Biofuel extraction by NH_3 from microalgal feedstock (II)

Takashi Nohmi (1,2) ,Taeko Fujii(1),Y.Sugawara(1),
Kiyoshi Sakuragi(3),H.Takiguchi(2),Toshio Mogi(2)
1. HysafeNohmi, Setagaya-Ku, Tokyo, Japan
2. The Uni. Tokyo Dept. of Chem. Syst. Eng., Hongo,Bunkyo-ku, Tokyo, Japan
3. Central Research Institute of Electric Power Industry, Yokosuka, Kanagawa, Japan

Novel Aspect: NH_3 extraction method, Hexane Soxhlet method, Bligh-Dyer method, ESI, Acetonitrile Water solvent, Microalgal feed stock, Nannochloropsis Oculata, Tisochrysis lutea, Pavlova, Tetraselmis chui, Raw material

Introduction:

Zero emissions and fossil alternative fuel studies have been developed. We have studied H_2 and NH_3 combustions. NH_3 combustion and their safe applications including mixed combustion with coal have been studied for next energy source. From the safety standpoint, NH_3 has priority to H_2 because NH_3 in liquid state is stable and transportable. Considering safety application NH_3 might be the next promising energy source. We can carry temperature and pressure to change state of NH_3 from liquid to gas reversibly by relatively small energy. So NH_3 extraction of biofuel component from microalgal feed stock are focused.

We tried to use Nannochloropsis Oculata, Tisochrysis lutea, Pavlova and Tetraselmis chui as raw material to extract fuel component and the extraction products analysis by ESI methods was carried out. A frozen solution (moisture 75.5%) was used as a microalgae material. Nannochloropsis belongs to the Eustigmatophyceae. It is a very small, spherical, single-celled alga, measuring 2-5 microns in size, green with chloroplasts, and its cell morphology resembles that of Chlorella in the Chlorophyceae order and it is also called marine chlorella. These are an oil-producing alga that accumulates more than 50% of oil in their body. It contains eicosapentaenoic acid (EPA), an omega-3 fatty acid.(Fig.7)

Table 1　Component of Raw material and Omega fatty acids(EPA)

Name	C14:0	C16:0	C16:1	C16:2	C16:3	C18:0	C18:1	C18:2	C18:3	C20:5	Total	Figure of Microalgal cultures
Nannochloropsis Oculata	6	20	27				2	3	1	35	94	Fig.1
Tisochrysis lutea	10.6	77.7	8.8	2.9							100	Fig.2
Pavlova	24.2	37.8	13.3				6.2	9.1		9.4	100	Fig.3
Tetraselmis chui		53.9				30.3	7.4	5.7	2.8		100	Fig.4
Chlorera		18	5	12	2		9	43	10		99	Fig.5
Palm Oil	3	35				7	37	11			93	Fig.6
Ω fatty acid EPA										EPA		Fig.7

