Biofuel production using Arctic psychrophilic microalga *Chlamydomonas* sp. KNM0029C

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ABSTRACT

In this study, 184 strains owned by KOPRI were used to find a candidate used as a feedstock for biofuels. The strain showing the highest lipid content was KNM0029C which was identified as genus *Chlamydomonas*. KNM0029C showed the highest cell concentration at 4 ˚C under 80 μmol photon $m^{-2}s^{-1}$ of light intensity in TAP medium. In order to efficiently produce biodiesel from biomass of KNM0029C, the production yield of FAME was compared between existing extraction methods and modified extraction methods. The modified A method yielded 0.16 g of FAME per g of biomass, which was 5.4 % lower than the conventional Lewis's method, but it reduced the process of removing moisture. After the biodiesel process, bioethanol was produced using the remaining residues. Bioethanol production using yeast fermentation was compared through a combination of physical, chemical, and biochemical (enzymatic) pretreatment methods. The highest yield of bioethanol was obtained by pretreatment with enzymatic treatment after ultrasonication and showed 0.22 g of bioethanol production per g of residual biomass. It was 38 % higher than the bioethanol production, which showed the highest yield in previous reports, using residual microalgae after de-oiled process.

MATERIALS & METHODS

Polar microalgae were collected from areas near Dasan Station (78˚55´N, 11˚56´E) in the Arctic and King Sejong Station (62˚13´S, 58˚47´W) in the Antarctic. The isolated microalgae strains were cultured in corresponding growth media of freshwater or seawater, depending on the sampling location. Each polar microalgal strain was classified following the systems of Korea Polar Research Institute (KOPRI) Culture Collection for Polar Microorganisms (KCCPM); KOPRI Arctic (North pole) Freshwater strain : KNF, KOPRI Arctic (North pole) seawater (Marine) strain : KNM, KOPRI Antarctic (South pole) Freshwater strain: KSF, KOPRI Antarctic (South pole) seawater (Marine) strain : KSM.

RESULTS

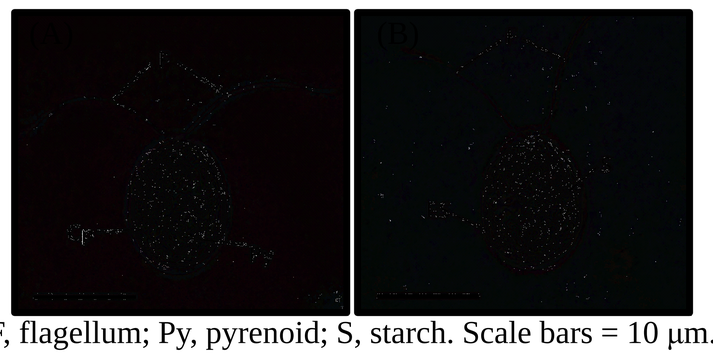

Fig. 1. Differential interference contrast of vegetative cells of the Arctic microalga *Chlamydomonas* sp. KNM0029C. (A) A vegetative cell featuring 2 flagella, a chloroplast and pyrenoid in optical section. (B) Surface sight of a cell presenting, a colored eyespot and starch. Cp, chloroplast; ES, eyespot; F, flagellum; Py, pyrenoid; S, starch. Scale bars = 10 μm.

Fig. 2. Tree constructed using Bayesian inference for the alignment of SSU rDNA from the Arctic green microalga KNM0029C and its relatives. Each species shown in bold characters is the type species of the genus.

Table 1. Comparison of FAME extraction methods for biodiesel production from *Chlamydomonas* sp. KNM0029C.

Method	Pretreatment	Lipid extraction	Catalyst; condition	Solvent	Fame yield (mg/g DCW)	Reference
Lewis	Freeze-drying biomass (100 mg)	Methanol /Chloroform (10:1)	HCl; 90°C, 2 hrs	Hexane /chloroform (1:1)	165.4	Lewis et al., 2000
Bligh & Dyer	Freeze-drying biomass (100 mg)	Methanol /Chloroform (2:1)	HCl; 90°C, 2 hrs	Chloroform	153.5	Bligh and Dyer, 1959
Sasser	Freeze-drying biomass (100 mg)	Methanol /Hexane /methyl tert-butyl ether (2:1:1)	HCl: 80°C, 10 min	Hexane /methyl tert-butyl ether (1:1)	143.5	Sasser, 1990
Modified FAME extraction A	Using wet biomass (100 mg as DCW), sonication treatment	Methanol /Chloroform (2:1)	HCl; 90°C, 1 hr	Chloroform	156.5	This study
Modified FAME extraction B	Using wet biomass (100 mg as DCW), sonication treatment	Methanol /Chloroform (10:1)	HCl; 90°C, 1 hr	Hexane /Chloroform (1:1)	140	This study

