Pretreatment Conditions for Hydrolysates from Unbleached Pulp Waste for Ethanol Fermentation with Xylose-fermenting Yeast, Candida intermedia 4-6-4T2

Masaru Saito, Hiroshi Nagasaki, Tomoaki Ikeda, Kiyotaka Saga, Koji Yoshida, and Shigeyuki Watanabe

Research & Development Center, Safety & Technology Management Unit, Cosmo Oil Co., Ltd., 1134-2 Gongendo, Satte, Saitama 340-0193, JAPAN
Biomaterial in Tokyo Co., Ltd., 5-23-32 Higashi Ooi, Shinagawa-ku, Tokyo 140-0011, JAPAN
Research & Development Dept., Safety & Technology Management Unit, Cosmo Oil Co., Ltd., 1-1-1 Shibaura, Minato-ku, Tokyo 105-8528, JAPAN

(Received October 2, 2017)

Steam explosion conditions were evaluated as pretreatment for unbleached pulp waste to prepare fermentable sugars with xylose fermenting yeast, Candida intermedia 4-6-4T2. The hydrolysates pretreated under low or moderate severity conditions (severity factor ($R_o$) = 3.53 or 4.12) did not contain known inhibitors to fermentation such as organic acids, furans and lignin-derived aromatic compounds in the LC-Mass (LC-MS) and HPLC analyses. C. intermedia 4-6-4T2 simultaneously converted both xylose and glucose in these hydrolysates to ethanol within 24 h. The ethanol yield was 0.40 (g/g) or 0.41 (g/g), respectively. In contrast, hydrolysates pretreated under high severity conditions ($R_o$ = 4.71) contained thirteen lignin-derived aromatic compounds, of which eleven were quantified by HPLC analyses. The concentrations of furans or lignin-derived aromatic compounds in the hydrolysate were less than 0.2 g/L or 0.02 g/L, respectively. The ethanol yield was 0.21 (g/g) after 24 h fermentation. C. intermedia 4-6-4T2 did not completely convert xylose and glucose to ethanol even during longer fermentation periods (48 h). Model sugar solutions containing higher concentrations of furans and lignin-derived aromatic compounds in 0.1 M KH$_2$PO$_4$ containing 3 g/L of acetic acid adjusted to pH 6 with KOH than in the high severity hydrolysate did not affect the fermentation of C. intermedia 4-6-4T2. Therefore, the inhibitory effect on the hydrolysate pretreated under high severity conditions ($R_o$ = 4.71) was not induced by previously identified compounds such as furfural, HMF and lignin-derived aromatic compounds, but may be induced by other unidentified compounds.

Keywords
Candida intermedia 4-6-4T2, Steam explosion, Acetic acid, Furan, Lignin-derived aromatic compound

1. Introduction

Pretreatment is an important part of ethanol production from lignocellulosic biomass resources. Hydrolysis of hemicelluloses is essential and removal of lignin is desirable to dissolve the cellulosic fiber structures and increase enzyme accessibility as well as enzyme hydrolysis activity. Typical pretreatments include hot water, steam explosion, acid or alkali explosion, and ammonia fiber expansion, all of which have specific advantages and disadvantages. Acid or alkali explosion requires lower processing temperatures than steam explosion but the acid/alkali must be neutralized before the enzymatic hydrolysis and fermentation processes. Unbleached pulp waste is a type of uncommercialized substrate obtained in the process of preparing cellulose, and is a relatively pure cellulosic material which could be easily saccharified by steam explosion pretreatment followed by enzymatic hydrolysis due to the low concentration of lignin.

The present study analyzed furans and lignin-derived aromatic compounds in the hydrolysate pretreated under very severe conditions ($R_o$ = 4.71) to assess the effect on ethanol production from xylose and glucose in unbleached pulp waste with C. intermedia 4-6-4T2.

2. Material and Methods

2.1. Materials
Sugars, acetic acid, formic acid, 4-oxopentanoic acid
(levulinic acid), 2-hydroxyfuroic acid, 2-furaldehyde (furfural), 5-(hydroxymethyl)-2-furaldehyde (HMF) and 4-hydroxybenzoic acid were purchased from Wako Pure Chem. Ind., Ltd.

