METHYLGLYOXAL EFFECTS ON CELL DIVISION OF SCENEDESMUS QUADRICAUDA (SCENEDESMACEAE)

DISSERTATION

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By

Kitae Rhie, B.S., M.S.
Denton, Texas

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Cell division of Scenedesmus quadricauda (Turp.) Breb. (Scenedesmaceae) is enhanced by methylglyoxal, a general inhibitor of cell division, at threshold concentration in conjunction with treatment timing related to growth stage of batch cultures. At 0.5 mM methylglyoxal concentration, cell division was significantly enhanced in algae treated in the logarithmic phase. Specific growth rates of methylglyoxal-treated cultures were rapidly increased at the beginning of logarithmic phase. Cultures inoculated with high cell numbers were less sensitive, but still showed high specific growth rates in logarithmic phase. Cell division in cultures which had low cell numbers was inhibited by 0.5 mM methylglyoxal treatment.

Both specific activity of Glyoxalase I and the ratio of Glyoxalase I to Glyoxalase II of methylgloxal-treated cultures were higher than those of controls (1.3 and 2.1-fold, respectively). Pyruvate concentration in treated cultures was increased after methylglyoxal treatment.

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF TABLES (CONTINUED)

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS (CONTINUED)

LIST OF ILLUSTRATIONS (CONTINUED)

LIST OF ILLUSTRATIONS (CONTINUED)

CHAPTER I

INTRODUCTION

Since 1965, the functions of methylglyoxal (MG) have been studied in relation to cell division, wound healing, and cancer. The growth-inhibiting effects of MG on a number of organisms and tissues has been shown (Egyud, 1965; Egyud and Szent-Gyorgy, 1966a and 1966b; Szent-Gyorgy *et al.*, 1967; Morris, 1969; Krymkiewicz *et al.*, 1971) and these studies suggested that MG strongly inhibited *in vivo* nucleic acid and protein synthesis, and arrested cell division in rapidly dividing cells at concentration of 1 to 2 mM.

The inhibitory mechanism of MG on cell division is not clear, but those workers concluded that methylglyoxal interacts with highly active sulfhydryl groups in the regulation of cell division in tissue and that this methylglyoxal-SH complex can arrest cell division in rapidly dividing cells. It was suggested that MG strongly inhibited *in vivo* deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis in *Escherichia coli* (Egyud and Szent-Gyorgy, 1966b; Krymkiewicz *et al.*, 1971). MG also caused some inhibition of respiration and nucleic acid degradation, and changed intracellular pools of guanine, guanosine

monophosphate and adenosine triphosphate (Krymkiewicz et al., 1971). Also, MG or some 2-ketoaldehydes is known to inhibit cell division by inhibiting protein synthesis at the translational level, probably react with the 7-methyl-guanosin 'cap' residue in mRNA (Kozarich et al., 1979; Carrington and Douglas, 1986). Methylglyoxal and similar aldehyde compounds exert marked effects on certain cancers by reducing their ascites fluid formation and prolonging the survival time of animals bearing those tumor cells (Jerzykowski et al., 1974; Fenselan and Long, 1976; Sato et al., 1980).

In spite of these toxic effects, there is still discussion of the identity and variety of 2-ketoaldehyde sources, some indigenous, some dietary or environmental, have been suggested (Carrington and Douglas, 1986). MG has been known for more than two decades to exist under unusual circumstances, such as in vitamin B_1 deficiency, in the urine (Salem, 1955), blood (Sato, 1964), or milk (Wako, 1951) of various species.

Formation of MG in animal tissue, some microorganisms, and yeast was reported from aminoacetone and dihydroxyacetone phosphate by amine oxidase or methylglyoxal synthase, respectively. As the bypath of glycolysis, methylglyoxal synthase which convert dihydroxyacetone phosphate to MG was isolated in goat liver (Ray and Ray,

1981), rat liver (Sato et al., 1980), Escherichia coli (Hopper and Cooper, 1972), Pseudomonas saccharophila (Cooper, 1974). Also, MG formation by degradation of amino acid, threonine or glycine, in animal tissue and yeast was reported. Aminoacetone from L-threonine by L-threonine dehydrogenase or glycine by aminoacetone synthase was reported to produce MG by amine oxidase in goat liver (Ray and Ray, 1987) or in yeast (Murata et al., 1986).

In biochemical studies, reported metabolic products of MG included lactate, pyruvate, and glucose (via gluconeogenesis) in animal tissues (Racker, 1951; Ting et al., 1965; Monder, 1967; Ray and Ray, 1982, 1984a, and 1984b; Saez et al., 1985) and in yeast (Inoue et al., 1985; Murata et al., 1985 and 1986) and E coli (Saikusa et al., 1987) and in mold (Inoue et al., 1988), via the glyoxalase system, α-ketoaldehyde dehydrogenase, or MG reductase. Also, H_2O_2 was reported as one of the products of MG metabolism by the enzyme glyoxal oxidase in basidiomycetes (Kersten and Kirk, 1987).

MG transformation into lactate and pyruvate is related to energy metabolism, catabolic and anabolic dissociation processes in carbohydrates and proteins, and, probably, to maintenance of asymmetrical entropy in vivo on the constant level (Alekseev, 1987).

Glyoxalase activity, which is capable of converting

methylglyoxal to lactic acid through a compound similar to pyruvic acid was described (Dakin and Dudley, 1913 a and b), this activity and methylglyoxal have been studied as the intermediates in the glycolytic pathway. Later it was suggested that glyoxalase was a two-enzyme complex, requiring glutathione, that resulted in the formation of lactate from methylglyoxal (Raker, 1951).

In plant and animal tissues, glyoxalase I (G I) (EC 4.4.1.5) catalyses the formation of S-D-lactoylglutathione from the non-enzymatically formed hemimercaptal adduct of methylglyoxal and reduced glutathione:

$$CH_3COCHO + GSH \quad <---> \quad CH_3COCH(OH)SG \quad ---> \quad CH_3CH(OH)CO\text{-}SG$$

Glyoxalase II (G II) (EC 3.1.2.6) catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate and reduced glutathione:

$$CH_3(OH)CO\text{-}SG + H_2O \quad ---> \quad CH_3CH(OH)CO_2H + GSH$$

In spite of its early discovery, little is known about the basic biological function of MG and the glyoxalase system as the glycolytic bypath. The glyoxalase system appears competent to receive and act on functional signals (chemotaxis, phagocytosis, degranulation, etc.), and an

intermediate product of MG metabolism *via* the glyoxalase system, S-D-lactoylglutathione, was considered to be a regulator or stimulator in microtubule assembly in brain cells (Gillespie, 1979) and human neutrophils (Thornalley and Bellavite, 1987; Thornalley *et al.*, 1987) and leukaemia cells (Hooper *et al.*, 1987) during the functional activation.

The glyoxalase system is widespread in biological tissues but the activity of glyoxalase II is lower in rapidly growing tissue than in tissue with slower growth kinetics (Principato *et al.*, 1982). Glyoxalase I is considered to regulate or to be related to cell proliferation (Principato *et al.*, 1982; Douglas *et al.*, 1982; Ueda *et al.*, 1984; Das *et al.*, 1987; Basu *et al.*, 1988; Sethi *et al.*, 1988; Murata *et al.*, 1988).

The glyoxalase system forms probably the main line of cellular defence against the cytotoxic ketoaldehydes, which are formed indogenously in a variety of cell types, e. g., by glycerol metabolism, from dihydroxyacetone phosphate by the action of methylglyoxal synthase. If such materials were allowed to accumulate intracellularly the inevitable result would be cell death. However, glyoxalase is not the only means of detoxification for MG. MG can be converted in bacteria and yeast to L- and D-lactaldehyde by methylglyoxal reductase and hence, by means of the appropriate lactate

dehydrogenase, to pyruvate:

Methylglyoxal ---> lactaldehyde ---> lactate <---> pyruvate

The enzyme, methylglyoxal reductase, is active in an irreversible conversion of methylglyoxal to lactaldehyde and appears to be dependent on NADH in animal tissue (Ray and Ray, 1984a). Lactaldehyde is oxidized to lactate by cytosolic aldehyde dehydrogenase in animal tissue (Ray and Ray, 1984b). In a later study, in yeast the NADPH-dependent methylglyoxal reductase was reported to convert methylglyoxal to L-lactaldehyde in an alternative route for methylglyoxal degradation by the glyoxalase system consisting of glyoxalase I and glyoxalase II (Murata et al., 1985). The L-lactaldehyde is then converted to L-lactate by NAD-dependent L-lactaldehyde dehydrogenase (Inoue et al., 1985).

In other forms of living organisms, MG can be converted directly to pyruvate by another enzyme. It was suggested that the α-ketoaldehyde dehydrogenase can convert methylglyoxal according to the following scheme (Monder, 1965):

Methylglyoxal ----> Pyruvate <----> L-Lactate

The direct oxidation of methylglyoxal to pyruvate by α-ketoaldehyde dehydrogenase has two enzyme systems, one NAD-dependent and the other NADP-dependent (Ray and Ray, 1982).

The relationship between the activity of α-ketoaldehyde dehydrogenase or MG reductase with their metabolic products and cell proliferation is not well studied.

Biotransformation of MG to pyruvate *via* α-ketoaldehyde dehydrogenase or MG reductase and a stimulatory relationship to microtubule assembly by metabolic intermediates of MG metabolism *via* glyoxalase system indicate a reconsideration of MG effects on cell growth and cell division, especially in unicellular or coenobial organisms, is needed. The only reported study of the effects of MG on algae (Morris, 1969) showed that MG, at less than 1 mM concentration, inhibited growth and delayed the onset of cell division of *Chlamydomonas reinhardii*.

Most of all studies on MG were done with enzymes as a catalyzer of MG metabolism in animal tissues and yeast. There was no study about MG effects related to physiological responses especially growth dynamics and its effective concentrations. This study is one approach to understanding the effect of MG on the growth and cell division of the coenobial planktonic green alga, *Scenedesmus quadricauda* with following objectives; physiological responses of green

algae, the effectiveness of MG to the cell division or growth, the threshold concentration of MG to inhibit cell division, MG metabolism in Scenedesmus quadricauda, stimulatory effects of MG on cell division with relation to the activity of glyoxalase I and II.

