# DEGRADATION PRODUCTS OF CHINESE SILVERGRASS BY $^{60}$CO Γ-RAY IRRADIATION PRETREATMENT

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After $^{60}$Co γ-ray irradiation pretreatment of Chinese silvergrass, a variety of products were identified by ion chromatography (IC) and gas chromatography–mass spectrometry (GC-MS). According to IC analysis, the degradation products included arabinose, galactose, glucose, xylose, D-galacturonic acid and D-glucuronic acid. The GC-MS analysis identified 22 carboxylic acids, 19 aromatic compounds and 3 furan compounds. The irradiation degradation mechanism was also discussed.

***Keywords***: Chinese silvergrass, $^{60}$Co γ-ray irradiation pretreatment, GC-MS, ion chromatography, degradation products

## INTRODUCTION

The depletion of fossil energy and the rising concerns about environmental pollution are clearly driving the demand for clean and renewable energy. Biomass is currently the most important renewable resource that accounts for 47% of all renewable energy consumption.[1]

*Miscanthus sinensis*, commonly known as Chinese silvergrass, is a perennial C4 Poaceae grass that has strong adaptability, high yield,[2] short life cycle and strong resistance to diseases. It is mainly composed of carbon, oxygen and hydrogen. As it has low nitrogen and sulfur contents, it generates less nitrogen- and sulfur-containing compounds during transformation, which is beneficial in reducing environmental pollution. The cellulose and hemicellulose contents of Chinese silvergrass can reach more than 80% and the ash content is less than 5%,[3] which makes it an excellent lignocellulosic material and thus draws increasing interest.

The production of ethanol fuel from lignocellulose materials mainly involves the hydrolysis of cellulose and hemicelluloses in the crude material to fermentable sugars and their subsequent fermentation to ethanol fuel.[4,5]

The γ-ray is a high-energy radiation released during the decay of radioactive nuclei that can activate molecules into ions or free radicals, which triggers dissociation of chemical bonds and alters the structure of substances.[6] It is well known that γ-ray can reduce the degree of polymerization of cellulose, enhance reaction accessibility,[7] significantly improve hydrolysis efficiency and thus increase the sugar yield.[8] Other advantages of γ-ray irradiation include short processing time, simple operation procedure and no environmental pollution.[9]

Lignocellulose is mainly composed of cellulose, hemicelluloses and lignin. During hydrolysis, the lignocellulose structure breaks to yield a series of fermentable sugars and some other products, such as carboxylic acids, aromatic compounds and furans. These non-sugar products have been studied using gas chromatography and liquid chromatography.[10,11] Common aromatic non-carbohydrate degradation products include 4-hydroxybenzoic acid, vanillic acid, syringic acid, 4-hydroxybenzaldehyde, vanillin and syringic aldehyde. Common furan products include furfural and 5-(hydroxymethyl)furfural, while common carboxylic acid products comprise acetic acid, formic acid and levulinic acid.[12,13]

In this paper, Chinese silvergrass was irradiated with $^{60}$Co γ-ray at different doses. The water extract of the irradiated Chinese silvergrass was analyzed by ion chromatography (IC) to determine the sugar components and by gas chromatography-mass spectrometry (GC-MS) to determine the other degradation products. The results provide experimental and theoretic information for the further processing of Chinese silvergrass to produce ethanol fuel.

## EXPERIMENTAL

### Raw material

Chinese silvergrass was collected from the *Miscanthus sinensis* research camp at Changsha, Hunan. The samples were dried for 8 h at 50 °C, crushed and filtered through a 40 mesh sieve, and then stored in a desiccator.

### Pretreatment

About 200 g of sample powder was placed in 250 mL jars and subjected to $^{60}$Co γ-ray irradiation at 400 KGy, 800 KGy, 1200 KGy 1600 KGy and 2000 KGy, respectively.

### Extraction of irradiated sample

The irradiated Chinese silvergrass sample was weighed and extracted in boiling water (at the ratio of 1 g grass sample to 10 mL water). The filtrate and solid residue were collected.

