Journal of Applied Phycology **7**: 59–64, 1995.

Influence of carbon source on growth, biochemical composition and pigmentation of *Ankistrodesmus convolutus*

Wan-Loy Chu, Siew-Moi Phang* & Swee-Hock Goh
Institute of Advanced Studies, University of Malaya, 59100 Kuala Lumpur, Malaysia
*(*Author for correspondence)*

Received 25 April 1994; revised 28 September 1994; accepted 2 October 1994

Key words: Ankistrodesmus convolutus, carbon source, glucose, fatty acids, carotenoids

Abstract

The unicellular chlorophyte *Ankistrodesmus convolutus* Corda was grown in the light using inorganic medium (Bold's Basal Medium, BBM) and BBM enriched with 0.1% w/v of glucose, sodium acetate, sodium citrate or sodium bicarbonate. Glucose supported the highest specific growth rate ($\mu = 0.93\ d^{-1}$) and gave the highest biomass (453 mg dry weight L^{-1}) at the time of harvest. Of four glucose concentrations (0.05, 0.1, 0.25, 0.5% w/v), best growth was attained at 0.1% w/v. At 0.5% w/v glucose, the cells had high carbohydrates but low lipids and proteins. The relative amounts of 16:0, 18:0, 18:1 and 18:2 increased at the expense of 18:3($n-3$) in the carbon-supplemented cultures and at glucose concentrations higher than 0.1% w/v. Cultures grown on glucose had less chlorophyll and carotenoid contents than cultures grown on other carbon sources. Chlorophyll and carotenoid contents decreased with increasing glucose concentrations in the medium.

Introduction

The addition of organic carbon may enhance the growth of certain microalgae. For instance the biomass of *Chlamydomonas humicola* increases 20-fold when grown on acetate (Laliberte & de la Noue, 1993), while *Scenedesmus acutus* increases 6-fold on molasses (Shamala *et al.*, 1982a). Acetate enhances growth and ammonium uptake in mixotrophic cultures of *S. obliquus* (Combres *et al.*, 1994).

Carbon sources assimilated through different metabolic pathways influence the biochemical composition of microalgae. Acetate is channelled into proteins and carbohydrates in *Chlamydomonas humicola* and may increase chlorophyll content of the mixotrophic cells (Laliberte & de la Noue, 1993). Glucose is mainly incorporated into lipids (particularly fatty acids) in *Chlorella prothecoides* grown heterotrophically (Matsuka *et al.*, 1969). To optimise growth and production of the desired chemicals from microalgae, it is essential to supply the right carbon source.

Screening of local microalgae showed that *Ankistrodesmus convolutus* contains the highest amount of carotenoids (Chu *et al.*, 1992), higher than other non-carotenogenic chlorophytes. The content decreases at high salinity and temperature, and under nitrogen limitation (Chu *et al.*, 1994b; Chu *et al.*, 1991). *A. convolutus* under nitrogen limitation contains more carbohydrates and less proteins than under nitrogen sufficiency (Chu *et al.*, 1991).

The present study investigated the effect of carbon source on growth, biochemical composition and pigmentation of *A. convolutus* including the influence of glucose levels.

Materials and methods

Organism and culture conditions

Ankistrodesmus convolutus Corda was isolated in axenic form from a freshwater lake and deposited in the Microalgal Culture Collection (isolate No. 101) at the Institute of Advanced Studies, University of Malaya. The culture is maintained in Bold's Basal Medium (BBM, Nichols, 1973).

Table 1. Biomass (mg dry weight L^{1}) and specific growth rate (d^{-1}) of *Ankistrodesmus convolutus* grown on different carbon sources and at different glucose levels (mean ± standard deviation, n=3).