CHAPTER II

MATERIALS AND METHODS

The alga, Scenedesmus quadricauda (Turp) Breb. (UTEX 614), was cultured in Bristol's medium as modified by Bold (Starr, 1978). The medium (50 ml in 250 ml side-armed flasks) was sterilized at 121°C and 1.1 $kg.cm^{-2}$ for 10 min. The alga was cultured at 25°C and agitated on a reciprocal shaker at 80 cpm. Cultures were grown in growth chambers under continuous light (cool-white fluorescent tubes) at 45 $\mu E \cdot m-2 \cdot sec^{-1}$.

Six sets of experiments were cultured to study the effect of different concentrations of methylglyoxal (MG) on growth dynamics of algae. Each set of algal cultures was inoculated with a different initial concentration of cells, and the cell concentrations after inoculation was 0.5×10^4 cells $\cdot$ ml^{-1} to 4.8×10^4 cells $\cdot$ ml^{-1}. In each experiment, MG (Sigma No. M-0252) was added directly to the treatment flask to achieve the desired final concentration (0.25, 0.5, 0.75, 1.0, or 2.0 mM) at the desired time (culture day 0, 1, 2, 3, 4, or 5). At the time of treatment, the cell concentration of the cultures ranged from 2.4×10^4 to 2.67×10^5 cells $\cdot$ ml^{-1}. Each set of cultures was replicated at

least in triplicate.

For enzyme studies, cultures were grown in 2 liter erlynmeyer flasks and 6 liter glass vessels. The vessels were attached to a MicroFerm Laboratory Fermentor equipped with a light bank. The algal cultures were aerated by filtered compressed air at the rate of 300 ml · l^{-1} · min^{-1} and stirred by rotating impellers at 100 rpm. The growth conditions were as described above.

Effects of MG on the physiology of Scenedesmus quadricauda were measured by the following parameters:

Growth Dynamics

Algal growth was measured by enumerating the cell number each day with the aid of an AO Spenser Bright-line Hemacytometer. To compare the rate of cell division of MG-treated cultures with controls, a specific growth rate (SGR) was calculated by dividing the difference of natural logarithmic cell numbers between two successive measurements by days on which two measurements were made (Toerien et al., 1971).

Photosynthetic and Respiratory Rate.

The photosynthetic and respiratory rates of methylglyoxal-treated and untreated cultures were determined

at different stages in the growth cycles of the algae with a YSI Oxygen Meter, Model 53. A vial in the water bath (25 °C) contained 3 ml of algal cultures from controls or MG treated cultures was monitored for 30 min.

Preparation of Cellular Extracts.

Cultures were harvested in their logarithmic phase and steady state phase by centrifugation (Beckman, J2-21) at 7,500 x g for 25 min. The cells were washed with distilled water and centrifuged again. The concentrated cell suspension was washed with 50 mM Tris buffer, pH 7.4, and recentrifuged. Algal cell paste was transfered to the homogenizer vial which was pre-cooled in a dry ice-acetone bath and the paste was homogenized in 50 ml aliquots in a Braun Cell Homogenizer, Model MSK, using 0.15 mm Glasperlen at 4,000 rpm for 2 min (for 4 times, 30 sec each) at a 3:1 ratio of suspension:beads. Glasperlen and cell debris were centrifuged in a Beckman Model J2-21 centrifuge at 0 °C at 8,000 x g for 25 min. The precipitate was discarded and the supernatant collected and designated "crude extract".

Methylglyoxal Assay.

MG in the algal cultures was measured by a modification of the method of Cooper (1974). A 0.10 ml of crude extract was mixed with 0.33 ml of 0.1 % 2,4-dinitrophenylhydrazine

in 2 M-HCl with 0.90 ml of DI water. After incubation at 30 °C for 15 min., 1.67 ml of 10 % NaOH was added. The absorbance of this preparation was then measured at 555 nm after 15 min in a Hitachi-Coleman Double Beam Spectrophotometer Model 124.

Protein and Enzyme Assay

Protein Assay. Total cell protein was determined spectrophotometrically by the method of Bradford (1976) from the standard curve with bovine serum albumin.

Glyoxalase I Activity. The glyoxalase I (GI) activity of algal cultures was measured by a modification of the method of Mannervik et al. (1982). The reaction mixture consisted of the following: 0.10 ml of the enzyme extract; 0.06 ml of 50 mM reduced glutathione : 2.34 ml distilled water; 0.10 ml of 40 mM MG; and 1.50 ml of 50 mM Tris buffer, pH 7.4. The initial rate of increase in concentration of S-D-lactoylglutathion was measured by absorbance at 240 nm at 25 °C.

Glyoxalase II Activity. The glyoxalase II (GII) activity of algal cultures was measured by a modification of the method of Thornalley et al. (1987). The activity of GII was

assayed by measuring the initial rate of decrease in concentration of S-D-lactoylglutathione on the addition of an aliquot of the cellular extract to 0.3 mM S-D-lactoylglutathione (from Sigma) in 50 mM Tris-HCl buffer, pH 7.4 at 25 °C. The rate of change is measured by absorbance at 240 nm.

Methylglyoxal Reductase Assay. The activity of methylglyoxal reductase (MG reductase) was measured by the spectrophotometric method of Murata et al.(1985) after modification. The assay mixture contained 100 mM Tris buffer (pH 7.0), 0.1 mM NADPH, 10 mM methylglyoxal and a requisite amount of the enzyme made to a volume of 3.0 ml, and measured at 340 nm.

α-Ketoaldehyde Dehydrogenase Assay. Enzymatic oxidation of methylglyoxal was measured at 340 nm following the formation of NADH (Ray and Ray, 1982). The assay mixture contained, in a total volume of 3.0 ml, 75 mM of Tris-HCl buffer (pH 8.6), 0.5 mM of NAD or 0.2 mM of NADP, 4 mM of methylglyoxal and the requisite amount of enzyme.

Product Assay

Pyruvate assay. Pyruvic acid determination was carried out by the spectrophotometric method described in Sigma Chemical

Co., Standard Procedure No. 726-UV (March, 1988).

Lactate assay. Lactic acid determination was carried out by the spectrophotometric method described in Sigma Chemical Co., Standard Procedure No. 826-UV (March, 1988).

Glucose assay. Glucose determination was carried out by the spectrophotometric method described in Sigma Chemical Co., Standard Procedure No. 16-UV (November, 1986) at 340 nm.

All chemicals were purchased from Sigma Chemical Co. except 2,4-dinitrophenylhydrazine (Estman Kodak No. 1866).

Data were analyzed by parametric ANOVA ($\alpha = 0.05$) and Duncan's Multiple range test ($\alpha = 0.05$) with SAS (Statistical Analysis System, SAS Institute Inc.).

CHAPTER III

RESULTS

Growth Dynamics

Methylglyoxal (MG) at concentration of 2.0 mM inhibited cell division of S. quadricauda regardless of the cell concentration in the cultures with high inoculation ($2.4 \pm 0.2 \times 10^4$ to $4.8 \pm 0.2 \times 10^4$ cells $\cdot$ ml^{-1}) at the time of treatment. The cell numbers of cultures inoculated with $2.4 \pm 0.2 \times 10^4$ cells $\cdot$ ml^{-1} and treated with 2.0 mM MG on day 2 when the cell concentration was $0.694 \pm 0.013 \times 10^5$ cells $\cdot$ ml^{-1}, were significantly lower than those of controls throughout all stages of the culture growth (Fig. 1). Even when algal cultures were inoculated with relatively high cell concentrations ($4.8 \pm 0.2 \times 10^4$ cells $\cdot$ ml^{-1}), and treated with 2.0 mM MG on day 2 when the cell concentration was $1.146 \pm 0.010 \times 10^5$ cells $\cdot$ ml^{-1}, the cell number was lower than that of controls (Fig. 2). The shape of growth curves of MG treated cultures was not a typical sigmoidal growth curve. The SGRs of those cultures treated with 2.0 mM MG were significantly decreased after MG treatment (Table 1 and 2). The SGR of cultures treated with 1.0 mM MG on day 1 when the cell concentration was $0.500 \pm 0.013 \times 10^5$ cells