(E)-3-(4-hydroxyphenyl)-2-propenoic acid (p-coumaric acid), 4-hydroxy-3-methobenzoic acid (vanillic acid), 4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid), (2E)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-enolic acid (ferulic acid), 3,4-dihydroxybenzaldehyde (protocatechualdehyde), 4-hydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde (vanillin), (E)-3-phenyl-2-propenoic acid (cinnamic acid) and 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde) were purchased from Tokyo Chemical Industry Co., Ltd.

2.2. Steam Explosion and Enzymatic Hydrolysis

Unbleached pulp wastes were shredded using an ASMIX S81C (ASKA Co., Ltd., Japan) cross shredder to 20 × 3 mm. Steam explosion experiments were carried out using a steam explosion apparatus (Nihon Dennetsu Co., Ltd.) at fixed residence time (15 min) with various temperatures (°C). In each experiment, 150 g (dry matter) of shredded unbleached pulp waste was soaked in 2 L of water overnight and then excess water was removed by filtration. Moisture content of the impregnate raw materials was about 60-70 %. Steam explosion samples were independently weighed out to obtain 10 % (w/v) substrate concentration in 20 L in the 30 L reactor.

For hydrolysis of cellulose, ten FPU of Cellic CTec2 (Novozymes Corp. Denmark) was added to one g of substrate (dry weight). Hydrolysis was carried out at 50 °C with shaking at 120 rpm on an orbital shaker in sodium acetate buffer (pH 5.0). Severity factors ($R_o$) were calculated from Eq. (1)$^4$.

$$R_o = \log_{10} t(\text{min}) \times e^{(T°C-100)/47.75} \quad (1)$$

For example, pretreatment at 180 °C for 15 min corresponded to $R_o = 3.53$.

2.3. Analyses of Aromatic Compounds by LC-MS

Phenolic compounds were identified by HPLC (Shimadzu LC-30A, Shimadzu Corp.) equipped with an Inertsil ODS-3 column (GL Sciences Inc.) or YMC-Triart PFP (YMC Co., Ltd.) column with photodiode array detection at 270 nm. The operating temperature was 50 °C, and mobile phase was 1 g/L of formic (A) acid-acetonitrile (B) gradient (v/v) (A/B = 95/5 (0-5 min), 95-50/5-50, (5-35 min), 50-5/50-95, (35-40 min), 5/95 (40-45 min)) at a flow rate of 1.0 mL/min. Electron spray ionization and mass spectra used a Q Exactive (Thermo Fisher Science) between 270-290 nm.

2.4. Analyses of Aromatic Compounds by HPLC

The concentrations of aromatic compounds (cinnamic acid, 4-hydroxybenzoic acid, ferulic acid, p-coumaric acid, syringic acid, vanillic acid, 4-hydroxybenzaldehyde, vanillin, syringaldehyde, coniferyl aldehyde and protocatechuic aldehyde) were analyzed and quantified by HPLC (Shimadzu LC-30A, Shimadzu Corp.). The system was operated in the same way as the LC-MS analyses and detected at 270 nm.

2.5. Preculture, Fermentation and Analyses

The detailed preculture and fermentation conditions have been described in a previous report$^3$. Briefly, C. intermedia 4-6-4T2 was precultured in Erlenmeyer flasks (1 L in a 2 L flask) containing YE-YNB media with 40 g/L of xylose plus 10 g/L of glucose as the carbon source for 72 h at 30 °C and the fermentation activity was tested with the following method. The obtained preculture was collected and transferred to a 200 mL flask (1 % (dry cell weight)) fitted with a sponge cap (Shin-Etsu Polymer Co., Ltd.) and incubated with shaking at 120 rpm for 48 h at 30 °C under limited aerobic conditions in 100 mL of each hydrolysate. At periodic intervals, the fermenting samples were collected and the concentrations of ethanol, sugars and aliphatic organic acids were analyzed by HPLC (Agilent HPLC 1100, Agilent Technologies) using a Bio-Rad Aminex HPX-87H lead column (300 by 7.8 mm) at 45 °C by refractive index detector, and furans were analyzed similarly by diode array detector at 260 nm. The mobile phase was 5 mM sulfuric acid at a flow rate of 0.6 mL/min.