	Biomass	Specific growth rate
Carbon source		
Control (BBM)	92.0±4.2	0.34
Glucose	453.3±6.3	0.93
Acetate	288.7±8.3	0.85
Citrate	87.3±2.3	0.41
Bicarbonate	88.0±4.0	0.30
Glucose level (% w/v)		
0	109.3±3.1	0.37
0.05	280.0±9.6	0.96
0.1	500.0±4.0	1.87
0.25	486.0±4.2	0.96
0.5	40.5±1.5	0.89

Four carbon sources namely sodium bicarbonate ($NaHCO_3$), glucose, sodium acetate (CH_3COONa $3H_2O$) and trisodium citrate ($Na_3C_6H_5O_7$ $2H_2O$) were used. Solutions containing the different carbon sources were autoclaved separately before adding to BBM to give a final concentration of 0.1% w/v. The cultures were also grown at 0.05, 0.1, 0.25 and 0.5% w/v glucose. The control contained only BBM with dissolved CO_2 from the air resulting from agitation. All media were buffered with 10 mM 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES).

Fourty mL of an axenic culture at exponential phase with an optical density of 0.2 at 620 nm (OD_{620}) was inoculated into each flask (cap. 1 L) containing 360 mL medium. The cultures were grown in a controlled-environment incubator shaker (28 °C, 150 rpm) under 12:12 h light-dark cycle (42 μmol m^{-2} s^{-1}).

Growth monitoring

Growth was monitored daily by cell count using an improved Neubauer haemacytometer. Specific growth rate was calculated from the linear portion of the semi-logarithmic plots (cell number versus day).

Chemical analyses

The cultures were harvested at stationary phase by filtration (Whatman GF/C, 0.45 μm). Cells were weighed after drying for 24 h at 100 °. Separate filtered samples were used for the extraction of lipids, proteins, carbohydrates and pigments.

Lipids were extracted by sonicating in methanol-chloroform-water (2:1:0.8, v/v/v) and determined gravimetrically (Bligh & Dyer, 1959). The lipids were transesterified using 1 N sodium methoxide (60 °C, 30 min) and the fatty acid methyl esters obtained were analysed by gas chromatography as described in Chu *et al.* (1994a). Quantification of fatty acids was based on integrated areas (Chromatopac RC8A) of the peaks using the external standards consisting of 16:0, 18:0, 18:1, 18:2, 18:3 and 18:4 (Sigma Chemicals).

Protein content of the cells was determined by the dye-binding method after extraction in 0.5 N NaOH (Bradford, 1976). Carbohydrates extracted from the cells in 2 N HCl were determined using the phenol-sulphuric acid method (Kochert, 1978). Pigments were extracted from the cells under dim light using 100% acetone (HPLC grade) before further analysis by HPLC.

The HPLC system consisted of a Rheodyne valve injection port (20 μL loop), a Shimadzu LC 7A pump and a Shimadzu M6A photo-diode array detector. The pigments were resolved isocratically using a reverse phase column (Shandon Hyperbond, 300 × 3.9 mm). The mobile phase consisted of acetonitrile:methanol:acetone (56:40:4, v/v/v) and the flow rate used was 1 mL min^{-1}. Quantification of the pigments was based on calibration of the integrated areas of authentic standards (Sigma and Fluka Chemicals).

Results

Growth trend

A. convolutus attained the highest specific growth rate and final biomass when grown on glucose (Table 1). Both glucose and acetate gave higher biomass than BBM. Both citrate and bicarbonate did not exert any marked effect on the growth of *A. convolutus* compared with BBM. All media were buffered with HEPES and no marked difference in final pH (7.00–7.60) was observed.

The cultures grown on glucose exhibited a lag of 24 h after inoculation. Only during this phase, enlarged granular cells of *A. convolutus* in contrast to the normally crescent-shaped cells were observed.

In the experiment to determine the suitable glucose level for growth of *A. convolutus*, enlarged cells

Table 2. Fatty acid composition (% total fatty acids) of *Ankistrodesmus convolutus* grown on different carbon sources (mean ± standard deviation, n=3).

Fatty acid	BBM	Carbon source			
		Glucose	Acetate	Citrate	Bicarbonate
16:0	1.9±0.2	7.7±1.0	7.1±0.6	5.9±0.3	9.1±1.0
16:1	–	–	–	–	0.4±0.1
16:4	1.2±0.2	0.7±0.1	0.7±0.1	0.9±0.2	0.6±0.1
18:0	–	–	–	0.8±0.1	1.1±0.2
18:1	1.3±0.3	8.7±0.6	7.1±0.5	6.5±0.4	13.9±0.3
18:2	1.3±0.2	2.5±0.4	3.2±0.3	3.9±1.0	3.0±0.4
18:3(*n*-3)	93.3±1.0	79.1±4.1	80.0±5.0	80.1±4.0	70.4±2.5
18:4	0.9±0.2	1.2±0.1	1.1±0.2	1.1±0.2	1.1±0.1

Table 3. Fatty acid composition (% total fatty acids) of *Ankistrodesmus convolutus* grown at different glucose levels (mean ± standard deviation, n=3).

