

The growth and lipid accumulation of *Scenedesmus quadricauda* under nitrogen starvation stress during xylose mixotrophic/heterotrophic cultivation

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Abstract

In order to conquer the block of high cost and low yields which limit to realize the commercialization of microalgal biodiesel, the mixotrophic and heterotrophic cultivation of *Scenedesmus quadricauda* FACHB-1297 fed on xylose was studied separately employing six forms of media: phosphorus sufficient, phosphorus restricted and phosphorus starvation were combined with nitrogen sufficient and nitrogen starvation conditions. The maximum oil percentage (about 41% of dry weight) was obtained on the 5th day (heterotrophic cultivation) and 8th day (mixotrophic cultivation) under the nitrogen starved and phosphorus sufficient (N₀&P) conditions, which was about 2-fold in comparison to the initial lipid content on the sufficient nitrogen condition (control). Under mixotrophic and heterotrophic modes, the highest lipid productions were both achieved in the N₀&P trial, with the value of 274.97 mg/L and 193.77 mg/L, successively. Xylose utilization rate of 30%-96% under heterotrophic modes was apparently higher than that of 20%-50% in mixotrophic modes. In contrast, phosphorus uptake rate of 100% under mixotrophic modes was significantly more than that of 60%-90% in heterotrophic cultivation. Furthermore, under xylose heterotrophic cultivation, the phosphorus had a positive impact on microalgae cell synthesis, and the oil content enhanced with the augmentation in phosphorus concentrations. We suggest that sufficient phosphorus should be supplied for obtaining more microalgal lipid production in the lack of nitrogen for xylose heterotrophic and mixotrophic conditions. This is a highly effective way to obtain efficient microalgae biofats production.

1.0 Introduction

The deteriorating living environment and the fossil energy crisis on the verge of exhaustion are the two most serious and urgent problems facing the world today (Rosli et al. 2020; Peter et al. 2022). It is urgent for mankind to accelerate the search for economic and environmental protection methods of recycling waste. At the meantime, studies are also required to develop renewable new energy. Compared with other biodiesel raw materials, microalgae has become the focus of current concern on account of its superiority for instance rich oil content, easy to cultivate, large yields per unit area, and not competing with agriculture for land (Yin et al. 2020; Rosli et al. 2020; Chu et al. 2021; Siddiki et al. 2022).

At present, the high cost and low energy conversion rate of microalgae oil production limit its large-scale industrial production. Therefore, how to increase the biomass, lipid productivity and lessen costs have become the focus of current research (Stephens et al. 2010; Togarcheti et al. 2017; Marangon et al. 2021; Chhandama et al. 2021). The microalgae fed with wastewater is a promising approach in the field of environmental protection and energy development. Due to the limitation of light, temperature and other factors (Brennan and Owende 2010), it is difficult to realize the full-scale culture of microalgae only by the autotrophic culture (Perez-Garcia et al. 2011; Castillo et al. 2021). The autotrophic mode of microalgae converts light energy into biomass through photosynthesis. As the culture progresses, a biofilm will form on the surface of the microalgae to reduce light transmittance and influence the development of microalgae. Heterotrophic culture of microalgae uses an external organic carbon source to synthesis biomass in the absence of light, which can eliminate the influence of light. Mixotrophic cultivation is

equivalent to associating heterotrophic with autotrophic cultures, and the interaction of these two processes will also increase the multiplication of microalgae. According to reports, the biomass and oil yield of autotrophic culture were both lower than heterotrophic and mixotrophic modes (Miao and Wu 2004; Pang et al. 2019; Gomaa and Ali 2021). But in fact, the consumption of organic carbon source takes up approximately 80% of the general culture medium consumption (Katiyar et al. 2017; Liang 2013). For reducing the expenditure of high organic carbon, it is imminent to search for a cheap substitute of carbon source.

In recent years, the utilization of lignocellulose has become the focus of researchers' attention. Xylose, as a product of lignocellulosic hydrolysis, is not only a renewable resource, but also has large promising development in the manufacture of chemicals and fuels. Studies have shown that pulp and paper wastewater contain 70% xylose and xylan (Wang et al. 2014). Song and Pei 2018 reported that *Scenedesmus quadricauda* FACHB-1297 had the ability to utilize xylose for mixotrophic and heterotrophic growth in xylose-rich papermaking wastewater.

Many studies had shown that nitrogen deficiency could promote lipid accumulation in algae, for example, *Chlorella vulgaris*, *Chlorella zofingiensis*, *Neochloris oleoabundans*, and *Scenedesmus obliquus*, synthesized exceed in 35% of their dry weight as lipid under nitrogen stress situation proved by Breuer et al. (2012). Morales-Sanchez et al. (2013) discovered that the oil percentage composition of *Neochloris oleoabundans* added up to 28% in the absence of nitrogen. Shen et al. (2015) investigated that *Chlorella vulgaris* NIES-227 nourished with glucose was obtained a large yield of fatty acids and a high percentage of fatty acid methyl ester under heterotrophic condition with nitrogen deficiency. Another research showed that after 20 days in the effluent with deprived nitrogen, both lipid and carbohydrate content of the chosen microalgae enhanced about 2 and 1.5-fold, respectively, compared with nitrogen sufficient condition (Nagappan and Kumar 2021). To sum up, so many researches demonstrated the feasibility of using nitrogen stress to increase oil percentage in microalgae. However, the characteristics of *Scenedesmus quadricauda* under different nitrogen and phosphorus content used xylose as carbon source have not been studied.

