Research Square

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Use of hemicellulose-derived xylose for starch production by mixotrophic duckweed

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Research Article

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Abstract

Background: Duckweeds are aquatic plants with considerable potential as substrates for environmentally sustainable production of bioenergy and protein because of extremely high starch and protein yield, and nutrient uptake capability from various wastewaters. D-xylose is a common sugar that exists on earth and the main sugar element of lignocellulosic hydrolysate. Efficient utilization of xylose is necessary to reduce the high cost of duckweed-based biofuel production.

Results: In this study, the potential use of D-xylose by duckweed was tested for heterotrophy and mixotrophy. The results showed that duckweed was capable of mixotrophic growth but not heterotrophic growth on D-xylose, which suggested that photosynthesis was necessary for D-xylose metabolism, with increases in biomass yields of 2.8-fold compared with that of photoautotrophy. Furthermore, D-xylose markedly boosted starch accumulation up to 9.7 times higher than photoautotrophic duckweed, and promoted nutrient recovery efficiency.

Conclusions: The present study showed that mixotrophic duckweed could use inorganic and organic carbon simultaneously, offering an attractive strategy to enhance the duckweed biomass production with a higher carbon capture efficiency from the surrounding environment, thus boosting starch and protein productivity for bioenergy and food conversion. The method developed in this study demonstrated novel applications in duckweed biomass-based renewable bioenergy production with inorganic and organic carbon capture combined with potentially organic carbon wastewater and flue gas (CO_2) treatment. This will enable the recycling of different carbons for various uses and realize a circular economy.

Highlights

1. Duckweed could natively use hemicellulose-derived xylose for mixotrophic growth.
2. Photosynthesis was necessary for the metabolism of xylose by mixotrophic duckweed.
3. Xylose enhanced biomass accumulation and total nitrogen and phosphorus recovery.
4. Starch yield was increased by 9.7-fold using xylose compared with photoautotrophy.
5. Mixotrophic duckweed offer a new option for biofuel production and carbon capture.

Background

Duckweed biomass is a sustainable substrate for biofuel production owing to its predominant superiority of fast growth rate, high starch, shorter culture periods, friendly effects on the environment, and less arable land demands [1, 2]. Nature photoautotrophic cultivation by photosynthesis uses light to fix atmospheric carbon dioxide into sustainable biomass, which benefits the global O_2 and CO_2 budget. However, this trophic mode always results in a limitation of plant densities given that duckweed frond self-shading decreases light penetration, which leads to the high operational cost of duckweed bioprocesses and restricts commercial biomass production. By contrast, mixotrophic cultivation is efficient in promoting biomass accumulation owing to the crosstalk effect on plant growth with the

exogenous supplementation of organic carbons to photosynthetic growth using the heterotrophic capability of photosynthetic microorganisms [3]. However, the capacity of duckweed growing under mixotrophic or heterotrophic conditions is species-specific, and exogenous supplementation with an organic substrate substantially raises the operational costs of duckweed bioprocesses. To avoid the abovementioned disadvantages, large-scale duckweed cultivation needs deliberate consideration of environmentally sustainable and economically acceptable organic carbon sources. Therefore, exploiting waste-driven low-cost organic carbon sources is essential for supporting the development of the duckweed biofuel industry, which not only recycles pollutants from wastewater, but also produces value-added biomass.

Hemicellulose biomass is a promising substrate for renewable sugar production because of its abundance and low cost in nature [4]. D-xylose is the main hemicellulosic hydrolysate carbohydrate and is abundant in waste lignocellulosic feedstocks produced from forestry and agriculture [5, 6]. D-xylose is also the main carbohydrate component of pulp and paper industry wastewaters [7, 8]. Glucose can be readily used by many species, whilst studies on D-xylose metabolism are relatively limited [9]. For example, only a few wild-type mixotrophic microalgae species can metabolise D-xylose [10, 11]. Moreover, D-xylose even inhibits microalgae growth via impairment of the photosynthetic pathway in some cases [12], which is accompanied by a marked decline in pigment content [10]. The utilisation of D-xylose by genetically engineered microorganisms such as fungi, bacteria, yeasts, and microalgae has been extensively studied [13], and great progress has been made in the metabolic pathway of D-xylose. However, studies on the use of D-xylose and its underlying mechanism in higher plants are few, and much less information on D-xylose metabolism in aquatic plant duckweed species is known. Duckweeds are potential candidates for biofuel production and phytoremediation; thus, studies on D-xylose metabolism by this plant will provide a theoretical foundation and new insights into renewable biofuel production and phytoremediation [1, 2]. Thus, determining whether duckweed species could grow on D-xylose is an interesting task.

The giant duckweed, *Spirodela polyrhiza* (*S. polyrhiza*), is attracting increasing attention owing to its application in the bioremediation of wastewater and biofuel production. It can accumulate starch levels as high as 75% in dry biomass [14]. Furthermore, it could recycle nutrients in a wide range of concentrations from various wastewaters, and the resulting duckweed biomass can be used for biofuel and animal feed [15, 16]. Notably, this strain can be grown mixotrophically and heterotrophically on diverse organic carbon sources [3]. Thus, the present work aimed to test the capability of *S. polyrhiza* growth using D-xylose as an economically viable carbon substrate under heterotrophic and mixotrophic cultivations. The impacts of D-xylose on biomass and starch accumulation, photosynthetic activity, protein profiles, and nutrient removal rates by duckweed were investigated.

Methods

Culture conditions and duckweed materials

S. polyrhiza 5543 was precultured in 250 mL beakers containing 200 mL 1/2 Murashige and Skoog (MS) medium for approximately 10 days at an initial pH of 5.8 for acclimatisation cultivation and accumulation of sufficient biomass for subsequent assays. The 1/2 MS media were autoclaved in an autoclave at 121 °C for 20 min before use. The glass beakers were kept in a plant growth chamber (HP600G-LED, Ruihua, China) (photoperiod of 16 h/8 h (light/darkness), light density of 150 μmol/m^2/s). The light intensity was tested with a quantum photometer (Digital Lux Meter, GM1020, Beneath).