Fig.1 Nannochloropsis Oculata

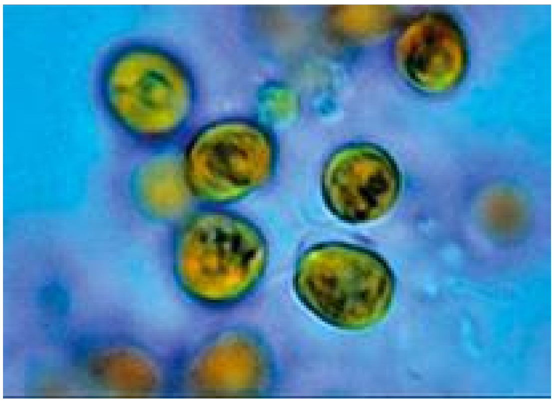

Fig.2 Tisochrysis lutea

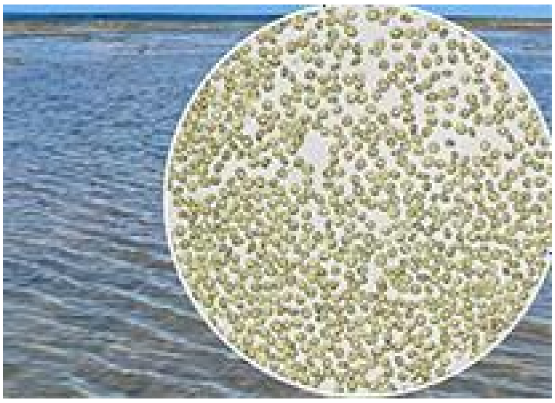

Fig.3 Pavlova

Fig.4　Tetraselmis chui

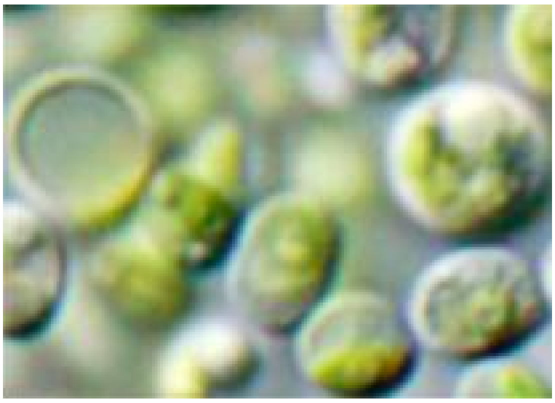

Fig.5 Chlorera

Fig.6 Palm Oil

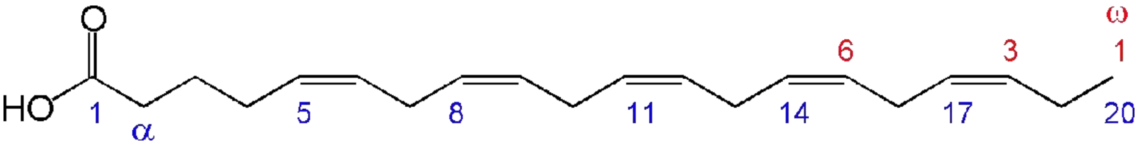

Fig.7 Structure of Ωfatty acid EPA

Experimental Methods:

1. Materials and methods

Lipid extraction from Nannochloropsis oculate as well as other raw material, Tisochrysis lutea, Pavlova and Tetraselmis chui were carried out in the same method. A wet sample of N. oculata, a commercially produced biomass feedstock for valuable components production, was obtained from Chlorella Industry Co. Ltd. (Tokyo, Japan). The sample was stored at -20 °C until further use. The water content of the wet N. oculata sample, estimated on the basis of the mass loss using the vacuum oven drying method at 40 °C, was 75.5 wt%. The characteristics of extraction using liquefied NH_3(l) were compared to those of two conventional extraction methods: the Bligh–Dyer and Hexane Soxhlet methods. A schematic of the plug-flow-type extractor that allows the passage of NH_3(l) through a microalgal sample filled vessel was shown in Fig.8.