In our previous work, it had been proved that *Scenedesmus quadricauda* could tolerate xylose, and the optimum concentration of xylose for the lipid synthesis was 4 g/L, which proved that *Scenedesmus quadricauda* was suitable for further research to use waste streams for biofuel production. In our research, the dominant energy microalgae (*Scenedesmus quadricauda* FACHB-1297) was chosen as the research object, and xylose was studied as the organic carbon source for the development of *Scenedesmus quadricauda*. In order to probe the lipid accumulation of this dominant algae species under nitrogen starvation stress, we set up six groups of cultivation modes with different nitrogen and phosphorus concentrations in mixotrophic/heterotrophic cultures. The utilization of xylose, oil accumulation and the removal of nitrogen and phosphorus by *Scenedesmus quadricauda* FACHB-1297 in media combined with diverse nitrogen and phosphorus content were researched.

2.0 Materials And Methods

2.1 Species and medium

The superior energy microalgal species *Scenedesmus quadricauda* (FACHB-1297) applied during the experiment was bought from Freshwater Algae Culture Collection at the Institute of Hydrobiology, China (FACHB-collection, China). Xylose with 4 g/L was selected as the external organic carbon source, BG-11 medium (nitrogen and phosphorus removal component) were prepares for the basic medium. The initial dry weight of microalgae was 0.5 g/L, the microalgae and the culture medium were mixed together, which were placed in the light incubator. The culture temperature was $25 \pm 1^{\circ}\text{C}$, the light intensity was 3000 Lx during 24 h, and one culture cycle was set as 8 days. Each set of tests was in triplicate.

2.2 Experimental scheme

Table 1 Initial concentrations of nitrate and phosphate in the six types of culture fluids

Table 1 Initial concentrations of nitrate and phosphate for the six media types						
Initial concentration	N&P	N&P ⁻	N&P ₀	N ₀ &P	N ₀ &P ⁻	N ₀ &P ₀
N(NO ₃ ⁻ -N) (mg/L)	250	250	250	0	0	0
P(PO ₄ ³⁻ -P) (mg/L)	45	5	0	45	5	0

Six media with diverse standards of nitrogen and phosphorus were applied during this experiment (Table 1). Respectively are: enough nitrogen and enough phosphorus (N&P), enough nitrogen and insufficient phosphorus (N&P⁻), enough nitrogen and phosphorus starved (N&P₀), nitrogen starved and enough phosphorus (N₀&P), nitrogen starved and insufficient phosphorus (N₀&P⁻), simultaneously nitrogen and phosphorus starved (N₀&P₀). The algal liquid was centrifugally suspended in the above six cultures (the concentrations of nitrogen and phosphorus were not consistent, and other nutrients were the same). The dry weight, pH, TN, TP and xylose concentrations of *Scenedesmus quadricauda* were measured every two days, and the lipid content of *Scenedesmus quadricauda* was measured on days 0, 2, 5 and 8. The growth of *Scenedesmus quadricauda*, the assimilation of nitrogen and phosphorus, xylose utilization and oil synthesis by *Scenedesmus quadricauda* under these six cultures were investigated respectively.

2.3 Analyses and calculations

2.3.1 Growth measurement of *Scenedesmus quadricauda*

The *Scenedesmus quadricauda* culture solution was extracted every day for biomass concentration as we said in the previous presentation (Song et al. 2013). The specific growth rate (K) of microalgae in exponential stage was figured up as the below formula (Song et al. 2013):

$$K(d^{-1}) = \frac{\ln C_2 - \ln C_1}{T_2 - T_1}$$

1

where C_1 and C_2 (mg/L) represent the biomass concentration on day T_1 and T_2 .

Biomass productivity during the exponential period of microalgal growth was acquired through the below equation (Song et al. 2013):

$$PDM_{DW}(\text{mg/L/d}) = DM \times K$$

2

where DM represents the biomass concentration (mg/L) in the last stage of the exponential period.

2.3.2 Lipid analysis of *Scenedesmus quadricauda*

The total lipid percentage was measured and analyzed by the chloroform/methanol extraction method, the ratio of the chloroform/methanol mixture used was 2:1 (v:v). The oil productivity (PL) was computed through the below equation (Song et al. 2013):

$$PL(\text{mg/L/d}) = PDM_{DW} \times LC$$

3

where PL represents the lipid productivity during the indicial stage. LC is the lipid content according to the dry weight.

2.3.3 Removal of total nitrogen and total phosphorus

The microalgal culture medium needed to be centrifuged at 4000 rpm for 10 min (-4°C) before total nitrogen (TN) and total phosphorus (TP) were detected, and the supernatant liquor was then filtered via a $0.45\ \mu\text{m}$ film so as to the measure of total nitrogen and total phosphorous by the method of PRC national standards GB 11894-89 and GB 11893-89, respectively.

2.3.4 Xylose concentration analysis

A certain amount of microalgae solution was extracted and conducted a centrifugation at 4000 rpm for 10 minutes. Afterwards, the DNS solution was added to the separated supernatant and cultivated at 90°C for 15 minutes in a water bath (Miller 1959). Then, cool down the system promptly. The absorbance of the xylose at 630 nm was received using a ultraviolet spectrophotometer and in comparison with the calibration curve made in the same modes (Leite et al. 2015).

2.3.5 Calculation of conversion coefficient of xylose

The biomass yield coefficient from xylose ($Y_{B/X}$), the lipid yield coefficient from xylose ($Y_{L/X}$) and the coefficient by a unit (g) of nitrogen consumption to a unit (g) of xylose intake ($Y_{N/X}$), were received on the basis of the below equation (Freitas et al. 2017) of (4)-(6):

$$Y_{B/X} (g/g) = \frac{B_{max} - B_0 - \Delta B_{ctrl}}{X_0 - X_f}$$

4

$$Y_{L/X} (g/g) = \frac{L_{max} - L_0 - \Delta L_{ctrl}}{X_0 - X_f}$$

5

$$Y_{N/X} (g/g) = \frac{N_0 - N_f - \Delta N_{ctrl}}{X_0 - X_f}$$

6

where B_0 , L_0 , N_0 and X_0 stand for the original values of biomass, lipid, nitrogen and xylose content, successively. B_{max} and L_{max} stand for the maximal values of the biomass and lipid content, successively. N_f and X_f represent the end values of nitrogen and xylose content, successively. ΔB_{ctrl} , ΔL_{ctrl} and ΔN_{ctrl} are the increment of biomass content, the increase in oil accumulation and the consumption of nitrogen content in the control group without xylose during the autotrophic culture, successively.

