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BIOMASS PRODUCTION AND NUTRIENT REMOVAL BY SPONTANEOUS GROWN GREEN ALGAE *Oedogonium* sp. FROM A POND IN UNIVERSITY OF TSUKUBA

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SUMMARY: As one of the methods for nutrient removal from eutrophicated lakes and ponds affected by nonpoint nutrient pollution, the conversion of nutrients into aquatic plant biomass (APB) has drawn increasing attention. In this study, the acquisition and sequestration of nutrients by APB and the application of APB as a resource were discussed. The study site was an eutrophicated inland pond at the University of Tsukuba, Amano-Gawa, which is occasionally covered with the submerged macrophyte green alga *Oedogonium* sp. This species was found to produce 1048 kg of total biomass in dry weight and 13.1 kg of lipid per harvest.

Keywords— aquatic plant biomass, Eutrophication, inland pond, nutrient and biomass acquisition, *Oedogonium* sp.

INTRODUCTION

Lake eutrophication is a major ecological and environmental problem, resulting in adverse ecosystem effects such as extinction of submerged aquatic plants, occurrence of microalgal blooms such as cyanobacteria and green algae, an increase in microbial biomass, and a reduction of biodiversity. Eutrophication results in a change within aquatic ecosystems to a simple biotic community structure, an instability of the ecosystem, and finally a change from the clear water and macrophyte dominant ecosystem to a turbid water and algae dominated ecosystem [1]. Because the level of eutrophication can be assessed by the average total phosphorus and inorganic nitrogen concentration, the phenomenon is triggered by an increasing input of nutrients from point sources such as sewage treatment plants and industrial plants and/or non-point sources such as agriculture and sewage [2]. Although it is possible to control point sources by laws and regulations, measures against eutrophication by non-point (diffuse) source pollution are difficult to define or implement because phosphate accumulated in soils and sediments is gradually released into water over a long period of time [3]. Therefore, the removal of nutrients from the watersystem is required in addition to the regulation of nutrient influx. Among the current nutrient removal technologies, macrophyte or aquatic plant biomass (APB) harvested from eutrophic water bodies has been investigated [4] and has drawn increasing attention. The utilization of APB is both profitable and can be used to remove problematic APB to maintain land drainage, flood conveyance, water quality, and visual appeal and recycle nutrients by producing a variety of value-added products from postharvest biomass. However, the most useful result of this technology is the removal of nutrients from water bodies, giving weight to the concept of APB as a global "resource" [5].

The aim of this study was to obtain data on the nutrient acquisition and production of biomass for the effective and further utilization of APB in the pond of the University of Tsukuba. The results of this research could provide useful information for the application of APB for the remediation of water systems.

2. MATERIALS AND METHODS

2.1 Materials

Fronds and water samples were collected from an artificial inland pond at the University of Tsukuba

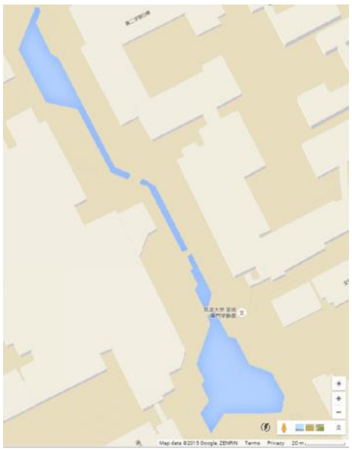

Fig. 1. Map of inland pond in University of Tsukuba (Map data© 2015 Google.ZENRIN). Scale bar indicates 20 m.

(36.1103779, 140.1012238). The total area of the water system was measured on the map and aerial photography provided by Google (Map data©2015 Google.ZENRIN).

Algal species were identified under a microscope. Algal fronds were harvested from a 2.5 m × 4 m area by hand in July 2014. After the mechanical dehydration and removal of contamination, APB was completely dried in the sun.

2.2 Separation and chemical analysis

Total lipids were extracted with hexane. After complete drying using a concentrating centrifuge, total lipids were analyzed gravimetrically. Proteins, polysaccharides, and nucleic acids were separated using trichloroacetic acid precipitation. Algal fronds were solubilized with potassium peroxydisulfate, and the concentration of nutrients and metals were analyzed.

3. RESULTS

An artificial inland water system, "Amano-Gawa," is located in the center area of the Tsukuba campus of the University of Tsukuba. It is approximately 230 m in length and consists of three parts: an upper pond, a lower pond, and a stream between the two ponds (Fig. 1). The water system is closed because the upper and lower ponds are connected by an underground pipe, and the water is cycled to the upper pond by a pump. Therefore, the water quality has been decreasing gradually, and the environmental index species of polluted water were present, such as pond snails and leeches (data not shown). As a result of eutrophication, blooms of the long filamentous green algae frequently occurred throughout the year, and the water area was completely covered by algae (Fig. 2A). Based on morphological observation under a microscope, the algal species was found to be *Oedogonium* sp. (Fig. 2B).

Table 1. Sample table (one column). Times New Roman, 8 points, table headings should appear above the tables. *, calculated from the lipid and mercury content, 1.25% and 0.05 ppm, respectively.

	size (m^2)	Biomass (kg)	Lipid* (kg)	Mercury* (mg)
sampling area	10	4.4	0.055	0.22
total water system	2383	1048.3	13.18	52.4

A total biomass of 4.4 kg (dry weigh) of algal fronds was obtained from the area after sun drying. Because the total area of the Amano-Gawa was estimated to be 2383 m^2, the total biomass obtained from the entire water system would be extrapolated to 1048 kg (Table 1). Total lipids contained in the biomass would be 13.18 kg according to the 1.25% of oil content in the dry algal fronds. Total mercury within the biomass was estimated to be 52 mg.

Fig. 2. A: flourish of submarged filamentous green alga at the inland pond in University of Tsukuba. B: the microscopic image of the alga Oedogonium. sp. Scale bar indicates 200 µm.

4. DISCUSSION

The exploitation of APB has gained increasing attention for the restoration of eutrophicated water systems affected by non-point source pollution; the recycling of nutrients; and the utilization of biomass as feedstock of energy, animals, and fertilizers. To determine the feasibility of the application of this technology, a preliminary survey was required on the prospective yield of biomass and nutrients as well as environmental and health risks. In the current research, the author obtained data of relevance to APB from the artificial inland pond at the University of Tsukuba. Floating weeds such as Spirodela and Lemna have been studied as potential APB species because they are characterized as low lignin plants and are easy to harvest using nets. The long filamentous green algae *Oedogonium* sp. harvested in the water system was found to be a good candidate for APB because green algae do not contain lignin, and this algal species is also easily harvested by mechanical techniques such as coiling or hooking. The spatial utilization of the water system as a submerged algae additionally offers a further advantage.

From the entire water system of Amano-Gawa, 1000 kg of biomass was calculated to be obtained. This species was found to be difficult to use as a feedstock for bio-diesel because the oil content was insufficient (1.25%) compared with oleaginous algae that contains more than 50%. With respect to alternative applications, composting, biochar, anaerobic digestion, fertilizer, and animal feed were considered. In the case of utilization as a fertilizer and animal feed, the study of APB was required to assess the environmental and human health risks such as those associated with invasive weeds, toxic compounds, hazardous bacteria, and microbes. The mercury content of 0.05 ppm was approximately one-tenth of the regulatory value for livestock feed in Japan.

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