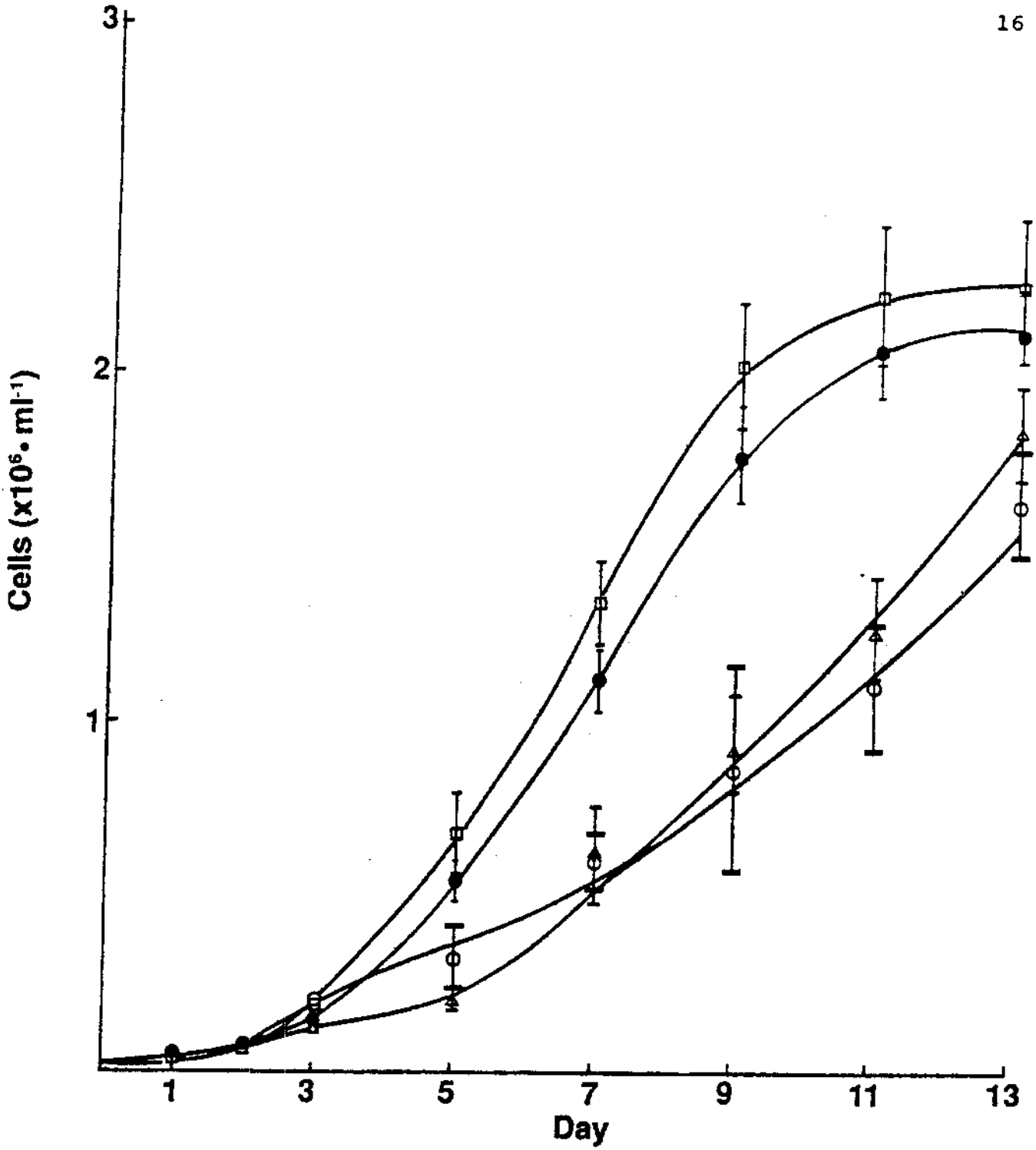

Fig. 1. Growth curve of methylglyoxal-treated (0.5, 1.0, or 2.0 mM MG) and untreated Scenedesmus quadricauda with initial inoculation of 2.4×10^4 cells $\cdot$ ml^{-1} (● - ● , Controls; □ - □ , 0.5 mM; △ - △ , 1.0 mM; ○ - ○ , 2.0 mM; symbol->mean, bar->standard deviation, n=3)

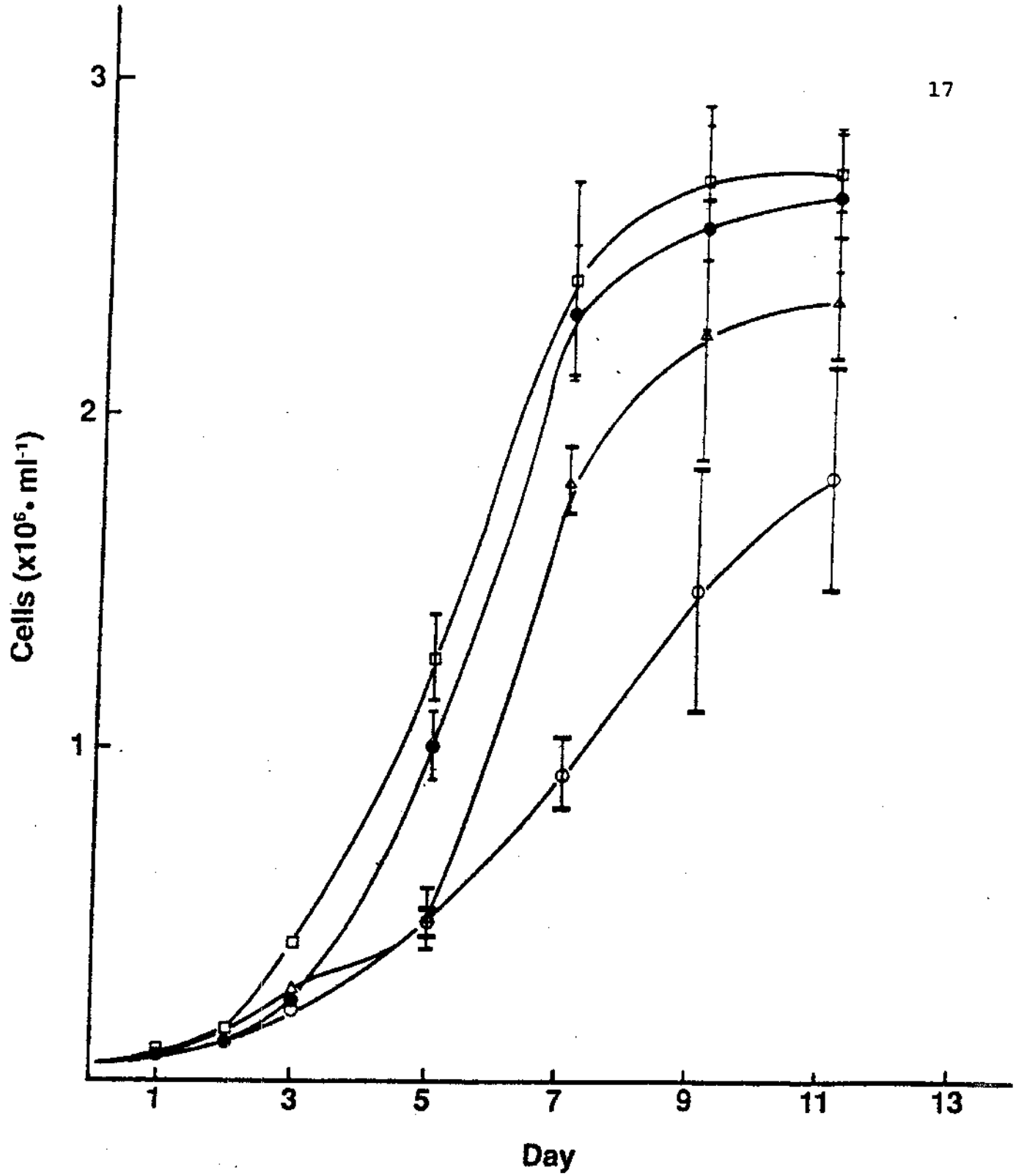

Fig. 2. Growth curve of methylglyoxal-treated (0.5, 1.0, or 2.0 mM MG) and untreated Scenedesmus quadricauda with initial inoculation of 4.8×10^4 cells · ml^{-1} (● - ● , Controls; □ - □ , 0.5 mM; △ - △ , 1.0 mM; ○ - ○ , 2.0 mM; symbol->mean, bar->standard deviation, n=3)

Table 1. Specific growth rate (SGR) of control and 0.5, 1.0, or 2.0 mM methylglyoxal (MG) treated cultures (2.4×10^4 cells · ml^{-1} inoculation)

Day / Culture	0 - 1	1 - 2	2 - 3	3 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	a 2) 1) 0.635 (0.068)	0.633 (0.034)	b 0.875 (0.003)	ab 0.483 (0.032)	b 0.366 (0.018)	0.226 (0.018)	ab 0.079 (0.012)	b 0.010 (0.001)
0.5 mM	b 0.248 (0.230) 3)	0.781 (0.304)	a 1.212 (0.039)	a 0.549 (0.018)	b 0.340 (0.018)	0.205 (0.026)	b 0.047 (0.003)	b 0.006 (0.002)
1.0 mM	a 0.605 (0.046)	0.485 (0.115) 3)	c 0.329 (0.155)	bc 0.411 (0.110)	a 0.490 (0.073)	0.212 (0.046)	a 0.155 (0.027)	a 0.190 (0.019)
2.0 mM	a 0.620 (0.068)	0.648 (0.037)	b 0.855 (0.193) 3)	c 0.352 (0.031)	c 0.195 (0.072)	0.166 (0.103)	a 0.138 (0.074)	a 0.197 (0.035)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) a day after MG treatment

Table 2. Specific growth rate (SGR) of control and 0.5, 1.0, or 2.0 mM methylglyoxal (MG) treated cultures (2.4×10^4 cells · ml^{-1} inoculation)

Culture \ Day	0 - 1	1 - 2	2 - 3	3 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	b 1) 0.367 (0.044)	c 2) 0.623 (0.049)	0.657 (0.076)	a 0.696 (0.010)	b 0.414 (0.016)	b 0.051 (0.019)	0.021 (0.030)	ab -0.072 (0.033)
0.5 mM	a 0.510 (0.050) 3)	a 0.830 (0.029)	0.796 (0.096)	b 0.563 (0.011)	b 0.322 (0.028)	b 0.060 (0.036)	0.004 (0.025)	b -0.125 (0.013)
1.0 mM	b 0.377 (0.049)	b 0.747 (0.060) 3)	0.619 (0.173)	d 0.260 (0.028)	a 0.680 (0.104)	b 0.103 (0.058)	0.026 (0.013)	a -0.012 (0.097)
2.0 mM	b 0.367 (0.033)	c 0.629 (0.021)	0.532 (0.024) 3)	c 0.377 (0.057)	b 0.334 (0.022)	a 0.223 (0.096)	0.116 (0.090)	a 0.017 (0.026)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) a day after MG treatment

· ml^{-1} was lower than controls until day 5 after treatment (Table 1). The cell number of cultures also were lower than those of controls throughout all stages of the culture growth (Fig. 1). Cell division of MG treated cultures with high inoculation ($4.8 \pm 0.2 \times 10^4$ cells · ml^{-1}) and treated with 1.0 mM MG on day 1 when algal concentration was $0.800 \pm 0.020 \times 10^5$ cells · ml^{-1} was inhibited until day 7 (Fig. 2). Also, SGR was lower than those of control until day 5 after treatment (Table 2). After day 5, SGRs of 1.0 mM MG treated cultures with inoculum concentration $4.8 \pm 0.2 \times 10^4$ cells · ml^{-1} recovered and there was no significant difference between controls and MG treated cultures. After day 7, the cell numbers were not significantly different from those of controls (Fig. 2).

When 0.5 mM MG was added to cultures on day 0 when the cell concentration was $4.8 \pm 0.2 \times 10^4$ cells · ml^{-1}, the logarithmic phase began sooner than for controls. The SGR and cell number of treated cultures were significantly higher than those of controls until day 5. After day 5, there was no significant difference between controls and treated cultures (Fig. 2 and Table 2). The cell number and SGR of cultures treated with 0.5 mM MG on day 0 when the cell number of the cultures was $2.4 \pm 0.1 \times 10^4$ cells · ml^{-1} were significantly lower than those of controls for one day after treatment. After day 2 the cell number and SGR

increased to the level of controls (Fig. 1 and Table 1). Furthermore, the cell number of treated cultures was significantly higher than those of controls (up to 1.26-fold of controls) during middle of logarithmic phase (day 5-7) (Fig. 1).

