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Stimulation of Lipid Extraction Efficiency from Sewage Sludge for Biodiesel Production through Hydrothermal Pretreatment

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Abstract: In this study, two types of sewage sludge (primary sludge and waste activated sludge) were hydrothermally treated at 125–250 °C to enhance the lipid extraction efficiency and obtain a higher biodiesel yield. The enhanced efficiency of the lipid extraction method was compared with the efficiency of the organic solvent extraction method. The results confirmed that a hydrothermal reaction could be an appropriate option for disrupting sludge cell walls and increasing the lipid extraction from sewage sludge. The highest lipid recovery efficiency was observed at 200 °C, and the lipid recovery efficiency of primary sludge and waste activated sludge increased from 7.56% and 5.35% to 14.01% and 11.55% by weight, respectively. Furthermore, transesterified lipids, such as biodiesel from sewage sludge, mostly consist of C16 and C18 methyl esters, and have features similar to those of jatropha oil-based biodiesel. During the hydrothermal treatment, the carbon content in the sludge decreased as the carbon transformed into lipids and the lipids were extracted. The volatile matter and fixed carbon content in the solid residue decreased and increased, respectively, through chemical dehydration and decarboxylation reactions under hydrothermal reaction conditions.

Keywords: hydrothermal reaction; lipid recovery; sewage sludge; biodiesel; renewable energy

1. Introduction

Sewage sludge generation has increased with the demand for an improved quality of life. Sewage sludge is a pollutant that affects human health and must be disposed of in an environmentally sound manner [1,2]. However, in recent years sewage sludge has gained attention as a promising source for biofuel production [3]. Many studies have suggested anaerobic digestion or fermentation and the direct use (through incineration after drying) of sewage sludge [4], but the low biological conversion efficiency and high energy consumption during sewage sludge drying are drawbacks of these methods. Recently, the high lipid content of sewage sludge has been gaining worldwide attention as a possible feedstock, owing to its low cost and availability, as it is an abundant organic waste [5]. The production of biodiesel, which is transesterified vegetable oils or animal fats with an alcohol

(usually methanol), using sewage sludge has received considerable attention owing to its lipid-rich characteristics (containing up to 15–20% lipid components) [6,7]. Thus, the large amount of sludge produced from wastewater treatment plants, which is readily available without cost or even with a subsidy, is highly valued as a stable supply of biodiesel feedstock [8–10]. Furthermore, many countries have encouraged the use of B20 (blending biodiesel and petrodiesel at a ratio of 20:80), and have established laws that enforce specific levels of biofuel content in fuel sources to replace fossil fuels [11].

Lipid extraction is the step preceding biodiesel production from sewage sludge, and its performance can determine the biodiesel yield. Sewage sludge originates from diverse sewage sources and is a complicated microbial cell mixture [12], which makes it difficult to extract lipids from sewage sludge [13,14]. Several lipid extraction methods are available, and a common approach is to extract the lipids from pretreated (by dewatering and drying) sludge using organic solvents (for example toluene, hexane, methanol, ethanol, and chloroform). The dewatering and drying of sludge, as a pretreatment for raw sludge, can affect the lipid extraction performance and consequently the biodiesel yield [15]. Raw sewage sludge has a high moisture content, which might lead to a high organic solvent consumption and an increase in the lipid extraction system size. After reducing the moisture content, the dewatered sludge becomes very adhesive and requires drying for lipid extraction. However, the dewatering and drying of sludge are energy-consuming processes, and account for more than 50% of the total biodiesel production cost. As sewage sludge has both polar and nonpolar lipids, the polarities of the extraction solution and lipids in the sludge must match and the lipid extraction efficiency is too selective [16]. In this regard, an appropriate pretreatment is required to improve the efficiency of lipid recovery from sewage sludge [7,17].