### Extraction of degradation by-products

The water extract was extracted with ethyl acetate for three times. The combined organic phase was washed with saturated sodium chloride solution, then dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

### Preparation of the silyl derivative of extracted by-products

The above extract (500 µL) was thoroughly mixed with N,O-trimethylsilyl trifluoroacetamide (BSTFA) (400 µL) and pyridine (100 µL), and the mixture was placed in an oven at 70 °C to react for 30 min. The obtained derivatization products were analyzed by GC-MS.

### Preparation of aqueous extract of irradiated Chinese silvergrass

The $^{60}$Co γ-ray irradiated Chinese silvergrass (1.00 g) was extracted in 50 mL distilled water for 30 min in a beaker using a boiling water bath. The solution was then filtered and the volume was set to 200 mL. An aliquot of 10 mL was passed through 0.22 µm nylon membrane and the OnGuard II RP column (2.5 cc, activated by methanol and water). The initial 6 mL discharge was discarded and the next 1 mL was collected for subsequent sugar composition tests.

### Preparation of mixed sugar standard solution

Glucose, xylose, galactose, arabinose, galacturonic acid and glucuronic acid were each accurately weighed (0.0500 g) and dissolved in exactly 10 mL ultrapure water in a volumetric flask. A 1.00 mL aliquot of each sugar solution was pooled into a 10 mL volumetric flask and the volume of the mixture was set to 10 mL to give the mixed sugar standard solution (5 mg/L for each sugar).

### Sugar composition analysis

The monosaccharides and disaccharides in the water extract of the irradiated Chinese silvergrass were quantified on an ICS-3000 ion chromatograph using a CarboPac PA20 analytical column (150 × 3 mm) and a CarboPac PA guard column (50 × 3 mm). The injection was performed using an autosampler and the injection volume was 25 µL.

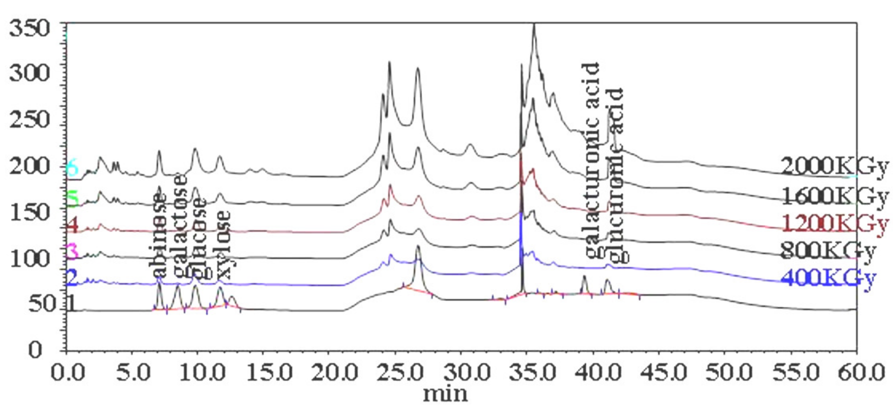


Figure 1: Ion chromatogram of sugars in the aqueous extract of irradiated Chinese silvergrass

**By-product analysis**

Non-sugar compounds were analyzed using a QP-2010 GC-MS (Shimadzu, Japan) on a DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm). The column temperature was controlled by a programmed gradient: initial temperature was maintained at 60 °C for 2 min, followed by heating at 20 °C/min to 200 °C and maintaining the temperature for 2 min, then heating at 15 °C/min to 310 °C/min and maintaining the temperature for 15 min. The MS conditions were: EI source, scanning range – 50-500 m/z, ion source temperature – 220 °C, interface temperature – 230 °C. Each sample was analyzed thrice.