3.0 Results And Discussion

3.1 Dry cell weight in mixotrophic/heterotrophic growth under six media types

Figure 1 The variations in (a) dry cell weight (DCW) (b) specific growth rate (K) of *Scenedesmus quadricauda* FACHB-1297 under different media in mixotrophic cultivation, and (c) dry cell weight (DCW) (d) specific growth rate (K) in heterotrophic cultivation

For assessing the mixotrophic and heterotrophic growth characters of *Scenedesmus quadricauda* under six different media types, diverse nitrogen and phosphorus concentrations in light conditions for mixotrophic culture simultaneously at the lack of light for heterotrophic culture were probed the effect of nitrogen and phosphorus for the development characteristics of *Scenedesmus quadricauda* (Fig. 1). Figure 1 (a) and (b) manifested that *Scenedesmus quadricauda* under six different media types entered the logarithmic growth phase after two days of adaptive phase, which was correspond with the research of Song et al (2018). Under the condition of mixotrophic culture, the specific growth rate of all the experimental groups reached the maximum on the sixth day, the reason was from the sixth day *Scenedesmus quadricauda* started to enter the period of stabilization, which was earlier than the

Scenedesmus obliquus, that needed approximately 40–45 days to reach a plateau (Yang et al. 2014). This suggested that xylose was well tolerated by our earlier domesticated *Scenedesmus quadricauda*.

In the conditions of mixotrophic modes, the biomass productivity under different nitrogen and phosphorus concentrations were 137.45 mg/L/d (N&P), 95.03 mg/L/d (N&P⁻), 83.45 mg/L/d (N&P₀), 83.83 mg/L/d (N₀&P), 80.04 mg/L/d (N₀&P⁻), and 75.01 mg/L/d (N₀&P₀) respectively (Table 2). The highest biomass productivity was achieved when nitrogen and phosphorus were sufficient, being 1.8-fold of the nitrogen and phosphorus deficiency group, which was accorded with the maximum value of dry cell weight (1.10 g/L) and the fastest particular growth velocity (0.14 d⁻¹) under N&P mode. This phenomenon can be explained that nitrogen and phosphorus were essential nutrients for the development of microalgae (Jiang et al. 2017; Chu et al. 2013; Han et al. 2014; Li et al. 2021).

Figure 1 (c) and (d) showed that the highest dry cell weight (0.60 g/L) and specific growth rate (0.13 d⁻¹) for *Scenedesmus quadricauda* were received at the N&P mode under heterotrophic cultivation. All of these values were lower than those obtained in the light under N&P mode, this phenomenon could be explained by the report of Manhaeghe et al. (2020), the mixotrophic development rate of microalgae was faster than those of the photoautotrophic mode and the heterotrophic mode. Furthermore, the highest biomass productivity of *Scenedesmus quadricauda* (137.45 mg/L/d) was not only more than the value of 61.11 mg/L/d got in the dark, but also more than that of 37.8 mg/L/d got by the *Chlorella* (PCH90) fed on xylose in mixotrophic condition (Leite et al. 2016). The reason why *Scenedesmus quadricauda* grew best under the mixotrophic conditions with nitrogen and phosphorus abundant was that in the mixotrophic culture, carbon sources could provide energy other than light and be synchronously absorbed for ATP yield (Pang and Chen 2017).

Figure 1 showed that the biomass and the specific growth rate of *Scenedesmus quadricauda* increased under mixotrophic and heterotrophic modes in diverse nitrogen and phosphorus content, which indicated that xylose could be absorbed and utilized by *Scenedesmus quadricauda*. The xylose was assimilated by microalgae via the cell membrane into the cells, in the mean time, the inducible hexose derivatives also facilitated xylose to entry the microalgae cells (Zheng et al. 2014). The endocellular xylose afterwards entranced the pentose phosphate pathway was decomposed via the NADPH-linked xylose reductase and NADP⁺-linked xylitol dehydrogenase in two steps to prepare for biomass and fatty acid synthesis (Alper and Stephanopoulos 2009). The energy and coenzymes produced by photosynthesis of microalgae under light could facilitate the above processes, so the biomass of microalgae in mixotrophic modes was more than that in heterotrophic modes.

Table 2

Biomass productivity, Lipid content and Lipid productivity of six treatment groups after 8-day cultivation in mixotrophic/heterotrophic cultivation

	Cultivation conditions	N&P	N&P ⁻	N&P ₀	N ₀ &P	N ₀ &P ⁻	N ₀ &P ₀
Biomass productivity (mg/L/d)	Mixotrophic	137.45	95.03	83.45	83.83	80.04	75.01
	Heterotrophic	61.11	72.68	64.38	61.77	61.01	60.25
Lipid content (%)	Mixotrophic	21.00	27.22	31.42	41.50	36.93	20.37
	Heterotrophic	32.23	17.34	14.40	35.72	34.01	16.70
Lipid productivity (mg/L/d)	Mixotrophic	28.86	25.66	26.25	34.37	28.82	15.00
	Heterotrophic	22.29	12.60	9.27	22.06	20.75	10.06

3.2 TN, TP and xylose changes under six media types in light/light-free

Figure 2 Assimilation profiles of xylose (a), TN (b), TP (c) by *Scenedesmus quadricauda* FACHB-1297 in mixotrophic cultivation, and xylose (d), TN (e), TP (f) in heterotrophic cultivation