The cell number of cultures inoculated with low cell number ($0.50 \pm 0.01 \times 10^6$ cells $\cdot$ ml^{-1}) and treated with 0.75 mM MG on day 3 when the cell concentration was $5.34 \pm 1.16 \times 10^4$ cells $\cdot$ ml^{-1} remained lower than those of controls throughout the experiment (Fig. 3). On day 7, the cell number of cultures treated with 0.5 mM MG was $0.527 \pm 0.012 \times 10^6$ cells $\cdot$ ml^{-1} which was significantly higher than controls ($0.387 \pm 0.031 \times 10^6$ cells $\cdot$ ml^{-1}) whereas the cell number of cultures treated with 0.25 mM MG ($0.427 \pm 0.012 \times 10^6$ cells $\cdot$ ml^{-1}) was not significantly different from controls. The SGR of controls and MG-treated cultures was not significantly different from each other until the stationary phase (day 11) (Table 3).

When algal cultures were inoculated with $0.80 \pm 0.06 \times 10^4$ cells $\cdot$ ml^{-1}, and treated with 0.5 mM MG on day 4 when cell concentration was $0.934 \pm 0.116 \times 10^5$ cells $\cdot$ ml^{-1}, the resulting cell number was significantly higher than that of controls (up to 1.81-fold of controls) after MG treatment until the stationary phase (day 11) (Fig. 4). Also, the SGR was increased (1.62-fold of controls) after treatment (Table

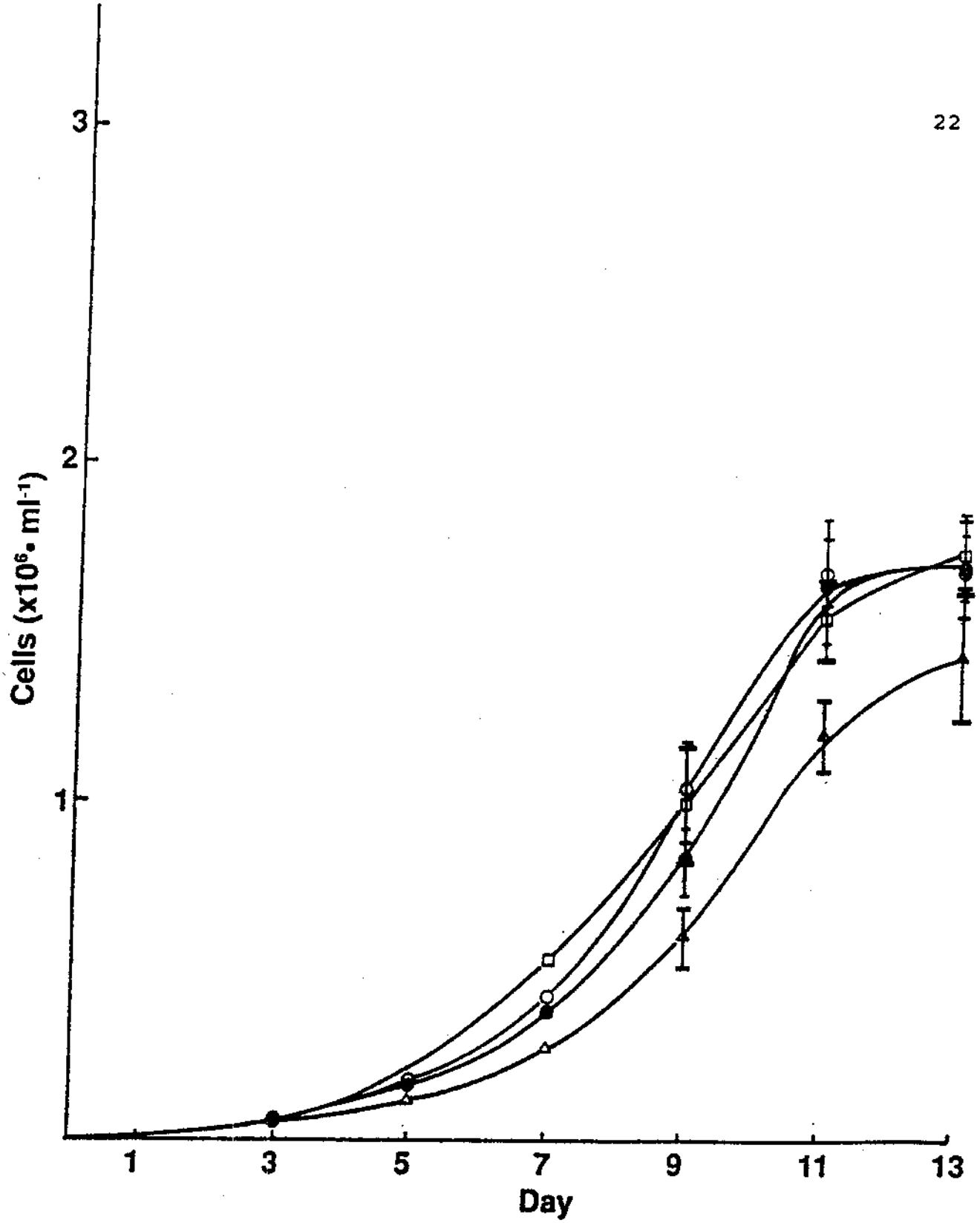

Fig. 3. Growth curve of methylglyoxal-treated (0.25, 0.5, or 0.75 mM MG) on day 3 and untreated Scenedesmus quadricauda with initial inoculation of 0.5×10^4 cells $\cdot$ ml^{-1} (● - ● , Controls; ○ - ○ , 0.25 mM; □ - □ , 0.5mM; △ - △ , 0.75mM; symbol->mean, bar->standard deviation, n=3)

Table 3. Specific growth rate (SGR) of control and 0.25, 0.5, or 0.75 mM methylglyoxal (MG) treated cultures (0.5×10^4 cells · ml^{-1} inoculation) on day 3

Culture \ Day	2 - 3	3 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	0.681 (0.078)[1]	0.573 (0.137)	0.425 (0.051)	0.378 (0.039)	0.344 (0.010)[a 2]	0.017 (0.078)
0.25mM	0.680 (0.041)	0.536 (0.079)[3]	0.513 (0.052)	0.443 (0.052)	0.240 (0.033)[b]	0.002 (0.060)
0.5 mM	0.680 (0.021)	0.595 (0.102)[3]	0.557 (0.047)	0.308 (0.071)	0.226 (0.052)[b]	0.058 (0.034)
0.75mM	0.682 (0.014)	0.364 (0.192)[3]	0.457 (0.182)	0.399 (0.060)	0.347 (0.038)[a]	0.086 (0.043)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) a day after MG treatment

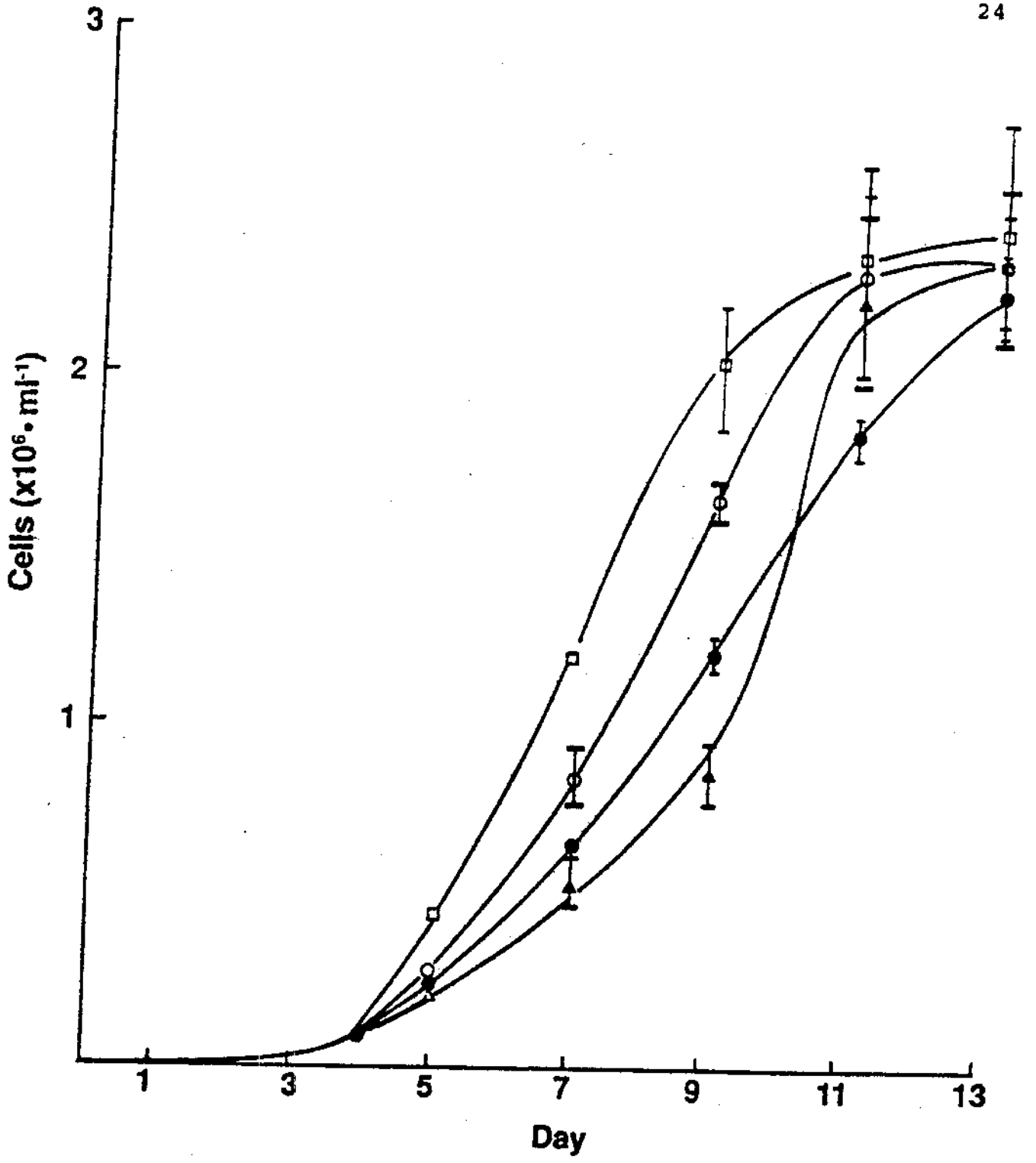

Fig. 4. Growth curve of methylglyoxal-treated (0.5, 0.75, or 1.0 mM MG) on day 4 and untreated Scenedesmus quadricauda with initial inoculation of 0.8 x 10^4 cells · ml^{-1} (● - ● , Controls; □ - □ , 0.5 mM; ○ - ○ , 0.75 mM; △ - △ , 1.0 mM; symbol->mean, bar->standard deviation, n=3)

4). The SGR and cell number of cultures treated with 0.75 mM MG were not significantly different from those of controls except day 9, while the cell number of cultures treated with 1.0 mM MG was lower than controls until day 9. However, the SGRs of MG-treated cultures were significantly higher than those of controls on day 11.