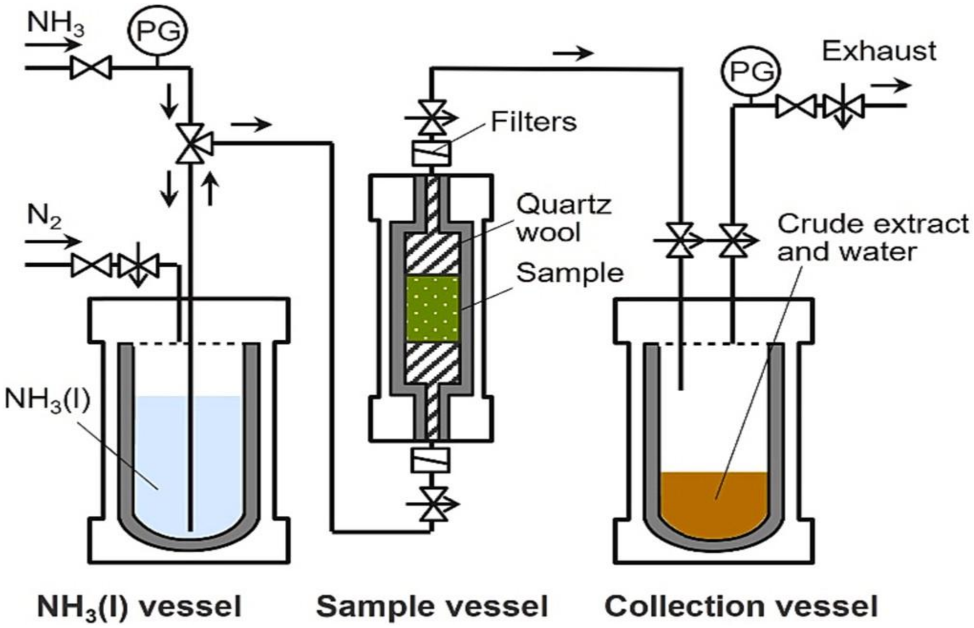

Fig. 8. Schematic of the lab-scale liquefied NH_3 extraction

Liquefied NH_3 extraction experiments were conducted in an apparatus with a plug-flow-type extractor, in which NH_3(l) was passed through the sample specimen bed and stored in a collection vessel at 20 °C and 0.85 MPa. After extraction, NH_3 was vaporized and removed by releasing the inside of the experimental apparatus back to atmospheric pressure to obtain the extract and residue. PG indicates the pressure gauge.

Liquefied NH_3 extraction method

The wet N. oculata (1 g) was filled into the pressure-resistant vessel (HPG-10-5, Taiatsu Techno Corp., Tokyo, Japan). Lipid extraction was performed by passing NH_3(l) through the sample at 20 °C, 0.85 MPa, and with a flow rate of approximately 10 $cm^3 \cdot min^{-1}$. The resultant NH_3(l) extract was then passed through a 0.1 μm glass fiber filter (GF-75, ADVANTEC Co., Ltd., Tokyo, Japan) followed by a 0.1 μm PTFE membrane (T010A013A, ADVANTEC Co., Ltd.), and collected in the collection vessel. After extraction, the solid fraction remaining in the sample vessel (extraction residue) and the liquid fraction in the collection vessel (crude extract and water) were returned to atmospheric pressure to vaporize the NH_3(l). To measure the crude extract yield, residual water and solvent in the extract were removed using vacuum distillation at 40 °C to a constant weight. Each extraction data point was represented as the mean of three measurements ± standard deviation. The yield was then calculated based on the weight of the crude extract after distillation as follows:

Crude extract yield (wt%)

= (wt.of the crude extract/dry wt.of microalgae) × 100

NH_3 which was expected to be widely used as an energy carrier, is a gas at room temperature and pressure, but turned into a liquid phase when pressurized (0.85 MPa at 20°C) or cooled (-33.5°C and normal pressure). By changing the pressure and temperature, it was possible to introduce gas into the raw material, harvest the extract in solid form, and discharge excess NH_3 and water as gaseous state.

Bligh–Dyer method

Lipid extraction using the Bligh–Dyer method was performed using a mixture of chloroform–methanol–water (1:1:0.9, v/v/v). Wet N. oculata was homogenized using a sawtooth generator probe (Dremel 300 Series; Robert Bosch Tool Corp., IL, USA; 10 mm [o.d.]) for 30 min at 10,000 rpm. Subsequently, 10 g of the sample was mixed with 12.5 mL of chloroform and 25 mL of methanol in the flask. The mixture was then shaken for 10 min, following which additional volumes of chloroform and water were poured into the flask. The mixture was shaken for 10 min and then centrifuged at 3000 rpm for 10 min (Centrifuge 5430 R, Eppendorf, Hamburg, Germany) to separate the upper and lower phases. The upper phase was rewashed with chloroform. The lower phase (chloroform layer with lipids) was recovered separately and washed with a chloroform–methanol–water mixture (3:48:49, v/v/v) before being centrifuged at 3000 rpm for 10 min to obtain the lipid-containing lower phase. The lipids were then separated from the chloroform using a rotary evaporator (N-1110, EYELA, Tokyo, Japan) and dried under vacuum at 40 °C. The extraction value was the mean of three measurements ± standard deviation.

