2011 International Conference on Food Engineering and Biotechnology
IPCBEE vol.9 (2011) © (2011)IACSIT Press, Singapore

AROMATIC BENZALDEHYDE FROM ORYZAE SATIVA

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Abstract. Most of the vanillins used nowadays come from the chemical synthesis of guaicol, which offers a high price rather than natural vanillin extracted from *Vanilla planifolia*. The natural vanillin derived from the plant is very expensive due to the tedious extraction process which takes a few months to produce a high quality vanillin. However, vanillin derived from biotransformation of lignocellulosic biomass also has a good potential to be exploited based on the large number of biomass which are under-utilized. In this study, the ferulic acid was extracted from the paddy straw by alkaline hydrolysis and was further utilized as a substrate for vanillin synthesis. The biotransformation study was carried out by *Aspergillus niger* and *Phanorochaete chrysosporium* through two steps of bioengineering. The optimum vanillic acid obtained in the first phase of fermentation was 50% while 12% of vanillin was obtained in the second phase of the fermentation.

Keywords: paddy straw, alkaline hydrolysis, A.niger, P.chrysosporium, vanillin

1. Introduction

Paddy straw in Malaysia is abundantly available and is under-utilized. The open burning practice of the straw has created several problems like the greenhouse effect, air pollution and health problem especially for asthmatic people. Thus, it is imperative to create a solution protocol on the waste management rather than burning it. One of the possible solutions is to extract the phenolic compound particularly ferulic acid which is attached in the plant cell wall through alkaline hydrolysis. The ferulic acid initiates the lignifications in the plant, increases the extensibility of the plant during the elongation period and protects the plant from diseases. It links with various carbohydrates like mono and disaccharide [1], polysaccharide [2], lignin [3] and other insoluble carbohydrate biopolymer of cell wall [4] to create a cross-linking between each other. The cross-linking seems to inspire the extensibility [5], plasticity, accessibility and digestibility of the cell wall [6]. Ferulic acid known as having a strong antioxidant level and has several potential applications, especially for food, health, cosmetic and pharmaceutical industries. Moreover, ferulic acid also seems to be as a feedstock for biovanillin production by selected fungus. The vanillin, which has intensely sweet aroma, has gained high attraction in related industries because of its wide applications in foods, beverages, fragrance ingredients in perfumes, and also in pharmaceuticals area. Most of the industries are dependent on the synthetic vanillin from guaicol as the natural vanillin is expensive [7].

The ferulic acid bioconversion to vanillin has been widely investigated from lignocellulosic materials such as sugar beet pulp, maize bran, wheat bran and a few more. The first biotransformation of sugar beet pulp to vanillin was discovered in 1999 [8]. They proposed two steps of fermentations, whereby, ferulic acid extracted from the lignocellulosic material was firstly converted to vanillic acid by *Aspergillus niger* I-1472 and the vanillic acid produced was then converted to vanillin by *Pycnoporus cinnabarinus* MUCL 39532. The vanillin obtained from the study was 357 mg/l corresponding to a molar yield of 50%. Laurence with other collaborators have investigated the potential of maize bran as a ferulic acid source [9] since ferulic acid content in maize bran was reported as high as 3.1% [10]. They found that with the addition of cellobiose, it

could inhibit the formation of methoxyhydroquinone as a by-product during the process, and increase the vanillin formation to 767 mg /l with a molar yield of 71%.

However, the biotransformation of paddy straw to vanillin is not researched extensively, and the conducted study has highlighted the probability of paddy straw to be a bio-vanillin source. The obtained ferulic acid was used as a substrate for biovanillin production by *A.niger* and *P.chrysosporium*. The study might minimize the lignocellulosic waste and the process maybe cheaper and used a readily procedure [9].

2. Materials and methods

2.1 Alkaline treatments

Alkaline treatment was carried out through mixing 1 g of straw with NaOH with solid to liquid ratio of 1:30 (g/ml) by refluxing in bath oil as was suggested [11]. After filtration and acidification to pH 2-3, the lignin was precipitated while the supernatant obtained was further extracted with ethyl acetate. After removing of ethyl acetate, the obtained ferulic acid was dissolved in methanol and further analyzed through RP-HPLC.

2.2 Fungus Strain

The strain of *Aspergillus niger* ATCC 16404 (Culture Collection Center of Bioprocess Engineering School, University Malaysia Perlis) and *Phanerochaete chrysosporium* ATCC 24725 (Culture Collection Center of Biotechnology Department, International University Islam Malaysia) were used. The strains from a working cell bank were stored under -80^{o}C in the 50% glycerol to prevent the cells from crystallization.