When algal cultures were inoculated with $2.40 \pm 0.14 \times 10^4$ cells $\cdot$ ml^{-1}, and treated with 0.5 mM MG on day 4 when the cell concentration was $2.667 \pm 0.114 \times 10^5$ cells $\cdot$ ml^{-1}, the SGR and cell number of treatment cultures were significantly higher than those of controls (up to 1.51-fold of controls) (Fig. 5 and Table 5) until end of the logarithmic phase (day 9). Both SGR and cell number of cultures treated with 1.0 mM MG were not significantly different from those of controls.

The cell number of cultures inoculated with $0.80 \pm 0.04 \times 10^4$ cells $\cdot$ ml^{-1}, and treated with 0.5 mM MG on day 2 when the cell concentration was $2.62 \pm 0.15 \times 10^4$ cells $\cdot$ ml^{-1}, was not significantly different from those of controls until day 5 which increased (up to 1.41-fold of controls) at the end of logarithmic phase (day 7-9) (Fig. 6). The cell number of cultures treated with 1.0 mM MG was significantly lower than controls after treatment. Also, the SGR was lower than controls one day after treatment, but significantly higher than that of controls after 2 days of

Table 4. Specific growth rate (SGR) of control and 0.5, 0.75, or 1.0 mM methylglyoxal (MG) treated cultures (0.8 X 10^4 cells · ml^{-1} inoculation) on day 4

Day / Culture	3 - 4	4 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	0.685 (0.032) [1]	0.967 (0.058) [b 2]	0.489 (0.025)	0.307 (0.051)	0.212 (0.002) [b]	0.100 (0.011)
0.5 mM	0.684 (0.030)	1.568 (0.042) [a] [3]	0.483 (0.039)	0.273 (0.038)	0.078 (0.015) [c]	0.013 (0.007)
0.75mM	0.684 (0.040)	1.044 (0.212) [b] [3]	0.581 (0.133)	0.330 (0.039)	0.171 (0.054) [b]	0.001 (0.038)
1.0 mM	0.685 (0.023)	0.845 (0.255) [b] [3]	0.494 (0.129)	0.239 (0.039)	0.478 (0.014) [a]	0.026 (0.086)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) a day after MG treatment

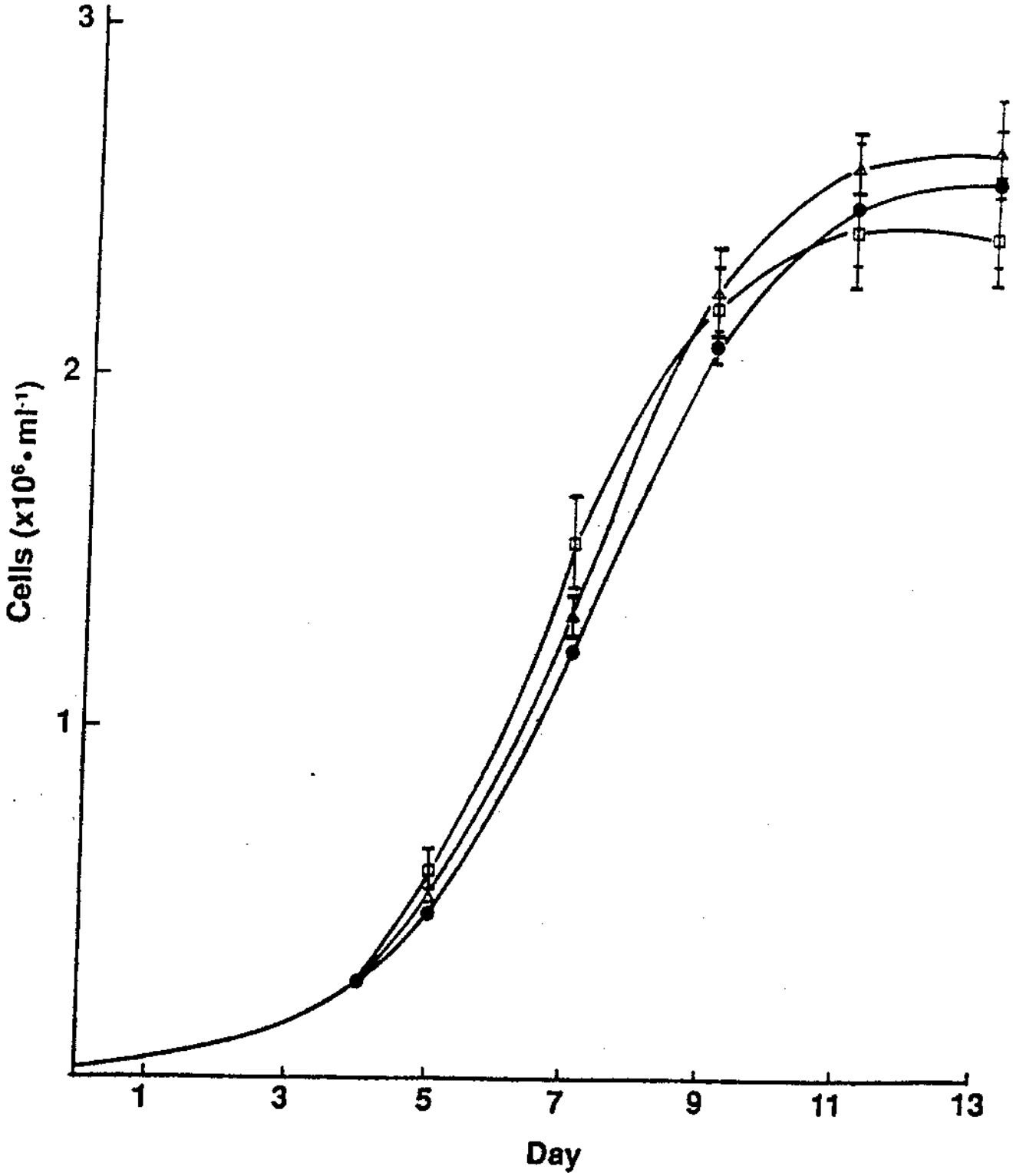

Fig. 5. Growth curve of methylglyoxal-treated (0.5 or 1.0 mM MG) on day 4 and untreated Scenedesmus quadricauda with initial inoculation of 2.4×10^4 cells · ml^{-1} (● - ● , Controls; □ - □ , 0.5 mM; Δ - Δ , 1.0 mM; symbol->mean, bar->standard deviation, n=3)

Table 5. Specific growth rate (SGR) of control and 0.5, or 1.0 mM methylglyoxal (MG) treated cultures (2.4×10^4 cells · ml^{-1} inoculation) on day 4

Culture \ Day	3 - 4	4 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	0.608 (0.021)[1]	0.536 (0.072)[a 2]	0.478 (0.036)	0.273 (0.005)[a]	0.087 (0.009)[a]	0.008 (0.050)
0.5 mM	0.602 (0.011)	0.809 (0.071)[a 3]	0.464 (0.032)	0.188 (0.025)[b]	0.046 (0.011)[b]	0.001 (0.009)
1.0 mM	0.614 (0.018)	0.645 (0.018)[b 3]	0.451 (0.006)	0.267 (0.021)[a]	0.074 (0.017)[a]	0.013 (0.035)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) a day after MG treatment

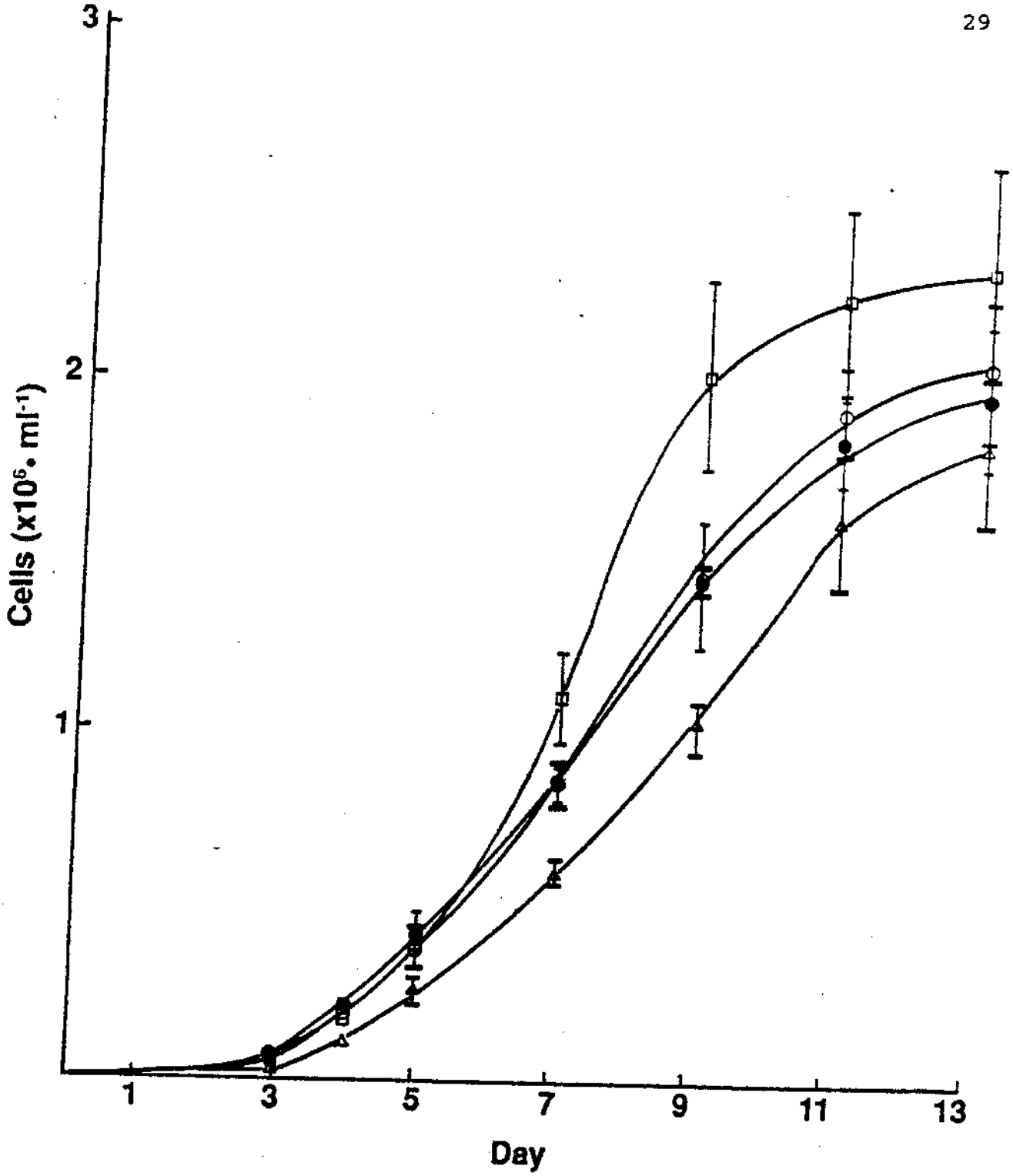

Fig. 6. Growth curve of methylglyoxal-treated (0.25, 0.5, or 1.0 mM MG) on day 2 and untreated Scenedesmus quadricauda with initial inoculation of 0.8 x 10^4 cells · ml^{-1} (● - ● , Controls; ○ - ○ , 0.25 mM; □ - □ , 0.5 mM; △ - △ , 1.0 mM; symbol->mean, bar->standard deviation, n=3)

MG treatment (Table 6).