## RESULTS AND DISCUSSION

### Impact of irradiation dose on the formation of reducing sugar

Upon $^{60}Co$ γ-ray irradiation, the cellulose and hemicellulose in Chinese silvergrass can be degraded to generate fermentable sugars. Figure 1 shows the ion chromatogram of sugars in the aqueous extract of irradiated Chinese silvergrass. It can be seen that after the irradiation, the Chinese silvergrass yielded arabinose, galactose, glucose, xylose, galacturonic acid and glucuronic acid. When the irradiation dose increased from 400 KGy to 2000 KGy, the yields of all arabinose, galactose, glucose, xylose and glucuronic acid increased. In particular, the yield of glucuronic acid increased from 0.312 mg/g to 7.280 mg/g, and the yield of glucose increased from 1.096 mg/g to 3.285 mg/g. The yield of galacturonic acid increased from 0.811 mg/g at 400 KGy to 4.870 mg/g at 1600 KGy, but decreased to 0.208 mg/g under 2000 Kgy irradiation.

### Impact of irradiation dose on degradation by-products

Irradiation degradation by-products include polar organic compounds such as phenols, aldehydes and carboxylic acids. In total, 44 degradation by-products were identified, which have carbon chain length in the range of C2–C18 and can be classified into three categories, i.e., carboxylic acids, aromatic compounds and furans.

### Influence of irradiation dose on the formation of carboxylic acids

A total of 22 carboxylic acids were identified among the Chinese silvergrass degradation by-products (Table 1). From Table 1, it can be seen that the yield of acids #1–#11 increased with rising irradiation dose, whereas the yield of acids #12–#15 decreased with rising irradiation dose. The yields of linoleic acid, myristic acid and pentanoic acid varied little with changing irradiation dose. Tartaric acid was detected only under 1600 KGy irradiation, and lauric acid was detected only under 400 KGy or 800 KGy irradiation. Maleic acid and 3-hydroxybutyric acid reached a maximum yield of 0.15% and 0.28% at 1200 KGy and 1600 KGy irradiation, respectively. The total relative peak area of carboxylic acids was 10.25% at 400 KGy and increased to 12.93% when the irradiation reached 2000 KGy.

The 22 detected carboxylic acids included long-chain saturated acids and short-chain or branched acids, the latter consisting of monocarboxylic acids and dicarboxylic acids. The long-chain saturated acids (e.g., lauric acid, myristic acid, palmitic acid, oleic acid, stearic acid and linoleic acid) came from the unmodified extract of the Chinese silvergrass.[14]

Table 1
Relative peak area (%) of carboxylic acids of Chinese silvergrass after irradiation pretreatment with different doses

| Sample | Compound | Irradiation dose (KGy) | | | | |
|---|---|---|---|---|---|---|
| | | 400 | 800 | 1200 | 1600 | 2000 |
| 1 | Lactic acid | 0.2 | 0.2 | 0.26±0.01 | 0.32±0.01 | 0.56±0.01 |
| 2 | Glycollic acid | 0.78±0.01 | 0.810.01 | 0.970.01 | 1.21±0.01 | 1.43±0.01 |
| 3 | Propionic acid | 1.13±0.01 | 1.34±0.01 | 1.57±0.01 | 1.69±0.01 | 1.79±0.01 |
| 4 | Succinic acid | 0.82±0.01 | 1.16±0.01 | 1.23±0.01 | 1.3±0.01 | 1.67±0.01 |
| 5 | Azelaic Acid | 0.22 | 0.57±0.01 | 0.6±0.01 | 0.69±0.01 | 0.79±0.01 |
| 6 | Glyceric acid | 0.15 | 0.21 | 0.29±0.01 | 0.33±0.01 | 0.5±0.01 |
| 7 | Malic acid | – | 0.25±0.01 | 0.35±0.01 | 0.35±0.01 | 0.39±0.01 |
| 8 | 2-Hydroxybutyric acid | 0.01 | 0.03 | 0.04 | 0.06 | 0.08 |
| 9 | 3,4-Dihydroxybutyric acid | 0.12 | 0.1 | 0.11 | 0.13 | 0.16 |
| 10 | Suberic acid | 0.18 | 0.18 | 0.2 | 0.41±0.01 | 0.49±0.01 |
| 11 | 5-Hydroxyvaleric acid | – | 0.01 | 0.02 | 0.05 | 0.13 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12 | Palmitic acid | 3.08±0.03 | 2.37±0.02 | 2.57±0.01 | 1.79±0.01 | 1.93±0.01 |
| 13 | Oleic acid | 0.7±0.01 | 0.6±0.01 | 0.74±0.01 | 0.53±0.01 | 0.47±0.01 |
| 14 | Stearic acid | 2.15±0.02 | 1.87±0.02 | 1.65±0.02 | 1.46±0.01 | 1.28±0.01 |
| 15 | Capric acid | 0.21 | 0.2 | 0.23±0.01 | 0.24±0.01 | 0 |
| 16 | Linoleic acid | 0.46±0.01 | 0.44±0.01 | 0.46±0.01 | 0.39±0.01 | 0.49±0.01 |
| 17 | Myristic acid | 0.21±0.01 | 0.28±0.01 | 0.28±0.01 | 0.21±0.01 | 0.23±0.01 |
| 18 | Pentanoic acid | 0.14 | 0.13 | 0.11 | 0.13 | 0.11 |
| 19 | Tartaric acid | – | – | – | 0.89±0.01 | 0.3±0.01 |
| 20 | Cis-Butene dioic acid | 0.08 | 0.12 | 0.15 | 0.11 | – |
| 21 | 3-Hydroxybutyric acid | 0.08 | 0.04 | 0.07 | 0.28±0.01 | 0.13 |
| 22 | Lauric acid | 0.16 | 0.18 | – | – | – |
| Total | | 10.25 | 11.09 | 11.9 | 12.57 | 12.93 |