Fatty acid	Glucose level (% w/v)				
	0	0.05	0.1	0.25	0.5
16:0	2.8±0.2	5.9±0.3	5.7±0.4	23.1±1.5	30.3±2.3
16:4	0.4±0.1	1.2±0.2	0.6±0.1	–	–
18:0	–	0.7±0.1	0.5±0.2	2.0±0.4	3.0±0.5
18:1	2.1±0.2	6.9±0.3	5.5±0.4	29.9±2.3	39.5±1.4
18:2	2.6±0.2	2.9±0.3	1.8±0.2	5.4±0.2	5.3±0.3
18:3(*n*-3)	91.6±3.0	81.0±2.5	86.0±5.0	32.7±5.5	21.5±2.5
18:4	0.9±0.2	1.0±0.1	–	1.1±0.2	0.8±0.2

Table 4. Pigment composition (mg g^{-1} dry weight) of *Ankistrodesmus convolutus* grown on different carbon sources (mean ± standard deviation, n-3).

Pigment	BBM	Carbon source			
		Glucose	Acetate	Citrate	Bicarbonate
Chl *a*	32.0±1.8	14.0±0.1	32.5±0.5	32.0±0.3	32.4±0.5
Chl *b*	12.0±0.9	7.2±0.2	12.0±0.3	13.0±0.4	11.8±0.3
Lutein	5.5±0.5	2.8±0.1	6.1±0.6	6.0±0.6	5.9±0.1
Neoxanthin	1.5±0.3	0.20±0.05	0.60±0.03	0.60±0.05	0.60±0.05
Violaxanthin	1.2±0.3	0.10±0.02	0.40±0.01	0.40±0.02	0.30±0.04
α-carotene	1.6±0.4	0.8±0.1	1.7±0.3	1.8±0.2	1.6±0.2
β-carotene	0.8±0.2	0.40±0.05	1.10±0.04	1.00±0.05	0.9±0.1
Total chlorophylls	44.0	21.2	44.5	45.0	44.2
Total carotenoids	10.6	4.3	9.9	9.8	9.3

Table 5. Pigment composition (mg g^{-1} dry weight) of *Ankistrodesmus convolutus* grown at different glucose levels (mean ± standard deviation, n=3).

Pigment	Glucose level (% w/v)				
	0	0.05	0.1	0.25	0.5
Chl *a*	20.5±1.5	10.9±0.3	13.7±0.3	3.4±0.1	4.9±0.2
Chl *b*	12.7±1.0	8.5±0.4	6.2±0.2	2.4±0.1	2.2±0.2
Lutein	4.7±1.2	3.3±0.4	2.8±0.1	2.4±0.3	3.6±0.2
Neoxanthin	1.3±0.5	0.20±0.04	0.20±0.05	0.10±0.02	0.10±0.03
Violaxanthin	1.0±0.2	0.10±0.02	0.10±0.03	0.10±0.03	0.10±0.03
α-carotene	0.9±0.2	0.9±0.1	0.8±0.1	–	–
β-carotene	0.70±0.15	0.50±0.10	0.40±0.10	0.30±0.05	0.8±0.1
Total chlorophylls	33.2	19.4	19.9	5.8	7.1
Total carotenoids	8.6	5.0	4.3	2.9	4.6

were again observed in the lag period. After the lag, the glucose-grown cultures attained higher cell number than the control (BBM). At high glucose levels (0.25 and 0.5% w/v) many enlarged cells persisted till the end of the experiment while final biomass attained was low. Cultures grown at 0.1% w/v glucose exhibited the highest specific growth rate and attained a final biomass almost five-fold of that attained in BBM (Table 1).