Experimental design

The 1/2 MS medium without organic carbons was set for photoautotrophic growth as a control group. The abovementioned media supplemented with 6, 4, 2 or 1 g/L D-xylose was selected for different mixotrophic culture media. For the heterotrophic growth assay, photoautotrophic media were added with 2 g/L D-xylose, and the beakers were kept in dark conditions and wrapped with tin foil paper. To determine the influences of photosynthesis on D-xylose metabolism, 2 g/L D-xylose mixotrophic media was supplemented with 2.5 mg/L 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). The initial inoculum plant density was 0.5 g fresh duckweed. The other culture conditions and working volume were in line with those described in the acclimatisation cultivation Section in all the cultures.
The experimental cycle was maintained for 25 days. The lost water was substituted with sterile distilled water across the experimental period to ensure a constant growth volume. All beakers were shaken two times every day to keep uniform light illumination of the plant. The duckweed growth, starch and pigment contents, chlorophyll fluorescence (*Fv*/*Fm*), Rubisco activity, D-xylose concentration and nutrient levels in the culturing medium were monitored at 5, 10, 15, 20 and 25 days after treatment.

Biomass parameters

Fresh duckweed fronds were sampled with a filter net and dehydrated using a paper towel before the determination of fresh weight using an electronic balance to test duckweed growth under different trophic modes. Subsequently, the fresh plant was washed with tap water several times to wipe off the residual medium solution, and then, the fresh sample was dried at 60 °C overnight in an oven before the dry weight was determined. The growth parameters were determined using the equation described by [3].

Photosynthetic parameters

The pigment contents were determined using the ethanol extraction method. In brief, fresh biomass (0.1 g) was extracted using 1 mL 95% ethanol and maintained in complete darkness at 4 °C overnight. Subsequently, the extracting solution was collected, and the volume was made up to 1 mL with 95% ethanol. The chlorophyll a and b and carotenoid contents were tested following the methods described by Liu [17]. For the *Fv*/*Fm* measurement, fresh duckweed was sampled and fully acclimated in dark conditions for 10 min. Thereafter, the *Fv*/*Fm* was tested at 4:00 pm of the day with a plant efficiency analyser (PEA, Hansatech Ltd., UK). The *Fv*/*Fm* value was calculated following the formula described by Baker [18].

Light microscopy observation

Intact and healthy duckweed fronds were collected, and pigments were completely extracted from plants using 95% ethanol at 4 °C overnight. Subsequently, the colourless plant samples were stained with 0.2% KI/I_2 solution at room temperature for 10 min. After that, the plants were photographed with a stereomicroscope (Leica M125, Leica).

Determination of starch content

The starch level of dry plant biomass was measured following the instructions of a starch assay kit (Grace Biotechnology, Suzhou, China). Briefly, the dry biomass sample obtained in Section 2.3 was ground into powder; then, exact 0.01 g powder was dissolved in 0.5 mL DMSO solution (dimethyl sulfoxide), and the homogenate was maintained in boiling water for approximately 15 min. Subsequently, the solutions were cooled to 25 °C, and then, they were mixed with 1.5 mL ethanol. The resulting suspension was centrifuged (25 °C, 6,000 rpm, 8 min). After that, the residual pellet was resuspended in 0.5 mL DMSO and was maintained in a boiling water bath for approximately 20 min. Thereafter, the suspension was centrifuged again (25 °C, 6,000 rpm, 10 min). Lastly, the hydrolysate was analysed following the instructions of a starch assay kit (Grace Biotechnology Co., Ltd., China). The starch parameters were determined following the methods determined by Guo [19].

Rubisco activity

For Rubisco activity determination, freshly harvested plants (accurate 0.1 g) were homogenised with a mortar and pestle in 5 mL lysis buffer on ice. The resulting homogenate was centrifuged (4 °C, 12,000 rpm, 10 min), and the resulting supernatant was analysed following the procedure described by the manufacturers (Grace Biotechnology Co., Ltd., China). Protein levels were quantified using a butyleyanoacrylate protein kit (Grace Biotechnology Co., Ltd., China).

D-xylose concentration

The assimilation of D-xylose in various culturing media was measured following the instructions of the manufacturer (Qiyi Biological Technology Co., Ltd., China). Briefly, the collected medium solutions at different sampling time points were filtered through a 0.45 μm microporous film, and the filtered liquid was mixed with DNS solution, which was composed of 10 g/L sodium hydroxide, 10 g/L 3,5-dinitrosalicylic acid, and 0.5 g/L sodium sulfate and maintained in the boiling water bath for approximately 5 min. Lastly, the mixed solution was rapidly cooled to room temperature using an ice-water solution. The absorbance of the solution was detected by a spectrophotometer (BioDrop uLite80-3006-51, UK) at 554 nm, and a standard curve of D-xylose was obtained under the same conditions.

Water quality analysis

Total nitrogen (TN) and total phosphorus (TP) concentrations in different media were detected following Chinese Standard Methods for Water and Wastewater Monitoring and Analysis [20]. Duckweed culture

solutions were harvested from flasks and filtered through a 0.45 μm filter membrane to quantify the residual nutrient concentration.

Statistical analysis

The value was shown as means of three replicates. SPSS (Version 21.0, SPSS, Chicago, USA) was used to analyse all the data. Statistical significance ($P \leq 0.05$) between the analysis variables was considered according to one-way analysis of variance.

