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Comprehensive evaluation of nitrogen removal rate and biomass, ethanol, and methane production yields by combination of four major duckweeds and three types of wastewater effluent

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ABSTRACT

To assess the potential of duckweeds as agents for nitrogen removal and biofuel feedstocks, *Spirodela polyrhiza*, *Lemna minor*, *Lemna gibba*, and *Landoltia punctata* were cultured in effluents of municipal wastewater, swine wastewater, or anaerobic digestion for 4 days. Total dissolved inorganic nitrogen (T-DIN) of 20–50 mg/L in effluents was effectively removed by inoculating with 0.3–1.0 g/L duckweeds. *S. polyrhiza* showed the highest nitrogen removal (2.0–10.8 mg T-DIN/L/day) and biomass production (52.6–70.3 mg d.w./L/day) rates in all the three effluents. Ethanol and methane were produced from duckweed biomass grown in each effluent. *S. polyrhiza* and *L. punctata* biomass showed higher ethanol (0.168–0.191, 0.166–0.172 and 0.174–0.191 g-ethanol/g-biomass, respectively) and methane (340–413 and 343–408 NL CH_4/kg VS, respectively) production potentials than the others, which is related to their higher carbon and starch contents and calorific values.

1. Introduction

Nitrogen removal from domestic, industrial, and agricultural wastewaters is necessary to prevent the eutrophication and pollution of aquatic environments. Conventional biological nitrogen removal methods used in tertiary treatment at wastewater treatment plants, although generally reliable and effective in nitrogen removal, are energy-intensive and quite costly. On the other hand, nitrogen is an essential nutrient for plants and is used in fertilizers to increase crop production. Recently, rather than removing nitrogen from wastewater by nitrification/denitrification, nitrogen recovery from wastewater has been recognized as a desirable technology for bioresource production. Due to their rapid nitrogen uptake and strong potential as a renewable bioresource, aquatic plants have been highlighted as promising tools for a sustainable system combining energy-saving and low-cost nitrogen removal and valuable resource production from wastewaters (Soda et al., 2013; Zhao et al., 2014a). Aquatic plants have several major advantages over terrestrial energy crops: They can take up nutrients directly from wastewater, do not need extra fertilization or irrigation, can grow throughout the year, and do not compete with food crop production and agricultural land use.

Duckweeds are the aquatic plants most studied for wastewater treatment because of their rapid growth and high nutrient uptake (Cheng et al., 2002; Dalu and Ndamba, 2003; Mohedano et al., 2012; Ran et al., 2004; Xu and Shen, 2011). Duckweeds are also an ideal feedstock for production of biofuels, especially of ethanol (Chen et al., 2012; Fujita et al., 2016; Ge et al., 2012; Soda et al., 2015; Takai et al., 2014; Xu et al., 2011, 2012), due to their soft biomass and high starch content that can be easily and effectively saccharified to glucose. Therefore, duckweed culture in wastewater treatment plants offers dual benefits of low-cost nitrogen removal and biofuel production. In addition, duckweeds are superior to microalgae with regard to the cost and ease of harvesting.

Duckweeds are classified into five genera, *Lemna*, *Landoltia*, *Spirodela*, *Wolffia*, and *Wolffiella*, and comprise about 37 species (Landolt, 1986). Several duckweed species have been examined for large-scale practical cultures or lab-scale experiments for nitrogen removal and/or biofuel production. The biomass production differs depending on duckweed species (Zhao et al., 2014b; Ziegler et al., 2015) and wastewater nutrient concentrations (Soda et al., 2015). Likewise, starch contents of duckweeds differ between species (Zhao et al., 2014b) and nutrient concentrations (Li et al., 2016; Zhao et al., 2014b). Previous studies evaluated the nitrogen removal capability, biomass production, or biofuel production of single duckweed species and/or one kind of wastewater. To develop an efficient duckweed-based nitrogen removal and biofuel production system, it is necessary to

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Table 1
Initial nitrogen concentrations in effluent samples.

Effluent sample	Nitrogen concentrations (mg/L)		
	NH_4-N	NO_2-N	NO_3-N
Secondary effluent of municipal wastewater	3.9	0.1	4.1
Secondary effluent of swine wastewater	75.1	2.6	2.3
Effluent of anaerobic digestion (1:1 diluted by using tap water)	30.1	0	3.3

compare both the nitrogen removal capability and biofuel production potential of different common duckweed species in different types of wastewater.

Methane fermentation by anaerobic digestion is the most feasible and cost-effective technology to produce biofuel from organic matter, including wastewater sludge, municipal solid waste, animal manure, food waste, and plant biomass (Appels et al., 2011; Chynoweth et al., 2001; Sawatdeenarunat et al., 2016). Application of anaerobic digestion to a wastewater treatment plant can turn the treatment plant into a net energy producer (McCarty et al., 2011; Scherson and Criddle, 2014). Co-digestion of duckweed biomass with wastewater sludge would increase methane productivity in wastewater treatment plants. Some recent studies demonstrated methane production by anaerobic digestion of duckweed biomass (Cu et al., 2015; Gaur et al., 2017; Ramaraj and Unpaprom, 2016; Yadav et al., 2017). To our knowledge, however, only one study has quantified the methane production potential of duckweed, *Spirodela polyrhiza* (Cu et al., 2015). To expand the application range of these dual benefits of growing duckweeds in wastewater treatment plants, it is important to evaluate the potentials of various common duckweed species in different types of wastewater for the production of methane as well as ethanol.