Biochemical composition

No significant difference in biochemical composition was observed in *A. convolutus* when grown on different carbon sources. Contents of lipids, carbohydrates and proteins were in the range of 19.3–25.0%, 14.7–20.0% and 17.2–21.1% dry weight respectively. A marked increase of carbohydrates which coincided with a decrease in proteins were observed at glucose levels higher than 0.1% w/v (Fig. 1). Cells grown at 0.5% w/v glucose contained high carbohydrates and low proteins and lipids.

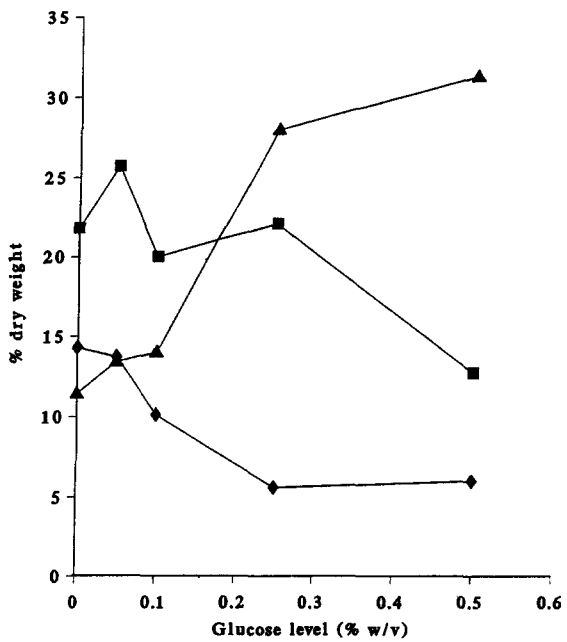

Fig. 1. Biochemical composition of *Ankistrodesmus convolutus* grown at different levels of glucose. Lipids (■), carbohydrates (▲), proteins (♦).

Fatty acid profiles

The predominant fatty acid of *A. convolutus* was 18:3($n-3$) (Table 2), irrespective of the carbon source supplied. The cultures supplemented with exogenous carbon especially bicarbonate had higher percentages of 16:0 and 18:1 than the control (Table 2).

At 0.1% w/v glucose and lower, 18:3($n-3$) was the predominant fatty acid (Table 3). At glucose levels higher than 0.1% w/v, the relative amount of 18:3($n-3$) decreased markedly with concomitant increases in 16:0 and 18:1. The monounsaturated fatty acid 18:1 predominated in the cultures grown at 0.5% w/v glucose.

Pigment composition

The predominant carotenoid of this chlorophyte is lutein (Tables 4 and 5). The contents of chlorophylls and carotenoids of the cultures grown on acetate, citrate, bicarbonate and BBM were similar (Table 4). In contrast, contents of all the pigments decreased markedly in the cells grown on glucose.

With increasing glucose level, chlorophylls decreased more markedly than carotenoids (Table 5). Cells grown at high glucose levels (0.25 and 0.5% w/v) had very low chlorophyll and carotenoid contents.

Discussion

Growth trend

The present study revealed that a five-fold increase in biomass of *A. convolutus* may be attained with the addition of 0.1% w/v glucose. In comparison, addition of 0.05% w/v glucose in mixotrophic culture of *Scenedesmus acutus* increases the biomass by three-fold (Shamala, 1982a). The growth enhancement of *Scenedesmus acutus* in the presence of glucose was attributed to the increased photosynthetic rate. *A. convolutus* did not grow on glucose under heterotrophic conditions, contrasting with other chlorophytes such as *Scenedesmus obliquus* and *Chlamydomonas humicola* which have heterotrophic growth capabilities (Combres *et al.*, 1994; Laliberte & de la Noue, 1993).

Polymorphism of *A. convolutus* cells observed in the present study may have resulted from the effects of glucose on cytokinesis, cell elongation and cell wall formation. The inoculum had not been pregrown in glucose. It is noteworthy to find out whether the cells will undergo similar morphological changes if they are preadapted to glucose before inoculation into glucose-enriched medium.