3. Results

3.1. Analyses of Hydrolysates from Unbleached Pulp Waste Pretreated by Steam Explosion

Figure 1 illustrates the total process from pretreatment to fermentation based on previous reports and our methods for ethanol production with C. intermedia 4-6-4T2$^{3,8}$. Table 1 summarizes the analytical findings of compounds in the hydrolysates from unbleached pulp waste pretreated by steam explosion with various severities considering the previous review of inhibitors. Figure 2 shows the lignin-derived aromatic compounds in the hydrolysate pretreated under severe conditions ($R_o = 4.71$) identified by LC-MS analyses$^3$. The concentration of the recovered sugars in the hydrolysates pretreated under different conditions was increased, but the ratio of xylose to glucose was decreased with more severe conditions (Fig. 3). Thirteen lignin-derived aromatic compounds were present in the hydrolysates treated under severe conditions, of which eleven compounds were quantified by HPLC analyses utilizing reference standard samples (Figs. 4 and 5). The concentration of all lignin-derived aromatic compounds was less than 0.1 g/L, even in the hydrolysate pretreated
Furfural, HMF and aliphatic acids are degraded compounds of sugars. Lignin-derived compounds are phenolic and non-phenolic aromatic compounds.

Fig. 1 Illustration of the Total Process for Cellulosic Biomass from Pretreatment by Steam Explosion to Fermentation with *C. intermedia* 4-6-4T2 and Production of Inhibitors

Table 1 Comparison of Compositions after Steam Explosion Pretreatment and Prepared Model Sugar Solutions

| Pretreatment conditions | 180 °C 15 min | 200 °C 15 min | 220 °C 15 min | 220 °C model solution-A | 220 °C model solution-B | 220 °C model solution-C | 220 °C model solution-D |
|-------------------------|---------------|---------------|---------------|------------------------|------------------------|------------------------|------------------------|
| Severity factor         | pKₐ           |               |              |                        |                        |                        |                        |
| Glucose                 | 77.5          | 83.5          | 98.9         | -                      | -                      | -                      | -                      |
| Xylose                  | 17.0          | 16.5          | 11.2         | 18.2                   | 18.2                   | 18.2                   | 18.2                   |
| 2-Furaldehyde (furfural)| 0.41          | <0.01         | 0.14         | 1.0                    | 0                      | 0.5                    | 0.5                    |
| 5-(Hydroxymethyl)-2-furaldehyde (5-HMF) | -0.37 | <0.01 | <0.01 | 0.016 | 0 | 1.0 | 0.5 | 0.5 |
| Fran-2-carboxyllic acid (2-furoic acid) | 3.16 | <0.01 | <0.01 | 0.089 | 0 | 0 | 0 | 0 |
| Acetic acid             | 4.76          | 3.0           | 3.3          | 3.2                    | 3.2                    | 3.2                    | 3.2                    |
| Formic acid             | 3.75          | <0.01         | <0.01        | <0.01                  | 0                      | 0                      | 0                      |
| 4-Oxopentanoic acid (levulinic acid) | 4.59 | <0.01 | <0.01 | <0.01 | 0 | 0 | 0 | 0 |
| 3-(4-Hydroxyphenyl)-2-propenoic acid (p-coumaric acid) | 4.63 | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | 0 |
| (2E)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-enolic acid (ferulic acid) | 4.56 | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | 0.1 |
| 4-Hydroxy-3,5-dimethoxybenzoic acid (syringic acid) | 4.33 | <0.001 | 0.003 | 0.003 | 0 | 0 | 0 | 0.1 |
| 4-Hydroxy-3-methobenzoic acid (vanillin acid) | 4.31 | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | 0.1 |
| 3-Phenyl-2-propenoic acid (cinnamic acid) | 4.44 | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | 0.1 |
| 4-Hydroxy benzoic acid | 4.48 | <0.001 | 0.006 | 0.003 | 0 | 0 | 0 | 0.1 |
| 3,4-Dihydroxybenzaldehyde (protocatechue aldehyde) | -0.01 | <0.001 | <0.001 | 0.055 | 0 | 0 | 0 | 0.1 |
| 4-Hydroxy benzaldehyde | 1.35 | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | 0.1 |
| 4-Hydroxy-3-methoxy-benzaldehyde (vanillin) | 1.21 | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | 0.1 |
| 4-Hydroxy-3,5-dimethoxybenzaldehyde | 0.99 | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | 0 |
| (2Z)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-enal (coniferyl aldehyde) | -0.01 | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | 0 |