Results And Discussion

Mixotrophic and heterotrophic growth

Four D-xylose concentrations (6, 4, 2 or 1 g/L) were set for mixotrophic cultivations in the light to analyse the capability of duckweed to use D-xylose. As depicted in Fig. 1a, supplementation with D-xylose in aquatic medium enhanced duckweed growth and biomass production relative to the photoautotrophic mode, but the specific variations in plant growth caused by D-xylose were different and dose-dependent. Exogenous supplementation with D-xylose in culturing medium led to a lag phase of growth, which suggested that duckweed might need to change its metabolic pathway or physiological state to accommodate the new D-xylose-enriched environment. Thereafter, D-xylose demonstrated a substantial promotion effect on duckweed biomass accumulation, especially at relatively low concentrations. However, the growth was arrested with a further extension of the culture time. On day 15, the 1 and 2 g/L D-xylose treatment groups obtained the highest level of exponential growth, after which the growth started to decrease, which suggested that D-xylose could boost duckweed growth to a certain extent and an adverse effect occurred when the plant was exposed to D-xylose for a long time. These negative effects on growth were possibly explained by pH variation or the accumulation of harmful metabolites in the medium. Moreover, immediate restrained growth was observed when the D-xylose concentration was larger than 8 g/L (data not shown), which was in line with previous studies showing that excessive D-xylose could greatly inhibit mixotrophic growth of microalgae [11, 21]. In the present study, xylose contributed to biomass accumulation, whereas it did not increase or even decrease above a threshold due to the death of plant cells. The highest growth rate was achieved under 2 g/L D-xylose conditions, and the resulting mixotrophic growth rate reached 4.3 g/m^2/day, which was 2.8-fold that under photoautotrophic conditions (1.5 g/m^2/day). These findings suggested that duckweed could withstand and utilise D-xylose within a certain concentration naturally in mixotrophic growth for a long time. The corresponding projected biomass yield under 2 g/L D-xylose conditions was 15.8 t/ha/year of mixotrophic duckweed in this study due to the year-round production capability. In the United States, the average dry biomass yields were 2.9 t/ha/year (wheat), 7.6 t/ha/year (rice), 9.6 t/ha/year (maize), 11.1 t/ha/year (potato), and 17.1 t/ha/year (sugar beet) in 2010 [22]. The present duckweed biomass yields were favourably comparable to and were even higher than the values obtained in the USA with land crops. These results indicated that duckweeds can produce much more biomass than land crops per area of cultivation. Therefore, *S. polyrhiza* 5543 is a potential feedstock for mixotrophic biomass production using D-xylose.

The optimal mixotrophic cultivation D-xylose level of 2 g/L was selected for heterotrophic cultivation to study the heterotrophic use capability of duckweed using D-xylose as the only organic carbon. As demonstrated by Fig. 1a, duckweed dry weight in heterotrophic conditions remained nearly constant throughout the culture period, which suggested that duckweed failed to utilise D-xylose in heterotrophic conditions. Thus, we proposed that the use of D-xylose by duckweed may require the involvement of photosynthesis or light. Therefore, DCMU was added in mixotrophic medium to block the electron transport of photosynthesis to further substantiate the abovementioned hypothesis. As shown in Fig. 1a, duckweed growth on D-xylose was completely eliminated with supplementation with DCMU. This result reconfirmed that duckweed failed to metabolise D-xylose without light illumination. DCMU blocks the production of NADPH by inhibiting electron flow from PSII, and lastly, photosynthesis is shut down. NADPH was proven to potentially participate in D-xylose metabolism as a coenzyme [13]. This study indicated that light, or more specifically, photosynthesis was a prerequisite for the metabolism of D-xylose by duckweed, as verified by its mixotrophic but not heterotrophic growth properties.
This phenomenon was in conformity with a study in microalgae [11]; it was proposed that the NADPH or energy produced by photosynthesis participates in D-xylose metabolism [11], and xylose reductase (XR) and xylitol dehydrogenase (XDH) have been proven to be involved in D-xylose metabolism [21], which are NADPH- and $NADP^+$-dependent enzymes, respectively (Fig. 1b). The detailed underlying mechanism of D-xylose metabolism in duckweed needs to be further explored in future work. Similarly, a few microalgae species showing the capacity to assimilate D-xylose under mixotrophic conditions have been reported previously [10, 11, 23], and few studies have shown that mixotrophic microalgae can use D-xylose under heterotrophic conditions [21]. The present findings provide new insights into D-xylose metabolism by duckweed and promising lignocellulose utilisation as a sustainable solution for mixotrophic duckweed biomass production. Notably, it is economically viable and environmentally friendly to bioremediate wastewater, which is rich in D-xylose (such as pulp and paper industry origin wastewater) and simultaneously produces a large amount of duckweed biomass.

Previously, no wild-type duckweed strains grown on D-xylose were reported. Herein, we validated that duckweed promoted biomass accumulation in mixotrophic growth using D-xylose compared with strictly photoautotrophic growth with compelling evidence. This study is the first to report that energy crop duckweed could absorb D-xylose from aquatic environments and use it for biomass production, which suggests that the metabolic pathways of D-xylose naturally exist in duckweed species. Therefore, D-xylose could be a low-cost and abundant organic carbon for high mixotrophic duckweed growth and biomass production. Notably, it may provide another valuable function in D-xylose-rich wastewater treatment. When combining simultaneous waste utilisation with biomass production, the operational costs of wastewater bioremediation and renewable duckweed biomass accumulation were concurrently reduced, which is a cost-effective and environmentally friendly method for sustainable biofuel production [24].