Hydrothermal reactions might have benefits, in terms of the energy balance and selectivity of the lipid extraction efficiency, in contrast with the above-mentioned lipid extraction methods, which use organic solvents with pretreatment [17–19]. The hydrothermal reaction is performed at a moderate temperature (up to 250 °C) for less than 1 h, and consumes a low amount of energy for the treatment of organic matter [20,21]. Furthermore, the dielectric constant (ϵ) of water significantly decreases from 80 F/m (standard water: 20 °C and 1 atm) to 25–35 F/m when hydrothermal pretreatment is performed. This ϵ value is similar to the dielectric constants of common organic solvents under standard conditions, such as acetonitrile ($\epsilon = 37.5$), dimethylformamide ($\epsilon = 36.7$), and acetone ($\epsilon = 20.7$) [22]. Thus, lipid extraction from sewage sludge combined with a hydrothermal reaction may be a feasible option to improve biodiesel yield.

This study focused on the enhancement of the lipid extraction efficiency of sewage sludge through a hydrothermal reaction, and compared the efficiency with those of various conventional organic solvent extraction methods, such as chloroform-methanol, dimethyl ether, and n-hexane. The effects of hydrothermal reaction temperatures on the lipid extraction efficiency were also investigated. Furthermore, the characteristics of biodiesel produced from lipids extracted from the sludge through the hydrothermal reaction were evaluated.

2. Materials and Methods

2.1. Materials

The sewage sludges were obtained from a municipal wastewater treatment plant (WWTP) in Seoul, Korea. The characteristics of the primary sludge (PS) and the waste active sludge (WAS) are shown in Table 1. The initial moisture and total solids (TS) of the sludges were observed as 97.4–98.4% and 1.6–1.7%, with 1.3–1.4% volatile solids (VSs) by weight (wet basis), respectively. The total chemical oxygen demand (TCOD_{Cr}) concentrations were 16,375 mg/L for PS and 21,440 mg/L for WAS. Furthermore, the soluble chemical oxygen demand (SCOD_{Cr}) concentrations of PS and WAS were 1070 and 6330 mg/L, respectively.

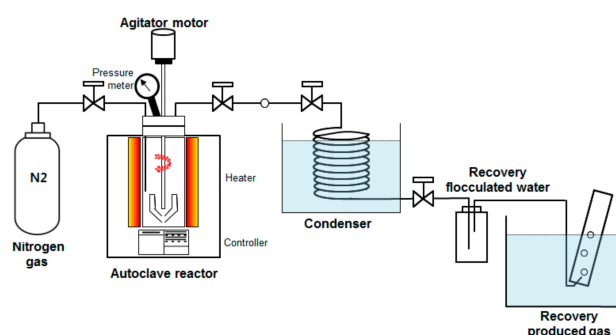
Table 1. Characteristics of PS and WAS as sludges.

Sludge	Moisture Content (wt. %) (a)	TS (wt. %) (a)	VS (wt. %) (a)	TCOD _{Cr} (mg/L)	SCOD _{Cr} (mg/L)
PS	98.3	1.7	1.4	16,375	1070
WAS	98.4	1.6	1.3	21,440	6330

(a) On a wet basis.

2.2. Hydrothermal Reaction Process

A lab-scale reactor with a volume of 1000 mL was used for the hydrothermal reaction (as shown in Figure 1). The reactor consisted of a reactor body, heater, steam condenser, and gas collector. Anaerobic conditions were achieved by purging the reactor with N₂ gas for 5 min before the hydrothermal reaction experiments began. The experiments were performed for a range of different temperatures (125–250 °C) for 30 min. The components in the reactor were mixed vigorously using an agitator at 200 rpm. When the hydrothermal reaction was complete, the reacted products were collected from the reactor.

**Figure 1.** Schematics of the lab-scale hydrothermal reaction process.

2.3. Lipid Extraction Using Solvents

The lipid extraction efficiency of the hydrothermal reaction was compared with that of the traditional solvent extraction methods using three different solvents such as chloroform-methanol, dimethyl ether, and n-hexane. The chloroform-methanol extraction method proposed by Bligh and Dyer [23] is as follows: 1 g of dried sludge and hydrothermal reaction products were mixed with 20 mL of mixed liquor (chloroform: methanol = 2:1, *v/v*) and then subjected to 60 °C for 4 h. Thereafter, the mixture was placed in a centrifuge for 15 min at 3000 rpm and the solvent phase was transferred to a glass vial. After evaporating the solvent at 60 °C, the vial was weighed. The dimethyl ether extraction method consisted of using 1 g of the dried sludge and hydrothermal reaction products, mixed with 20 mL of dimethyl ether, and sustaining for 30 min. Then, the samples were placed in the centrifuge for 10 min at 3000 rpm and the solvent phase was moved to a glass vial. After evaporating the solvent at 60 °C, the vial was weighed. The n-hexane extraction method consisted of using 1 g of the dried sludge and mixing hydrothermal reaction products with 10 mL of n-hexane at pH 4 (as H₂SO₄). This was then sustained for 30 min at 150 rpm. Afterwards, the mixture was placed in a centrifuge for 10 min at 3000 rpm, and the solvent phase was transferred to a glass vial. After evaporating the solvent at 60 °C, the vial was weighed.

