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In situ mass spectrometry of glucose decomposition under hydrothermal reactions

Pattasuda Duangkaew*, Shuhei Inoue, Tsunehiro Aki***, Yutaka Nakashimada***, Yoshiko Okamura***, Takahisa Tajima***, and Yukihiko Matsumura**,†**

*Department of Mechanical Science and Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima, 739-8527, Japan
**Division of Energy and Environmental Engineering, Institute of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima, 739-8527, Japan
***Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, 739-8530, Japan
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Abstract−We designed an in situ mass spectrometry (in situ MS) analysis method and developed to identify the products of glucose decomposition under hydrothermal condition for the first time. The in situ MS analysis was performed by coupling a tubular batch reactor with a quadrupole mass analyzer via custom-built connection fittings. The products of glucose decomposition were investigated by in situ MS, mass spectrometry of cold effluent, and high-performance liquid chromatography (HPLC) analysis of cold effluent and the results were compared. At 140 °C, in situ MS and mass spectrometry of cold effluent showed that the decomposition of glucose does not proceed; this was confirmed by comparison with the mass spectral database for glucose. At 180 °C or higher, a clear base fragmentation peak of 5-hydroxymethylfurfural (5-HMF) at position *m/z* 97 and that of furfural at *m/z* 96, formic acid (*m/z*=46) and levulinic acid (*m/z*=116) were observed by mass spectrometry. No levulinic acid or furfural was observed through conventional HPLC analysis under any condition; only glucose, formic acid, and 5-HMF could be detected. The effectiveness of in situ MS analysis is clear, compared to mass spectrometry analysis of cold effluent and HPLC analysis.

Keywords: In Situ Analysis, Mass Spectrometry, Glucose, Hydrothermal Reaction, Quadrupole Mass Analyzer

INTRODUCTION

The increase in global world energy demand is causing a depletion of fossil fuel resources and leading to global warming; therefore, potential sources of renewable energy other than fossil fuels are required. One of the most promising renewable energy sources is marine macroalgae the third generation of renewable biomass resources [1,2] that could be a realistic source of sustainable feedstock for renewable energy production. Marine biomass mainly consists of carbohydrates, and lacks lignin; its low hemicellulose content allows for an easy processing for valuable marine products such as bioethanol, bio-oil, and bio-gas [1-3]. To improve the process efficiency, a high-performance pretreatment process is needed. Hydrothermal pretreatment is suitable for this process. This is a thermochemical process used to convert biomass into small molecules using high-temperature and high-pressure water to break the bonds of the long-chain polymeric structure. The process is simple and economical [3-6]. For commercialization, though, details of the reaction mechanism and kinetics need to be understood.

In the fast pyrolysis process to produce bio-oil, many researchers have investigated the primary products such as levoglucosan to study the decomposition of biomass related organic compounds. Many researchers have used coupled gas chromatography/mass spectrometry (GC/MS) [7-11]. However, the problem of GC/MS is that only thermally stable volatile compounds can be detected and that the monomeric unit of carbohydrate larger than 162 Da cannot be detected without derivatization [12,13]. To detect other compounds that exist in the reaction field at high temperatures with a short lifetime, in situ mass spectrometry (in situ MS) analysis is essential.

MS is attractive because of its high selectivity, sensitivity, high throughput, small sample size, and potential combination with others instrument [14]. Previous researchers successfully elucidated the effect of hot-compressed water on cellobiose, its primary reaction products, and demonstrated the production of sugar and anhydrosugar oligomers from cellulose by using a high performance anion exchange chromatography with pulsed amperometric detection and mass spectrometry (HPAEC-PAD-MS) [15,16]. However, the in situ MS analysis of biomass-related organics during hydrothermal reactions has not been well studied and developed. This is mainly because of the high pressure atmosphere in the hydrothermal reactor. MS, by nature, requires low pressure for sampling. Once the sample is collected under atmospheric pressure, it is not difficult to send the sample to the MS analysis, but in situ MS for hydrothermal reaction has been a challenge.

