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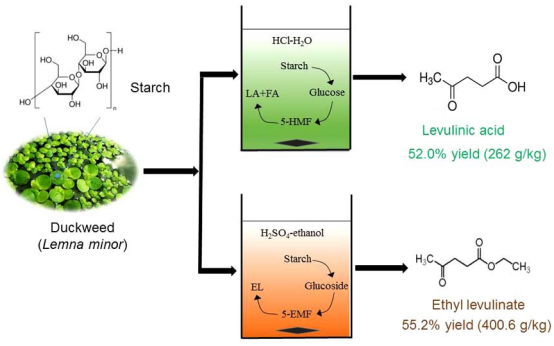
Catalytic production of levulinic acid and ethyl levulinate from uniconazole-induced duckweed (*Lemna minor*)

Chunguang Liu[b], Qingna Feng[a,b], Jirui Yang[a], Xinhua Qi[a,*]

[a] *Agro-Environmental Protection Institute, Chinese Academy of Agricultural Sciences, Tianjin 300191, China*
[b] *Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Nankai University, Tianjin 300350, China*

GRAPHICAL ABSTRACT



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ABSTRACT

Duckweed (*Lemna minor*) with a high starch content of 50.4% was cultivated by uniconazole-induction method. The cultivated duckweed was used to produce value-added chemicals such as glucose, levulinic acid and formic acid in diluted HCl aqueous solution. A high glucose yield of 93.4% (471 g/kg based on loading duckweed mass) could be achieved at 180 °C in short reaction time, and the generated glucose was converted into levulinic acid and formic acid with yields of 52.0% and 34.1%, respectively, for 150 min, corresponding to 262 g/kg levulinic acid yield and 171 g/kg formic acid yield based on the mass of loading duckweed, respectively. Moreover, the duckweed was efficiently converted to ethyl levulinate with 55.2% yield (400.6 g/kg) at 200 °C in ethanol. This work provides a promising strategy for the production of value-added chemicals from phytoplankton that is able to purify the wastewater containing high content of P and N.

1. Introduction

With the gradual depletion of traditional fossil resources such as coal, oil and natural gas, the search for alternative sustainable energy sources has become an important research topic in recent years. Biomasses are renewable and abundant resources, and are regarded as promising alternative for the fossil resources. Thus, the study on the transformation of biomass into various chemicals and fuels such as 5-hydromethylfurfural, lactic acid, 2,5-dimethylfuran, and levulinic acid, has attracted increasing concerns during the past decades (Antonetti et al., 2015; Antonetti et al., 2017a; Antonetti et al., 2017b; Koh et al., 2014; Papendiek & Venus, 2014; Raspolli Galletti et al., 2012; Rivas et al., 2016). Among these bio-based compounds, levulinic acid is identified as one of the twelve key platform chemicals by National

* Corresponding author.
E-mail address: qixinhua@nankai.edu.cn (X. Qi).

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Renewable Energy Laboratory (Denver, USA), since levulinic acid can be used to produce a series of bio-chemicals through esterification, halogenation, hydrogenation, condensation and other chemical reactions, and these chemicals are widely applied in solvents, medicine, food, biodegradable surfactants, agriculture, and biofuels (Rackemann & Doherty, 2011; Antonetti et al., 2016; Freitas et al., 2016; Rivas et al., 2015; Shen et al., 2017; Weingarten et al., 2012). For examples, levulinic acid can be converted into ethyl levulinate via esterification with ethanol, and ethyl levulinate can be used for the production of biodegradable surfactants (Freitas et al., 2016), or directly used as fuel additives (Cirujano et al., 2015; Fernandes et al., 2012; Patil et al., 2014). Levulinic acid can also be converted to γ-valerolactone by hydrogenation with hydrogen or formic acid as hydrogen donor, which is suitable to replace ethanol in gasoline-ethanol mixtures and is a kind of sustainable liquid transport fuel (Braden et al., 2011; Galletti et al., 2012; Shimizu et al., 2014). If γ-valerolactone continues to be hydrogenated, it can be used to produce valeric acid that can be transferred into a series of cellulosic transportation fuels in the alcohol reaction system (Raspolli Galletti et al., 2013; Xin et al., 2014).

Generally, levulinic acid is manufactured from biomass substrates with acid catalysts (Chen et al., 2017b). In this approach, the feedstocks could be wheat, corn, cassava, bagasse and other food crops (Chang et al., 2007; Elumalai et al., 2016; Yan et al., 2008) or food waste (Chen et al., 2017b; Jeong & Park, 2010) or lignocellulose form plant (Chen et al., 2017a), which contains sugar, starch, cellulose or other ingredients. These ingredients consist of glucose units linked by β-1,4-glucosidic bonds. When the reaction is performed under acidic conditions, the β-1,4-glucosidic bonds are broken and these ingredients are firstly converted into water-soluble glucose, then the glucose is transformed to 5-hydroxymethylfurfural (5-HMF) by removing three molecules of water, following with the formed 5-HMF being converted into levulinic acid and formic acid through the hydration and rearrangement process (Ding et al., 2014; Su et al., 2017). However, most of these biomass feedstocks are terrestrial plants whose production is limited by land resource. In addition, the usage of some of them as feedstock will compete valuable food resource with human or animals, so the discovery of new biomass feedstocks for the production of value-added chemicals and biofuels can further promote the development of biorefinery industry.