There was no difference in cell number or SGR between controls and cultures treated with 0.25 mM MG.

The cell number of cultures inoculated with 1.20 ± 0.08 x 10^4 cells · ml^{-1}, and treated with MG on day 2 when the cell concentration was 3.9 ± 0.15 x 10^4 cells · ml^{-1}, was significantly higher than that of controls throughout all stage of growth cycle of algae. Cell number of MG treated cultures was increased (up to 2.48, 2.27, or 1.94-fold of controls, respectively) after MG treatment with 0.25, 0.5, or 1.0 mM (Fig. 7). Also, SGR was increased in the cultures treated with 0.25 mM (up to 2.02-fold of controls), and in the cultures treated with 0.5 or 1.0 mM (1.89-fold of controls), one day after MG treatment (Table 7).

Cultures inoculated with 1.80 ± 0.13 x 10^4 cells · ml^{-1} and treated with MG on day 2 produced significantly higher cell numbers than those of controls until day 5 (Fig. 8). Cell number of MG treated cultures was increased (up to 1.95-fold of controls) 2 days after MG treatment with 1.0 mM. The SGRs of treated cultures were also significantly higher than those of controls (up to 1.92-fold of controls) one day after MG treatment with 1.0 mM (Table 8). After day 3, SGRs of MG treated cultures were not significantly different from those of controls.

When the algal cultures were treated 0.5 mM MG which is

Table 6. Specific growth rate (SGR) of control and 0.25, 0.5, or 1.0 mM methylglyoxal (MG) treated cultures (0.6 X 10^4 cells · ml^{-1} inoculation) on day 2

Day / Culture	1 - 2	2 - 3	3 - 4	4 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	1) 0.638 (0.031)	a 2) 0.989 (0.155)	b 1.109 (0.032)	0.716 (0.076)	0.373 (0.044)	0.250 (0.032)	b 0.130 (0.027)	0.029 (0.018)
0.25mM	0.638 (0.030)	a 3) 1.071 (0.071)	b 0.989 (0.055)	0.651 (0.120)	0.425 (0.049)	0.262 (0.022)	b 0.136 (0.021)	0.029 (0.024)
0.5 mM	0.638 (0.030)	a 3) 0.756 (0.234)	b 1.134 (0.191)	0.882 (0.134)	0.509 (0.052)	0.306 (0.025)	c 0.051 (0.036)	0.019 (0.080)
1.0 mM	0.683 (0.030)	b 3) 0.103 (0.085)	a 1.395 (0.047)	0.852 (0.028)	0.427 (0.065)	0.259 (0.010)	a 0.221 (0.041)	0.067 (0.009)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) a day after MG treatment

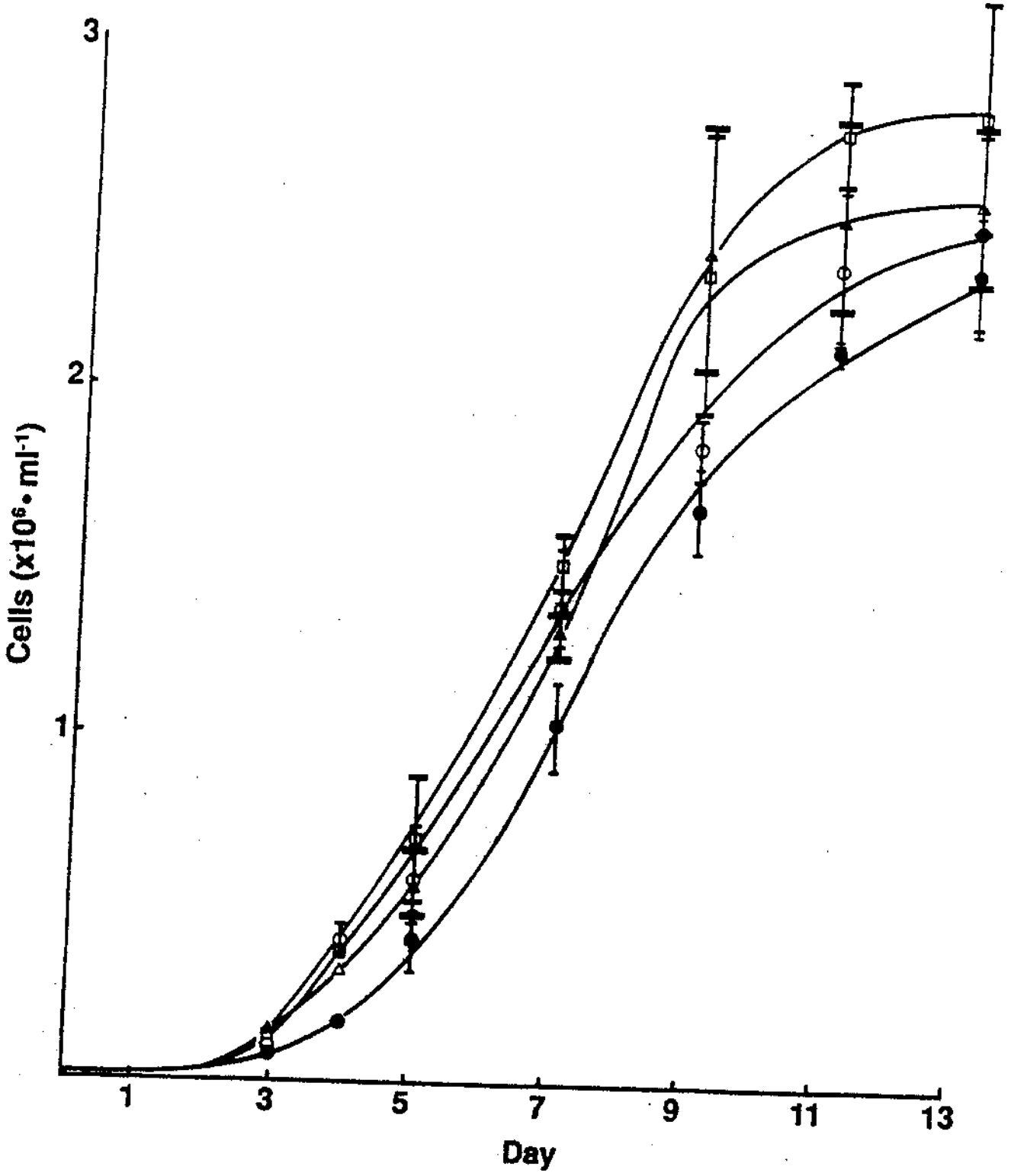

Fig. 7. Growth curve of methylglyoxal-treated (0.25, 0.5, or 1.0 mM MG) on day 2 and untreated Scenedesmus quadricauda with initial inoculation of 1.2 x 10^4 cells • ml^{-1} (● - ● , Controls; ○ - ○ , 0.25 mM; □ - □ , 0.5 mM; △ - △ , 1.0 mM; symbol->mean, bar->standard deviation, n=3)

Table 7. Specific growth rate (SGR) of control and 0.25, 0.5, or 1.0 mM methylglyoxal (MG) treated cultures (1.2×10^4 cells · ml^{-1} inoculation) on day 2

Day / Culture	1 - 2	2 - 3	3 - 4	4 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	1) 0.629 (0.031)	c 2) 0.713 (0.157)	c 0.730 (0.109)	a 0.895 (0.197)	0.467 (0.078)	ab 0.238 (0.032)	0.123 (0.027)	0.051 (0.033)
0.25mM	0.628 (0.030)	b 0.873 (0.012) 3)	a 1.478 (0.099)	b 0.347 (0.168)	0.427 (0.082)	b 0.148 (0.050)	0.126 (0.042)	0.024 (0.017)
0.5 mM	0.628 (0.030)	a 1.345 (0.025) 3)	b 1.116 (0.163)	ab 0.608 (0.119)	0.395 (0.123)	ab 0.217 (0.067)	0.085 (0.082)	0.010 (0.038)
1.0 mM	0.629 (0.030)	a 1.345 (0.039) 3)	b 0.973 (0.020)	ab 0.587 (0.175)	0.408 (0.082)	a 0.300 (0.054)	0.021 (0.032)	0.005 (0.047)

1) Mean (standard deviation)

2) Same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) a day after MG treatment