Our purpose was to develop an in situ MS analysis system that can detect products during hydrothermal pretreatment and verify its effectiveness in reaction analysis. This is the first report to apply

†To whom correspondence should be addressed.
E-mail: mat@hiroshima-u.ac.jp

mass spectrometry to in situ observation of hydrothermal reactions.

EXPERIMENTAL SECTION

1. Chemicals and Reagents

Glucose (≥99.5%; CAS 50-99-7), furfural (99%; CAS 98-01-1), 5-hydroxymethylfurfural (≥99%; CAS 67-47-0), levulinic acid (98%; CAS 123-76-2), and formic acid (≥99.5%; CAS 64-18-6) were obtained from Sigma-Aldrich and Nacalai Tesque. All chemicals and solvents, acetonitrile (≥99.5%, CAS 75-05-8, Sigma-Aldrich), and distilled water used in this study were of analytical purity and used without further purification. Glucose was used as the model compound for biomass feedstock.

2. Hydrothermal Reaction

Fig. 1 shows the experimental set-up. In this study, a tubular batch reactor made of SS316 steel (15.7 m length, 4.35 mm inner diameter) was used. After the temperature was adjusted with the empty reactor, a glucose solution of 0.5 wt%, prepared with deionized water, was fed to the reactor until the pressure in the reactor got to he desired value. The large surface area of the tubular batch reactor allowed rapid heating of the glucose solution. The solution was kept at the reaction temperature for the desired reaction time, and then by opening the valve V1, the reactor content was released into the analyzers by rapid expansion. When it was not for the in situ analysis, the reactor content was cooled by the heat exchanger. This cold effluent was sampled for high-performance liquid chromatography (HPLC) and MS analyses. This MS analysis of the cold effluent is called "once-cooled mass spectrometry (once-cooled MS) analysis" in this study. The reaction temperature was controlled with the furnace, and the temperature ranged from 140 to 220 °C. The reaction pressure was set at 5 MPa for all experimental runs. Reaction time in the reactor ranged from 5 to 20 min. Table 1 shows the reaction conditions.

Table 1. Hydrothermal pretreatment conditions

Parameters	Reaction conditions
Feedstock	Glucose
Concentration	0.5% wt
Temperature	140, 180, and 220 °C
Pressure	5 MPa
Reaction time	5, 10 and 20 min

3. In Situ MS Analysis Setup

In this study, the in situ MS analysis system was newly developed to directly detect the reaction products under hydrothermal conditions. Fig. 1 also shows this set-up. A quadrupole mass spectrometer (ULVAC Co., USA) was used as detector, and electron ionization (EI) at 70 eV of ionizing electron energy was employed for all runs. It was coupled with a hydrothermal tube reactor before the heat exchanger by using custom-made connection fitting parts so that the hot effluent could be analyzed without cooling. In detail, a 3-way valve V1 was connected at the end of the tubular batch reactor so that the reactor content could be released into the cooling-down system described above or to the in situ MS system. The line for the in situ MS system was further connected to a small orifice nozzle spray. The diameter of the orifice was 0.1 mm, and it was used to reduce the high pressure of the reactor to achieve the