Duckweed is a kind of common aquatic plant that is easy to multiply in the rich nutrition condition. The excessive proliferation of duckweed normally leads to low oxygen content in the water body, and greatly threats the growth of other aquatic plants and the survival of aquatic animals, thus causing serious environmental and ecological issues. On the other hand, it was found that duckweed could efficiently absorb N and P in water, and could be applied to treat wastewater containing high content of P and N (Iatrou et al., 2017; Liu et al., 2017). The total nitrogen and total phosphorus recovery rate could reach 0.4 and 0.1 g/m^2/d, respectively, by duckweed in a pilot-scale wastewater treatment system (Zhao et al., 2014). Moreover, duckweed has fast growth rate, low requirements for the growth environment and high starch content above 30% on the basis of dry mass, and duckweed contains only low content of lignin. Therefore, duckweed should have great potential as one of the non-grain biomass resources in biorefinery (Cui & Cheng, 2015).

Recently, duckweed has been used to produce liquid fuels such as ethanol, butanol, and butanediol by enzymatic zymotechnics (Gusain & Suthar, 2017; Zhao et al., 2015a). For example, a kind of duckweed *Wolffia globosa* was firstly pretreated with 1% hydrogen peroxide for 1 h and 1% sodium hydroxide for 1 h, following with fermentation by 0.1% yeast in 30 mM ammonium sulfate aqueous solution, and an ethanol yield of 69 g/L was obtained (Fujita et al., 2016). Su et al. explored the production of butanol from *Wolffia globosa* by enzymatic fermentation, and a highest butanol yield of 13.56 g/L was achieved (Su et al., 2015). Although enzymatic fermentation is efficient for the production of liquid fuels, it needs long reaction time and high enzyme cost, and the enzymatic activity is highly sensitive to the reaction conditions, which limits its large-scale application.

In this work, duckweed was used as the feedstock for the catalytic production of value-added chemicals levulinic acid and ethyl levulinate. To improve the starch content in the duckweed, uniconazole was employed as a plant growth regulator to induce the starch accumulation in duckweed by weakening the respiration. The cultured duckweed gave high yields of levulinic acid and ethyl levulinate in aqueous solution and ethanol, respectively. This work provides a useful strategy for the conversion of phytoplankton, which is able to purify the wastewater containing high content of P and N, into value-added chemicals and fuels.

2. Materials and methods

2.1. Materials

Duckweed (*Lemna minor*) colonies were collected from Xiqing Lake in Tianjin (China), and were washed many times with running water before cultivation. The harvested duckweed after cultivation was dried at 85 °C for 24 h and grounded to pass through a 100 mesh sieve, and the obtained duckweed powder was then stored in a sealed bag for use. Uniconazole was purchased from Solaibao Technology Co Ltd. (Beijing, China). Hydrochloric acid and sulfuric acid were bought from Sinopharm Chemical Reagent Co Ltd (Beijing, China). Ethanol was received from Refining Fine Chemical Research Institute (Tianjin, China).

2.2. Duckweed cultivation and induction

The collected duckweed from the lake was placed in a plastic box (16 × 11 × 6 cm) containing 100 ml standard Hoagland's solution (N: 100 mg/L, P: 150 mg/L), and then the plastic box was placed in an intelligent light incubator to pre-culture for a period of time. The cultivation temperature was set at 25 and 15 °C for daytime and nighttime, respectively, and the corresponding lighting period was 16 and 8 h, respectively. The light source was provided by eight incandescent lamps. The pre-cultured duckweed was washed using distilled water.

The starch induction experiment was carried out using 2 L plastic boxes (23 × 15 × 7 cm). Each box was filled with 600 ml of 1/2 strength Hoagland's solution and inoculated with 3.5 g fresh duckweed to cover the entire water surface with a single layer of fronds. Different concentrations of uniconazole solutions (100, 300, 600 and 1000 mg/L) was prepared with 10 wt% methanol solution and then 3.5 ml uniconazole solution were uniformly sprayed on the surface of duckweed. The duckweed was cultured in the light incubator for 10 days and the cultivation conditions were consistent with the pre-cultivation conditions. The evaporated water was replenished with distilled water every day. Each experiment was performed in three replicates for different uniconazole concentrations.

2.3. Hydrolysis of duckweed in diluted HCl aqueous solution

A certain amount of duckweed powder was mixed with 10 ml of different concentrations of HCl aqueous solutions, and the mixture was placed in a 50 ml Teflon lined stainless steel autoclave. Then the closed autoclave was heated to the desired temperature with magnetically stirring at 1000 r/min, and kept for a given reaction duration. After reaction, the reactor was quickly cooled with cold water, and the solid residue was separated from reaction mixture by centrifugation at 14,000 r/min. The supernatant was analyzed with high performance liquid chromatography (HPLC).

2.4. Alcoholysis of duckweed to produce ethyl levulinate in ethanol solution

The ethyl levulinate was produced from 0.1 g duckweed powder in 10 ml ethanol solution in the presence of 0.04 mol/L sulfuric acid at