2.3 Medium and culture conditions for Aspergillus niger and Phanerochaete chrysosporium

The cultures were grown in a basal medium fermentation reported with maltose (20 g/l) as the carbon source and diammonium tartrate (1.82 g/l) as the nitrogen source. Other nutrients included KH_2PO_4 (0.2 g/l), $CaCl_2$ (0.0132 g/l), $MgSO_4$ (0.5 g/l) and 5 g/l yeast extract [12]. The pH of the medium was adjusted to 5.5 before autoclaving. The maltose as a carbon source was sterilized separately and mixed to the culture prior to inoculate accordingly. The inoculation was carried out with 1 x 10^6-10^7 spore/ml of conidiospores in 0.9% Na_2Cl.

The cultivation for the seed culture was carried out in a 250 ml baffled flask with a working volume of 100 ml and was grown for 3 days at 30^{0}C. After 3 days, the seed cultures of *A.niger* and *P.*chrysosporium were transferred to a basal medium and incubated at 30^{o}C separately, with an agitation of 150rpm. In the first part of fermentation, 0.3 g/l of crude ferulic acid obtained from alkaline hydrolysis of paddy straw was added to the culture as an inducer cum substrate for *A.niger* to transform it to vanillic acid on the third day. In the second step of fermentation, 0.3 g/l of crude vanillic acid obtained from the first fermentation part was introduced to the *P.chrysosporium* followed with 0.5 g/l sterilized cellobiose to transform it to vanillin on the third day.

2.4 HPLC analysis of phenolic metabolites.

Three flasks were removed and filtered through 0.45 µm nylon filters (Whatman, UK) on day 3 to day 8 of the fermentation. The filtrate was analyzed by HPLC model (Shimadzu), equipped with a variable UV/VIS detector. The separation was achieved on a Fisher C_{18} column (RP-18, 125 X 4 mm) as described earlier [13]. The column temperature was fixed at 40^{o}C and the detector wavelength was 250 nm. The chromatograph was connected to the HP Lab Solutions for chromatographic data handling and the quantification was performed using an external standard. The mobile phase, at a flow rate of 0.90 ml/min, comprised of an isocratic linear solvent system of methanol: aqueous trifluoroacetic acid (1 mM) in the ratio of 28: 72 for 30 min. The solvent used were of HPLC grade.

3. Results and Discussion

3.1. Fermentation of ferulate to vanillic acid

The fermentation of ferulic acid obtained after alkaline hydrolysis to vanillic acid was carried out by *A.niger* as illustrated in Figure 3.1. An amount of 0.3 g/l of crude ferulic acid after alkaline hydrolysis of

paddy straw was supplied for three consecutive days started on day 3. The production of vanillic acid starts to increase on day 4 followed by an optimum production on day 6 to give 0.280 g/l and slowly reduced on day 7 to day 8 as a result of low concentration of substrate in the culture. Furthermore, the fungus might metabolized the vanillic acid produced. Total ferulic acid consumed on day 6 was 0.800 g/l which corresponds to 50% molar yield. The obtained value of vanillic acid was in agreement with a study conducted before from rice bran oil which recovered 63.5% vanillic acid by *A.niger*. Experimentation from sugar beet pulp produced approximately 60.5% of molar yield of vanillic acid [8].

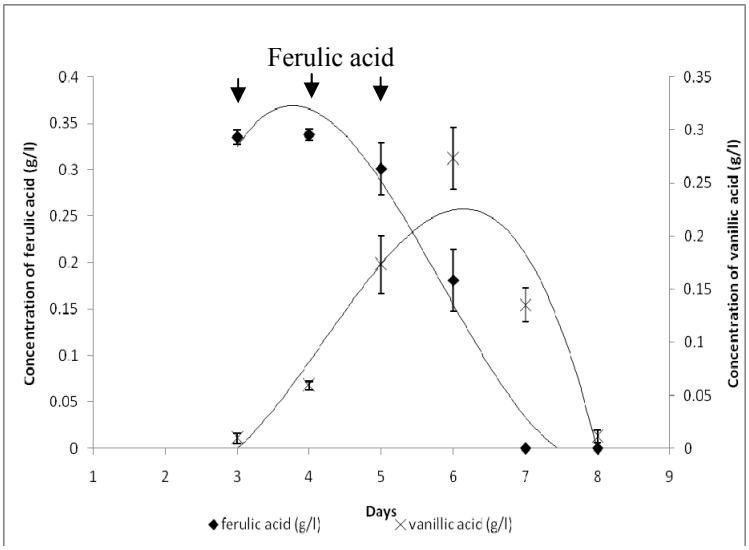

Figure 3.1: The formation of vanillic acid by A.niger in the 3 days daily supplemented with 0.3 g/l crude ferulic acid.

