Study of biofuel derived from satsuma mandarin *(Citrus unshiu)* peel

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SUMMARY

Bio-ethanol production from carbohydrates, crops containing starch, and cellulosic forest thinning are receiving increasing attention as renewable alternative energies to counteract the exhaustion of fossil fuels and to improve the environment against global warming. This research has examined the utilization of Satsuma mandarin (*citrus unshiu*) peels, obtained via a normal disposal process in Japan, to produce bio-ethanol, including identifying the most suitable diastatic fermenting yeast. As a result, we found that a combination of *Aspergillus niger* as an enzyme for saccharification and standard bread yeast for alcohol conversion during the fermentation produced ethanol with a good inversion rate. Furthermore, the experiment demonstrated that, as a raw material, the peels of Satsuma mandarin (*citrus unshiu*) require a dryness of over 99% to function in an appropriate reaction scheme. In addition, combustion experiments that ethanol mixed with varying amounts of kerosene petroleum demonstrated the possibility of using the ethanol obtained as a blended fuel.

Key word : Bioethanol, Citrus peel, Enzyme

INTRODUCTION

In an effort to prevent fossil fuel depletion and global warming, cellulosic bio-ethanol as a renewable fuel is attracting significant attention[1 – 14]. Although sugar and starch crops have been the traditional sources of cellulosic raw material, 1.6 million tons of mandarin orange (*Citrus unshiu*) peels are disposed of annually in Japan (about 8.5 million tons globally) and might provide another source of cellulose. This work optimized the amount of enzyme and yeast required for ethanol production from this material, as well as an analysis of the quality of the resulting ethanol.

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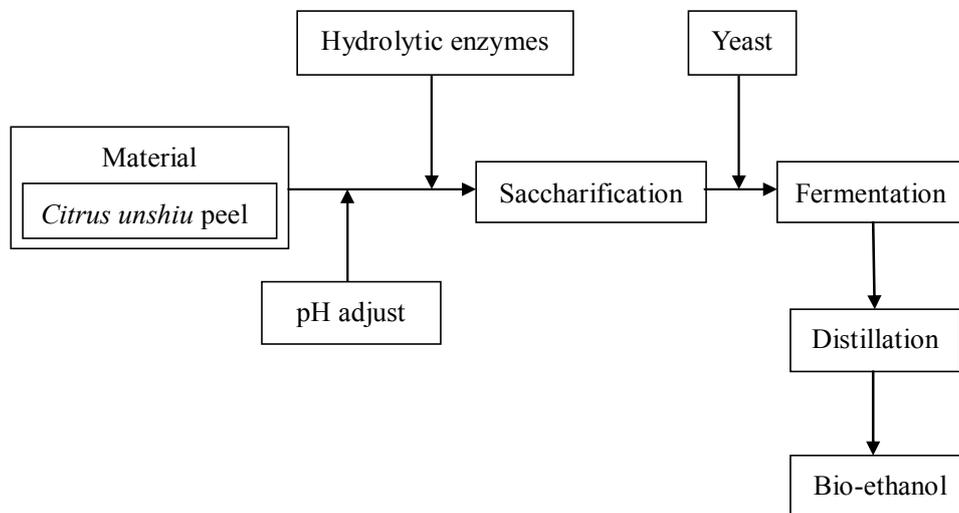
EXPERIMENTAL

Fig. 1　Reaction scheme

Materials

Peels from mature Satsuma mandarin oranges were harvested and dried in the sun (5% moisture content), and the essential oil was extracted. The dried peel was ground to a fine powder using a grinder.

Optimization of enzymatic hydrolysis

Enzyme was added to the dried peel in 100mL distilled water at 1%, 5% or 10% (w/w) (5%, 10%, 20% (w/v)). The pH was adjusted to ~ 4.0 with 10M NaOH. A bio-shaker was used to agitate the mixture at $55 \pm 2°C$ and 120 rpm for 96 h.

Estimation of glucose

After hydrolysis was complete, the samples were centrifuged for 5 min at 6000 rpm, and the superna-tant was diluted and decanted twice with a 1:1 (v/v) acetonitrile : water solution.

The determination of sugars was quantified via high performance liquid chromatography (HPLC, Shimadzu Corporation, Japan) with a YMC-Pack NH$_2$ (4.6×150 mm I.D.) column, and a refractive index (RI) detector. 75:25 acetonitrile:water mixture was used as the mobile phase at a flow rate of 1.0 $^{mL}/_{min}{}^{-1}$.

Fermentation optimization

After enzymatic hydrolysis, the solution was cooled to room temperature and the fermentation yeast was added at 1%, 1.5%, 2%, 2.5% or 3% (v/v) on a dry matter basis. The mixture was agitated on the bio-shaker at $35 \pm 2°C$ and 120 rpm for 48 to 72 h.