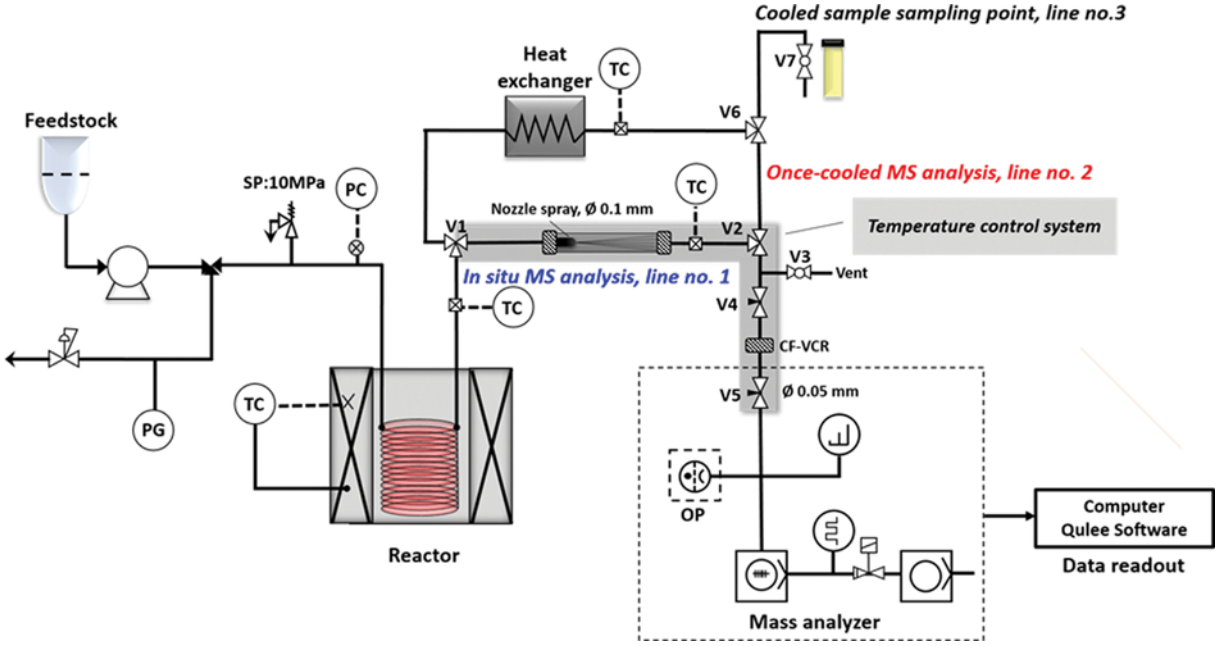

Fig. 1. Schematic of experimental apparatus. The quadrupole mass spectrometer was directly coupled to the tubular batch reactor by connection fittings consisting of small orifice spray nozzle with 0.1 mm in diameter. Passing the spray nozzle, pressure of liquid hot sample was decreased; small amount of the sample was passed though the flow line until mass analyzer, pressure of room before MS-detection zone is lower than 1 mPa (PG: pressure gauge, PC: pressure controller, TC: temperature controller, SP: static pressure and CF-VCR: conflat flange to female VCR adapter, V1-V7: Valves).

vacuum required for MS analysis. Stainless steel tubing with an outside diameter of 19.1 mm and an inner diameter of 16.6 mm was used as the spraying box. The hot effluent was then delivered to another 3-way valve V2 and then to the line connected to the mass spectrometer. Valve V4 at the inlet of the MS system and valve V5 incorporated in the MS system were used to adjust the sample amount fed to the MS system, and the other part of the effluent was released through valve V3. The flow line leading to the in situ MS system was kept at the same temperature as the reactor by using a temperature control system. A thermocouple (TC) was placed in the flow line, and a PID controller (proportional-integral-derivative controller) was used to control the power of the heating jacket of the line. Data collection was processed using software for gas analysis Qulee QCS ver. 3.0, provided by ULVAC Co. The mass spectra were first determined for glucose, 5-hydroxymethylfurfural (5-HMF), furfural, formic acid, and levulinic acid using the standard chemicals. Each standard compound was diluted in distilled water to an approximate concentration of 1 kg/m^3 (1 mg/mL) and was then analyzed by MS directly. The mass spectrometer was operated under the scanning mode with an operating pressure of 1 mPa.

4. Conventional HPLC Analysis

To compare the in situ MS analysis results with the conventional analysis results, the cold effluent was analyzed by HPLC (Shimadzu, Japan). The cold effluent samples were filtered before injection with a 0.2 µm syringe filter (Sartorius, Germany). The SCR 102 HG column (Shimadzu Corporation) was used to quantify the amount of organic acids produced from glucose decomposition under hydrothermal reaction. The analysis was conducted at an oven temperature (40 °C) using a 5 mol/m^3 (5 mM) perchloric acid ($HClO_4$) aqueous solution as the eluent at a flow rate of 0.7 cm^3/min with a refractive index detector (Shimadzu RID 10-A). Ring compounds like 5-HMF and furfural were quantified by using a column from Showa Denko K.K., DE413L using a UV-vis detector from Shimadzu, SPD 10-A; 5 mol/m^3 (5 mM) $HClO_4$ aqueous solution mixed with acetonitrile (ratio 1 : 1) was used as the eluent. The oven temperature was 40 °C, and the flow rate of the eluent was 0.7 cm^3/min.