3.2. Fermentation of vanillic acid to vanillin

The consumption and the production of vanillic acid and vanillin respectively are presented in Figure 3.2. It shows that the optimum vanillin which was obtained on day 6 after 3 days consecutively feeding with 0.5 g/l of cellobiose followed by 0.3 g/l of crude vanillic acid 2h later to the culture of *P.chrysosporium.* However, the formation of vanillin was slowly reduced as the fermentation proceeds to day 8 to produce only 0.008 g/l of vanillin. Optimum vanillin production was 0.085 g/l which corresponds to 12% molar yield. The amount was considered low compared to the vanillin obtained from maize bran (0.767g/l) [9], sugar beet pulp (0.357 g/l) [8] and rice bran oil (1.09 g/l) [14]. Even though the rice bran oil has recorded to give the highest concentration of vanillin, however; the rice bran oil itself as a ferulic acid sources was expensive due to the numerous nutritious compound in it and it also competes with other profitable product based on its strong antioxidant.

The ferulic acid from paddy straw was offered at low price and optimization of the biotransformation process might enhanced the production of vanillin. The purification of crude ferulic acid obtained might improved the vanillin production as it could eliminate the inhibitors like furfural and hydrxymethyl-furfural (HMF) [15-17] to the biotransformation process. Furthermore, the use of selective absorbent to trap the vanillin produced might increased the vanillin production.

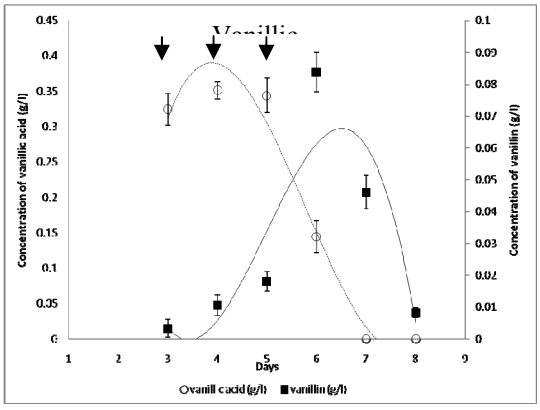

Figure 3.2: The formation of vanillin from vanillic acid obtained in the second step of the fermentation

3.3. Characterization of crude vanillin

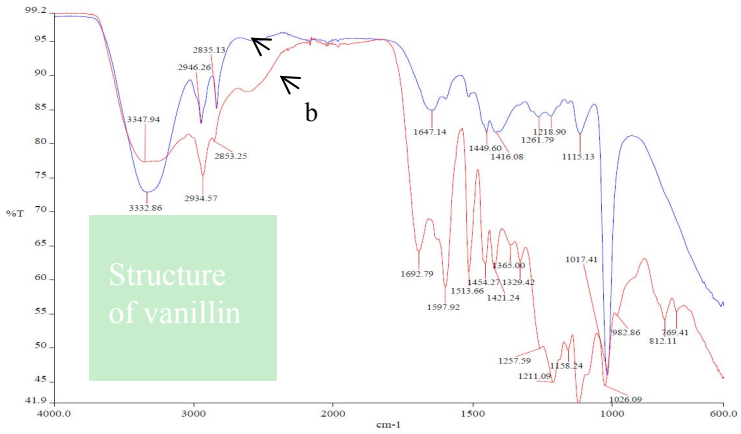

Figure 3.3 : Comparison of FT-IR spectra between (a) standard vanillin and (b) crude vanillin

The crude vanillin obtained after fermentation was further characterized by FT-IR (Figure 3.3). The comparison band between crude vanillin and standard vanillin was in the frequency range of 4000cm^{-1} to 600cm^{-1}. The broad band at 3347.94cm^{-1} for crude vanillin and band at 3332.86cm^{-1} for standard vanillin indicated the characteristic of OH group, whereby, the carbonyl group of vanillin (C = O) was designated at 1647.14cm^{-1} and at 1692.79cm^{-1} for standard vanillin and crude vanillin respectively. The methyl group of the vanillin was presented by the C-H vibration at band 1449cm-1 and 1416cm-1 for standard vanillin and band at 1421cm^{-1} and 1421cm^{-1} for crude vanillin. Strong band at 1017cm^{-1} and 1026.09cm^{-1} for standard vanillin and crude vanillin respectively indicated for C-O stretch in the ester group of vanillin compound [18]. The crude vanillin obtained in the study has the characteristic which was almost similar to the commercial vanillin, thus; this proved that vanillin produced by biotransformation of ferulic acid from paddy straw also could be one of the vanillin sources.

4. Acknowledgment

The author would like to gratefully acknowledge the School of Bioprocess Engineering, University Malaysia Perlis for providing the facilities to undertake the research.