The results are reported as the mean value plus or minus standard deviation (not determined where value was not indicated); – not identified

Table 2
Relative peak area (%) of aromatic compounds of Chinese silvergrass after irradiation pretreatment with different doses

| Sample | Compound | Irradiation dose (KGy) | | | | |
|---|---|---|---|---|---|---|
| | | 400 | 800 | 1200 | 1600 | 2000 |
| 1 | Vanillin | 1.57±0.01 | 1.67±0.01 | 1.66±0.01 | 1.74±0.01 | 2.42±0.02 |
| 2 | Vanillic acid | 1.94±0.01 | 2.23±0.01 | 2.35±0.01 | 2.73±0.02 | 2.78±0.02 |
| 3 | p-Hydroxybenzoic acid | 1.82±0.01 | 2.23±0.02 | 2.46±0.02 | 3.02±0.02 | 4.09±0.04 |
| 4 | Protocatechuic acid | 0.24±0.01 | 0.36±0.01 | 0.39±0.01 | 0.56±0.01 | 0.67±0.01 |
| 5 | Syringic acid | 0.93±0.01 | 1.18±0.01 | 1.22±0.01 | 1.45±0.01 | 1.49±0.01 |
| 6 | Salicylic acid | 0.22±0.01 | 0.33±0.01 | 0.39±0.01 | 0.48±0.01 | 0.54±0.01 |
| 7 | Syringaldazine | 0.54±0.01 | 0.58±0.01 | 0.63±0.01 | 0.67±0.01 | 0.73±0.01 |
| 8 | 3-Hydroxy-3-(4-hydroxyphenyl)-propionic acid | – | 0.46±0.01 | 0.68±0.01 | 0.66±0.01 | 0.67±0.01 |
| 9 | 3'-Hydroxyacetophenone | 0.29±0.01 | 0.32±0.01 | 0.58±0.01 | 0.62±0.01 | 0.68±0.01 |
| 10 | p-Hydroxybenzaldehyde | 2.17±0.01 | 2.35±0.02 | 2.54±0.02 | 2.11±0.01 | 2.29±0.01 |
| 11 | 2-Hydroxy-2-(4-meyhoxyphenyl)-propionic acid | – | 2.04±0.01 | 0.48±0.01 | 1.75±0.01 | 2.12±0.01 |
| 12 | 2-(4-Methoxy-3-hydroxyphenyl)-ethylene glycol | 0.16 | 0.34±0.01 | 0.56±0.01 | 0.62±0.01 | 0.49±0.01 |
| 13 | Hydroquinone | 2.02±0.02 | 1.90±0.01 | 1.97±0.01 | 1.92±0.01 | 1.98±0.01 |
| 14 | Benzoic acid | 0.07 | 0.05 | 0.06 | 0.08 | 0.10 |
| 15 | p-Coumaric acid | 4.52±0.03 | 4.68±0.03 | 4.34±0.02 | 3.31±0.02 | 3.14±0.02 |
| 16 | p-Chlorophenol | 0.09 | 0.02 | 0.02 | – | – |
| 17 | 2-(3-Methoxy-4-hydroxyphenyl)-ethylene glycol | 0.60±0.01 | 0.57±0.01 | 0.59±0.01 | 0.54±0.01 | 0.62±0.01 |
| 18 | p-Hydroxyphenylacetic acid | 0.13 | 0.16 | – | – | – |
| 19 | Ferulic acid | 1.03±0.01 | 0.91±0.01 | 0.85±0.01 | 0.82±0.01 | 0.62±0.01 |
| Total | | 18.34 | 22.38 | 21.77 | 23.08 | 25.43 |