5. Once-cooled MS Analysis

The cold effluent was also analyzed by using MS (once-cooled MS). To compare the MS analysis of the hot effluent and that of the effluent after cooling, a line was also prepared so that the effluent after cooling, could be fed to the MS system (Fig. 1). The cold effluent was fed to the line connected to MS, line no. 2, via the 3-way valve V6, and then fed to the MS system via valve V2.

RESULTS AND DISCUSSION

Fig. 2 shows the mass spectra of the standard compounds. The mass spectra of the glucose standard showed base peak fragmentation at signal *m/z* 73, minor fragments at signal *m/z* 60, 43, and 31, but no molecular ion peak at signal *m/z* 180. The mass spectra of the 5-HMF standard showed the molecular ion peak at signal *m/z* 126 and the base peak at signal *m/z* 97 ($C_5H_5O_2$). Furfural standard showed the molecular ion peak (which is the base peak) at signal *m/z* 96 ($C_5H_4O_2$). The formic acid standard mass spectrum

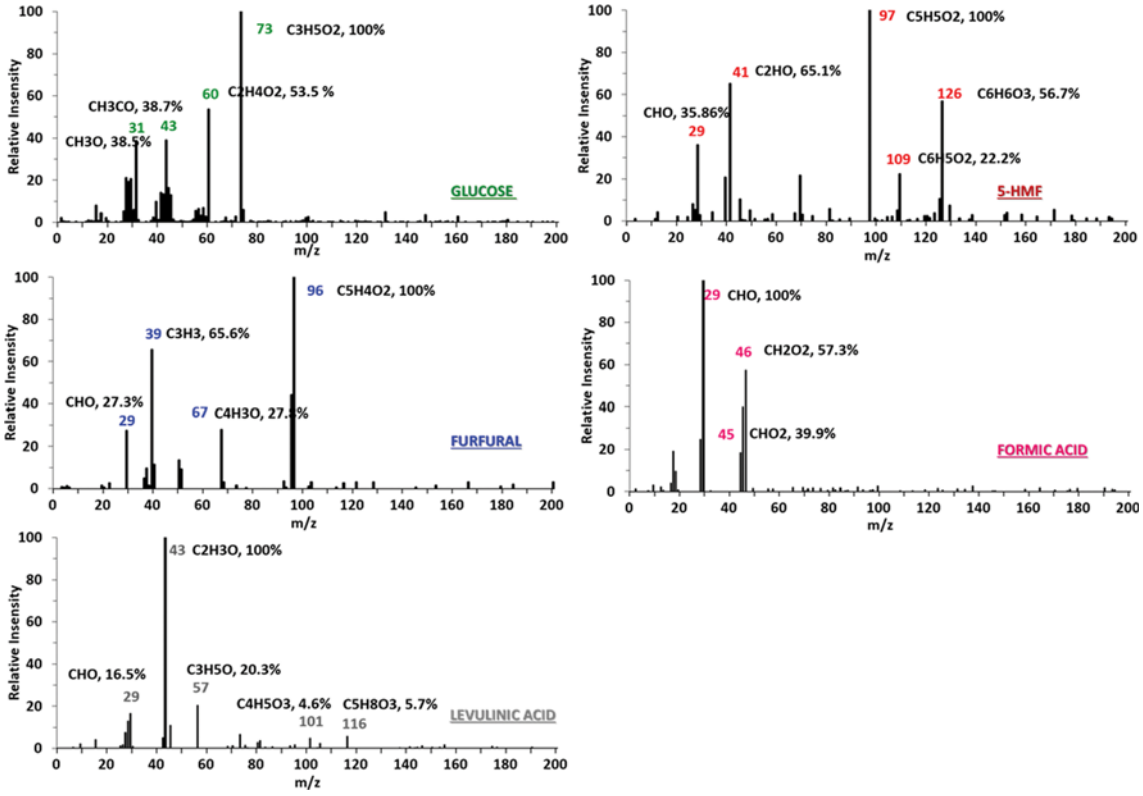

Fig. 2. Mass spectrum of standard compounds at concentration 1 kg/m^3 obtained from in situ MS analysis apparatus, room temperature.