Estimation of ethanol concentration

After the fermentation process, the solution was centrifuged for 5 min at 6000 rpm, and the super-natant was diluted and decanted twice with a 1:1 (v/v) acetonitrile : water solution.

The ethanol concentration was determined via gas chromatography (GC, GC-8AT, Shimadzu) using a C-R5A column, He (150 kPa) carrier gas, an oven temperature of 130°C, and an injector and detector temperature of 300°C.

RESULTS AND DISCUSSION

Estimation of ethanol concentration

Hydrolysis conditions are important in bio-ethanol production; thus, a variety of yeasts from dried mandarin peel were studied. The optimized mass of mandarin peel used in this reaction was 30% (v/v); and therefore, the maximum amount was 5g on this scale. A cellulase from *Onozuka* (Ono.), *Aspergillus niger* (Asp.) or *Tricoderma reesei* (Ree.) was added at 5% or 10% (v/v), the pH was adjusted to 4.5 (using 10M NaOH), and the glucose concentration was optimized. An enzyme concentration of 10% (v/v) resulted in the most efficient glucose conversion

rate ranging from 33 to 70% after 72 h of enzymatic action (Table 1 and Fig. 2). The optimal yeast concentration for the fermentation was 3% (w/w), and the maximum amount of bio-ethanol produced was ~23% (v/w) after 48 h. Hydrolysis using *Aspergillus niger* (Asp.) with pectinase (pectinase G) and α-amylase (Type V1-B, from porcine pancreas) was also carried out using the optimized enzymatic conditions. The sugars obtained after enzymatic hydrolysis included glucose, fructose, sucrose and maltose, proving that sucrose is hydrolyzed to glucose and fructose. The rate of sugar conversion was above 100%.

Table 1 Bio-ethanol production from *Citrus unshiu* peel [%(v/v)].

Time (h)	Enzymes					
	10%-Ono.	5%-Ono.	10%-Asp.	5%-Asp.	10%-Ree.	5%-Ree.
0	7.4	7.4	12.6	7.4	7.4	7.4
3	12.6	12.6	22.4	22.4	12.3	12.3
10	14.8	24.9	24.9	24.9	15.9	14.7
24	20.9	19.5	35.5	35.5	24.6	21.9
72	39.7	21.0	70.6	30.3	33.3	31.3

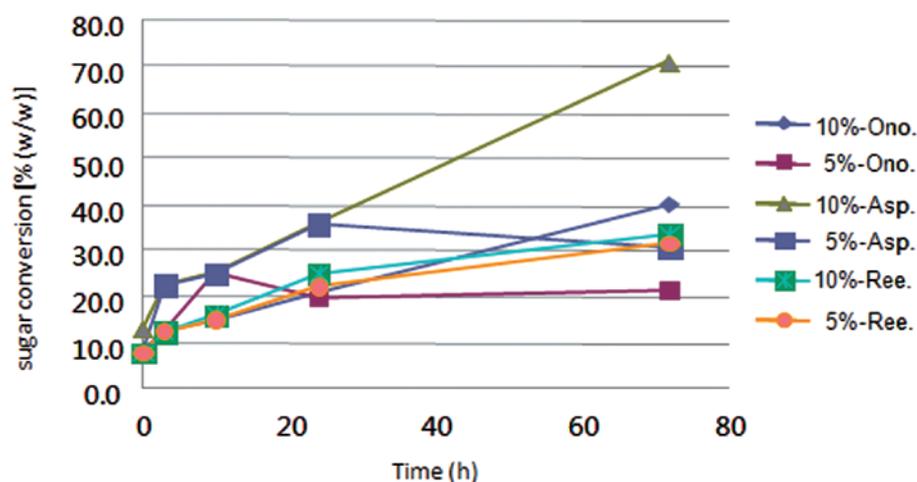Ono. ; *Onozuka*, Asp. ; *Aspergillus niger*, Ree. ; *Tricoderma reesei*.

Fig. 2 Time course of changes in the rate of sugar conversion during enzymatic hydrolysis.

Combustion of bio-ethanol

Combustion of the crude product was carried out by atmospheric distillation and the purity (%) of the bio-ethanol was evaluated using GC-MS analysis. The bio-ethanol was found to have a purity of 85.5%. The other components of the crude product were moisture, 7.2%; 2-amino-1-propanol, 5.2%; 2-methyl -3-buten-2-one, 0.3%; furfural, 0.8%; and limonene, 1.0%. A simple method to improve the purity was considered. The bio-ethanol was combined with commercial ethanol (purity, 98%) in a ratio of 0:100, 10:90 or 25:75 (vol. %), and then used in home heating equipment (kerosene heater) in a combustion experiment (Table 2).

The gas temperature decreased (from 420 to 260°C) with an increasing content of commercial ethanol. NO_X, CO and CO_2 resulting from inorganic hazardous substances were inhibited by about 1/4 to 1/3. The caloric values (cal) of bio-ethanol and commercial ethanol were also compared (6340 cal and 6940 cal, respectively).