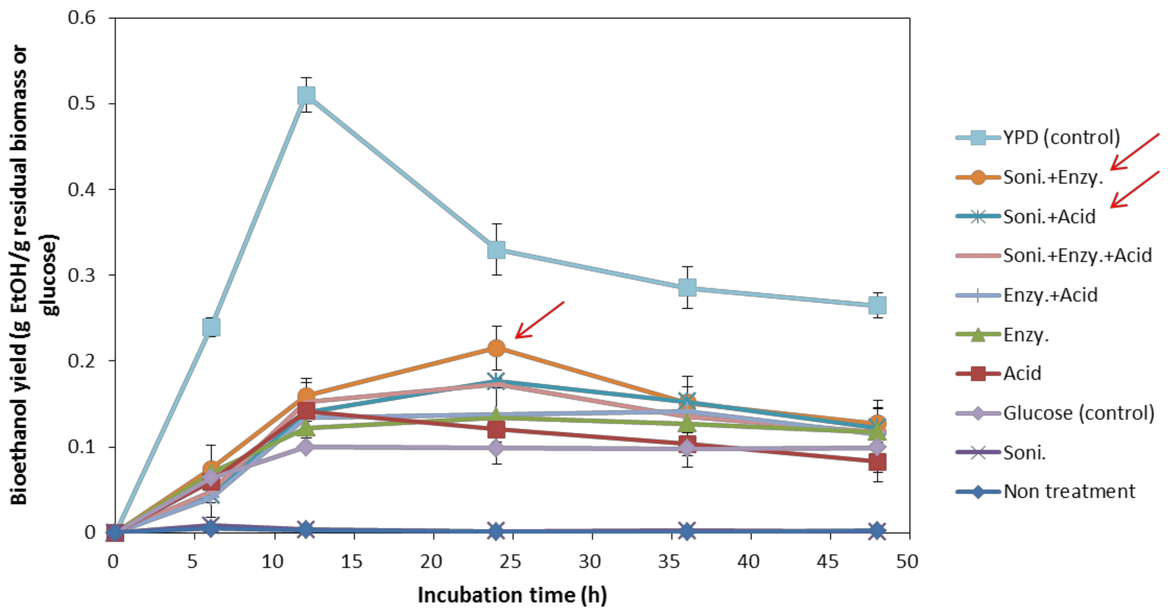

Fig. 3. Effects of different hydrolysis methods on bioethanol yield in polar microalgal biomass. Sonication, acid and α-amylase were used for pretreatment, and pretreated biomass was fermented by *Saccharomyces cerevis*iae to produce ethanol. Data shown are the average ethanol content ± standard deviation (SD) from triplicates.

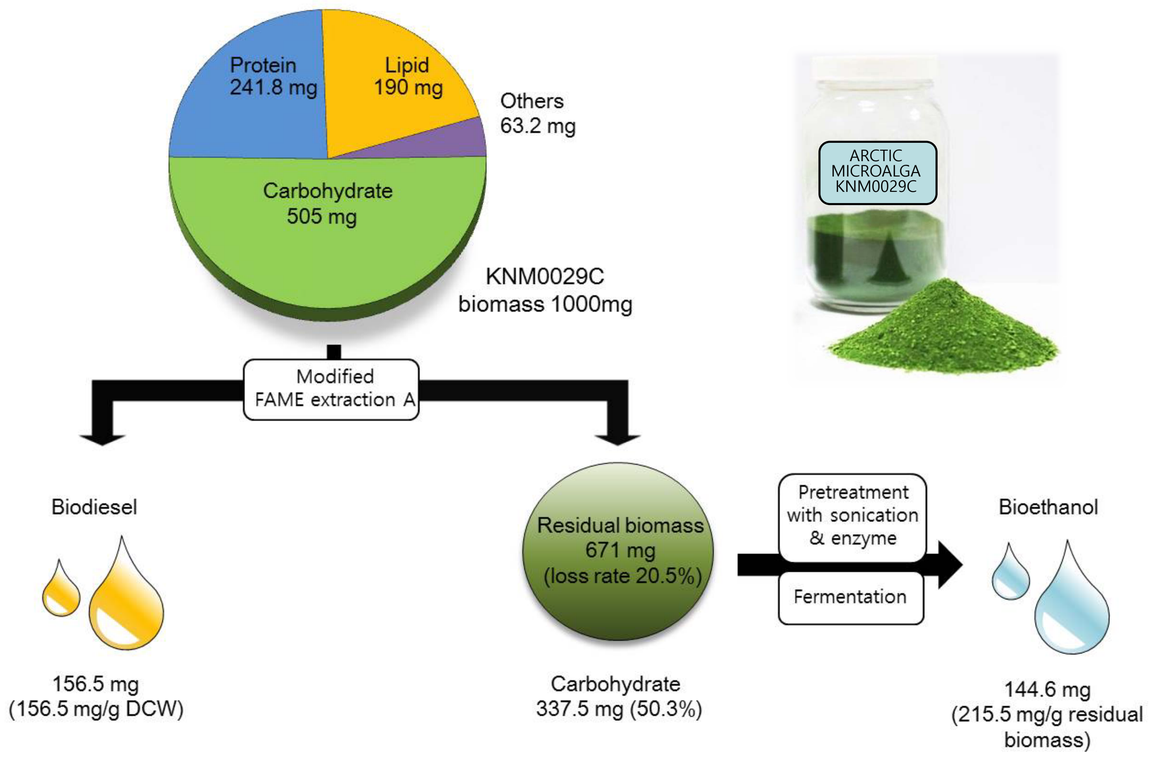

Fig. 4 Conversion of biomass of Arctic *Chlamydomonas* sp. KNM0029C into biofuels.

CONCLUSION

The FAME content of KNM0029C was 15.7 %, which was low for biodiesel production. However, carbohydrate content was 50.5%, which would be suitable for use as a strain for ethanol fermentation. In this study, various methods of FAME extraction were compared to produce biodiesel and modified methods extracting FAME from wet biomass have been proposed to reduce cost and procedure. These results showed that using wet biomass reduced the process of freeze-drying. From microalgae, lipid is extracted to make biodiesel fuel, and then residual biomass is generated as a byproduct. The biomass is fermented by yeast to produce ethanol. The psychrophilic *Chlamydomonas* sp. KNM0029C was used as a feedstock for biofuels, and 156.5 mg of biodiesel and 144.6 mg of bioethanol were produced from 1000 mg of biomass. These results can be utilized in the process of efficiently producing biodiesel and bioethanol from polar microalgae.

Acknowledgement

This work was supported by grants of KOPRI (PE19180 and PE19270)