a) pKₐ values of protocatechuic aldehyde and coniferyl aldehyde have not been published.
under the most severe conditions \((R_o = 4.71)\) (Table 1). The hydrolysate contained quite small amounts of inhibitors except acetic acid used as the buffer for enzymatic hydrolysis after pretreatment under mild or moderate conditions \((R_o = 3.53 \text{ or } 4.12)\) (Fig. 4 and Table 1). On the other hand, the hydrolysate contained small amounts of furans and lignin-derived aromatic compounds (Fig. 5 and Table 1) after pretreatment under very severe conditions \((R_o = 4.71)\), in addition to unidentified compounds in the HPLC analysis (Fig. 5).

### 3.2 Fermentation for Hydrolysates from Unbleached Pulp Waste Pretreated by Steam Explosion

We previously reported preculture and fermentation methods with acetic acid (3 g/L) at pH of 5.5 using xylose-fermenting yeasts\(^3\). Figure 6 shows the present results for the fermentation of hydrolysates. *C. intermedia* 4-6-4T2 converted xylose and glucose completely to ethanol within 24 h after pretreatment under low or moderate conditions \((R_o = 3.53 \text{ or } 4.12)\). In contrast, *C. intermedia* 4-6-4T2 did not consume most xylose and did not completely consume glucose resulting in low concentration of ethanol, even during 48-h fermentation periods, after pretreatment under very severe conditions \((R_o = 4.71)\) (Fig. 7).

Four different model sugar solutions were prepared in 0.1 M KH\(_2\)PO\(_4\) containing 3 g/L of acetic acid adjusted to pH 6 with KOH that contained much higher concentrations of furfural (0.5-1 g/L) and/or HMF (0.5-1 g/L) with/without inhibitor mixtures (0-0.1 g/L) than those in the hydrolysate. *C. intermedia* 4-6-4T2 mostly consumed and converted the sugars in the model solutions were to ethanol within 48 h (Fig. 7).

![Figure 2](image1.png)

**Fig. 2** Comparison of the Recovered Sugars in Each Hydrolysate after Pretreatment under Different Conditions Followed by Hydrolyzation by the Same Method

![Figure 3](image2.png)

**Fig. 3** Compounds Identified by LC-MS Analyses in the Hydrolysate Pretreated under High Severity Conditions and Compounds Quantified by HPLC Analyses in the Hydrolysates
4. Discussion

The main disadvantage of steam explosion pretreatment is the partial degradation of hemicelluloses and the formation of inhibitory compounds for growth and fermentation such as furans and lignin-derived aromatic compounds\(^9,10\)). Although unbleached pulp waste is a relatively pure material that contains small amounts of lignin abundant in the original cellulosic biomass resources\(^11\)), but lignin-derived aromatic compounds were formed as byproducts of pretreatment under the very severe conditions used in this study.