Hexane Soxhlet method

N. oculata was subjected to drying and cell disruption for extraction using the hexane Soxhlet method. The sample was vacuum-dried for 8 h at 40 °C. The sample was then disrupted in hexane using a homogenizer equipped with a sawtooth generator probe (Dremel 300 Series; Robert Bosch Tool Corp.) for 30 min at 10,000 rpm. After cell disruption, the sample was subjected to the hexane Soxhlet method for 18 h at 70 °C. To measure the yield, the extract was vacuum-dried at 40 °C to remove the solvent and subsequently weighed. The crude extract yield obtained using the NH_3(l) extraction method (48.5 %) was higher than that obtained using the Bligh–Dyer (21.2 %) and hexane Soxhlet (17.1 %) methods, although the NH_3(l) extraction method did not require drying and cell-disruption processes.

Sample preparation

N. oculata solutions (moisture content 75.5%) was used as the raw material microalgae. Samples were prepared by 3 methods: Ammonia extraction method, Bligh-Dyer method by Chloroform and Methanol solvent extraction and Soxhlet Extraction method using Hexane as solvent. Samples are dissolved in aqueous solution of acetonitrile/H20=50%/50% solvent and the fatty acid and triglyceride analysis by negative ESI method was performed.

ESI:

ESI method was applied to in negative and positive mode in acetonitrile aqueous solvent. Analysis of fatty acids and the products by three solvent extraction methods are carried out. Oleic acid, monooleic, dioleic, trioleic acid were used as standard samples. Ammonia extraction, Bligh-Dyer method by Chloroform and Methanol solvent extraction, Soxhlet Extraction method using Hexane as solvent samples were used as extraction samples. Measurements were carried in negative ion mode and positive ion mode of ESi. But ESI in the positive mode showed unclear peaks based on fragmentations, so we will focus on the results in the ESI negative mode.

In addition, since neutral molecules and fragments of glycol units 44 were observed in the cationization method, it was found that glycol molecules fragmentation and water molecules were taken away during ionization. Dehydration reactions are likely to proceed. Ionization in negative mode gave the relatively easier anions analysis.

In the analysis of microalgae extracts, the main component of the ammonia extraction was m/z256 palmitic acid, with the other m/z439 and 431 Fragments of triolein were seen.

On the other hand, from the hexane extraction, the components including triglycerides appeared with m/z more than 800.

From this result showed that liquid NH_3 acts as a solvent to dissolve various organic compounds, so it was the best solvent for extraction.

We also believe that it was effective that useful components could be obtained from the hydrous microalgae by dehydration and drying after extraction.