2.4. Transesterification

The process of converting lipids extracted by the hydrothermal treatment into biodiesel proceeds in three steps as shown in Figure 2. In the first step, lipids from the wastewater sludge were extracted through a hydrothermal process. In the next step, 25 g of lipid extract produced by a hydrothermal treatment was added to a 500 mL flask containing 150 mL of methanol solution and 100 mL of n-hexane.

The methanol solution contained 1 volume% sulfuric acid as an acid catalyst. The reaction was carried out for 12 h at 55 °C using a water bath. After the reaction was completed, the mixtures were separated into two layers: n-hexane containing transesterified lipids in the upper layer, and methanol containing glycerol by-products in the lower layer. The by-products of the lower layer were carefully discarded, and we recovered the transesterified lipids along with n-hexane. In the final step, biodiesel was recovered from n-hexane using a rotary evaporator (R-124, Buchi, Switzerland) at 50 °C under vacuum conditions.

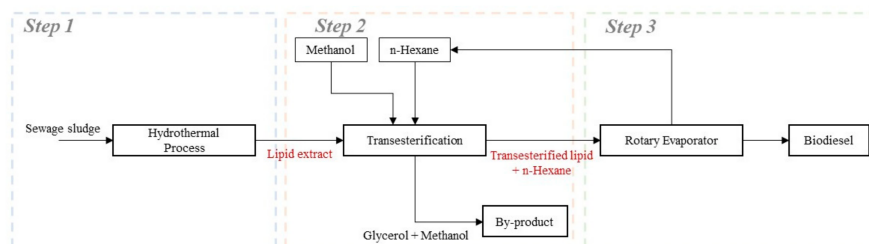


Figure 2. Schematics of the transesterification process coupled with hydrothermal lipid extraction.

2.5. Analytical Procedures

The experiment was conducted in triplicate. The extracted lipid from the sludge was then converted to biodiesel by a reaction with methanol, and the methanol to lipid molar ratio was 6:1 in the presence of H_2SO_4 (1% *v/v* methanol). The extraction had a 12 h reaction time. The property of the biodiesel was analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS) (Clarus 500, Perkin Elmer, Waltham, MA, USA). The transesterification efficiency was estimated based on the GC-MS results. After lipid extraction from the sludge, all the samples were prepared into a powder. The powders were sieved to separate particles between 177 and 250 μm for solid property analysis. An elemental composition analysis of solid products was conducted using a PerkinElmer CHN organic elemental analyzer (2400 Series II, PerkinElmer, Waltham, MA, USA). The proximate analysis was conducted using a thermogravimetric analyzer (TGA) (D-50 simultaneous, SHIMADZU, Kyoto, Japan). The calorific value was determined using a standard method of bomb calorimetry.

3. Results and Discussion

3.1. Lipid Recovery from Sewage Sludge through Solvent Extraction

Among the solvent extraction methods, the highest lipid recovery efficiency was observed using chloroform-methanol extraction. The values were 14.01% for PS and 11.55% for WAS, respectively (as shown in Figure 3). Previous studies have reported that the lipid recovery efficiency of sewage sludge, via a solvent extraction, is typically in the range of 10–16% for PS and WAS. The lipid recovery efficiency can vary due to the characteristics of sludge and the type of treatment plant. The observed lipid recovery efficiency is comparable to the efficiencies reported in previous studies [3,6,7,18]. Solvents with a high polarity are required to rupture the phospholipid layer and release the lipid drops. In terms of polarity, methanol (polar) is more suitable than hexane (non-polar) for the aforementioned reason [24]. Accordingly, the lipid recovery with chloroform and methanol was higher than the lipid recovery rates of other solvents. Dimethyl ether is a more polar solvent than n-hexane and has a similar extraction yield to that of a chloroform-methanol solvent. Since chloroform is toxic, dimethyl ether could be a suitable alternative for the chloroform-methanol solvent.