As shown in Fig. 2, the removal rate of TN and xylose were a bit slow, nevertheless the removal rate of TP was faster especially in the first two days (60%-92%) and almost all of the TP was removed by *Scenedesmus quadricauda* on the eighth day. Under mixotrophic mode, the TP content of phosphorus sufficient assays rapidly reduced from 37.00 to 0 mg/L in 8 days (100%), and these of phosphorus restrictive groups likewise lessened from 4.00 to 0 mg/L in 8 days (100%). However, the assimilate efficiency of TP in heterotrophic mode was not as high as under the condition of mixotrophic culture, which was 60% of phosphorus sufficient assays and 90% of phosphorus restrictive conditions. The rapid absorption of TP in the beginning may be due to the surface area of microalgae, and the higher uptake efficiency of TP in the mixotrophic culture may be caused by photosynthesis (Song et al. 2014; Song et al. 2018).

Whether there was light or not, the removal rate of TN decreased with the phosphorus concentration decreased by N&P, N&P⁻ and N&P₀, which was also consistent with the variations of maximum dry cell weight of 1.10 g/L, 0.76 g/L, 0.66 g/L, of N&P, N&P⁻ and N&P₀ group, successively. In the heterotrophic mode, the removal rates of TN achieved at 99% in the N&P trial, following by N&P⁻ of 95% and N&P₀ of 74%, those were much higher than that got under mixotrophic mode with the value of 43%, 24% and 21% under N&P, N&P⁻, N&P₀ conditions, successively. The xylose removal rate of 96% under heterotrophic mode was also better than that under mixotrophic conditions. However, the biomass of heterotrophic mode were lower than that of mixotrophic mode, the absorption of xylose might promote the absorption of

nitrogen, and the adsorbed nitrogen and xylose might be transeferred to lipid (Leite et al. 2016), and similar to the loss of biomass under the conditions of heterotrophic condition also appeared in the report of Miao and Wu (2006). Those suggested that *Scenedesmus quadricauda* could use xylose both in and out of the light, which was in line with the report of Leite et al. (2016), who showed that only *Scenedesmus quadricauda* could use xylose to grow in mixotrophic and heterotroph modes.

3.3 Lipid accumulation in mixotrophic/heterotrophic growth under six media types

Figure 3 Lipid content and the production variations of lipid under six treatment during 8-day cultivation in (a) mixotrophic cultivation and (b) heterotrophic cultivation

Figure 4 The mechanism of the effect of xylose assimilation and nitrogen starvation on the lipid accumulation in mixotrophic/heterotrophic cultivation

Table 2 Biomass productivity, lipid content and lipid productivity of six treatment groups after 8-day cultivation in mixotrophic/heterotrophic cultivation

When *Scenedesmus quadricauda* was cultured under nitrogen-deficient conditions, the maximum oil percentage (about 41%) was obtained on 5th day (heterotrophic cultivation) and 8th day (mixotrophic cultivation) under the nitrogen starved and phosphorus sufficient (N_0P) condition, which was about 2-fold compared with the initial lipid content under nitrogen-sufficient condition (control) (Fig. 3). The groups with the highest oil production had similar results with oil content, which achieved at 8 days of mixotrophic culture (274.97 mg/L) and 5 days of heterotrophic culture (193.77 mg/L) under N_0P groups (Fig. 3). It was interesting to find that the highest lipid content was reached faster under heterotrophic conditions, in addition, this result was accorded with the faster absorption rate of xylose under heterotrophic conditions (30%-96%). Figure 3 (b) showed that the lipid content began to decrease slowly from the fifth day, which illustrated *Scenedesmus quadricauda* began consuming lipid after the fifth day in the dark. However, the maximum lipid production was obtained in the mixotrophic cultivation under N_0P condition (274.97 mg/L) but not in the heterotrophic cultivation (178.34 mg/L), which suggested that the presence of light was beneficial for *Scenedesmus quadricauda* to absorb xylose to synthesize biomass, so that the lipid productivity reached high. Similar conclusions have been proved by Perez-Garcia et al. (2011), that the photosource was beneficial to oil synthesis of microalgae in the heterotrophic mode. It could be attributed to the photosynthesis provided enough energy for Acyl-CoA to malonyl coenzyme A (Mayl-CoA) for formation of fatty acids.

Another finding of this experiment was that in both mixotrophic and heterotrophic modes, the maximum lipid content were received in nitrogen starvation media. The general content of lipid in microalgae perhaps was on a scale of 1–85% (Chisti 2007), when nutrition was restricted, it could usually reach more than 40%, which was consistent with the lipid content (41%) obtained by restricting nitrogen in this study. And this data (41%) was more than the oil content (37%) of the accumulation of *Chlorella vulgaris* SDEC-3M in nitrogen lack of conditions within NSE nutrient solution (Qi et al. 2016). As shown in Fig. 4, when

the microalgae was under stress, such as nitrogen deficiency, salt stress, light limiting and other conditions, some stress markers like superoxide dismutase/reactive oxygen species (SOD/ROS) would be produced, which stimulated triglyceride (TAG) production in reply to environmental force (Yu et al. 2018). In addition to that, the carbon storage mechanism was triggered by the restricted conditions of nitrogen to adapt to the continuous consumption of carbon and the growth of cells, which could inhibit the protein synthesis and transfer redundant carbon to amyllum and/or lipid to accelerate oil accumulation (Farooq et al. 2022). To sum up (according to Fig. 4), conditions of xylose and nitrogen stress co-promoted fatty synthesis. One of the reasons was nitrogen restriction increased the microalgae content of fatty acid acetyl-CoA (Takagi et al. 2000; Qi et al. 2016), which was a crucial ferment and the precursor of certain energy-storing substances. In the meantime, when xylose was absorbed by microalgae, the substance (xylulose-5-phosphat) involved in the regulation of lipogenesis genes synthesis was also produced, which also stimulated more oil synthesis (Leite et al. 2016). However, there are few studies on the use of nitrogen and phosphorus in xylose mixotrophic cultivation and heterotrophic cultivation to produce biodiesel.