showed a molecular ion peak of formic acid of *m/z* 46 (CH_2O_2), and at signal 45 and 44 were CHO_2 and CO_2, respectively. Base peak fragmentation was observed at signal *m/z* 29 referring to the CHO fragmentation molecule. Levulinic acid showed a peak at signal *m/z* 116 and base peak at *m/z* 43 referring to fragmentation of C_2H_3O. Peaks were also found at signal *m/z* 101, 56, 29, 28, and 27, referring to $C_3H_5O_2$, C_3O, C_2H_5, CO, and C_2H_3, respectively. The major fragmentations, which were either the base peak or the molecular ion peak of each standard, were used to interpret the mass spectra of the products from glucose decomposition under various hydrothermal conditions. A summary of the major fragmentation and element compositions is shown in Table 2.

The effect of the reactor temperature on the decomposition products is shown in Fig. 3. The experiments were conducted at 10 min of residence time for each temperature. At a low temperature of 140 °C, the fragmentation peaks were observed only at signal *m/z* 73, 60, 43 and 31, which represent the mass spectral pattern of the glucose standard. This implies that the decomposition of glucose did not proceed at this temperature. However, when the temperature was increased to 180 °C and 220 °C, in addition to the peak at signal *m/z* 73, which is the base peak of glucose fragmentation, the relative intensity of the peaks at signals *m/z* 97, 96, 29, and 116 were clearly observed, which represent 5-HMF, furfural, formic acid and levulinic acid, respectively. At 180 °C, the fragmentation peaks of small acid molecules like levulinic acid and ring compounds like 5-HMF and furfural are observed. At 220 °C, formic acid was also observed at the peak *m/z* 46 and 45. There

Table 2. Summary of fragmentation and element composition of each standard compound

Standard compounds	*m/z*	Element composition	Relative abundance (%)
Glucose	73	$C_3H_5O_2$	100.0
	60	$C_2H_4O_2$	53.48
	43	CH_3CO	38.71
	31	CH_3O	38.46
5-HMF	126	$C_6H_6O_3$	56.70
	109	$C_6H_5O_2$	22.18
	97	$C_5H_5O_2$	100.0
	41	C_2HO	65.07
	29	CHO	35.86
Furfural	96	$C_5H_4O_2$	100.0
	67	C_4H_3O	27.75
	39	C_3H_3	65.55
	29	CHO	27.27
Formic acid	46	CH_2O_2	57.28
	45	CHO_2	39.87
	29	CHO	100.0
	17	OH	18.99
Levulinic acid	116	$C_5H_8O_3$	5.69
	57	C_3H_5O	20.31
	43	C_2H_3O	100.0
	29	CHO	16.54