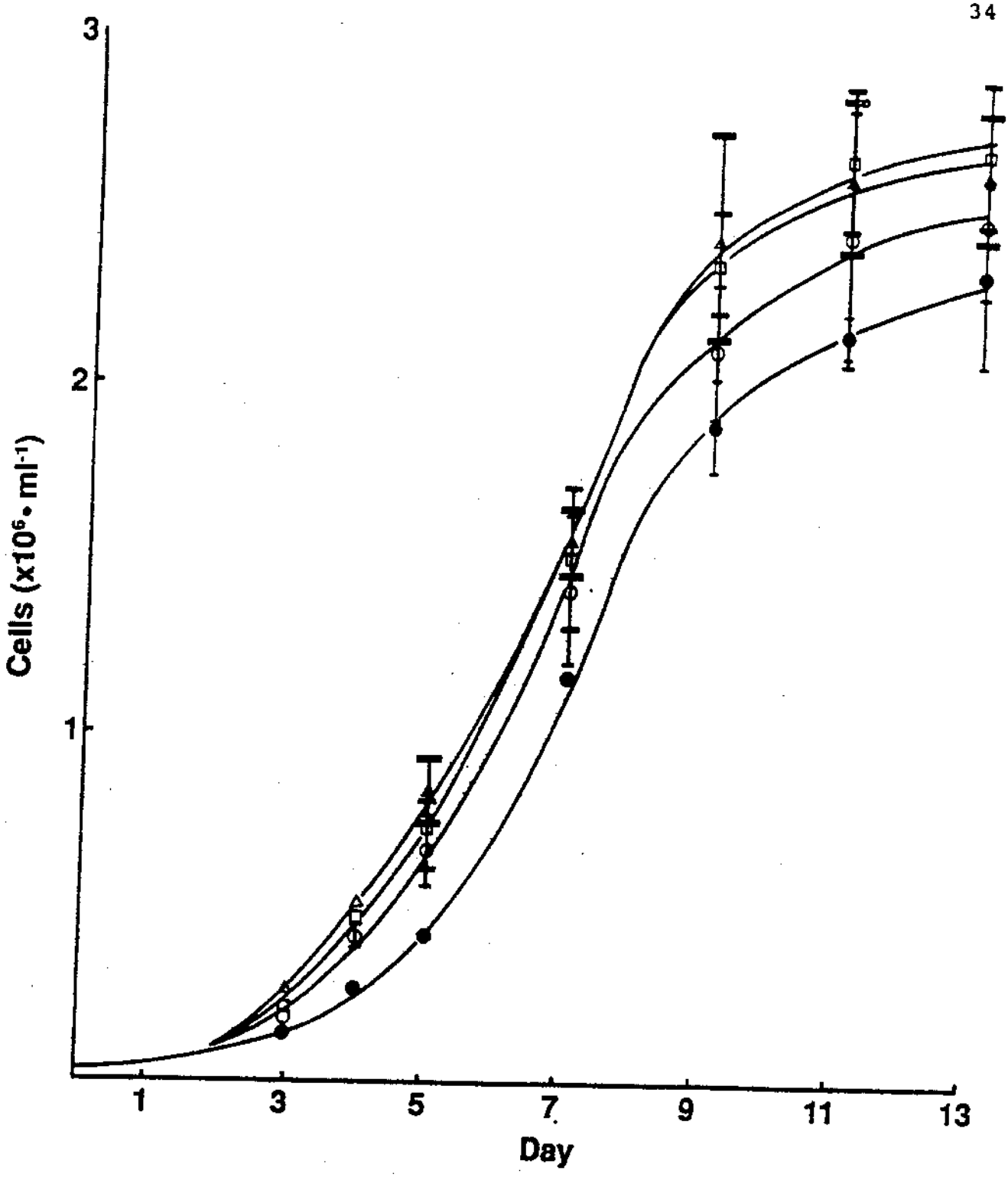

Fig. 8. Growth curve of methylglyoxal-treated (0.25, 0.5, or 1.0 mM MG) on day 2 and untreated Scenedesmus quadricauda with initial inoculation of 1.8×10^4 cells $\cdot$ ml^{-1} (● - ● , Controls; ○ - ○ , 0.25 mM; □ - □ , 0.5 mM; △ - △ , 1.0 mM; symbol->mean, bar->standard deviation, n=3)

Table 8. Specific growth rate (SGR) of control and 0.25, 0.5, or 1.0 mM methylglyoxal (MG) treated cultures (1.8×10^4 cells · ml $^{-1}$ inoculation) on day 2

Day								
Culture	1 - 2	2 - 3	3 - 4	4 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	1) 0.650 (0.031)	c 2) 0.699 (0.061)	0.692 (0.097)	0.485 (0.166)	a 0.508 (0.059)	0.240 (0.047)	0.068 (0.030)	0.040 (0.063)
0.25mM	0.650 (0.030)	b 1.043 (0.086) 3)	0.833 (0.083)	0.454 (0.118)	b 0.374 (0.023)	0.200 (0.038)	0.072 (0.026)	0.012 (0.035)
0.5 mM	0.650 (0.030)	ab 1.212 (0.147) 3)	0.816 (0.049)	0.376 (0.117)	b 0.371 (0.011)	0.226 (0.042)	0.058 (0.014)	0.005 (0.069)
1.0 mM	0.650 (0.030)	a 1.344 (0.017) 3)	0.700 (0.089)	0.480 (0.042)	b 0.321 (0.036)	0.221 (0.050)	0.040 (0.054)	-0.004 (0.014

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) a day after MG treatment

the most effective concentration for algal cell division, on day 2, 3, 4, or 5, cell numbers and SGRs of treated cultures were significantly higher than those of controls (Fig. 9 and Table 9). Cell number of treated cultures was increased (up to 1.91-fold of controls) one day after MG treatment on day 4. The SGRs of treated cultures were increased (up to 1.66-fold of control) one day after MG treatment on day 5 (Table 5).

Biodegradation of Methylglyoxal

Generally, MG in algal cultures was not detected after day 6 at 0.25 or 0.5 mM treatment and day 8 at 0.75 mM treatment concentration (Fig. 10). At 1.0 mM MG treatment, MG degradation rate was different, depending on the cell number of the cultures. When the most effective MG concentration (0.5 mM) for cell division stimulation was added on day 7, the degradation rate of MG was faster than that of day 3 or day 5 treatment (Fig. 11).

Enzyme Activity

The specific activity of glyoxalase I (G I) of 0.5 mM MG treated cultures was significantly higher than that of controls while specific activity of G I of 1.0 mM treated cultures was significantly lower than that of controls after MG treatment (Fig. 12). As the growth of 1.0 mM MG treated

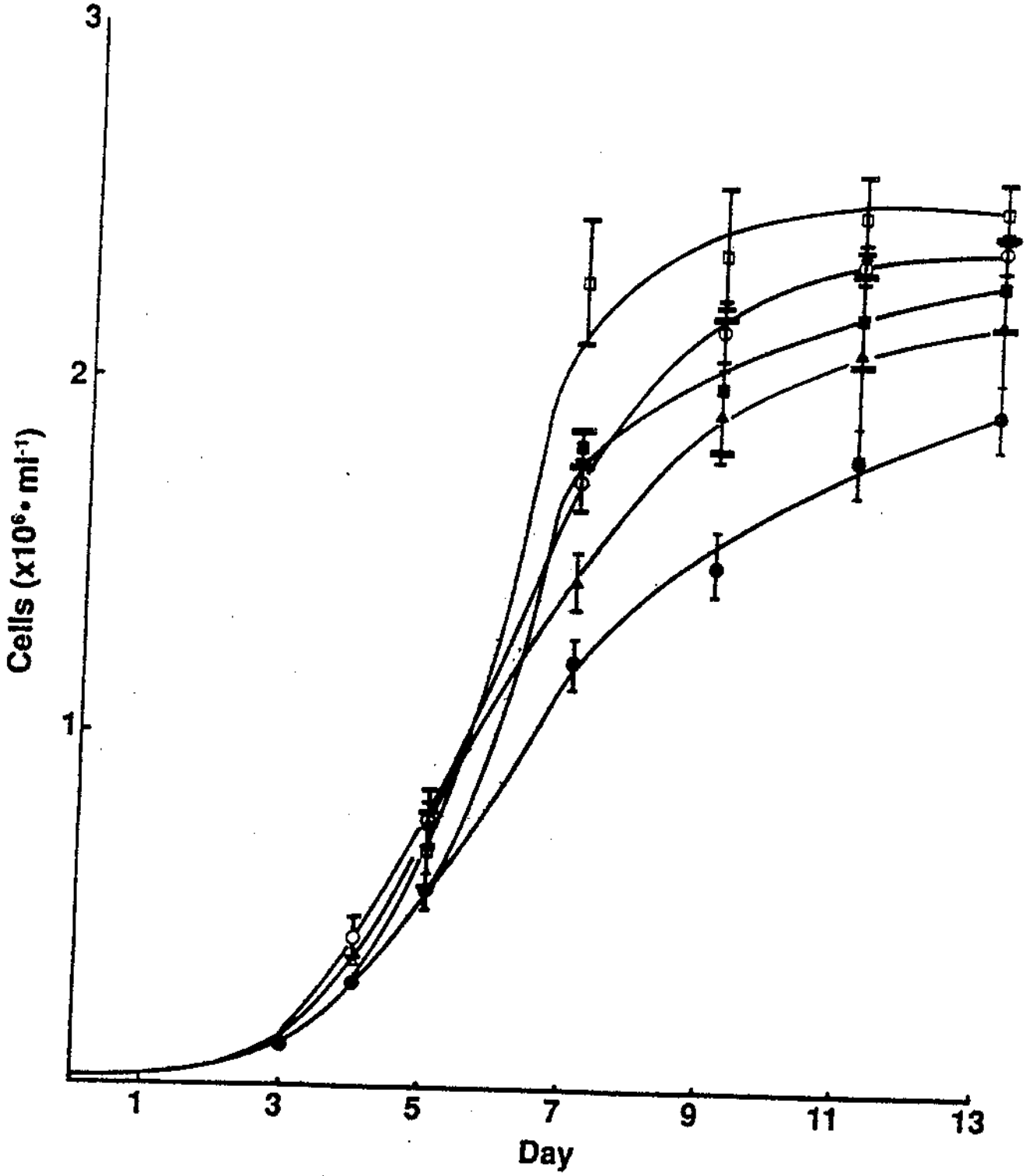

Fig. 9. Growth curve of controls and methylglyoxal-treated (0.5 mM MG on day 2, 3, 4, or 5) cultures of Scenedesmus quadricauda with initial inoculation of 0.8 x 104 cells · ml^{-1} (● - ● , Controls; △ - △ , day 2; ○ - ○ , day 3; □ - □ , day 4; ■ - ■ , day 5; symbol->mean, bar->standard deviation, n=3)

Table 9. Specific growth rate (SGR) of control and 0.5 mM methylglyoxal (MG) treated cultures (0.8×10^4 cells · ml $^{-1}$ inoculation) on day 2, 3, 4, or 5