As shown in Fig. 3, the lipid percentage reduced from 41–20% with the phosphorus content decreased under nitrogen deficiency in mixotrophic cultivation, the similar pattern was discovered under heterotrophic conditions (with the decrease of phosphorus content, the lipid percentage decreased from 35–16%). This result demonstrated that phosphorus promoted lipid accumulation in the case of nitrogen starvation under mixotrophic cultivation and heterotrophic cultivation. In the case of insufficient nitrogen, phosphorus played a vital part during the lipid production capacity of microalgae in autotrophic growth cultivation (Chu et al. 2013; Shen et al. 2020). During the growth of microalgae, phosphorus in the culture solution was converted into Poly-P and stored in the microalgae cells (Chu et al. 2013). Soto et al. (2019) demonstrated that Poly-P as an energy storage material, could accumulate in large quantities at the condition of nutrient lack. In the case of nitrogen lack, the increase of phosphorus content led to the increase of Poly-P, which promoted the accumulation of lipid.

3.4 Relationship between xylose conversion factor and lipid accumulation

Table 3 Biomass yield (Y_B/X), Lipid yield (Y_L/X) and Nitrogen depletion (Y_N/X) of six treatment groups after 8-day cultivation in mixotrophic/heterotrophic cultivation

Table 3
Biomass yield ($Y_{B/X}$), Lipid yield ($Y_{L/X}$) and Nitrogen depletion ($Y_{N/X}$) of six treatment groups after 8-day cultivation in mixotrophic/heterotrophic cultivation

	Cultivation conditions	N&P	N&P ⁻	N&P ₀	N ₀ &P	N ₀ &P ⁻	N ₀ &P ₀
$Y_{L/X}(g \cdot g^{-1})$	Mixotrophic	0.63	0.57	0.36	0.83	0.73	0.17
	Heterotrophic	0.67	0.59	0.38	0.93	0.75	0.19
$Y_{B/X}(g \cdot g^{-1})$	Mixotrophic	0.85	0.61	0.48	0.36	0.25	0.16
	Heterotrophic	0.73	0.56	0.32	0.28	0.22	0.13
$Y_{N/X}(g \cdot g^{-1})$	Mixotrophic	0.31	0.23	0.13	-	-	-
	Heterotrophic	0.24	0.25	0.27	-	-	-

For further research the relationship between the xylose assimilation, lipid production, nitrogen removal and development characteristics, the $Y_{L/X}$, $Y_{N/X}$ and $Y_{B/X}$ were calculated (Table 3). The highest transfer coefficient of xylose to the lipid ($Y_{L/X}$) under mixotrophic cultivation in N₀&P mode was 0.83 g/g, which was 5 times more than the conversion factor of 0.17 g/g without nitrogen and phosphorus addition, and this maximum value was also 1.4 times higher than the factor of 0.63 g/g in the light under N&P group. These indicated that nitrogen deficiency was an important factor to promoting lipid accumulation. This result was correspond with that observed by Breuer et al. (2012) for *Chlorella vulgaris*, *Chlorella zofingiensis*, *Neochloris oleoabundans*, and *Scenedesmus obliquus*, which synthesized over 35% of their dry weight as triglycerides under nitrogen starvation condition. Similar result was obtained under heterotrophic condition, the highest $Y_{L/X}$ (0.93 g/g) was acquired in N₀&P condition as well.

According to Table 3, it could be seen that under mixotrophic cultivation the maximum values of $Y_{L/X}$ (0.83 g/g) and $Y_{N/X}$ (0.31 g/g) were both lower than $Y_{L/X}$ (0.92 g/g) and $Y_{N/X}$ (0.47 g/g) under heterotrophic condition, but the value of $Y_{B/X}$ (0.73 g/g) under heterotrophic mode was lower than $Y_{B/X}$ (0.85 g/g) under mixotrophic mode. These conversion coefficients were consistent with higher nitrogen and xylose absorption rates under heterotrophic condition, which also indicated that heterotrophic condition was more conducive to nitrogen absorption by microalgae and xylose conversion to oil. In the dark, microalgae only relied on absorbing xylose as carbon source, while under light conditions, microalgae still obtained energy through photosynthesis. Therefore, the conversion factor of xylose into lipid was higher under dark than that in light. But in the fact, in terms of total oil production, microalgae accumulated more lipid and biomass under light, owing to the energy and the coenzyme (NADPH) produced by photosynthesis could promote xylose metabolism (Zheng et al. 2014), which could facilitate the expression of ACCase to induce the synthesis of fatty acids (Song and Pei 2018).

By comparing the data obtained by the experiment, the highest lipid percentage (41%) was received faster under heterotrophic condition (5 day) than mixotrophic condition (8 day), which suggested that in order to obtain lipid, microalgae was cultured more economically under heterotrophic conditions. We could get the same content of lipid in a short period of cycle. In these two cultural modes, the energy supplied by light and/or xylose was assimilated by *Scenedesmus quadricauda* and then converted to ATP for various energy requirements within cells. Yang et al. (2000) certified that the ATP production rate under mixotrophic and heterotrophic conditions were 12% and 18% respectively, this could explain the cause of this interesting phenomenon. In heterotrophic culture, due to the conversion capacity to ATP (18%) and the transforming factor of xylose to oil (0.92 g/g) were both higher than that of mixotrophic culture, these factors had jointly caused faster lipid accumulation under heterotrophic condition.