Effect of D-xylose on starch accumulation

Starch-enriched biomass is a vital consideration for duckweed biofuel production. Therefore, the duckweed starch content was analysed, as presented in Fig. 2. After xylose treatment, the starch content changed dramatically (Fig. 2). Obviously, greater starch accumulation levels were generated in mixotrophic duckweed growth on D-xylose than in photoautotrophic duckweed. Photoautotrophic duckweed maintained a low starch content throughout the cultivation time. Under 1 and 2 g/L D-xylose conditions, the starch content gradually increased, reached a maximum level at day 20 and then decreased in the following days. Notably, much higher starch content was observed under higher D-xylose concentrations, which indicated that a higher D-xylose level was conducive to starch accumulation. Specifically, the maximum starch level with 2 g/L D-xylose was 8.7% of dry mass, which was 4.6-fold higher than that of duckweed grown under photoautotrophic conditions (1.9% of dry weight). The starch content of duckweed under 1 g/L D-xylose (4.7%) was substantially lower than that under 2 g/L D-xylose but still 2.5-fold larger than that of photoautotrophic duckweed. In this study, supplementation with D-xylose substantially boosted the synthesis of starch in duckweed. Similarly, glucose increased starch accumulation in mixotrophic duckweed relative to photoautotrophic plants [3]. The same phenomena were reported in mixotrophic microalgae in which D-xylose increased the lipid content [7]. These results indicated that more carbon was allocated to starch biosynthesis with exogenous supplementation with xylose in the medium. However, the mixotrophic duckweed starch content obtained here was lower than that under stress conditions shown in other studies [25, 26], but higher than that in photoautotrophic duckweed [3]. Generally, duckweed starch content is low when grown under rich nutrition conditions without organic carbon and is much higher under nutrient limitation conditions [1, 2]. For example, under various nutrient starvation conditions, the duckweed strain *Lamna punctata* demonstrated starch levels ranging from 30% to 53% on a dry weight basis in different reports [19, 27, 28]. When the duckweed strain *S. polyrhiza* was exposed to well water, a starch content of 26.6% of dry biomass was achieved in previous reports [29]. Similarly, another duckweed strain, *Lemna aequinoctialis*, was cultured in tap water, and the starch accumulation was 35% of dry weight [30]. The highest starch content of mixotrophic duckweeds (*Spirodela oligorrhiza*) was obtained under phosphate-limiting conditions with the addition of sucrose to the culturing medium [14]. These starch contents of duckweed under different nutrient deficiency conditions were substantially higher than the level obtained in this study. Therefore, further optimising mixotrophic parameters for starch synthesis in duckweed to use xylose as an organic carbon is necessary.

The morphology of duckweed at day 10 was analysed by an iodine staining assay to further validate the starch level of duckweed. As described in Fig. 2c, the iodine staining results demonstrated that duckweed fronds in 2 g/L D-xylose showed the deepest blue appearance after iodine staining, followed by 1 g/L D-xylose-treated duckweed, whilst the control fronds were brown. These results visually confirmed the increased starch accumulation after D-xylose treatment, which corresponded with the starch measurement results (Fig. 2a).

Starch yield, which depends on starch content and biomass accumulation, is critical in biofuel production. Duckweed starch yields were determined by combining biomass and starch content results. As shown in Fig. 2b and Table 1, D-xylose greatly boosted starch yield in mixotrophic duckweed

compared with the control group. Under mixotrophic conditions, the starch yield increased with the extent of culturing time, reached a maximum level on day 20 and then decreased because of the decreased biomass accumulation and starch content. The starch yield was kept at a low level during the cultivation period in photoautotrophic duckweed. The maximum starch yields were 2.6 and 6.8 g/m^2 under 1 and 2 g/L D-xylose conditions, respectively. In the meantime, the control group was only 0.7 g/m^2. The corresponding starch yields under mixotrophic conditions were 3.7- and 9.7-fold that in the control group, respectively. Sun [3] demonstrated a mixotrophic duckweed starch yield of 2.2 g/m^2/day using glucose. Xiao [31] determined a starch value of 0.55 g/m^2/day in *Landoltia punctata* under field conditions. In another duckweed species (*Lemna japonica*), a high starch accumulation rate of 2.1 g/m^2/day was reported by Zhao [32]. These values were much higher than the results measured in the current work. These variations in starch productivity rates may be due to the different duckweed species and culture conditions used. Overall, our findings allow mixotrophic duckweed to use xylose as a renewable substrate for biofuel production.

Table 1 Duckweed biomass parameters.

Culture condition	Maximum biomass yield (g/m^2)	Maximum starch content (%)	Maximum starch yield (g/m^2)
Photoautotrophic	42.2 ± 1.3	1.9 ± 0.2	0.7 ± 0.1
Mixotrophic+1 g/L	81.5 ± 5.2	4.7 ± 0.5	2.6 ± 0.2
Mixotrophic+2 g/L	80.4 ± 2.8	8.7 ± 1.4	6.8 ± 0.4

Values represent means of triplicates ± standard deviation. Different lowercase letters indicate significant differences among different conditions ($P < 0.05$).

Photosynthetic parameters

The pigment level was analysed to show the variations in the physiological state of duckweed after xylose treatment. As shown in Fig. 3a, b and c, carotenoid, chlorophyll a and b contents declined gradually under 1 g/L D-xylose conditions and rapidly under 2 g/L D-xylose conditions compared with those in photoautotrophic cultivation across the growth period. The effect of D-xylose on pigment levels was dose-dependent. Under 2 g/L D-xylose conditions, the total chlorophyll content declined from the starting level of 0.68 mg/g to 0.18 mg/g on a fresh weight basis at day 25 (Fig. 3d), and a marked decrease was also observed in the carotenoid content dropping from 0.19 mg/g to 0.06 mg/g (Fig. 3c). Under 1 g/L D-xylose conditions, the total chlorophyll level changed from the starting content of 0.68 mg/g to 0.46 mg/g, and the carotenoid content decreased from 0.19 to 0.13 mg/g. These findings implied that D-xylose substantially inhibited duckweed photosynthetic performance. Similar findings have also been reported in mixotrophic microalgae using D-xylose, glucose or acetate as organic carbons [12, 33].

The photosynthetic activity was also reflected by *Fv/Fm*. As depicted in Fig. 3e, the *Fv/Fm* value declined under mixotrophic conditions compared with the control group, with a drop from the initial value of 0.82 to 0.77 and 0.72 under 1 g/L and 2 g/L D-xylose, respectively, which was indicative of lower photosynthetic activity in mixotrophic duckweed after D-xylose treatment. However, *Fv/Fm* remained constant under photoautotrophic conditions in this study. These results suggested a progressive decline in PSII potential after xylose treatment. The *Fv/Fm* value in photosynthetic plants is generally constant under optimal growth conditions and decreases when suffering from environmental stress stimuli [34]. The decreased *Fv/Fm* was largely attributed to xylose treatment in this study. Similarly, microalgae demonstrated a decreased photochemical efficiency of photosystem II in the presence of glucose [35, 36].

Rubisco activity, the key rate-limited enzyme of photosynthesis, was determined to further analyse the impacts of D-xylose on photosynthetic activity. As depicted in Fig. 3f, the Rubisco activity of mixotrophic duckweed showed a marked decrease compared with photoautotrophic plants. These results indicated that the duckweed photosynthetic activity decreased with supplementation with D-xylose in the culture medium. These results further confirmed the conclusions drawn by pigment parameters and *Fv/Fm* results. On the basis of the abovementioned results, we proposed that D-xylose could support duckweed mixotrophic growth but inhibited photosynthetic growth simultaneously.