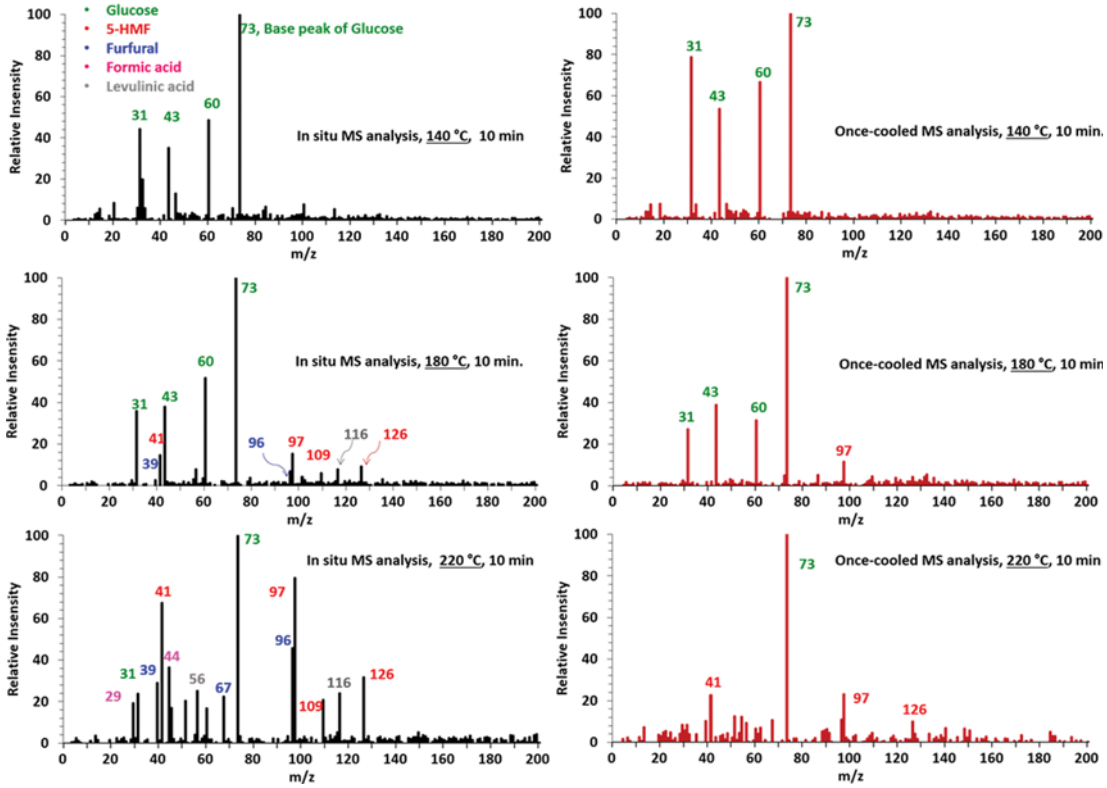

Fig. 3. Mass spectrum of glucose decomposition under various temperatures of hydrothermal pretreatment process in rang of 140-220 °C by in situ MS analysis compared with once-cooled MS analysis (10 min).

are some difficulties in interpreting the whole data such as absence of base peak of levulinic acid at 220 °C and further improvement is needed. Still, in situ measurement of mass spectrum was successfully obtained.

For the once-cooled MS analysis, similar behavior was observed, but some differences were found. First, the small acid molecules formed during the decomposition of glucose at 180 and 220 °C could be detected only by in situ MS analysis. Second, the peaks of ring compounds such as 5-HMF and furfural were also observed in once-cooled MS analysis, but they are much weaker. The reason for this difference is unclear, but the significance of in situ MS analysis is clear because it can detect what once-cooled MS analysis cannot. One of the differences between in-situ and once-cooled analyses is injection. For in-situ analysis, the sample was supplied by passing through the nozzle, while for once-cooled analysis, the sample was supplied directly. However, in both cases the sample was evaporated by high temperature and low pressure. At the inlet of the MS, there should not be a difference. No solid product and no gas product were observed by in situ MS analysis or once-cooled analysis.

The effect of residence time is shown in Fig. 4. It shows that the ion current for glucose (m/z=73) decreases with residence time, while those of 5-HMF (m/z=97), furfural (m/z=96), formic acid (m/z=46) and levulinic acid (m/z=116) increase. The highest peak shifts from glucose to 5-HMF at 20 min of residence time, indicating 5-HMF production by the decomposition of glucose. Low-molecular-weight acids are also formed during the hydrothermal pretreatment process, which is shown by growing peaks for these compounds with residence time. Again, the peaks for acids and ring compounds are much smaller for the once-cooled MS analysis than that for in situ MS analysis.

