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Turion, an innovative duckweed-based starch production system for economical biofuel manufacture

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ABSTRACT

Turion is a kind of dormant tissue from *Spirodela polyrrhiza* (L) Schleid and represents another duckweed-based starch production system, which owns several potential technical merits than the conventional one. This paper systematically investigated the physiological, biochemical and production characteristics of turion and preliminarily evaluate its feasibility for bioethanol production. Turion productivity of 3.78 g/m^2/d and starch productivity of 2.90 g/m^2/d was achieved in strain 0196. Full-component analysis revealed that turion is high-quality substrate as it contained high starch content (65.63%) and low lignocellulose content (12.82%). Besides, turion can be continuously produced by strain 0196 up to 6 weeks, indicating a sustainable manufacture of them is possible. Finally, harvested turion was used for ethanol fermentation for the first time with an ethanol yield of 0.34 g g^{-1}(turion), resulted in an annual yield of 4.69 t/ha. This research elucidated that turion from duckweed is a novel biomass for biofuel production.

1. Introduction

With the increasing energy demand and the exhausting of fossil fuels, human society are facing big challenges from energy production (Harun et al., 2010; Mandotra et al., 2014). Photosynthesis of plants play a crucial role in energy supply and serves as an important source of renewable energy (Silva et al., 2015). Notably, non-grain energy crops contribute a lot to biofuel production and, at the same time, avoiding the competition with food crops (Feng et al., 2014; Saikia et al., 2015; Xu et al., 2011b; Zhang et al., 2016).

Hydrophyte duckweed has also been suggested to be a novel option due to its numerous biomass and efficient starch accumulation ability (Huang et al., 2014; Xiao et al., 2013; Xu et al., 2011a; Zhao et al., 2012), combined with outstanding capability in wastewater purification (Xu and Shen, 2011; Zhao et al., 2015b). Among the duckweed family, several species have shown huge starch production capability during laboratory- and pilot-scale experiments (Yin et al., 2015; Zhao et al., 2015b,), and the harvested biomass was used as feedstock for biofuel production (Ge et al., 2012c; Zhao et al., 2015a). For *Spirodela polyrhiza*, however, accumulating starch in frond seems impossible since it's characteristic of special developmental pattern as high-starch dormant tissue, namely turion, will be formed when confronted with stress.

Unlike the vegetative frond, turion is a kind of vegetative propagules and featured smaller size, less aerenchyma and thicker cell wall compared to normal fronds (Appenroth and Bergfeld, 1993). As a dormant tissue, turion serves as a survival strategy of duckweed under unfavorable environment and germinates to produce normal frond when the condition turns suitable (Wang et al., 2014). Whereas, it's much different from the common seeds as it's asexually produced (Landolt and Kandeler, 1987). One of the similarities with seeds is that ripe turion has a generally high starch content over 60% DW, and in the meantime, starch grains with a smaller size can be easily hydrolyzed for biofuel production (Wang and Messing, 2012). Mature turion usually sinks into the water bottom and can be directly harvested from the floating fronds. More importantly, higher weight/area ratio and lower water content compared with normal frond contribute to more economic after-harvest treatment. These features distinguished turion from traditional duckweed biomass as a neoteric biofuel feedstock.

Turion has been widely used as a model system in the study of ecotoxicological indicating, starch degradation, dormancy and germinate survey (Appenroth and Bergfeld, 1993; Appenroth et al., 1996;

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Oláh et al., 2016; Reimann et al., 2004). However, its status as candidate biofuel feedstock remains ambiguous although it's characteristic of high starch. Other physiological traits, such as starch productivity, production pattern and ethanol conversion efficiency, should be further assessed. Kuehdorf et al. (2013) has put forward the possibility of turion for bioethanol production, and it's also reminded that the current turion-based starch yield is insufficient. Thereinto, biomass productivity is the key limiting factor. As reported, turion productivity of *S. polyrhiza* is strains-dependent and varied substantially between different strains (Appenroth, 2003, 2002a; Appenroth and Adamec, 2015). The yield gap between different strains can reach as high as 27-fold (Kuehdorf et al., 2013). On the other hand, the current productivity evaluation was processed in vitro by calculating the turion number formed by one frond, which is inefficient in revealing the actual turion and starch productivity (Appenroth, 2002b). Therefore, strains screening and further evaluation is necessary.

This study aimed to examine the potential of different *S. polyrhiza* ecotypes in turion and starch production, and to investigate the physiological features, biochemical compositions, production pattern and fermentative efficiency of turion. Twelve *S. polyrhiza* ecotypes were applied for biomass evaluation and the best-performance strain was selected for further study. Turion production of the elite strain was processed in nutrients-free medium for 6 weeks and the production pattern was recorded. Finally, turion was first-time used as feedstock for fermentative bioethanol production by yeast.

