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DECOMPOSITION OF NON–WOODY FIBRE FROM NAPIER GRASS BY INDIGENOUS ISOLATE POTENTIAL FOR BIOPULPING PROCESS

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Abstract

Biopulping of a non-woody fiber could be a sustainable process towards a greener and safer process compared to the conventional pulping methods. Current research is focused on isolation of potential bacterial from soil for biopulping activity. Thirty two isolates were successfully recovered in the first stage of isolation on a nutrient agar supplemented with the non-woody fiber. Further isolation was performed by culturing the 32 isolates in a broth containing 1% of non-woody fiber at 45 ± 5°C. Only five isolates (isolate 5, 7, 8, 27 and 28) were able to grow under the aforementioned condition. Among the five isolates, isolate 28 showed the highest percentage of decomposition which was 74.8%. The isolate was identified as a Gram negative bacteria, had a coccus shaped and occurred in a streptococci of cells arrangement. In addition, the environmental factors that may affect the decomposition of the non-woody fiber such as pH and raw material size were examined. It was found that pH in the range did not significantly affect the decomposition ability of the bacteria. But the size of raw material of 0.5 cm may assist in improving the decomposition ability compared to the 2 cm and 1 cm sizes.

Keywords: Biopulping; Decomposition; Non-woody fiber; Napier grass; Pennisetum purpurem;

1.0 INTRODUCTION

Fiber from wood is the main source for pulp in the conventional paper making industry. Due to deforestation issue, an alternative fiber from non-wood sources will provide a good solution to prevent environmental destruction from cutting down trees. Agro waste materials such as Napier grass which are high in fiber contents are abundance in Malaysia (Shahida Hanum et al., 2015) [1]. The grass has the advantages of high cellulose content and fiber compactness that make this non-wood material a suitable alternative pulp for paper industry (Zawawi et al., 2013) [2]. Pulp and paper manufactured from wood is a chemical intensive process (Torres et al., 2012) [3]. Chemical pulping gives superior paper quality but the impact on environment is very high as it produces a strong liquid effluent that needs to be treated (Singh et al., 2010) [4]. Hence, biological pulping has the potential to reduce energy costs and environmental impact related to traditional pulping operations (Malherbe and Cloete, 2002) [5].

Although white-rot fungi was a well-known microorganism as a lignin biodegrader for biopulping (Bajpai, 2012) [6] but they are sometimes not practical under extreme environmental and substrate conditions (Wang et al., 2013) [7]. Therefore, the main objective of this paper is to isolate the bacteria that are capable of decomposing of the non-woody fiber from Napier grass. The environmental factors that may support the decomposition process were also studied. Isolates from this study will be assessed in a later study to identify their potential for biopulping.

2.0 METHODOLOGY

Isolation of non-wood fiber decomposing bacteria (Huang et al., 2007) [8]

Soil from local area was used as the source of the potential bacteria. A solution containing of 10% of soil and 10% of sterile and grinded grass was streaked on a sterile and solidified nutrient agar (Merck). The agar was incubated at 37.5°C for 24 hours. Further isolation was done by inoculating the isolates that grew on nutrient agar into LBY broth and incubated at 45±5°C for 24 hours. Measurement of the bacterial growth was determined through the optical density reading at 600nm of wavelength. Next, the bacteria that grew at 45±5°C were cultured in LBY broth containing 1% of sterile grass and percentage of decomposition was determined. Gram staining was done to the isolate with the highest percentage of decomposition only.

Decomposition percentage formula:

$$\left(\frac{\textit{Average of fermentative leaves} - \textit{Average of intact leaves}}{\textit{Average of fermentative leaves}}\right) \times \mathbf{100\%}$$

Effect of pH and raw material sizes on decomposition process

The effect of pH on decomposition process was determined at pH 5.0, 6.5 and 8.0, while the raw material size was kept constant at 2.0 cm length. For the effect of raw materials, the sizes were varied from 0.5 cm to 1 cm and 2 cm of length. At the same time, pH of the culture was maintained at pH 8.0. In both experiments, the bacteria were cultured in LBY broth containing 1% of sterile raw material and incubated for 1 week at 45±5°C and 200 rpm of agitation. A 10% of a 24 hours inoculum was used to inoculate the broth and each experiment was performed in triplicate.

3.0 MAIN RESULTS

Isolation of decomposing bacteria

In the first step of isolation, 32 isolates were identified to be able to grow on nutrient agar supplemented with 1% of fiber source. All isolate was denoted as isolate 1 to isolate 32. When the 32 isolates were cultured in LBY broth at 45±5°C, only 5 isolates showed a significant growth compared to control (Figure 1). Among the five isolates, isolate 28 showed the highest reading of optical density absorbance which was 0.363 compared to 0.089 abs for the control. While the lowest absorbance was 0.113 abs by isolate 8. Another three isolates that showed significant growth was isolate 5, 7 and 27 with the absorbance reading of 0.171, 0.13 and 0.307 abs respectively. In a subsequent experiment, isolate 28 displayed the highest decomposition with 74.8% percent of decomposition of the non-woody fiber (Figure 2). While isolate 5 was able to decompose 58.7% of the fiber, followed by isolate 7 with 52.3% decomposed. The lowest decomposition percentage was shown by isolate 8 and 27 with 33.9% and 24.3% respectively. Gram staining on isolate 28 showed that the bacteria was a gram negative type of bacteria (Figure 3). It was observed that it had a coccus shaped and from the cells arrangement it was determined as a streptococci cells. Since isolate 28 demonstrated the highest percentage of decomposition, so, it was used in a subsequent study to determine the effect of pH and raw material sizes on decomposition of the non-woody fiber.