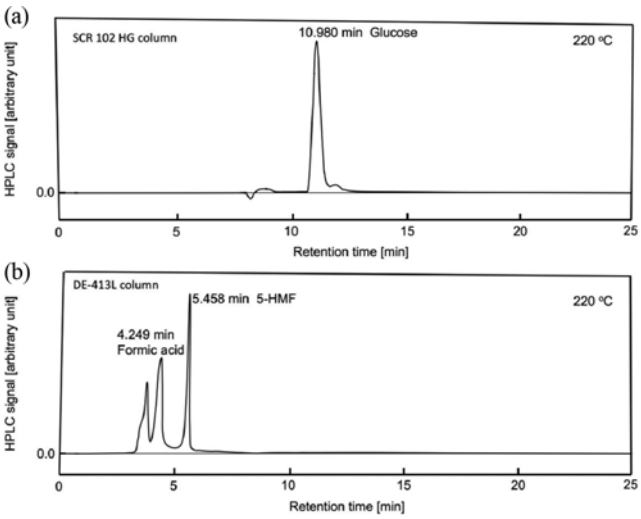

Fig. 5. HPLC results of glucose decomposition products at 220 °C, 10 min of residence time by using: (a) SCR 102 HG column; (b) DE-413L column.

Fig. 5 shows the typical analysis result of the cooled liquid sample by conventional HPLC analysis. Only three kinds of products

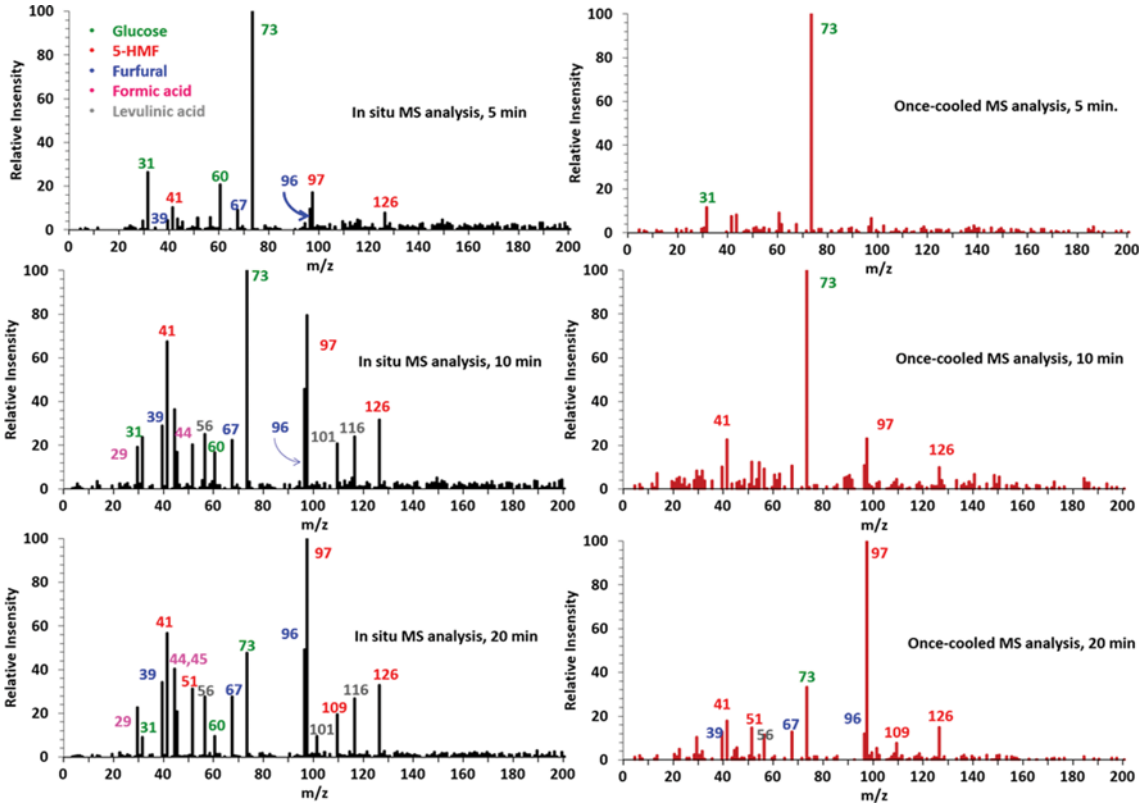

Fig. 4. Mass spectrum of glucose decomposition under various residences of hydrothermal pretreatment process time in the range of 5-20 min by in situ MS analysis compared with once-cooled MS analysis (220 °C).