Acetic acid predominantly remains as the undissociated form (CH\(_3\)COOH) at pH below the pK\(_a\) value, so can permeate the plasma membrane by simple diffusion\(^12\)–\(^15\)). In the presence of glucose, acetic acid enters cells in its undissociated form by facilitated diffusion through Fps1p (aquaglyceroporin channel), then the dissociated form of acetic acid leads to the release of protons (H\(^+\)) and anions (CH\(_3\)COO\(^-\)), which induce intracellular acidification and accumulation of ROS (Reactive Oxygen Species) and the ROS induce mitochondrial dysfunction resulting in apoptosis\(^16\)). Table 1 shows the pK\(_a\) values adopted from the previous published study and the DRUG BANK\(^14,17\)). The concentration of acetic acid was much higher than those of the other compounds, and the pK\(_a\) value of acetic acid is the highest in this study. As reported previously, C. intermedia 4-6-4T2 could convert about 44 g/L of xylose and 88 g/L of glucose in a sugar solution containing 3 g/L of acetic acid to ethanol at pH 5.5 within 48 h\(^19\)). Most of the acetic acid was the dissociated
form at pH 6 and did not affect the fermentation of C. intermedia 4-6-4T2. Therefore, pH adjustment of the hydrolysates to 6 could avoid the effect of acetic acid on fermentation. This could be one of the reasons why fermentation with the hydrolysates pretreated at mild or moderate conditions (Ro = 3.53 or 4.12, respectively) was successful with 3 g/L of acetic acid within 24 h. In addition, more than 30 times lower concentrations of other weak acids such as formic acid and lignin-derived aromatic acids did not seem to affect fermentation. The furans, furfural and HMF are derived from the degradation of pentose sugars and hexose sugars, respectively, and have inhibitory effects on the growth of yeasts and bacteria\(^\text{5,14}\). The explanation of aldehyde inhibition to xylose-fermenting yeasts is that aldehyde dehydrogenase uses nicotinamide adenine dinucleotide (phosphate) (NADH or NADPH) as a cofactor for reducing furfural and HMF to the corresponding alcohols. NADH or NADPH is also a key cofactor for consumption of xylose by xylose-fermenting yeasts so introduc-

![Graphs showing ethanol, glucose, and xylose production](image-url)
ing competition between cell growth and reduction of furans for NADH or NADPH\(^5,6\). The YNB medium contains higher concentration of nicotinamide (about 10 mg/L) as vitamin B\(_3\) than the well-defined medium (1 mg/L)\(^7\). Nicotinamide is a component of NADH or NADPH. Preculture with higher concentrations of nicotinamide may be helpful for reducing the inhibitory effects of furfural, HMF and other aldehydes derived from lignin-derived aromatic compounds, and we previously reported the superiority of preculture methods for fermentation with furans and acetic acid as a USA patent\(^9\). Therefore, the inhibitors in the hydrolysate after pretreatment under severe conditions \((R_o = 4.71)\) were apparently not known compounds in this study.

5. Conclusion

The present study could not identify the compounds that inhibited the fermentation of glucose and xylose by \(C.\) intermedia 4-6-4T2 in hydrolysate pretreated under severe conditions \((R_o = 4.71)\). The conventional inhibitory mechanism based on weak organic acids and furans could not explain this finding. One possibility is that unidentified compounds might affect the fermentation function of \(C.\) intermedia 4-6-4T2. In contrast, \(C.\) intermedia 4-6-4T2 converted xylose and glucose simultaneously to ethanol within 24 h in hydrolysate pretreated by steam explosion \((R_o = 3.53 \text{ or } 4.12)\).

Acknowledgment

This research was partially financed by the New Energy and Industrial Technology Department Organization (NEDO). We express our gratitude to Dr. Motoshi Onoda (Toray Research Center, Inc.) for discussion on the analyses of lignin-derived aromatic compounds.