Both citrate and bicarbonate did not enhance growth of *A. convolutus*. Citrate was probably not taken into the cells. Various intermediates of the tricarboxylic acid cycle, such as pyruvate, citrate, succinate and malate have been tried unsuccessfully as carbon sources for algal growth (Danforth, 1962). The failure has been attributed to their impermeability to the cells rather than enzymatic deficiency. These molecules fail to penetrate the cell membrane due to their strongly ionised and hydrophilic nature (Danforth, 1962).

A. convolutus was probably not able to utilise HCO_3^- efficiently, because it lacked a CO_2-concentrating mechanism involving carbonic anhydrase as found in chlorophytes where the enzyme is located on the cell membrane, converting HCO_3^- to CO_2 which is then 'pumped' into the cell for carbon-fixation process (Tsuzuki & Miyachi, 1989).

Biochemical composition

The fate of the carbon source incorporated into microalgal cells vary with species and is controlled by other factors such as light-dark cycle and nitrogen level. Acetate in *Nannochloropsis* sp. and glucose in *Chlorella prothecoides* are mainly incorporated into lipids (Sukenik & Carrneli, 1990; Matsuka *et al.*, 1969). Mixotrophic cells of *Chlamydomonas humicola* grown on acetate accumulate proteins at the expense of carbohydrates (Laliberte & de la Noule, 1993). Only at high glucose levels (0.25 and 0.5% w/v), carbohydrate synthesis was favoured in *A. convolutus*, contrasting with *Scenedesmus acutus* which produces high amount of carbohydrates even at 0.05% w/v glucose. (Shamala *et al.*, 1982b).

Fatty acid profiles

Fatty acid profiles of *A. convolutus* resembled the general pattern shown in chlorophytes which contain high amounts of diunsaturated and triunsaturated C16 and C18 fatty acids but lack C20 and C22 polyunsaturated fatty acids (Dunstan *et al.*, 1992). Recently, Renaud *et al.* (1994) reported that 18:3($n-3$), 16:1($n-7$) and 16:3($n-4$) were equally abundant in *Ankistrodesmus* sp. However, at glucose levels lower than 0.1% w/v, 18:3($n-3$) was predominant in *A. convolutus*.

In the present study, the relative amounts of other fatty acids increased at the expense of 18:3($n-3$) when exogenous carbon sources were added especially at increasing glucose levels. This was also observed at high NaCl concentrations (Chu *et al.*, 1994b). Changes in the relative amounts of 18:3($n-3$) may be attributed to effects on the desaturation pathways of fatty acids.

Pigment composition

The array of pigments identified in *A. convolutus*, except for the absence of loroxanthin, resembled the pigment composition of *Ankistrodesmus braunii* (Sange & Senger, 1990). With the addition of glucose, the pigment content decreased markedly, resulting in the 'bleaching' of the cells, most likely attributed to a degradative process. Cell 'bleaching' did not appear to have any adverse effect on its growth. In comparison, cell 'bleaching' with decreased growth occurs when

A. convolutus is under nitrogen-limitation or grown at high salinity and temperature (Chu *et al.*, 1991; Chu *et al.*, 1994b).

The total carotenoid yield (2.2 mg L^{-1}) of cultures grown on 0.1% w/v glucose was doubled that in BBM. The enhanced growth in glucose probably led to depletion of other nutrients, especially nitrate which may have caused the decreased pigment content. A doubling of nitrate level led to an increase in carotenoid yield when the cultures were supplemented with glucose (unpublished results).

Potential applications

Renaud *et al.* (1994) showed that *Ankistrodesmus* sp. may not be suitable for aquaculture feed as it lacks 20:5($n-3$) and 22:6($n-3$) fatty acids. However, the pigment content of their species of *Ankistrodesmus* was not characterised. Our present study showed that *A. convolutus* contains appreciable amounts of carotenoids especially lutein and this alga may be a potential poultry feed. It is advantageous to work with *A. convolutus* which is capable of using organic carbon sources (especially glucose) due to the high biomass attained. A cheap glucose source such as molasses may be used to produce biomass economically for animal feed. Further manipulative studies are in progress to enhance the biomass and carotenoid yield of this alga.