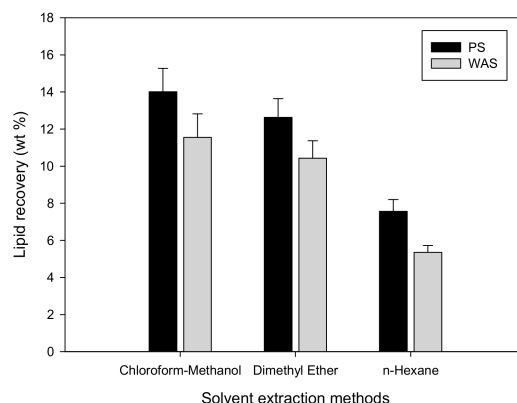


Figure 3. Lipid recovery efficiency from sludges through differential solvent types.

The lipid content of WAS was lower than that of PS. Most of the lipid components of the sludge are microbial membranes; therefore, WAS is mostly composed of microbial flocs. These results are similar to those from previous research, and highly correlated with changes in lipid content according to water treatment plants, recent economic activity, and eating habits.

3.2. Improving Lipid Recovery Efficiency with Hydrothermal Reaction

WAS was used to determine the effects of the hydrothermal reactions of sewage sludge on lipid recovery via solvent extraction. The hydrothermal reactions are an appropriate option for disrupting sludge cell walls [20,25]. To investigate the effects of differential hydrothermal reaction temperatures on the lipid extraction from WAS, the hydrothermal reaction process was conducted as a pretreatment at 125, 150, 175, 200, 225, and 250 °C. As shown in Figure 4, according to the increase in the hydrothermal reaction temperature, the lipid recovery efficiency via chloroform-methanol extraction increased until 225 °C, and then experienced a decrease at 250 °C. However, there was no significant difference in the lipid recovery efficiency between 200 and 225 °C. The results showed that the hydrothermal reaction raised the recovery efficiency by 225% at 200 °C and 235% at 225 °C, when compared to the efficiency without hydrothermal reaction. The chemical structure of the lipids from sludge was disrupted due to thermal decomposition. However, when the temperature increased past 250 °C in the hydrothermal reaction, the lipid content was decreased. This is because the lipid decomposed, or, in other words, the long chain of lipids was converted to a shorter chain and further decomposed to smaller molecules of organic matter because of the increasing temperature in the hydrothermal reaction [18,26,27].

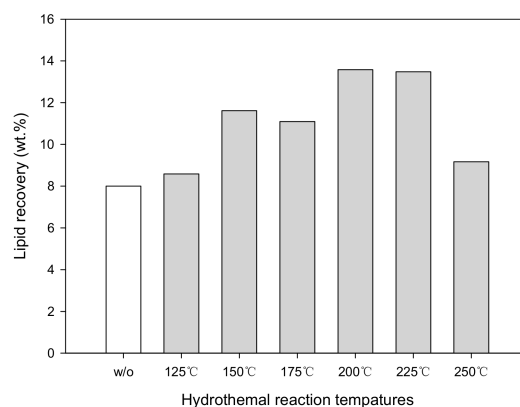


Figure 4. Effect of the hydrothermal reaction temperatures on the lipid recovery efficiency.

Figure 5 shows that the optimal temperature for the hydrothermal reactions applied to the sewage sludge was between 220 and 225 °C. The lipid recovery efficiency showed that there was a 150%

to 172% increase in PS and WAS, respectively, compared to the results without the hydrothermal reaction. Hydrothermal treatment is an appropriate option for disrupting the sewage sludge cell walls, which results in an increase in the lipid extraction from sludge with PS and WAS.

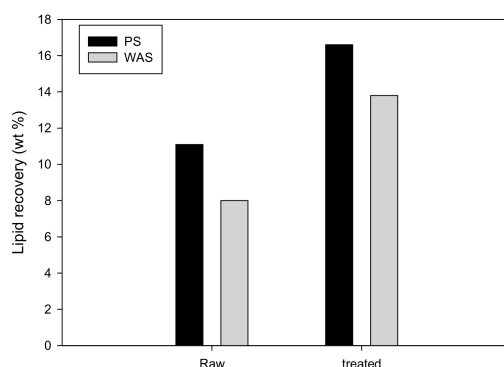


Figure 5. Improvement of the lipid recovery efficiency of sludges through hydrothermal reaction.