Protein profiles

Duckweeds are also an important feedstock for sustainable protein production as food or feed. As demonstrated in Fig. 4a, the protein content gradually declined in all cultivations. In general, D-xylose substantially reduced the protein accumulation of duckweed compared with photoautotrophic growth. Specifically, the final protein contents were 182.4, 162.5 and 131.1 mg/g dry weight under photoautotrophic, 1 g/L and 2 g/L D-xylose conditions on day 25, respectively. In this study, lower protein content was observed in mixotrophic duckweed, whereas the starch level was higher than that of photoautotrophic plants (Figs. 4a and 2a), which was in agreement with a previous study [3]. In terms of protein yield, mixotrophic duckweed gained the highest protein yield of 13.24 and 9.49 g/m^2 under 1 and 2 g/L D-xylose conditions, respectively, because of the advantage of higher biomass production, which was larger than the protein yield determined under photoautotrophic conditions (7.38 g/m^2). These results suggested that the addition of D-xylose greatly reduced the protein content but boosted protein production. Similarly, mixotrophic duckweed using glucose generated much more protein yield than photoautotrophic plants [3].

Xylose, total nitrogen and total phosphorus variations

The residual D-xylose amount in the medium was determined and analysed to confirm that D-xylose was utilised by duckweed. The time course of D-xylose concentrations is presented in Fig. 5. The amount of D-xylose gradually decreased under mixotrophic conditions. By the end of cultivation, D-xylose dropped to below 0.25 g/L under 1 g/L D-xylose conditions compared with 0.78 g/L under 2 g/L D-xylose conditions, with corresponding removal rates of 75.0% and 61.8%, respectively. The greatest removal rate of xylose

(1092.3 mg/m^2/day) was observed under 2 g/L D-xylose conditions, which was 1.6-fold higher than that under 1 g/L D-xylose conditions (663.6 mg/m^2/day). The continuously reducing D-xylose concentration corresponded well with the increase in biomass in Fig. 1a, which indirectly validated that xylose was metabolised by duckweed for biomass accumulation. These results again confirmed that the D-xylose metabolic pathway existed naturally in duckweed. By contrast, the D-xylose level kept little variation in the dark- or DUCM-treated condition. This finding suggested that duckweed in heterotrophic and mixotrophic + DCMU conditions could not metabolise D-xylose and further substantiated the abovementioned conclusions that light was necessary for D-xylose metabolism by duckweed.

Phosphorus and nitrogen are essential nutrient elements for duckweed growth. TP and TN were determined and compared to analyse these nutrient variations under different culture conditions. Table 2 showed the removal efficiency of TP and TN under the different conditions. As shown in Fig. 1 and Table 2, the rapid biomass accumulations resulted in a marked decrease in TP and TN concentrations. The uptake rates of TN were 103.7 and 76.0 mg/m^2/day under 1 and 2 g/L D-xylose conditions on the 25th day, respectively, which were 2.4-fold and 1.7-fold higher than that in photoautotrophic control group of 43.8 mg/m^2/day. Similarly, approximately 16.6 and 13.9 mg/m^2/day of TP was effectively removed under 1 and 2 g/L D-xylose conditions, respectively, which was higher than that in photoautotrophic culture (12.3 mg/m^2/day). These results indicated that the addition of xylose to the media substantially promoted nutrient uptake efficiency. In the presence of xylose, the removal rate of these elements obtained in this work was lower than the value gained in mixotrophic duckweed using glucose due to the greater biomass accumulation [3].

Table 2 Xylose, total phosphorus (TP) and total nitrogen (TN) recycling rates.

Cultivation mode	Xylose removal rate (mg/m^2/day)	TN removal rate (mg/m^2/day)	TP removal rate (mg/$m^{2/}$day)
Photoautotrophic		43.8 ± 1.2c	12.3 ± 0.5c
Mixotrophic+1 g/L	663.6 ± 35.8b	103.7 ± 4.4a	16.6 ± 0.3a
Mixotrophic+2 g/L	1092.3 ± 19.8a	76.0 ± 2.4b	13.9 ± 0.5b

Values represent means of triplicates ± standard deviation. Different lowercase letters indicate significant differences among different conditions ($P < 0.05$).

Conclusions

This study provided a promising strategy using waste-derived xylose as a low-cost organic carbon source for duckweed biofuel production. The results showed that duckweed could metabolise D-xylose for mixotrophic growth via photosynthesis. Mixotrophic duckweed accumulated 2.8-fold more biomass than photoautotrophic duckweed. The enhanced starch and protein yields after xylose treatment make

duckweed a valuable feedstock for biofuel and feed production. Xylose also boosted nutrient recovery rates. The present results emphasise the possibility of mixotrophic biomass production using D-xylose as a sustainable organic carbon feedstock and develop the feasibility of using D-xylose-enriched wastewater for the growth of duckweed and biofuel production.

Declarations

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Authors' contributions

All the authors read and approved the final manuscript.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