Conclusion

The supplementation of 0.1% w/v glucose enhanced biomass *A. convolutus* by five-fold compared with BBM. The relative proportions of 16:0 and 18:1 increased at the expense of 18:3($n-3$) when grown at high glucose levels. Carotenoid content of the cells decreased while its yield doubled when grown on 0.1% w/v glucose.

Acknowledgments

This study was supported by a research grant (R & D 1/026/01) from the Ministry of Science, Technology and Environment of Malaysia.

References

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248–254.

Chu WL, Phang SM, Goh SH (1991) Effect of nitrate concentration on the growth and chemical composition of an *Ankistrodesmus* isolate. Trans. mal. Soc. Pl. Physiol. 2: 113–117.

Chu WL, Phang SM, Goh SH (1994a) Studies on the production of useful chemicals especially fatty acids in the marine diatom *Nitzschia conspicua* Grunow. Hydrobiologia 285: 3340.

Chu WL, Phang SM, Goh SH, Blakebrough N (1992) Promising microalgae for production of useful chemicals. In Shaari K, Kadir AA, Ali ARM (eds), Proceeding of the Conference on Medicinal Products from Tropical Rain Forests. Forest Research Institute of Malaysia, Kuala Lumpur: 338–345.

Chu WL, Phang SM, Goh SH, Blakebrough N (1994b) Environmental effects on growth and biochemical composition of *Ankistrodesmus convolutus*. In Phang SM, Lee YK, Borowitzka MA, Whitton BA (eds), Proceeding of the 1st Asia-Pacific Conference on Algal Biotechnology, University of Malaya, Kuala Lumpur: 16–27.

Combres C, Laliberte G, Reyssac JS, de la Noue J (1994) Effect of acetate on growth and ammonium uptake in the microalga *Scenedesmus obliquus*. Physiol. Pl. 91: 729–734.

Danforth WF (1962) Substrate assimilation and heterotrophy. In Lewin RA (ed.), Physiology and Biochemistry of Algae. Academic Press, New York: 99–123.

Dunstan GA, Volkman JK, Barret SM, Garland CD (1993) Changes in the lipid composition and maximisation of the polyunsaturated fatty acid content of three microalgae grown in mass culture. J. appl. Phycol. 5: 71–83.

Kochert AG (1978) Carbohydrate determination by the phenol- sulfuric acid method. In Hellebust JA, Craigie JS (eds), Handbook of Phycological Methods – Physiological and Biochemical Methods. Cambridge U.P., Cambridge: 95–97.

Laliberte G, de la Noue J (1993) Auto-, hetero- and mixotrophic growth of *Chlamydomonas humicola* (Chlorophyceae) on acetate. J. Phycol. 29: 612–620.

Matsuka M, Miyachi S, Hase E (1969) Further studies on the metabolism of glucose in the process of 'glucose-bleaching' of *Chlorella protothecoides*. Pl. Cell Physiol. 10: 513–529.

Nichols HW (1973) Growth media-freshwater. In Stein JR (ed.), Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge U.P., Cambridge: 7–24.

Renaud SM, Parry DL, Thinh LV (1994) Microalgae for use in tropical aquaculture I: Gross chemical and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. J. appl. Phycol. 6: 337–345.

Senge M, Senger H (1990) Response of photosynthetic apparatus during adaptation of *Chlorella* and *Ankistrodesmus* to irradiance changes. J. Pl. Physiol. 136: 675–679.

Shamala TR, Drawert F, Leupold G (1982a) Studies on *Scenedesmus acutus* growth I. Effect on autotrophic and mixotrophic conditions on the growth of *Scenedesmus acutus*. Biotech. Bioengng 24: 1287–1299.

Shamala TR, Drawert F, Leupold G (1982b) Studies on *Scenedesmus acutus* growth II. Effect of autotrophic and mixotrophic growth on the amino acid and the carbohydrate composition of *Scenedesmus acutus* Biotech. Bioengng 24: 1301–1317.

Sukenik A, Carnneli Y (1990) Lipid synthesis and fatty acid composition in *Nannochloropsis* sp. (Eustigmatophyceae) grown in a light-dark cycle. J. Phycol. 26: 463–469.

Tsuzuki M, Miyachi S (1989) The function of carbonic anhydrase in aquatic photosynthesis. Aquat. Bot. 34: 85–104.