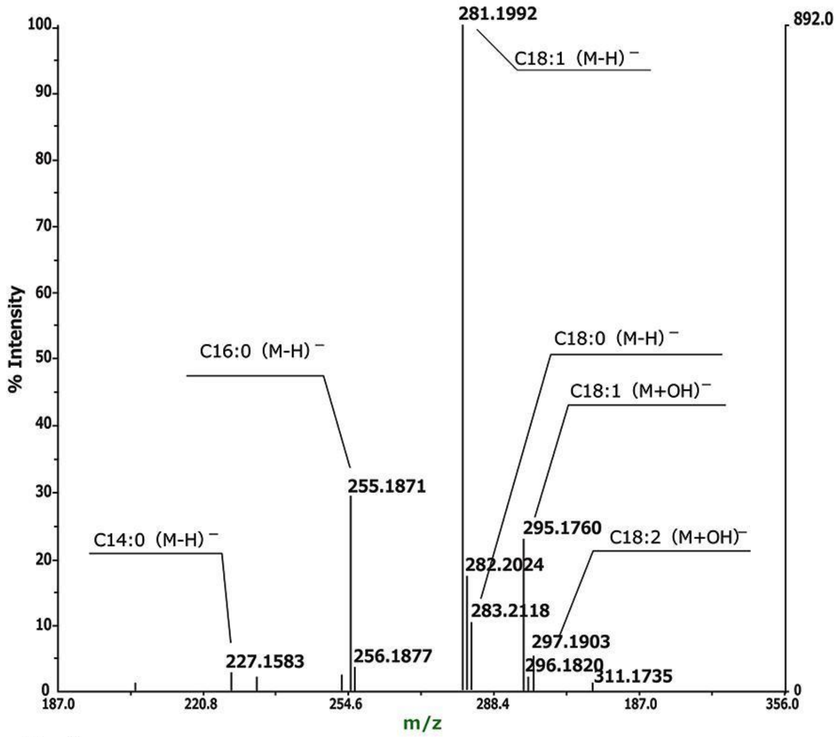

Fig.9 Negative ESI Spectrum of Dioleic Acid in ACN/H20 = 50/50 Solution

In Fig.9
Negative ESI Spectrum of Dioleic acid was shown.
From the peak of dioleic acid, the order of the largest peaks was C18:1, C16:0, C18:0, C18:2, C14:0, and it could be seen that diolein contained three types of fatty acids

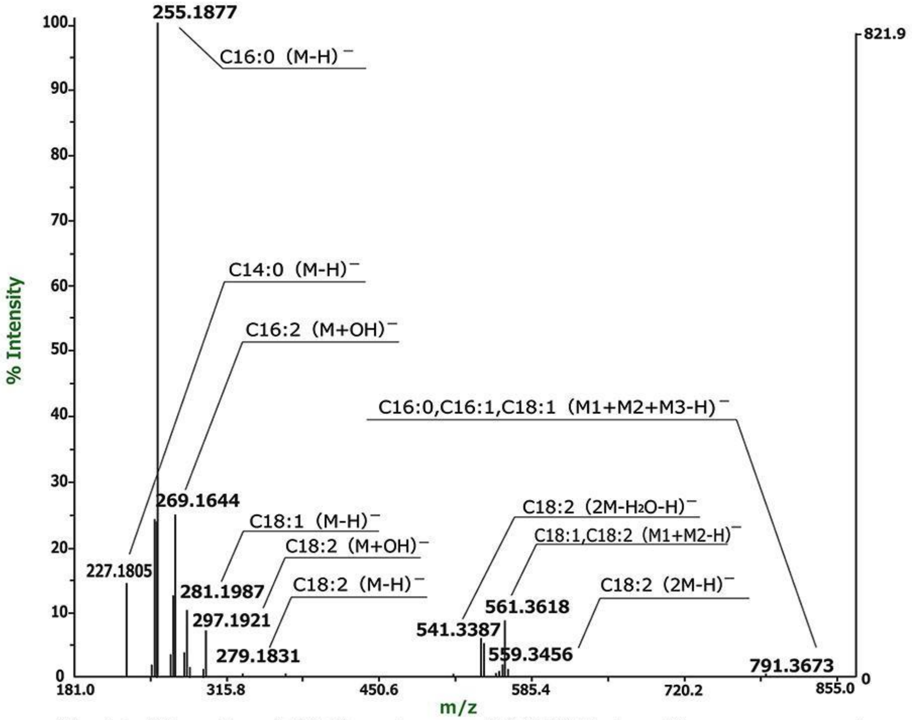

Fig.10 Negative ESI Spectrum of NH3 Extraction components from microalgae.