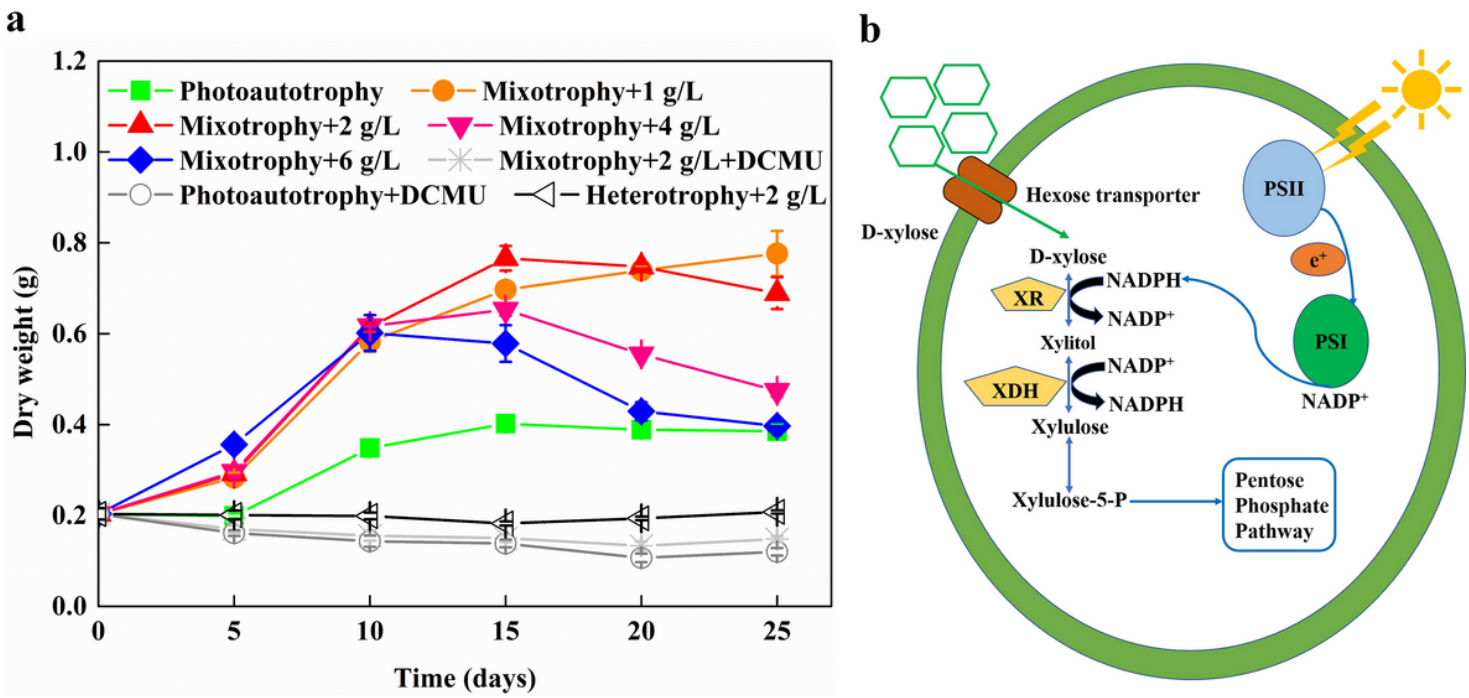

Figure 1

Growth characteristics of duckweed in medium containing different concentrations of D-xylose. a, growth curve; b, the proposed D-xylose metabolic pathway of duckweed. When D-xylose was transported into the cell, the expression of NADPH-linked XR and NADP + -linked XDH were activated, which converted xylose to xylulose and the end product entered into the Pentose Phosphate Pathway. NADPH produced by photosynthesis might act as the coenzyme for D-xylose metabolism. The values presented are the mean ± standard error.

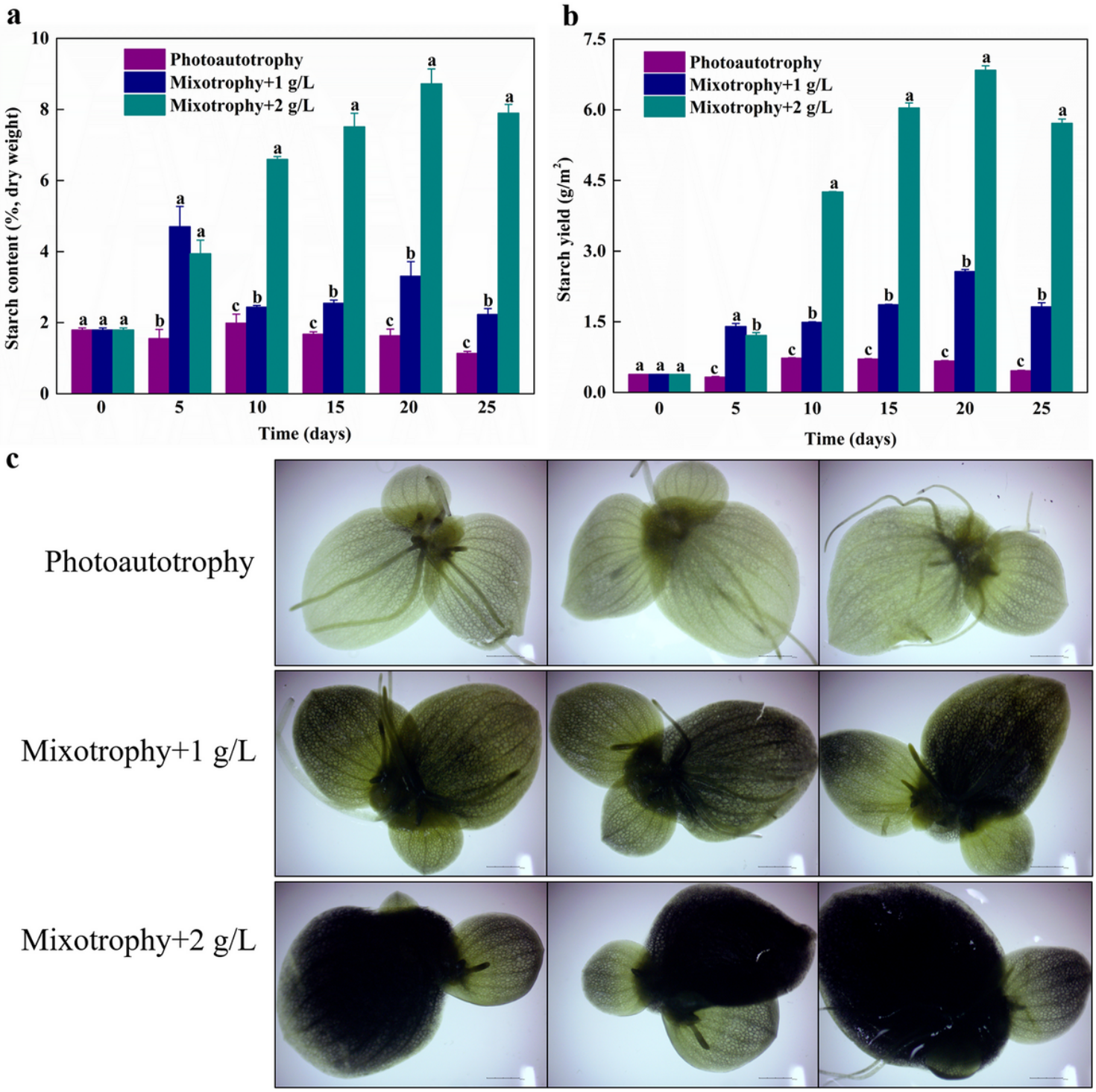

Figure 2

Starch characteristics of duckweed at various D-xylose concentrations. Starch content (a); starch yield (b); iodine staining (c). The values presented are the mean ± standard error. Different lowercase letters indicate significant differences among different conditions ($P < 0.05$).