Effect of pH and raw material sizes

Investigation had demonstrated that pH had no significant effect on decomposition of the non-fiber wood (Figure 4). At all pH value, the decomposition of non-woody fiber was in the range of 84.9% and 87.1%. While, the smallest size which is 0.5 cm was shown to significantly improved the decomposition of the non-woody fiber. The calculated decomposition percentage was 91.4%. This percentage was 36.3% and 31.0% higher than percentage of decomposition at 1 cm and 2 cm length (Figure 5).

4.0 DISCUSSION

White-rot fungi is a well-known microorganism that is being utilized for biopulping in the paper making industry. Nonetheless, there are limitations which greatly hindered the application of biodelignification by the fungi in industry such as long incubation times, poor delignification selectivity, and degradation of large of amount carbohydrates (cellulose and hemicellulose) which decreased the yield of pulp (Li et al., 2008) [9]. In contrary to white-rot fungi, bacteria may have the potential as lignin degrader because of their immense environmental adaptability and biochemical versatility (Wang et al., 2013) [7]. Bacteria which have the potential for biopulping had been reported elsewhere. For instance, Klebsiella aerogenes NCIM 2098 could significantly reduce lignin content of the bagasse (Harit and Mandakini, 2013) [10]. Besides, a lignin degradation bacterial consortium also showed the capability to degrade lignin and was efficient for biopulping (Wang et al., 2013) [7]. In this study, the isolated bacteria was determined as Gram negative bacteria with a coccus shape. However, Huang and Peng, (2007) demonstrated that in their study, all potential isolates were a Gram positive bacteria and from the genus of Bacillus. Still, isolate 28 from current study did demonstrate its ability to decompose the Napier grass fiber at the investigated conditions. However, further investigation are required to determine lignin degradation ability of these isolate since this ability will further confirm its potential for biopulping.

5.0 CONCLUSION

In this study, a gram negative bacteria denoted as isolate 28 with coccus shaped and in a streptococci arrangement was successfully isolated from soil. The bacteria showed a high non-woody fiber decomposition among other isolates. Environmental condition such as pH did not significantly affect the decomposition process. However, when the smallest size of raw materials was used which was 0.5 cm, the decomposition percentage was improved compared to size 1 and 2 cm. Thus, in a further research, the identity of the isolated bacteria will be identified and to explore its full potential as a non-woody fiber decomposing bacteria for biopulping purpose.

6.0 FIGURES

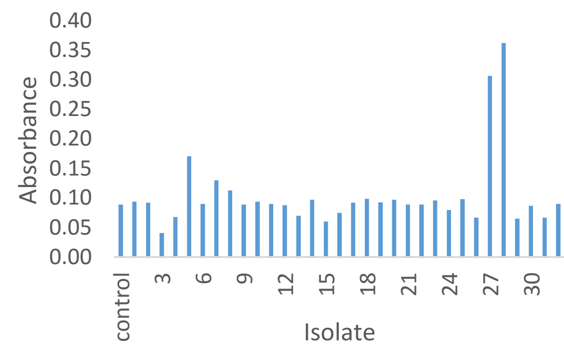

Fig. 1. Optical density (Abs) reading of 32 isolates cultured in LBY broth.

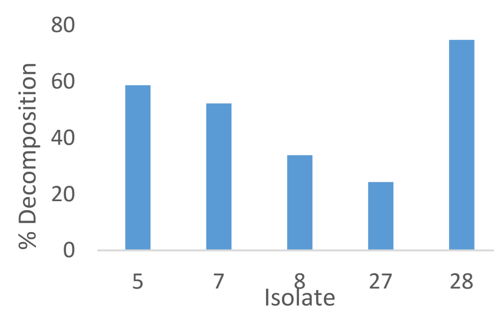

Fig. 2. Percentage of decomposition of five isolates in LBY broth containing 1% of grass.

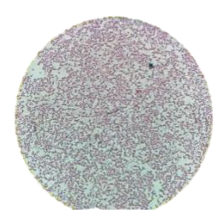

Fig. 3. Gram staining of isolate 28 indicated that the isolate was a gram negative bacteria.

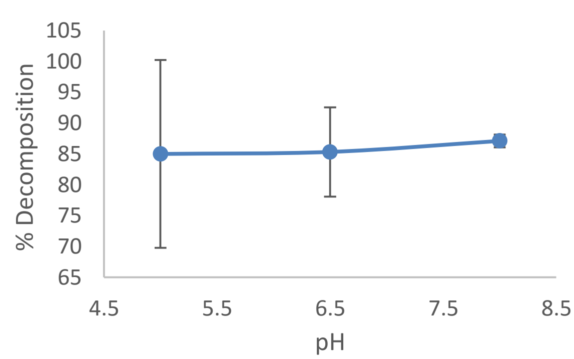

Fig. 4. Effect of pH on decomposition of the nonwoody fiber, Napier grass.

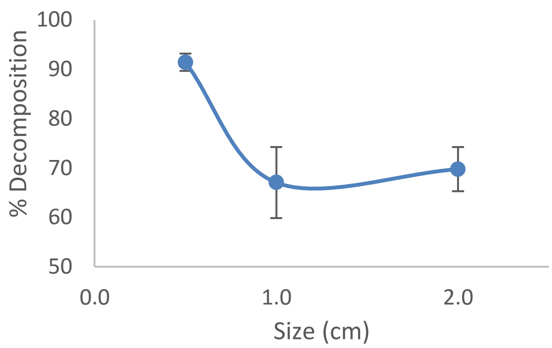

Fig. 5. Effect of raw material sizes on decomposition of the nonwoody fiber, Napier grass.

Appendix

Figures of decomposed non-woody fiber. On the left is fermentative leaves and on the right is intact leaves that had been separated from fermentative leaves.

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