3.3. Transesterification of Lipids from the Sludge

The lipids extracted from the sludge contain various components, including fat, oil, and fatty acids. The triglycerides (TG) and phospholipid fatty acids (PFLAs), which are the major lipid components of the sludge, are able to be converted to biodiesel fatty acid methyl esters (FAMES) via transesterification [23]. On the other hand, about 20–36% of inert lipids (such as free fatty acids, wax esters, and paraffins) remain in the crude biodiesels [28].

The composition and content of FAMES are two of the key indicators used to determine the quality of biodiesel. Biodiesel is identified through saturated and unsaturated FAMES with carbon backbones from C12 (lauric acid) to C24 (lignoceric acid). In particular, the main component of animal oil-derived biodiesel consists of saturated fatty acids such as C16 (palmitic acid) and C18 (stearic acid), and vegetable oil-derived biodiesel contains a large number of unsaturated fatty acids such as C16:1 (palmitoleic acid), C18:1 (oleic acid), and C18:2 (linoleic acid) [29]. The composition of individual FAMES is an important factor in determining the fuel characteristics of biodiesel, such as combustion rate, ignition temperature, fluidity, and viscosity [3]. Although large amounts of saturated FAMES found in animal fats have a high combustion rate, they can degrade the quality of biodiesel by reducing the fluidity. Even though the optimal ratio of saturated and unsaturated FAMES has not been established, containing a high proportion of unsaturated fatty acids is more favorable for biodiesel quality.

Figure 6 shows the 13 FAMES from C12 to C24 identified in the biodiesel that were produced by the transesterification of lipids extracted by a hydrothermal reaction. Among the identified FAMES, sludge-derived biodiesel has predominantly C16:0 (palmitic acid), C18:0 (stearic acid), and some C18:1 (oleic acid) methyl ester, besides C19:0, which is used as an internal standard. The composition was complex because the sewage sludge lipids originate from both animal fats and vegetable oils. Nevertheless, the FAME compositions of the sludge-derived biodiesel were more similar to those of jatropha oil (Table 2), and comprised 38.6% of the total weight of the extracts.

3.4. Characteristics of Solid By-Products after Lipid Extraction by Hydrothermal Treatment

Table 3 compares the characteristics of the raw solids and lipid extracted solid residue (by-products) of sludge. After lipid extraction, the properties of solid residue decreased the carbon content by transferring the volatile matter and carbon source into lipid content. However, the volatile matter of the byproduct decreased while the fixed carbon increased due to the chemical dehydration and decarboxylation reactions of the hydrothermal carbonization reaction [25,31,32]. As a result, the fuel ratio (FC/VM) increased from 0.18 to 0.19 with PS and 0.16 to 0.23 with WAS, respectively. The decrease in the carbon source, lost during the lipid extraction effect, also decreased the calorific value.

The calorific value of solid residue decreased to 17.2 MJ/kg of PS and 16.2 MJ/kg of WAS, respectively. Consequently, the calorific value of the solid by-products decreased due to the lipid extraction, but this was supplemented by hydrothermal carbonization.

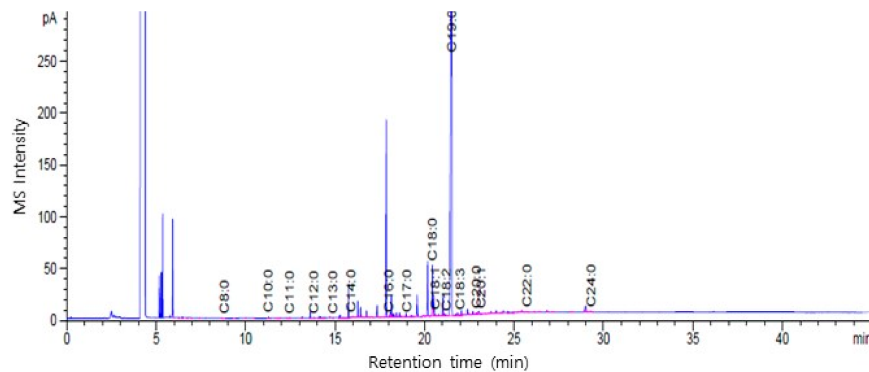


Figure 6. GC-MS chromatograms of the analysis data of the biodiesel from the extracted lipids of sludge.