Fig10
In the ammonia extraction sample, three types of fatty acids were observed, in order of abundance: C16:0>C16:1>C16:2>C14:0>C18:2. the ammonia extraction sample could be seen that products contained three types of fatty acids.

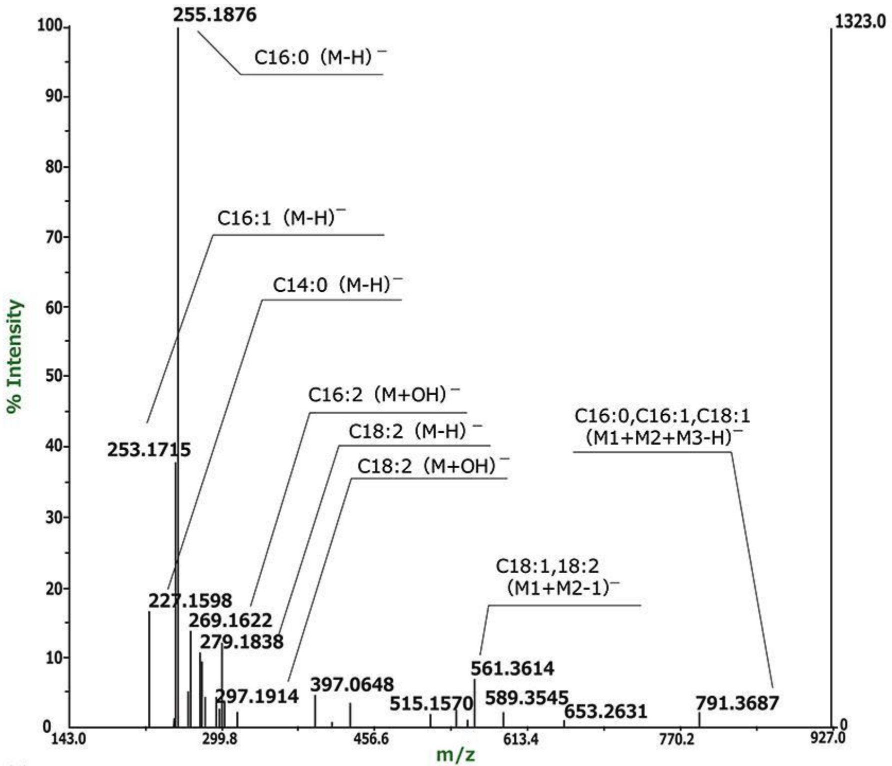

Fig.11 Negative ESI Spectrum of Chloroform methanol Extraction components from microalgae.

Fig11

In the chloroform/methanol extraction method, the order of abundance was C16:0＞C16:1> C14:0>C18:2.