The results are reported as the mean value plus or minus standard deviation (not determined where value was not indicated); – not identified

For the short-chain or branched acids, the monocarboxylic acids (*e.g.*, lactic acid, glycolic acid, 3-hydroxypropionic acid, glyceric acid, 2-hydroxybutyric acid, 3,4-dihydroxybutyric acid, 5-hydroxypentanoic acid, capric acid, pentanoic acid, 3-hydroxybutyric acid) came from the monosaccharides that formed through the irradiation of cellulose and hemicellulose.

There are two possible formation pathways for succinic acid, azelaic acid, malic acid, suberic acid, tartaric acid and maleic acid: (1) the cellulose and hemicellulose degrade to form pentose, which further dehydrates to form furfural; the intermediates of furfural condensation reaction may form organic dicarboxylic acids upon further transformation; (2) the benzene ring and the side chain of lignin structural units may break simultaneously to give dicarboxylic acids.

Table 3
Relative peak area (%) of aromatic compounds of Chinese silvergrass after irradiation pretreatment with different doses

| Sample | Compound | Irradiation dose (KGy) | | | | |
|---|---|---|---|---|---|---|
| | | 400 | 800 | 1200 | 1600 | 2000 |
| 1 | Furoic acid | – | 0.11 | 0.27±0.01 | 0.62±0.01 | 0.97±0.01 |
| 2 | 5-Hydroxymethyl-2-furoic acid | – | 0.03 | 0.07 | 0.07 | 0.10 |
| 3 | Kojic acid | – | 0.17 | 0.27±0.01 | 0.55±0.01 | 0.59±0.01 |
| Total | | – | 0.31±0.01 | 0.61±0.01 | 1.24±0.01 | 1.66±0.01 |

The results are reported as the mean value plus or minus standard deviation (not determined where value was not indicated); – not identified

**Influence of irradiation dose on the formation of aromatic compounds**

Under $^{60}Co$ γ-ray irradiation, the lignin macromolecules in the Chinese silvergrass depolymerize to give a series of complex aromatic compounds. A total of 19 aromatic compounds were detected in this experiment (Table 2). It can be seen from Table 2 that the contents of coumaric acid, p-hydroxybenzaldehyde and hydroquinone (4.52%, 2.17% and 2.02% respectively) are the highest at 400 KGy, but at 2000 KGy the contents of hydroxybenzoic acid, coumaric acid and vanillic acid reach the highest (4.09%, 3.14% and 2.78% respectively).