5. Conclusion

The ferulic acid obtained from alkaline hydrolysis used as a substrate for the biotransformation to vanillic acid was carried by *A.niger*. The vanillic acid was detected to be optimum on day 6 to give 0.28g/l corresponding to 50% molar yield of vanillic acid. In the second phase of fermentation carried by *P.chrysosporium,* the optimum of vanillin produced was observed on day 6 which gave 0.085 g/l of vanillin corresponding to 12% molar yield. The crude ferulic acid used during the first phase of the fermentation may be one of the factors that contributed to the low production of vanillin. Although, the vanillin obtained in the study was comparably low with other investigation of the same nature, however; ferulic acid extracted from paddy straw was a good substrate for biotransformation of vanillin.

6. References

[1] Bokern, M., Heuer, S., Wray, V., & Macek, T. Ferulic acd conjugates and betacyanins from cell cultures of *Beta vulgaris*. P*hytochemistry.* 1991,30, 3261-3265.

[2] Meyer, K., Kohler, A., & Kauss, H. Biosynthesis of ferulic acid esters of plant cell wall polysaccharides in endomembranes from parsley cells . *FEBS Letters,* 1991, 290, 209-212.

[3] Scalbert, A., Monties, B., & Rolando, C. Formation of ether linkage between phenolic acid and graminae

lignin: A possible mechanism involving quinone methides. *Holzforschung,* 1986, 40, 191-195.

[4] Hartley, R., Morrison, W., & Borneman. Cross-linking of cell wall phenolic arabinoxylans in graminaceous plant. *Phytochemistry,* 1990, 29, 3705-3709.

[5] Tan, K., Hoson, T., Masuda, Y., & Kamisaka, S. Correlation between cell wall extensibility and the content of diferulic and ferulic acids in the cell walls of oryza sativa coleoptiles grown under water and in air. Physiology *of Plant,* 1991,83, 397-403.

[6] Lam, T., & Liyama, K. Characteristics of senescent straw cell walls of dwarf, semidwarf and normal strains of rice (Oryzae sativa) plants. *Journal of Wood Science,* 2000, 46, 376-380.

[7] Priefert, H., & Steinbuchel, A. Biotechnological production of vanillin. *Applied Microbiology and Biotechnology,* 2001, 56, 296-314.

[8] Laurence Lesage Meesen, C., Anne, L., Delphine, C., & Michele, A. Fungal transformation of ferulic acid from sugar beet pulp to natural vanillin. *Journal of Agricultural and Food Chemistry,* 1999, 79, 487-490.

[9] Laurence Lesage Meesen, L. A., Bonnin, E., Thibault, J., Buleon, A., Roller, M., Asther, M., et al. A biotechnological process involving filamentous fungi to produce natural crystalline vanillin from maize bran. *Applied Biochemistry and Biotechnology,* 2002, 102, 141-153.

[10] Saulnier, L., Marot, C., Elgorriaga, M., & Bonnin, E. Thermal and enzymatic treatments for the release of free ferulic acid from maize bran. *Carbohydrate Polymers,* 2001, 45, 269-275.

[11] Sun, R., Sun, X., Wang, S., & Zhu, W. Ester and ether linkages between hydroxycinnamic acids and lignin from wheat, rice, rye and barley straws, maize stems and fast growing poplar wood. *Indstrial Crops and Products,* 2002, 15, 179-188.

[12] Laurence Lesage-Meessen, Delattre, M., Haon, M., & Thibault, J. A two step bioconversion process for vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporous cinnabarinus*. *Journal of Biotechnology,* 1996, 50, 107-113.

[13] Sachan, D., Ghosh, H., & Mitra, A. Detection of major phenolic acids from dried mesocarpic husk of mature cocunut by thin layer chromatography. *Industrial Crops and Products,* 2004,18, 171-176.

[14] Lirong, Z., Zheng, P., Bai, Y., Wang, J., & Guo, X. Production of vanilln from waste residueof rice bran oil by *Aspergillus niger* and *Pycnoporus cinnabarinus*. *Bioresource Technology,* 2007, 98, 1115-1119.

[15] Laser, M., Schulman, D., Allen, S., & Lichwa, J. A comparison of liquid hot water and steam pretreatments of sugarcane baggase for bioconversion to ethanol. *Bioresource Technology,* 2002, 81, 33-44.

[16] Mosier, N., Wyman, C., Dale, B., & Elander, R. Features of promising technologies for pretreatment of lignocelluloisc biomass. *Bioresource Technology,* 2005, 96, 673-686.

[17] Demirbas, A. Mechanisms of liquefaction and pyrolysis reactions of biomass. *Energy Conversion and Management,* 2000, 41, 633-646.

[18] Mohamad Nasir, M., & Nor Nadiah, M. N. Separation and characterization of the vanillin compound from soda lignin. In *Innovations in Chemical Biology,* 2009.