2. Materials and methods

2.1. Duckweed strains and material preparation

Duckweed strains 0003, 0043, 0085, 0192, 0196, 7498, 9502, 9256, 9617, 9560, 9333 and 9497 of *Spirodela polyrrhiza* were obtained from the Duckweed Germplasm Bank, Chengdu Institute of Biology, Chinese Academy of Science. The renewing culture was processed in whole-strength Hoagland medium (Hoagland and Arnon, 1950) with 1.5% sucrose after duckweed was sterilely transferred from the stock culture. Then they were transferred to 1/5 Hoagland medium for adaption culture and to accumulate sufficient biomass for the following experiment. Meanwhile, environmental and nutritional conditions were ensured to be suitable for duckweed's normal growth and won't lead to the formation of turion. In this experiment, room temperature of 25 ± 1 °C, light intensity of 85 ± 10 μmol /m^2/s and timely renewal of culture medium won't result in turion formation.

2.2. Experimental setup

2.2.1. High-turion-productivity strain screening

Turion productivity evaluation was conducted with two methods. The first method was processed in exoteric environment to investigate the turion biomass and starch productivity of tested strains. Nutrients starvation was used as the inducing factor, fluorescent lamp with photon intensity of 110 μmol/m^2/s served as light source, room temperature was 25 ± 1 °C. Twelve *S. polyrhiza* strains were respectively inoculated in 250 mL beaker with an initial biomass of 1.2 g (fresh weight). Fronds and turions were separately harvested 14 days later for biomass and starch content evaluation. Each treatment was processed with three replicates.

Another method was established by Appenroth (2002b) by calculating the STY (specific turion yield) value, which is defined as the turion amount formed by one frond. Briefly, after necessary pre-cultivation, two strong 3-frond colonies were axenically inoculated in 100 mL Erlenmeyer flask containing 75 mL 0.3 × Hoagland medium. The experimental temperature was set at 25 °C ± 1 °C. Continuous light with the intensity of 60 μmol/m^2/s was provided by a fluorescent lamp. After 50 days, frond number and turion number of each strain were counted and the STY (specific turion yield) value was calculated. Each treatment was processed with three replicates. *S. polyrhiza* strain with the highest turion productivity was selected and served as the material for the following experiment.

2.2.2. Production pattern of turion

S. polyrhiza strain with the highest turion yield was used for investigating its production pattern. Experimental condition was in accordance with 2.2.1. Duckweeds were harvested every 7 days, turions and fronds were separately collected and weighed.

2.3. Measurement of biomass and growth rate

Fronds and turions were separately collected at the end of the experiment. Turions sank into the bottle bottom and those stick to the mother frond were both harvested and dried by filter paper. The fresh weight (FW) was weighed by an analytical balance. After dried in an oven at 60 °C for overnight to consistent weight, the dry weight(DW) was also measured and recorded.

Growth rate was calculated as follows:

$$G_b(gm^{-2}d^{-1}) = \frac{W_t - W_0}{t \times s} \quad (1)$$

Starch accumulate rate was calculated as follows:

$$G_s(gm^{-2}d^{-1}) = \frac{W_t \times S_t - W_0 \times S_0}{t \times s} \quad (2)$$

W_t: dry weight (g) of final biomass; W_0: dry weight (g) of initial biomass; t: culture period (d); s: surface area of the container (m^2); S_t: starch content of the dry final biomass (%); S_0: Starch content of the dry initial biomass (%)

2.4. Composition analysis

The starch content was determined according to the methods described by Xiao et al. (2013). Total glucose, cellulose, hemicellulose (xylose, galactose and arabinose) and lignin were detected based on the technical report of NREL (NREL/TP-510-42618, July 2011). Lipid was assayed by National Standard (GB/T 5512-2008). Pectin was analyzed according to Yang et al. (2011) and Blumenkrantz and Asboehansen (1973). Ash content was estimated according to Ge et al. (2012a). Protein content was calculated based on the nitrogen element (N) with a coefficient of 6.25, while the N content was automatically measured by Elemental Analyzer (Vario Macro Cube, Elementar Analysensysteme, Germeny) with TCD detector, C/N mode. Flavonoids content was measured according to the method of Qiao et al. (2011).

2.5. Enzymatic hydrolysis and ethanol fermentation

Hydrolysis of the sample was processed according to a two-step method recorded by Xu et al. (2011a). Briefly, dry turion was grinded thoroughly by a mortar, turion powder (starch content = 65.99%) was homogenized with 45 mL distilled water in a 100-mL beaker and boiled in water bath. Then 0.1% (v/v) α-amylase (Novozymes, Denmark) was added and the mixture was maintained at 90 °C for 45 min with temporary stirring. Thereafter, the mixture was sterilized at 121 °C for 30 min. After cooling to room temperature, the pH of the mixture was adjusted to 4.5 and 0.1% (v/v) amyloglucosidase (Novozymes, Denmark) was added, then the mixture was further hydrolyzed for another 2 h at 60 °C. Finally, the hydrolysate was diluted with distilled water to reach a final volume of 60 ml for the following experiment. Three replicates were conducted.

Each Erlenmeyer flask was inoculated with 1% (v/v) *Saccharomyces cerevisiae* (CCTCC M206111) (isolated from wine lees by our laboratory) and the ethanol fermentation was performed under an anaerobic condition at 30 °C for nearly 24 h. Ethanol content of the fermentation solution was determined by GC (Agilent Technologies 7820 A,