# The R&D of microalgae-based biofuel production at University of Tsukuba

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## ABSTRACT

Microalgae have been attracting an attention as an alternative feedstock for biofuel production, because some microalgae show high oil productivity, and that microalgal cultivation does not conflict with food production. Many microalgae accumulate lipids such as triacylglycerol (TAG). Although TAG can be converted into biodiesel (FAMEs), it would be more straightforward if we could extract hydrocarbons from algal biomass. To address this issue, we focus on the biofuel production using two hydrocarbon-producing algae, *Botryococcus braunii* and *Aurantiochytrium* sp.; the former is a phototroph whereas the latter is a heterotroph. One of the big challenges toward the hydrocarbon production using the algae is the cost of cultivation (e.g., nutrient supply). By combining the cultivation of the algae into a wastewater treatment, we expect that the cost of the biofuel production can be greatly reduced. We are developing a combinatory culture system in the wastewater treatment plant in Sendai city, which is on the way to restoration from the devastating damage caused by the 2011 Tohoku earthquake and tsunami.

Keywords**:** microalgae, hydrocarbons, *Botryococcus*, *Aurantiochytrium*, Tohoku earthquake

Microalgal biofuel attracts increasing attention worldwide as alternative biofuel resources. Several microalgae accumulate larger amount of lipids, and the potential productivity of microalgal fuel is more than an order of magnitude higher than those of other crops like corns or soybeans (Chisti 2007). Most microalgae accumulate lipids in the form of triacylglycerols (TAGs), which can be converted via trans-esterification to fatty acid methylesters (FAMEs) that can be used as biodiesel. One of the drawbacks of FAMEs production from TAGs is the massive recovery of glycerin, which is less useful, as a byproduct. It is thus much better if we can extract hydrocarbons, not TAGs, from algal biomass. In addition, hydrocarbons are more compatible than TAGs with the present oil refinery infrastructures, allowing us to convert hydrocarbons into liquid transportation fuels such as gasoline and jet fuel without developing a refinery system *de novo*. These are the reasons we investigate hydrocarbon-accumulating algae. At present, we focus on two algal species, *Botryococcus braunii* and *Aurantiochytrium* sp., both of which accumulate hydrocarbons (Yoshida et al. 2012).

*Botryococcus braunii* is a green algae belonging to Trebouxiophyceae, Chlorophyta. *B. braunii* forms a well-developed colony, in which cells are surrounded by an extracellular matrix mainly consisting of polysaccharides and high molecular weight hydrocarbon-derivatives (Weiss *et al*. 2012). Notably, *B. braunii* accumulates high amount of hydrocarbons within the extracellular matrix, reaching 75% of dry cell weight (Chisti 2007). The hydrocarbons produced by *B. braunii* vary among strains and the hydrocarbons can be classified into three groups, n- alkadiene, botryococcenes and lycopadienes. The specific production of different hydrocarbon groups correlates with the phylogeny of the strains (Kawachi *et al*. 2012), indicating that the specific hydrocarbon production is stable. *B. braunii* is phototrophic alga and thus requires light, CO2 and nutrients (e.g. N, P) for growth. We deal with the strain *B. braunii* BOT-22, which shows the fastest growth with highest hydrocarbon contents (Watanabe & Tanabe 2013).

BOT-22 accumulates $C_{34}$ botryococcene as a major hydrocarbon. Analyses of physical property of $C_{34}$ botryococcene indicated that this hydrocabons are comparable to LGO, but require hydrocacking for practical use (Nagano *et al*. 2012).

Thraustochytrids are heterotrophic algae, do not perform photosynthesis, and thus requires organic carbon sources (e.g., glucose) other than $CO_2$ for growth. Traditionally, thraustochytrids have been investigated for commercial DHA production. In 2010, we have obtained a novel thraustochytrid strain *Aurantiochytrium* sp. 18w-13a, which accumulates high amount of a hydrocarbon squalen inside the cells, up to 20% of its dry cell weight (Kaya *et al*. 2011). Subsequent study indicated that squalen accumulation is a characteristic feature of the genus *Aurantiochytrium,* although the hydrocarbon contents are different from strain to strain (Nakazawa *et al*. 2014). *Aurantiochytrium* is not a carbon-neutral bioresource, but is still attractive because its growth rate is remarkably high compared to other algal counterparts, yielding a hydrocarbon productivity of 0.3 $gL^{-1}day^{-1}$. Nitrogen, phosphates, and other micronutrient such as amino acids and vitamins should be also supplied for cultivation.

Although both algae are attractive resources for microalgal hydrocarbon production, we face with several challenges: 1) the hydrocarbon productivity of *B. braunii* is low particularly in outdoor open ponds; 2) the hydrocarbon productions using both algae are not cost-effective in their present form. To address the first issue, we are evaluating the possible utility of genetic engineering approach to improve the productivity of *B. braunii*. To address the second issue, we consider a hybrid culture system, which combines phototrophic and heterotrophic algal cultures with a waste-water treatment. The basic idea is that by using the residues and run-off from waste-water treatment plant, we can cultivate both algae at low cost.

To this end, we are setting up a bench plant at Minami-Gamo waste-water treatment center in Sendai city, which is devastatingly damaged by the tsunami following the 2011 Tohoku earthquake. This research project is granted by a national foundation which aims at recovering commnunities and infrastructures in Tohoku area. By 2016, we hope to propose a refined model of microalgal biofuel production mixed with waste-water treatment that can be applicable to other waste-water plants in Japan as well as those in other countries.

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