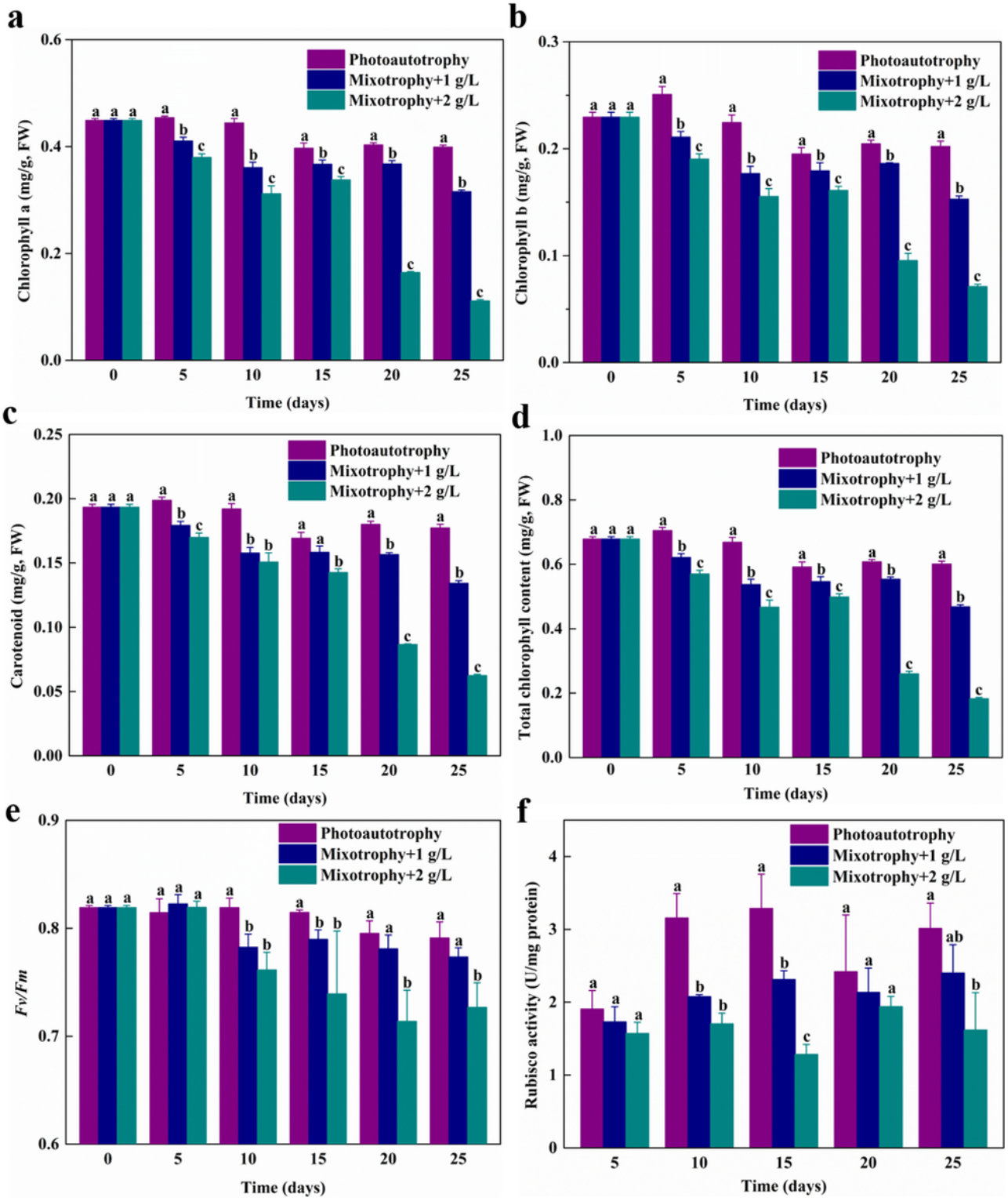

Figure 3

Effects on photosynthetic parameters. Chlorophyll a (a); chlorophyll b (b); carotenoid (c); total chlorophyll (d); maximum photochemical efficiency of PSII (*Fv*/*Fm*) (e); Rubisco activity (f). The values presented are the mean ± standard error. Different lowercase letters indicate significant differences among different conditions ($P < 0.05$).