3.5 Implications of this work

It was reported that the discharge of papermaking wastewater would cause certain pollution to the environment (An et al. 2022), while there was a lot of lignocellulosic biomass such as xylose in the waste liquid of papermaking, and the yield of xylose could reach 21.91–57.15 g/L (Shi et al. 2021) by using the existing technology. The cost of extracting bio-oil from lignocellulosic biomass was about \$47/ton (Huang et al. 2013). According to the highest oil yield obtained in this experiment (274.97 mg/L), it consumed about 14.55 tons of xylose to synthesize one ton of bio-oil, so the cost of producing one ton of bio-oil was about \$1100-\$ 2860, which had a great advantage in price than employing glucose (\$3400/ton) to produce oil (Singh et al. 2022). Therefore, this study could not only economically utilize xylose in papermaking wastewater, but also produced microalgae oil, which had double economic benefits.

4.0 Conclusions

This research indicated nitrogen hunger with mixotrophic/heterotrophic conditions fed on xylose seem to be a promising way for *Scenedesmus quadricauda* accumulating lipid. The maximum oil content (41%) was obtained in the N₀&P group on the fifth day of heterotrophic and the eighth day of mixotrophic culture, respectively. In terms of oil yield, mixotrophic culture had more preponderance with the value of 274.97 mg/L over that of 193.77 mg/L in heterotrophic mode. But heterotrophic condition could shorten the culture cycle to achieve the highest oil content (41%). The maximum absorption rate of xylose was 96% (in heterotrophic mode), and the maximum phosphorus removal rate was 100% (in mixotrophic mode). Phosphorus played a critical part of increasing lipid synthesis of *Scenedesmus quadricauda* in nitrogen depletion condition. With the increase of phosphorus content, the oil content increased by 21% and 19% under mixotrophic and heterotrophic conditions, respectively. This study demonstrated the vast perspectives of *Scenedesmus quadricauda* to efficiently produce algal lipids in heterotrophic/mixotrophic culture using xylose in wastewater combined with nitrogen stress.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials

Code availability

Not applicable

Author's Contribution

All authors contributed to the study conception and design. Material preparation and data collection were performed by Na Liu, Kunyang Su, Xue Li and Tianxiang Lu. Data analysis, the first draft of the manuscript were written by Yiwen Mou and revised by Mingming Song and Yu Ze. All authors read and approved the final manuscript.

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Figures

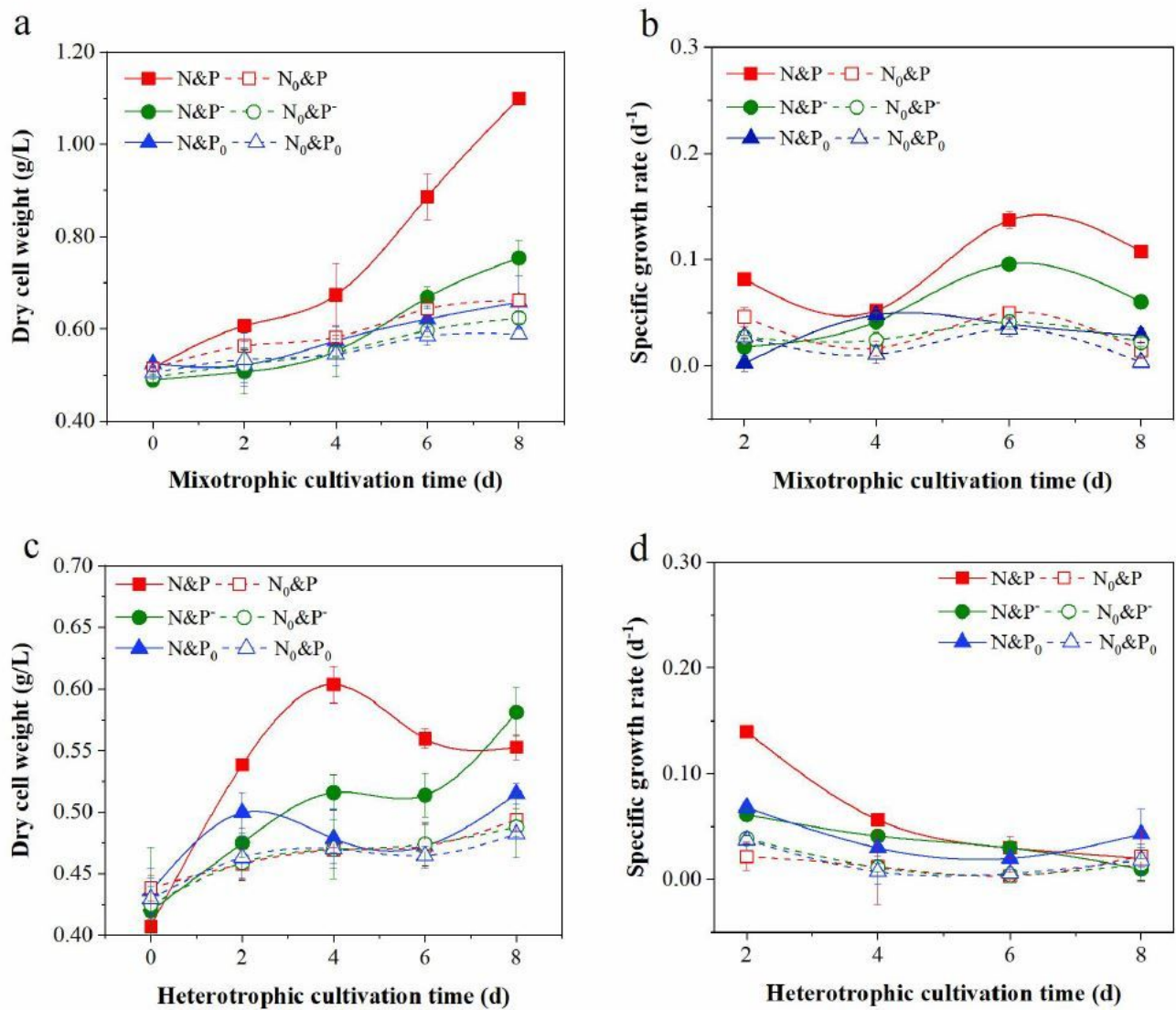


Figure 1

The variations in (a) dry cell weight (DCW) (b) specific growth rate (K) of *Scenedesmus quadricauda* FACHB-1297 under different media in mixotrophic cultivation, and (c) dry cell weight (DCW) (d) specific growth rate (K) in heterotrophic cultivation