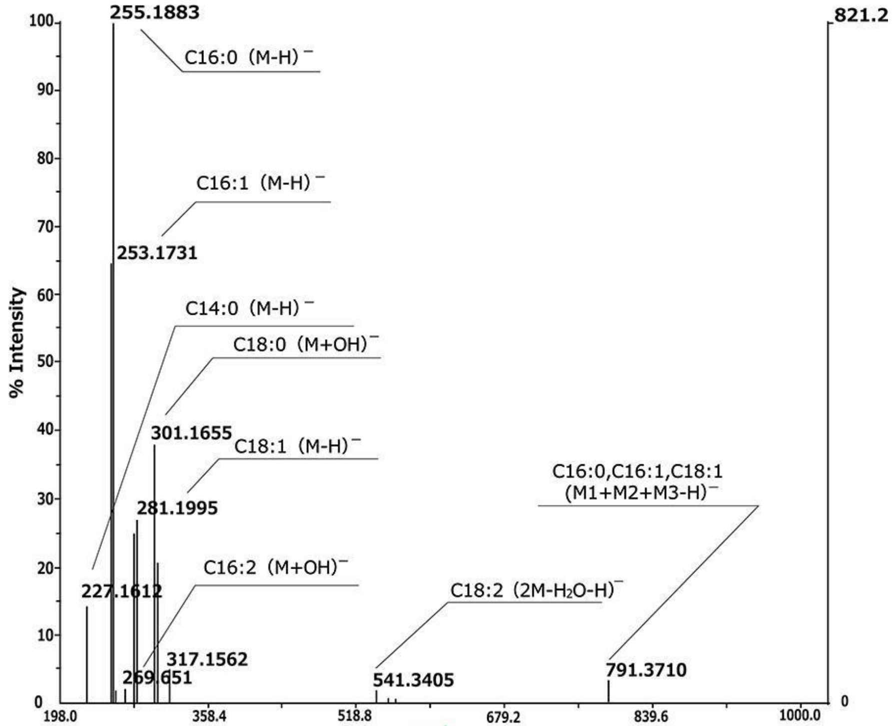

Fig. 12 Negative ESI Spectrum of Hexane Extraction components from microalgae.

Fig12

In the hexane extraction method, the order of abundance was C16:0>C16:1>C18:0>C18:1>C14:0

From the results, it was demonstrated that the fatty acid extraction results using the extraction solvent were in the order of abundance from the ESI results

NH3 C16:N>C14:0>C18:2 (N=0,1,2)

Chloroform/Methanol C16:N> C14:0>C18:2 (N=0,1)

Hexane C16:N>C18:N>C14:0 (N=0,1)

Result showed the extraction performance of NH_3 was similar to the Bligh–Dyer method of Chloroform/Methanol solvent and showed the excellent yield of product.

Table2 Extraction methods and pretreatment process and yield

No	Name	Yeild(%) of NH3(l) Ext.	Yeild(%) of Bligh and Dyer Ext.	Yeild(%) of Hexane Soxhlet Ext.
1	Nonochloropsis oculata	48.5	21.2	17.1
2	Tisochrysis lutea	39.5	16.1	18.8
3	Pavlova sp	54.7	15.8	18.1
4	Tetraselmis chui	70.9	12.9	8.5
5	Chlorella.vulgaris	39.7	11.1	18.8