Table 2　Combustion of bio-ethanol (instead of using commercial ethanol).

Ethanol／Kerosense (voL.%)	Gas Temp. (℃)	Exhaust Gas Components									
		Organic					Inorganic				
		CH_4*	C_2H_5*	C_3H_5*	iso-C_4H_{10}*	n-C_4H_{10}*	NO_X*	O_2**	CO*	CO_2**	N_2**
0：100	420-440	4.0	<1	<1	<1	<1	30.0	13.1	40.0	5.4	81.4
10：90	360-380	4.0	<1	<1	<1	<1	20.0	14.5	30.0	4.5	80.9
25：75	260-270	4.0	<1	<1	<1	<1	10.0	16.2	10.0	3.3	80.4

*vol. ppm, **vol. %

Lawn mower combustion experiment

Combustion raw material was used in atmospheric distillation and the purity (%) of the bio-ethanol was evaluated using GC-MS analysis. A purity of 93.6% was obtained. Various ratios of bio-ethanol to gasoline (8%, 12%, and 20%) were also examined in additional combustion experiments (Table 3), and a compositional analysis of the exhaust gas was carried out (Table 4). The caloric value (JIS K2301) and specific gravity of each ratio are shown in Table 5.

The hybrid fuel was burned in a 2-cycle lawn mower engine. The amount (vol. ppm) of methane (CH_4) and ethylene (C_2H_4) increased by about 20-30% and 10-15%, respectively, in the exhaust gas from the pure bio-ethanol, but the concentrations of other organic gases was almost the same. On the other hand, there was significant variation in the amount of nitrogen oxides (NO_X) in the exhaust gas. Whereas other inorganic exhaust gas components remained relatively constant with increased bio-ethanol content, NO_X increased 1.5-2-fold.

Table 3　The mixing ratio of ethanol and gasoline (volume ratio).

Condition	Bio-ethanol ratio (%)	Gasoline (vol. %)	Bio-ethanol (vol. %)	2-Cycle engine gas (vol. %)
1	0	25	0	1
2	8	23	2	1
3	12	22	3	1
4	20	20	5	1

Table 4 Analysis of organic and inorganic exhaust gases in each mixing ratio.

Gas Components		Condition			
		No.1 (0%)	No.2 (8%)	No.3 (12%)	No.4 (20%)
Organic	CH_4*	560	710	680	700
	C_2H_4*	330	370	360	680
	C_2H_6*	40	40	40	40
	C_3H_6*	80	80	80	80
	C_3H_8*	10	10	10	10
	iso-C_4H_{10}*	380	440	440	430
	n-C_4H_{10}*	240	270	270	270
	i-C_5H_{12}*	900	990	960	980
	n-C_5H_{12}*	730	800	770	790
Inorganic	H_2**	0.9	1.2	1.3	1.4
	NO_x*	18.0	30.0	32.0	36.0
	O_2**	15.2	14.5	14.2	13.2
	CO**	2.6	3.1	3.1	3.4
	CO_2**	3.1	3.3	3.5	3.9
	N_2**	77.8	77.5	77.6	77.6
Gas Temperature (℃)		89.0	96.0	96.0	96.0

*vol. ppm, **vol. %

Next, the specific gravity and caloric value of the exhaust gases were compared (Table 5). With increased bio-ethanol, the gross calorific value (kJ/m^3) and calorific value increased about 15-20% and 16-22%, respectively. The specific gravity (air = 1) and density (kJ/m^3) showed no significant difference between the pure and the mixed fuels.

Table 5 Calorific value and specific gravity of exhaust gas in each mixing ratio.

Components	Unit	Condition 1 (0%)	Condition 2 (8%)	Condition 3 (12%)	Condition 4 (20%)
Gross calorific value	kJ/m^3	870	1030	1010	1060
Net calorific value	kJ/m^3	820	960	950	1000
Specific gravity	air=1	1.001	0.999	0.999	0.999
Density	kJ/m^3	1.295	1.292	1.292	1.291

CONCLUSIONS

This study, which was undertaken to encourage research appropriate for the University of the 21st Century, has investigated the conditions for bio-ethanol production over a period of three years, and has achieved a maximum rate of bio-ethanol addition of 20% in the production of a bio-plastic. On the basis of combustion experiments of bio-ethanol in both heating equipment and a lawn mower, the potential of bio-ethanol as alter-native energy has been revealed with respect to exhaust gas components and caloric value. In addition, the residue obtained after bio-ethanol production has been examined with respect to its use in the production of a bio-plastic, as well as the bio-degra-dability of this prototype bio-plastic in   soil (with-out the addition of a decomposition inducing com-pound). Slow decomposition of the bio- plastic was observed. Ongoing work is investigating the effects of increasing the percentage bio-ethanol residue added to the bio-plastic, as well as ways to promote degradation.

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