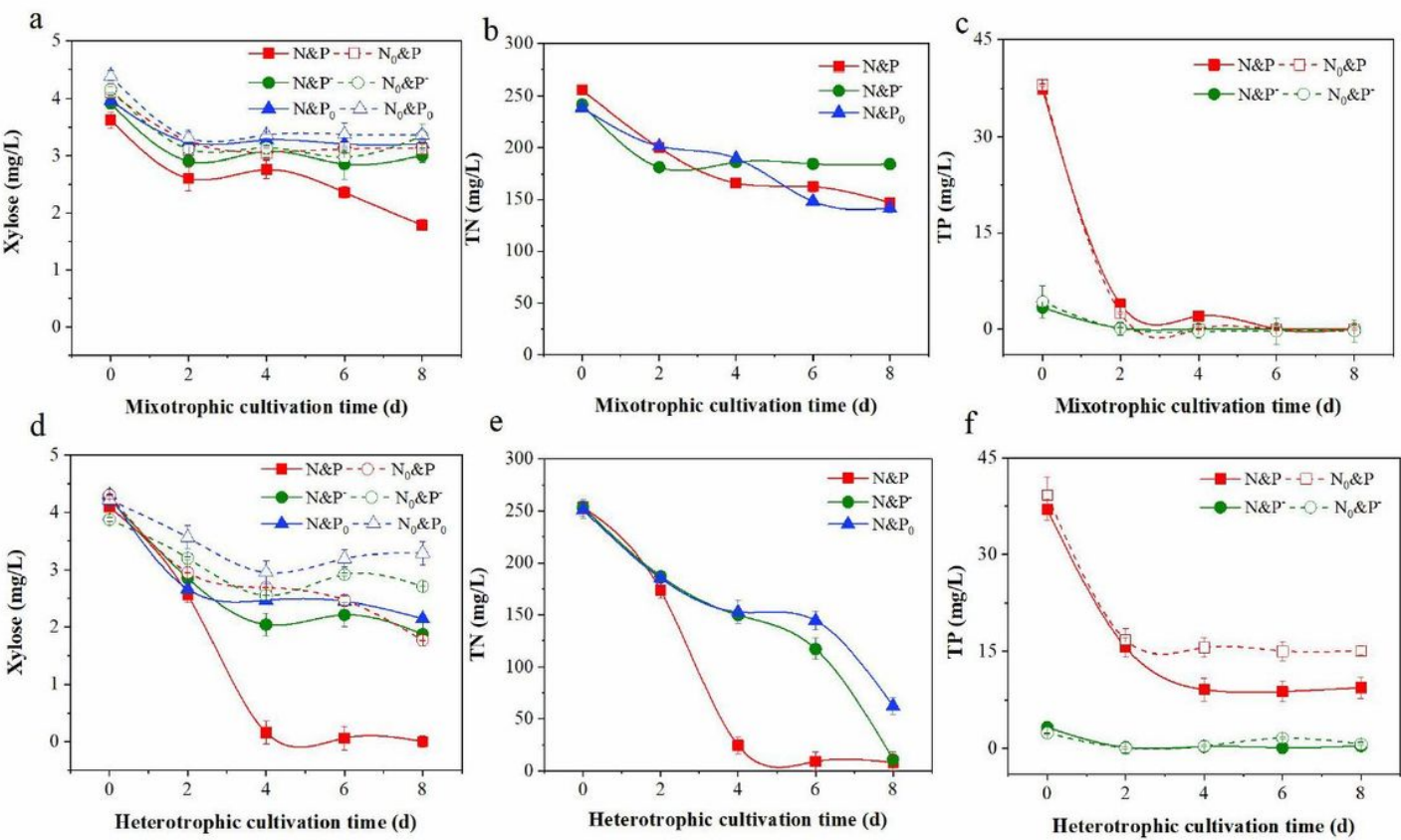


Figure 2

Assimilation profiles of xylose (a), TN (b), TP (c) by *Scenedesmus quadricauda* FACHB-1297 in mixotrophic cultivation, and xylose (d), TN (e), TP (f) in heterotrophic cultivation