References

1) Maurya, D. P., Singla, A., Negi, S., Biotech., 5, 597 (2015).
2) Kumar, A. K., Sharma, S., Biore sourc. Bioprocess, 4, 7 (2017).
3) Saito, M., Nagasaki, H., Fujimoto, T., Watanabe, S., J. Jpn. Petrol. Inst., 60, (3), 127 (2017).
4) Overend, R. P., Chornet, E., Gascoligne, J. A., Mathematical and Physical Science, 321, 523 (1987).
5) Liu, Z. L., Appl. Microbiol. Biotechnol., 90, 809 (2011).
6) Jonsson, L., Allriksson, B., Nilvebrant, N.-O., Biotechnol. Biofuels, 6, 16 (2013).
7) Caspeta, L., Castillo, T., Nielsen, J., Frontiers in Bioengineering and Biotechnology, 3, 184 (2015).
8) Alvira, P., Negro, M. J., Ballesteros, I., Gonzalez, A., Ballesteros, M., Bio ethanol, 2, 66 (2016).
9) Oliva, J. M., Saez, F., Ballesteros, I., González, A., Negro, M. J., Manzanares, P., Ballesteros, M., Appl. Microbiol. Biotechnol., 105, 141 (2003).
10) Jurado, M., Prieto, A., Martínez-Alcalá, Á., Martínez, Á. T., Martínez, M. J., Biore sourc. Technol., 100, 6378 (2009).
11) Elliston, A., Wilson, D. R., Wellner, N., Collins, S. R. A., Roberts, I. N., Waldron, K. W., Biore sourc. Technol., 187, 136 (2015).
12) Palmqvist, E., Hahn-Hägerdal, B., Biore sourc. Technol., 121, 25 (2000).
13) Pampulha, M. E., Loureiro-Dias, M. C., Appl. Microbiol. Biotechnol., 31, 547 (1989).
14) Franden, M. A., Pilath, H. M., Mohagheghi, A., Pienkos, P. T., Zhang, M., Biotechnol. Biofuels, 6, 99 (2013).
15) Guo, Z., Olsson, L., FEMS Yeast Res., 14, 1234 (2014).
16) Giannattasio, S., Guarangnella, N., Ždralevic’, M., Marra, E., Front. Microbiol., 33, 1 (2013).
17) https://www.drugbank.ca/drugsDB.
18) Nilsson, A., Gorwa-Grauslund, M. F., Hahn-Hägerdal, B., Lidén, G., Appl. Environ. Microbiol., 71, 7866 (2005).
19) Nagasaki, H., Suzuki, Y., U.S. Pat. US20160002675.
要 旨

Candida intermedia 4-6-4T2株によるエタノール発酵のための燃料精製前処理条件検討

齋藤 優†1, 長崎 宏†1, 池田 智明†1, 佐賀 清崇†2, 吉田 浩代†2, 渡邉 緑幸*3

†1 コスモ石油(株)安全技術統括ユニット 中央研究所, 340-0193 埼玉県幸手市権現堂1134-2
†2 (株) BIOMATERIAL IN TOKYO, 104-0014 東京都品川区東大井5-23-32
*3 コスモ石油(株)安全技術統括ユニット 研究部, 105-8528 東京都港区芝浦1-1-1

未精製燃料を原料に用いたエタノール発酵用紡錘液の調製のため、Candida intermedia 4-6-4T2による発酵実験を実施し、水蒸気爆発条件を評価した。湿和(Severity factor (R.) = 3.53)、または中程度 (R. = 4.12) の水蒸気爆発条件では、LC-MS あるいは HPLC による分析の結果、糖液中の既知の阻害物質(有機酸、フラン化合物、リグニン由来の芳香族化合物)の濃度は低く、C. intermedia 4-6-4T2を用いた発酵実験では、糖液中のある阻害およびグルコースは24時間以内に消費され、エタノールに変換された。水中爆発条件で調製した糖液を発酵した場合のエタノール収率は0.40 (g/g)，また中程度の爆発条件で調製した糖液を発酵した場合のエタノール収率は 0.41 (g/g) であった。一方、酸性の水蒸気爆発条件 (R. = 4.71) では、LC-MS 分析により 13 種類のリグニン由来の芳香族化合物が同定され、11 種類の化合物については HPLC 分析による定量が可能であった。定量の結果、フラン化合物の濃度は 0.2 g/L 以下、またリグニン由来の芳香族化合物の濃度は 0.02 g/L 以下であった。この糖液中での発酵実験では、24時間経過した時点でのエタノール収率は 0.21 (g/g) と低く、発酵時間到達時までキシロースがほとんど消費されず、またグルコースも全てになされてはなかった。発酵阻害物質の影響を検証するために、3 g/L の酢酸を含む 0.1 M リン酸緩衝液に糖液中で定量された以上の濃度のフラン化合物、リグニン由来の芳香族化合物を添加したモデル糖液を数種類調製し (pH 6.0)，発酵実験を行った。いずれの系においても、糖はほぼ完全に消費され、エタノールに変換された。このことから酸性の水蒸気爆発条件 (R. = 4.71) での発酵阻害物質は酢酸、既知のフラン化合物あるいは、リグニン由来の芳香族化合物とは異なる化合物である可能性が示唆された。