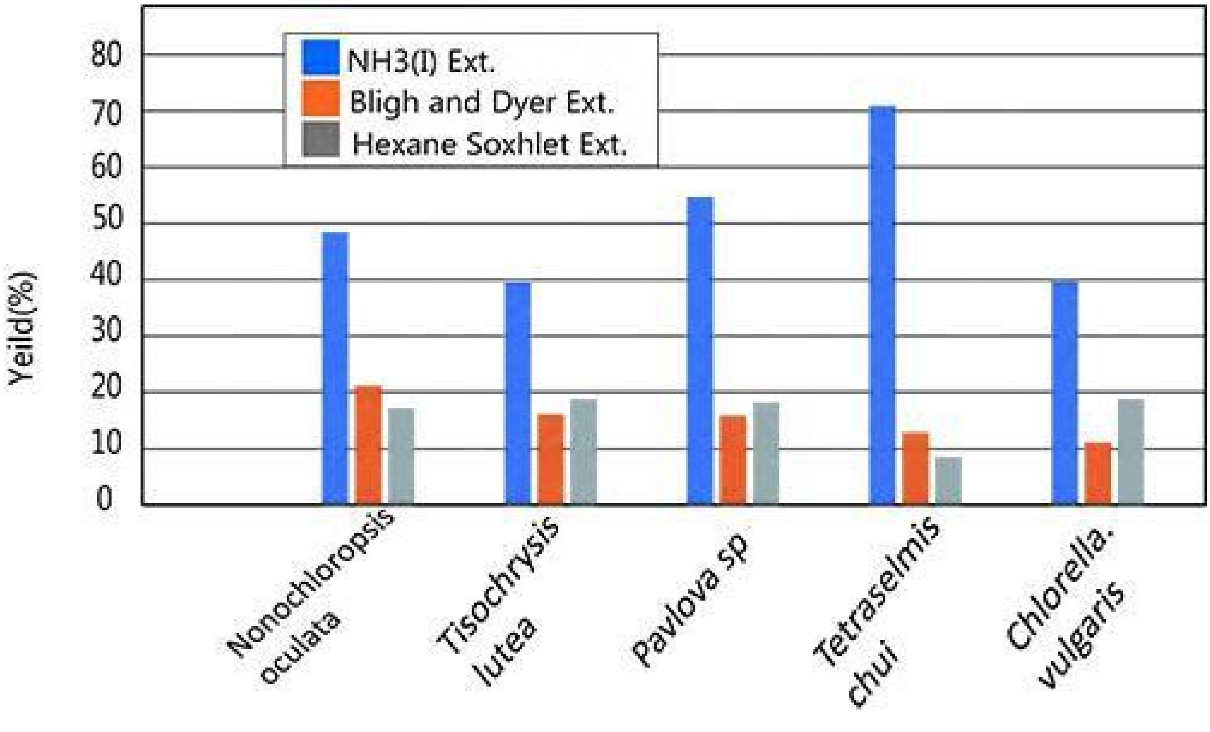

Fig.13 Comparison of Crude Extract Yield by 3 extracting methods

The results in Table 2 showed that the liquid ammonia extraction method could （extract fatty acids more efficiently than the Bligh–Dyer method of chloroform/methanol solven and the hexane Soxhlet method.

Conclusion

This liquidified NH$_3$(l) extraction method achieved a high yield of crude extract from raw material (Nannochloropsis Oculata, Tisochrysis lutea, Pavlova and Tetraselmis chui) removing more than 99 wt% of the water to obtain a dry residue. Although the extract contained compounds other than fatty acids with small molecular weights, the total FAME（Fatty Acid Methyl Ester）yield obtained using the method was comparable to that obtained by conventional extraction methods. The extracts obtained via our method contained valuable components such as saturated and unsaturated fatty acids (12 to 20 carbons). The method was a promising extraction technique for obtaining valuable components from microalgae, comparable to conventional methods but without requiring energy-consuming processes

We found that by using liquefied ammonia as an extraction solvent, fatty acid components could be extracted from unbroken wet algae particles with a yield comparable to that of existing methods that required drying and cell disruption. Since the liquefied ammonia extract contained many components other than fatty acids, this demonstrated the possibility of applying this technique as an extraction technology for microalgae components, not limited to fuel applications.

References

1) K.Sakuragi,M.Otaka, Bioresource Technology Reports,24,101623(2023)

2)T.Nohmi,J.B.Fenn,J.Am.Chem.Soc.,114,No.9,3241-3246(1992) Electrospray Mass Spectrometry of Poly(ethyleneglycoles with Molecular weight up to Five Million,

3) T.Nohmi,M.Maekawa,JohnB.Fenn,Membrane,35,(3)105-112(2010),From Electrospray to Electrosppining,

4) Soxlet,F.V.,Die gewichtsanalytische bestimmung des milchfettes. Dinglers Polytechnisches Journal, 232,461-465(1879)

5) Demirbas,A., Production of biodiesel from algae oils.,Energ.Source.A:Recovery,Unit., Environ.Eff.31,163-168(2008), http://doi.org/10.1080/15567030701521775.

6)K.Sakuragi,M.Otaka, ACS Omega,24,101623(2023)

7) K.Sakuragi,M.Otaka, Bioresource Technology Reports,24,101623(2023)

8) Biofuel extraction by ammonia from microalgal feed stock N.O.,T. Nohmi , K.Sakuragi, T.Mogi, MP142,ASMS (2024)