The lignin in Chinese silvergrass consists of phenylpropanoids in the form of p-hydroxyphenyl, guaiacyl and syringyl units. During irradiation, the aliphatic hydroxy group in the branched chain of lignin can fracture to generate volatile small molecules and the ether bond breaks to generate various phenolic compounds. At the same time, the phenolic benzene ring in lignin and the carbonyl and enal side chains will oxidize to break the side chain and oxidize the aromatic ring, thus forming a series of aromatic acids. It can be seen from Table 2 that 12 aromatic acids were detected, which account for 63.2% of the total aromatic compounds. Upon $^{60}Co$ γ-ray irradiation, lignin produced ferulic acid and coumaric acid as primary products, and their yield reached 1.03% and 4.52% at 400 KGy, respectively. Both acids could be oxidized further to give vanillin, vanillic acid, p-hydroxybenzaldehyde and p-hydroxybenzoic acid as secondary products, whose yield reached 1.57%, 1.94%, 2.17% and 1.82% at 400 KGy, respectively. At 2000 KGy, the yield of ferulic acid and coumaric acid decreased to 0.62% and 3.14%, whereas the yield of vanillin, vanillic acid, p-hydroxybenzaldehyde and p-hydroxybenzoic acid increased to 2.42%, 2.78%, 2.29% and 4.09%, respectively.

**Influence of irradiation dose on the formation of furans**

In the aqueous extract of irradiated Chinese silvergrass, three furan derivatives were detected, which were furoic acid, 5-hydroxymethyl furoic acid and kojic acid (Table 3). The further reaction of xylose, galactose and glucose, which are degradation products of hemicellulose and cellulose, could yield furfural and 5-(hydroxymethyl)furfural, but they were not detected in this experiment. The three detected furans were all noted only when the irradiation was no less than 800 KGy. The furoic acid had the greatest content, which reached 0.97% at 2000 KGy. When processing lignocellulose, the cellulose mainly degrades to generate glucose. Glucose undergoes cyclization and dehydration to form 5-hydroxymethylfurfural, which further decarboxylates to form levulinic acid and formic acid. The relatively active hydroxymethyl group in 5-(hydroxymethyl)furfural breaks off to generate furfural, or pentose may dehydrate during the reaction to give furfural. Using liquid chromatography,[15] furfural, 5-(hydroxymethyl) furfural, levulinic acid and formic acid were detected in the hydrolyzate of biologically pretreated lignocellulose. In this work, furfural and 5-(hydroxymethyl)furfural were not detected by GC-MS, and furoic acid and 5-(hydroxymethyl) furoic acid were detected instead. It is possible that under irradiation, the furfural and 5-(hydroxymethyl)furfural that were generated from glucose and pentose dehydration were oxidized to furoic acid and 5-(hydroxymethyl) furoic acid, or GC-MS sample may have been oxidized during sample pre-treatment. Besides, in this work, the carboxylic acid by-products mainly contained the inherent acids of Chinese silvergrass (e.g., stearic acid and palmitic acid) and the 3-hydroxypropanoic acid that were from glucose degradation; levulinic acid and formic acid were also not detected. It is possible that the 2000 KGy irradiation is still too mild to generate formic acid, acetic acid and levulinic acid. Palmqvist *et al.*[11] have shown that furfural, 5-(hydroxymethyl)furfural, formic acid, acetic acid and levulinic acid are all major fermentation inhibitors. The absence of these substances in the detected degradation products of this test indicates that compared with other lignocellulose degradation processing methods, irradiation may reduce the formation of fermentation inhibitors.

## CONCLUSION

In this experiment, the degradation products of Chinese silvergrass after $^{60}Co$ γ-ray irradiation have been analyzed by ion chromatography and GC-MS. The results showed that the carbohydrates in the irradiation degradation products contained arabinose, glucose, galactose, xylose, galacturonic acid and glucuronic acid. The yield of all of them, except galactose, increased with increasing radiation dose. The yield of glucuronic acid increased by 6.968 mg/g when the irradiation increased from 400 KGy to 2000 KGy. Other irradiation by-products mainly included carboxylic acids, aromatic compounds and furans, and their yield also increased at higher irradiation dose. Major fermentation inhibitors, such as furfural, 5-(hydroxymethyl)furfural, formic acid, acetic acid and levulinic acid, were not detected in the degradation products, indicating that the $^{60}Co$ γ-ray irradiation of lignocelluloses can reduce the formation of the major fermentation inhibitors, which is beneficial to the subsequent fermentation processes.

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