This study aimed to compare the capabilities of four common duckweed species for nitrogen removal, biomass production, and ethanol and methane production. Nitrogen removal rates and biomass production rates of *S. polyrhiza*, *Lemna minor*, *Lemna gibba*, and *Landoltia punctata* were examined under three different wastewater cultures: secondary effluent of a municipal wastewater treatment plant, secondary effluent of swine wastewater, and effluent of anaerobic digestion of human fecal sludge. Productivities of ethanol and methane were determined for the duckweed biomass to assess the four species' potential use as feedstock for biofuels. Our results provide the first comprehensive dataset on the potentials of nitrogen removal, ethanol production, and methane production of common duckweeds.

2. Materials and methods

2.1. Plant materials

Bacteria-free *S. polyrhiza*, *L. minor*, *L. gibba*, and *L. punctata* were prepared by washing in 0.5% sodium hypochlorite for 3 min, then in 70% ethanol for 1 min, and finally in sterilized water three times for 1 min. Duckweeds were aseptically and routinely cultured in flasks

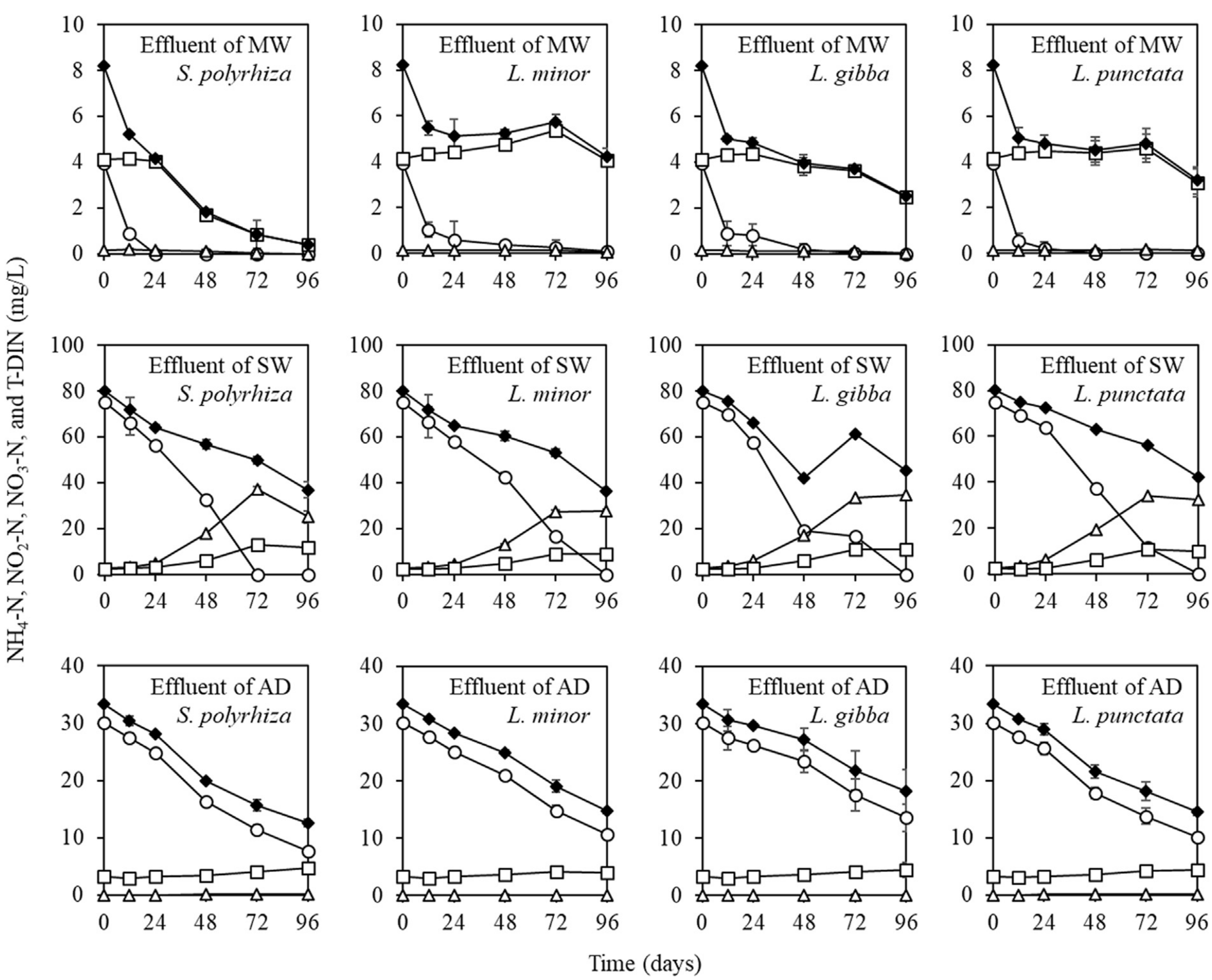

Fig. 1. Changes in NH_4-N (open circles), NO_2-N (open triangles), NO_3-N (open squares), and T-DIN (closed diamonds) concentrations in three effluent samples (MW: municipal wastewater; SW: swine wastewater; AD, anaerobic digestion) during 4 days of duckweed cultivation. Values are mean ± SD (*n* = 2).

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