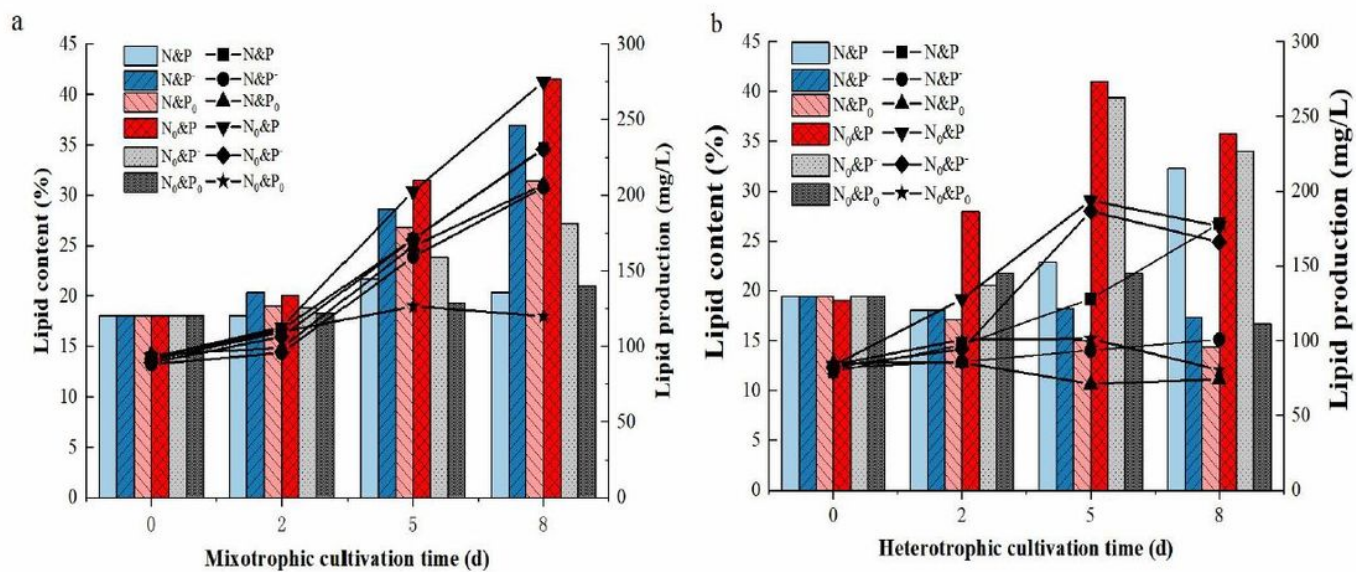


Figure 3

Lipid content and the production variations of lipid under six treatment during 8-day cultivation in (a) mixotrophic cultivation and (b) heterotrophic cultivation

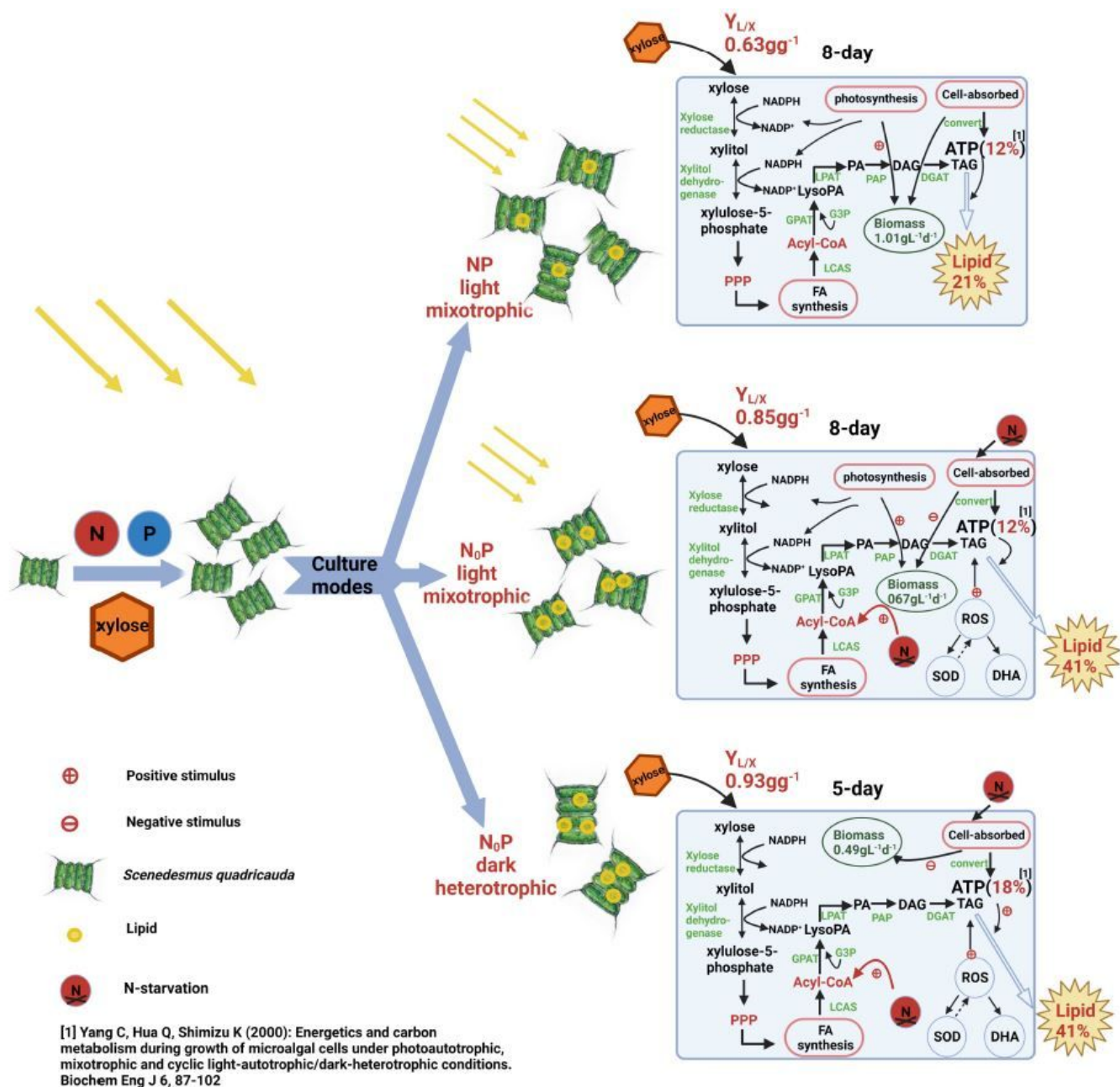


Figure 4

The mechanism of the effect of xylose assimilation and nitrogen starvation on the lipid accumulation in mixotrophic/heterotrophic cultivation

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