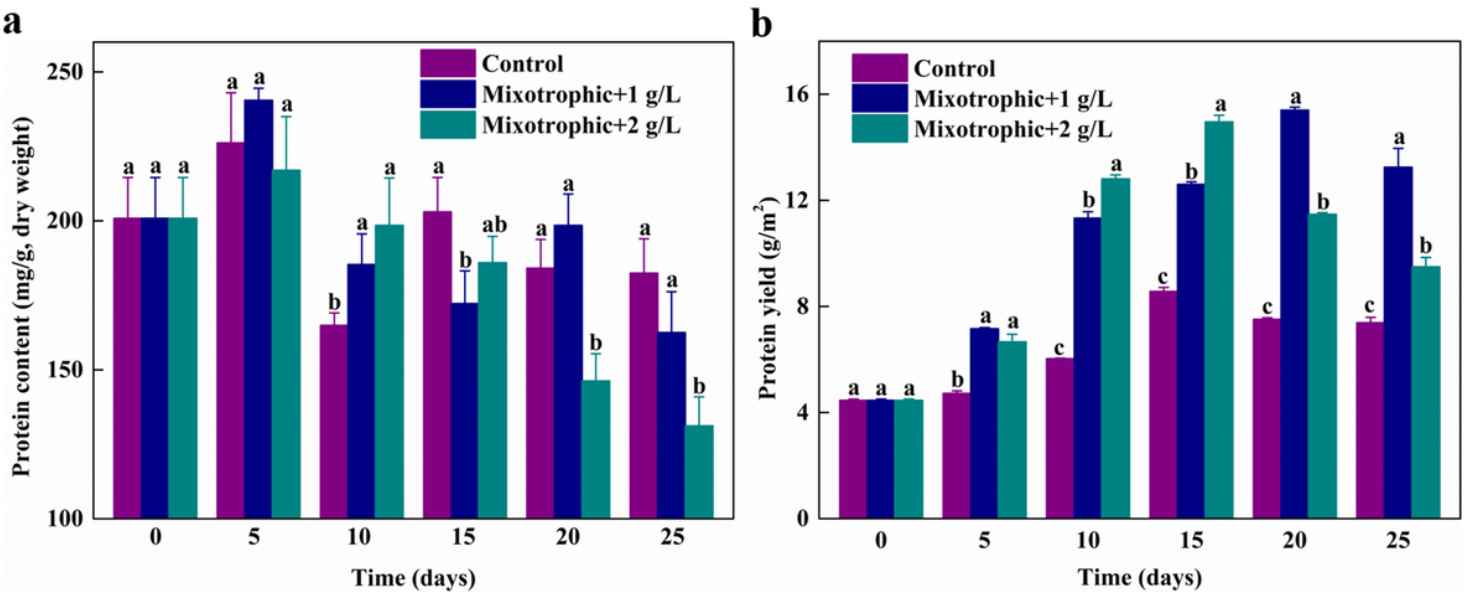

Figure 4

Protein content and yield of duckweed. Protein content (a); protein yield (b). The values presented are the mean ± standard error. Different lowercase letters indicate significant differences among different conditions ($P < 0.05$).

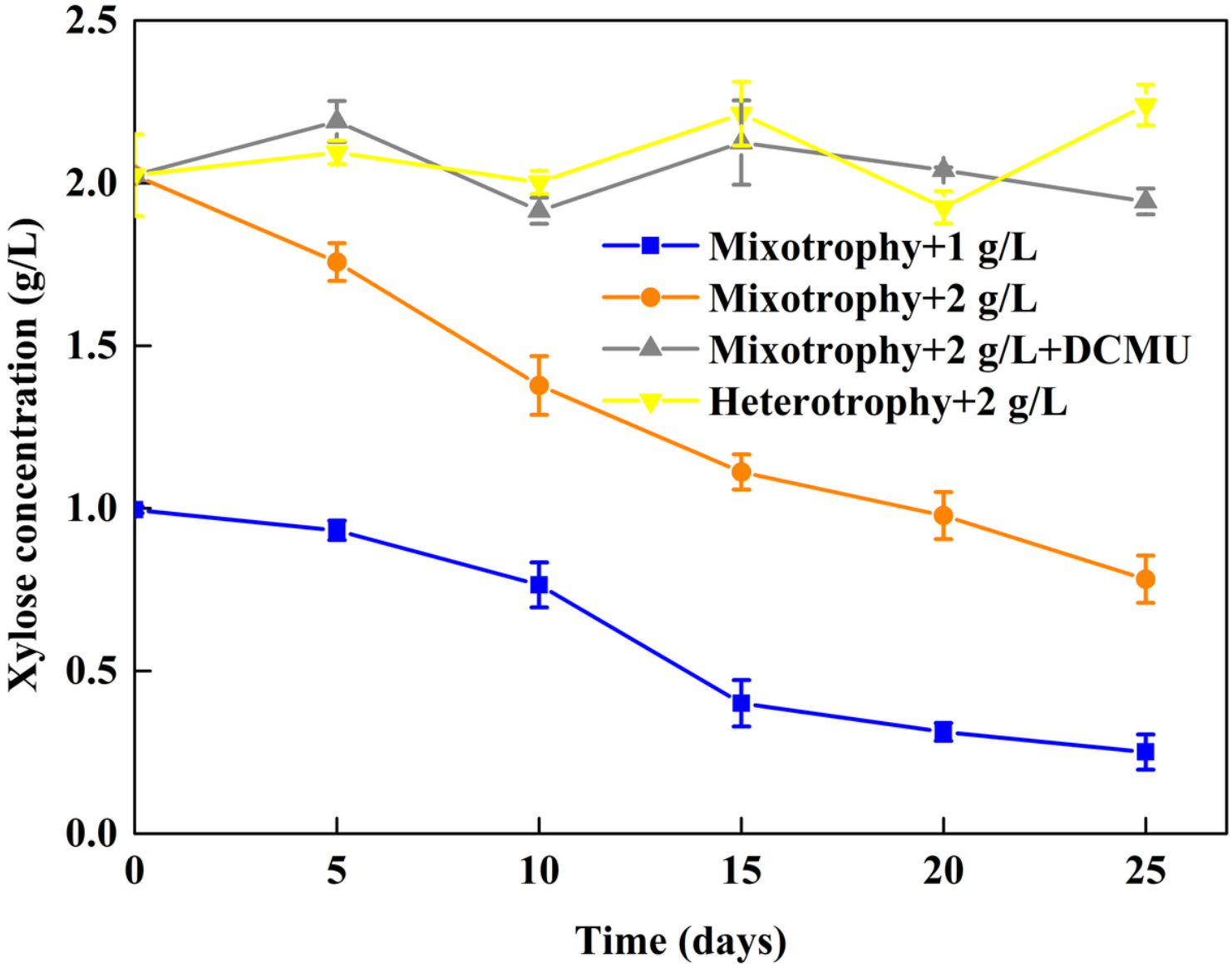

Figure 5

Time courses of D-xylose concentration by duckweed at various dosages of D-xylose. The values presented are the mean ± standard error.

Supplementary Files

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