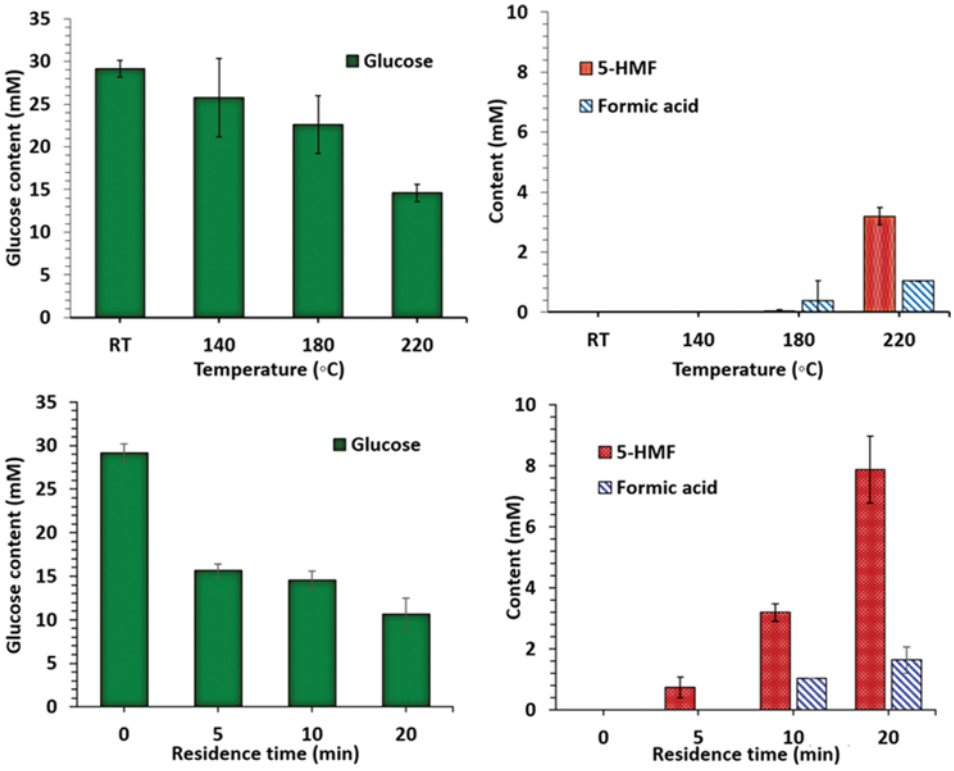

Fig. 6. Effect of temperature (at 10 min of residence time) and residence time (at 220 °C) on the content of glucose and its decomposition products obtained by HPLC.

can be observed: glucose, 5-HMF, and formic acid. The observed amount of 5-HMF is in good agreement with the previous study [17]. Fig. 6 shows the effect of temperature and residence time on the yield of these compounds determined with this analysis. The glucose concentration decreases with residence time and temperature, while those of 5-HMF and formic acid increase. This tendency is in good agreement with the trend observed by in situ MS analysis. However, furfural and levulinic acid could not be detected. Many studies have been conducted on glucose as a model compound of biomass under hydrothermal conditions [17-21]. They reported results for glucose hydrothermal decomposition products at temperatures ranging between 175 °C to 400 °C, and at 25 MPa to 40 MPa. Glucose decomposed to fructose, erythrose, glycolaldehyde, glyceraldehyde, 1,6-anhydroglucose, 5-hydroxymethylfurfural (5-HMF) where any kind of acid was not observed using HPLC, illustrating the effectiveness of in situ MS analysis in this study.

CONCLUSIONS

A new method of in situ MS analysis of compounds under hydrothermal conditions by mass spectroscopic methodology was developed successfully. The analysis method employed a quadrupole mass analyzer coupled with a tubular batch reactor through a custom-built connection fitting with a small orifice nozzle spray. The results agreed with conventional analysis by HPLC and once-cooled MS analysis, for the behavior of the main compounds, including glucose, 5-HMF, and formic acid. The yield of glucose decreased with temperature and residence time, while those of 5-HMF and formic acid increased. However, a difference was observed for minor products. In situ MS analysis was effective at detecting the minor products such as furfural and levulinic acid, which were not detected or whose peaks were much smaller by conventional HPLC and once-cooled MS analysis.

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