Table 2. FAME compositions of the vegetable oil and sludge-derived biodiesel.

Fatty Acid	Content	Amount (%)				
		Soapnut ⁽¹⁾	Rapeseed ⁽¹⁾	Palm ⁽¹⁾	Jatropha ⁽²⁾	Sludge ⁽³⁾
Lauric acid	C12:0	0	0	0	0.31	0.61
Myristic acid	C14:0	0	0	0.5–6	0	1.45
Palmitic acid	C16:0	4.67	1–3	32–45	13.38	18.89
Palmitoleic acid	C16:1	0.37	0.2–3	0	0.88	0
Stearic acid	C18:0	1.45	0.4–3.5	2–7	5.44	5.62
Oleic acid	C18:1	52.64	12–24	38–52	45.79	8.78
Linoleic acid	C18:2	4.73	12–16	5–11	32.27	1.39
Linolenic acid	C18:3	1.94	7–10	0	0	0.26
Arachidic acid	C20:0	7.02	0.5–2.4	0	0	0.45
Eicosenoic acid	C20:1	23.85	4–12	0	0	0.65
Behenic acid	C22:0	1.45	0.6–2.1	0	0	0.25
Erucic acid	C22:1	1.09	40–50	0	0	0
Lignoceric acid	C24:0	0.47	0	0	0	0.26

⁽¹⁾ Chhetri et al., 2008 [29]; ⁽²⁾ Gobitz et al., 1999 [30]; ⁽³⁾ this study.

Table 3. Characteristics of the solid byproduct after the lipid extraction.

	PS	WAS	Solid from PS ^(a)	Solid from WAS ^(a)
Moisture content (wt. %) ^(b)	98.3	96.2	–	–
Ultimate analysis (wt. %) ^(c)				
Carbon	41.42	37.48	37.82	35.15
Hydrogen	6.75	5.77	6.04	6.01
Oxygen	23.08	22.97	24.8	22.77
Nitrogen	6.33	7.5	6.02	6.91
Atomic H/C ratio	1.78	1.68	1.74	1.87
Atomic O/C ratio	0.42	0.46	0.49	0.48
Proximate analysis (wt. %) ^(c)				
Fixed carbon	11.88	10.39	12.02	13.07
Volatile matter	67.68	63.31	62.66	57.77
Ash	22.42	26.28	25.32	29.16
Fuel ratio ^(d)	0.18	0.16	0.19	0.23
Calorific value (MJ/kg) ^(c)	19.4	16.8	17.6	16.2

^(a) After lipid extraction by hydrothermal reaction at 200 °C; ^(b) as received; ^(c) on a dry basis; ^(d) fixed carbon/volatile matter.

4. Conclusions

Sewage sludge with a certain level of lipid content has been considered as an attractive biodiesel feedstock. The biodiesel productivity of sewage sludge can be improved by increasing the lipid recovery efficiency. A hydrothermal reaction is a thermal pretreatment process that disrupts sludge cell walls and effectively releases lipids. As the temperature of the hydrothermal reaction increased to 225 °C the lipid recovery efficiency improved, and an extraction efficiency similar to that of the conventional solvent extraction method was achieved. However, the efficiency decreased again under higher temperatures (from 250 °C) as the chemical and physical structure of the sludge was decomposed. Biodiesel is produced through the transesterification of the lipids extracted from the sewage sludge. The components of the biodiesel were analyzed by GC-MS. The results indicated that the most dominant FAMES among the sludge biodiesel were C16 and C18, and that their characteristics were similar to those of the biodiesel recovered from jatropha oil. After lipid extraction, the produced solid residue tends to decrease the carbon content by converting the volatile matter and carbon content into lipid content. However, the volatile matter from the by-products decreased while the fixed carbon increased due to the chemical dehydration and decarboxylation reactions of the hydrothermal carbonization process.

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