure or analysis of functional groups and is most widely used for practical purposes, is the medium infrared region with a wavenumber between 4000–500 cm⁻¹ (Silverstein & Bassler, 1963). The FTIR spectrum of lignin shows several prominent absorption bands that can be assigned empirically to the groups present in the lignin molecule. Several bonds or extensions that should exist in lignin are OH stretching, C=O stretching, aromatic ring vibration, guaiacil ring vibration, and ring vibration (Kim et al., 2017; Nada et al., 1998). The lignin-based inhibitor shows several peaks subjected to FTIR (Figure 1). Some are OH stretching at wavelength 3343, C=O stretching at wavelength 1635, aromatic ring vibration, guaiacil ring vibration, and ring vibration (Kim et al., 2017; Nada et al., 1998). The lignin-based inhibitor shows several peaks subjected to FTIR (Figure 1). Some are OH stretching at wavelength 3343, C=O stretching at wavelength 1635, aromatic ring vibration at wavelength 1558, cylindrical ring vibration at wavelength 1370, and guaiacil ring vibration at wavelength 1111. These results indicate that lignin was successfully recovered from black liquor. This is because the syringyl and guaiacil rings are the main parameters of a lignin-containing material, these bonds...
are obtained from the hardwood part of the plant (Kim et al., 2017).

The TGA curve for waste-based lignin, i.e., black liquor after isolation, can be seen in Figure 2. An initial weight loss of about 10% occurs near 10 °C due to moisture loss remaining in the extracted lignin (Kim et al., 2017). Primary weight loss occurs after about 200 °C. This can be explained that around 250 °C, lignin can soften, melt and then unite the lignin particles (Baliga et al., 2003). At this thermal stage which is about 250 °C to 500 °C, the guaiacyl or syringyl components of the lignin and their chemical structure begin to change, resulting in some weight loss. Due to phenol degradation further weight loss occurring above 500 °C is possible (Haykiri-Acma et al., 2010).

**Ammonia release**

After testing the urease enzyme inhibition with waste-based lignin, the results are as shown in Figure 3. In this inhibition test, two tests were carried out. The first treatment was the urea and urease enzyme, which was to assess the performance of the urease enzyme when mixed with urea without any inhibitors or lignin. The second test performed urea and urease enzymes mixed with lignin-based waste, which determines the performance of lignin-based waste inhibition on ammonia concentrations.

The ammonia change is plotted against time as lignin starts in contact with the urease enzyme. Changes in ammonia concentration were significant differences by addition of lignin-based inhibitor. The addition of lignin-based inhibitor can prevent hydrolysis of urea to ammonia. The effect of waste-based lignin on ammonia concentration can be seen significantly in each experiment (Table 1).

The result in Figure 3 shows that lignin based waste effectively inhibits the urease enzyme, which usually accelerates the urea hydrolysis reaction (Ramli et al., 2014). The parent compounds
in lignin such as p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol can inhibit the hydrolysis reaction, thereby inhibiting the response between urea-urease (Davin & Lewis, 2005; Ramli et al., 2014). Meanwhile, the highest ammonia concentration was found at 80-minute treatment, 0.189 mg/L. In this treatment, the urease enzyme accelerates the formation of ammonia. This is because urease enzyme plays an essential role as a catalyst for the hydrolysis of urea into ammonia and carbamic acid, which takes place spontaneously (Sujoy & Aparna, 2012).

**CONCLUSION**

The use of urea is associated with adverse environmental impacts and is a key source of greenhouse and harmful gas emissions. FTIR data revealed the presence of lignin in isolated black liquor. The addition of enzymes to urea tends to increase the release of ammonia. On the other hand, the use of black liquor can inhibit the release of ammonia.

**Aknowladgement**

This research was a collaboration Universitas Pertamina and Universiti Teknologi PETRONAS with contract number 010/UP-WR3/SK/II/2019. In addition, this research was also supported by final project with contract number 0001A/UP-DKN3/SK/I/2020.

**REFERENCE**

3. Apriani, R., Manik, N.N., Mahardhika, E.H., &