Culture \ Day	1 - 2	2 - 3	3 - 4	4 - 5	5 - 7	7 - 9	9 - 11	11 - 13
Control	1) 0.600 (0.014)	b 2) 0.621 (0.023)	b 0.971 (0.126)	b 0.608 (0.092)	bc 0.379 (0.014)	a 0.112 (0.015)	0.086 (0.005)	0.040 (0.008)
0.5 mM Day 2	0.600 (0.014)	a 0.774 (0.070)	b 0.964 (0.030)	a 0.737 (0.041)	c 0.332 (0.026)	a 0.146 (0.023)	0.042 (0.046)	0.019 (0.017)
0.5 mM Day 3	0.600 (0.014)	b 0.615 (0.020)	a 1.289 (0.153)	b 0.599 (0.069)	b 0.412 (0.027)	a 0.106 (0.043)	0.045 (0.058)	0.010 (0.002)
0.5 mM Day 4	0.600 (0.014)	b 0.591 (0.048)	b 1.005 (0.061)	a 0.762 (0.069)	a 0.621 (0.039)	b 0.020 (0.013)	0.023 (0.016)	0.004 (0.018)
0.5 mM Day 5	0.600 (0.014)	b 0.651 (0.020)	b 1.004 (0.065)	b 0.504 (0.067)	a 0.629 (0.044)	b 0.038 (0.048)	0.054 (0.028)	0.021 (0.028)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

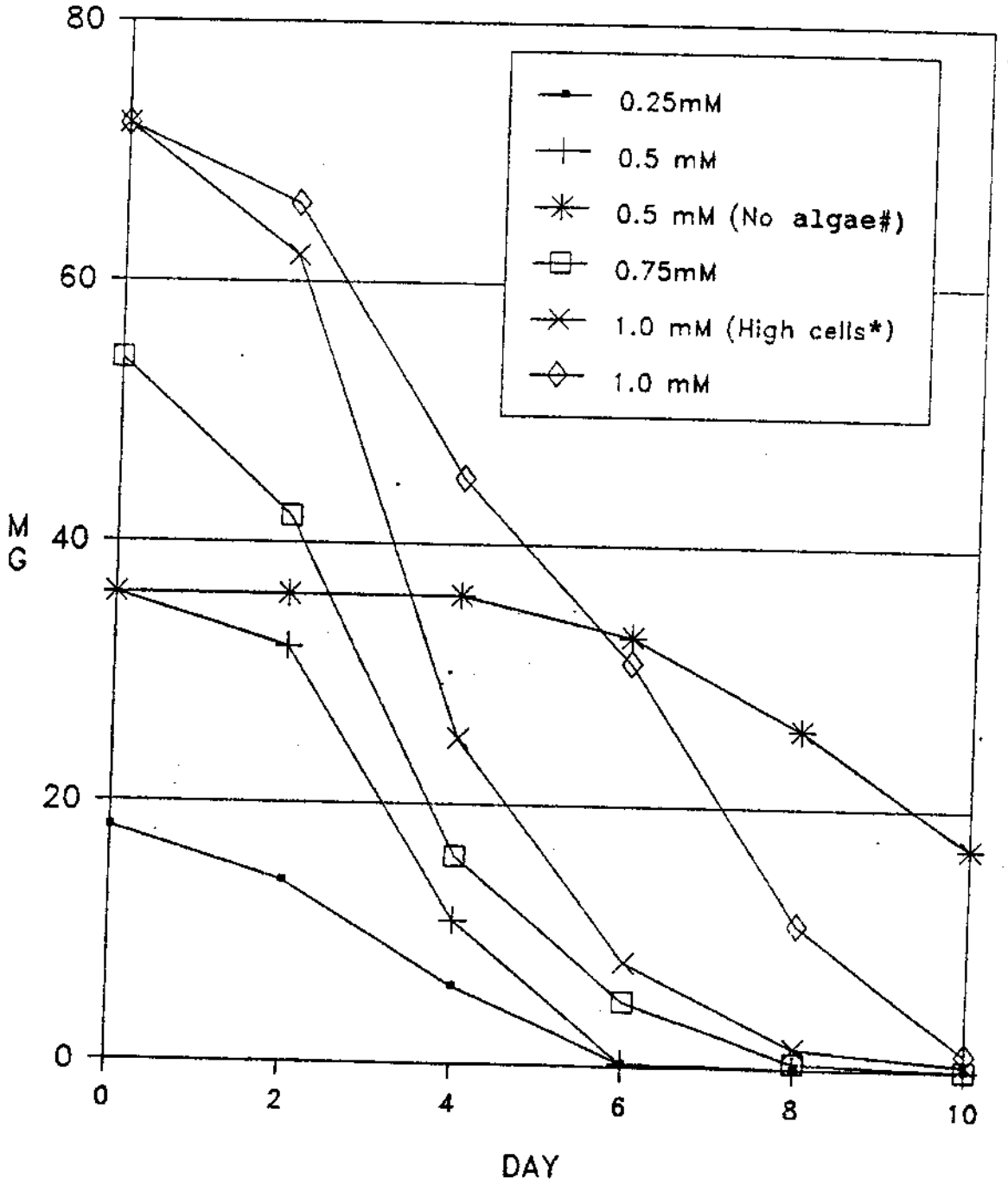

Fig. 10. Degradation of methylglyoxal (MG: $\mu g \cdot ml^{-1}$) in Scenedesmus quadricauda cultures (0.8×10^4 cells $\cdot ml^{-1}$ inoculation) with 0.25, 0.5, 0.75, 1.0, 1.0 (high inoculation*), or 0.5 mM (without algae#) MG treatment (mean and standard deviation -> appendix B1)

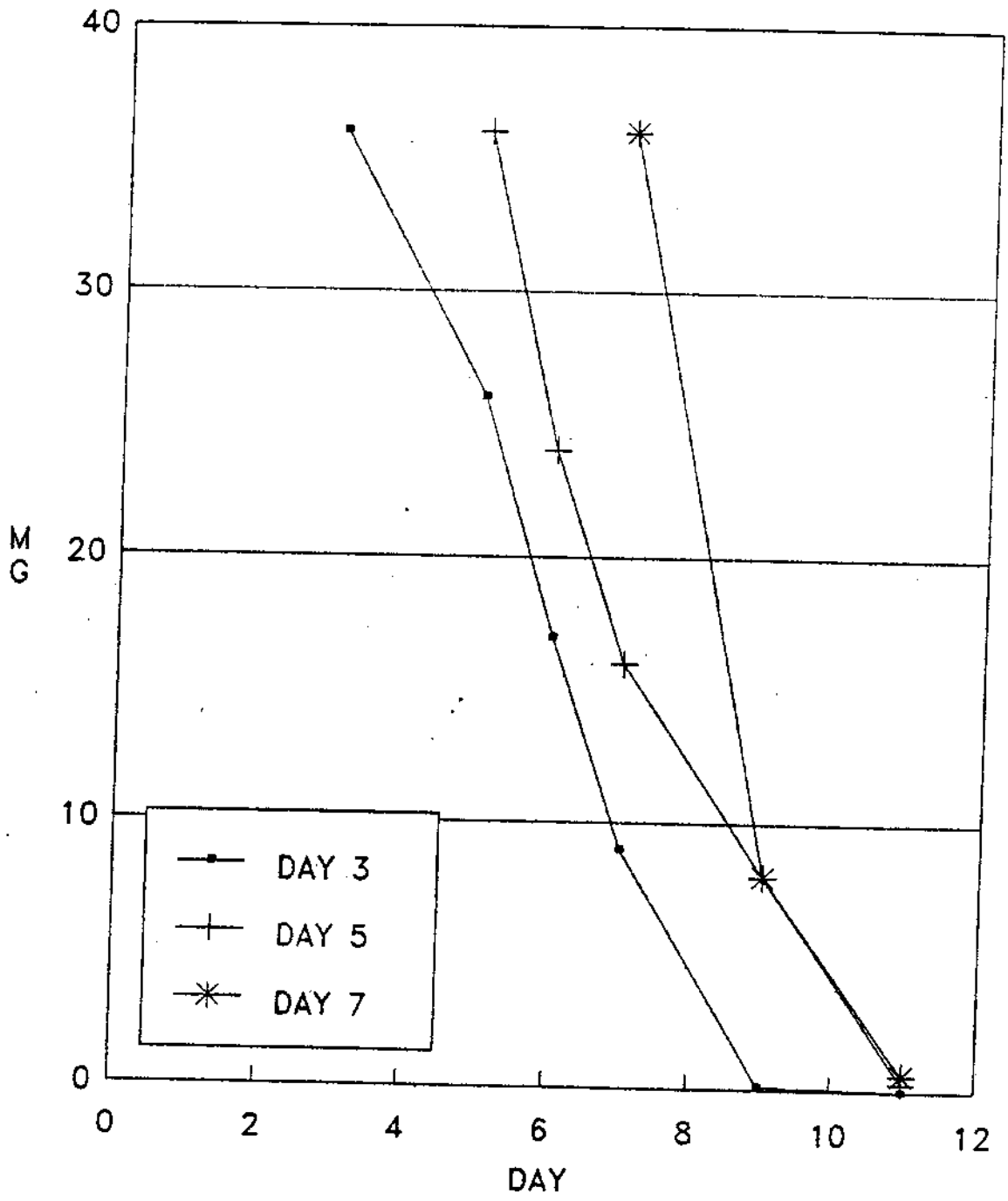

Fig. 11. Degradation of methylglyoxal (MG: $\mu g \cdot ml^{-1}$) in Scenedesmus quadricauda cultures (0.8×10^4 cells $\cdot ml^{-1}$ inoculation) treated with 0.5 mM MG on day 3, 5, or 7 (mean and standard deviation -> appendix B2)

cultures recovered, the specific activity of G I was increased after middle of logarithmic phase, while those of both control and 0.5 mM MG treated cultures were decreased on day 9. There was no significant difference in G I activity at steady state phase (day 13) between controls and MG treated cultures. The specific activity of glyoxalase II (G II) in 1.0 mM MG treated cultures was significantly lower than those of controls and 0.5 mM MG treated cultures after MG treatment (Fig. 13), while specific activity of G II of controls was significantly higher than those of MG treated cultures on day 9. There was no difference in G II activity at the end of the algal growth in the batch cultures, which was day 13.

The ratio of specific activity of G I to G II of 0.5 mM MG treated cultures was higher than those of controls and 1.0 mM MG treated cultures after MG treatment (day 5), while that of 1.0 mM MG treated culture was higher before steady state phase of algal growth (day 9) (Fig. 14). Once again, there was no difference in ratio of G I to G II activity between controls and MG treated cultures on day 13.

There was no measurable activity of α-ketoaldehyde dehydrogenase or MG reductase.

Metabolic Products

Pyruvate concentration in control cultures was none or

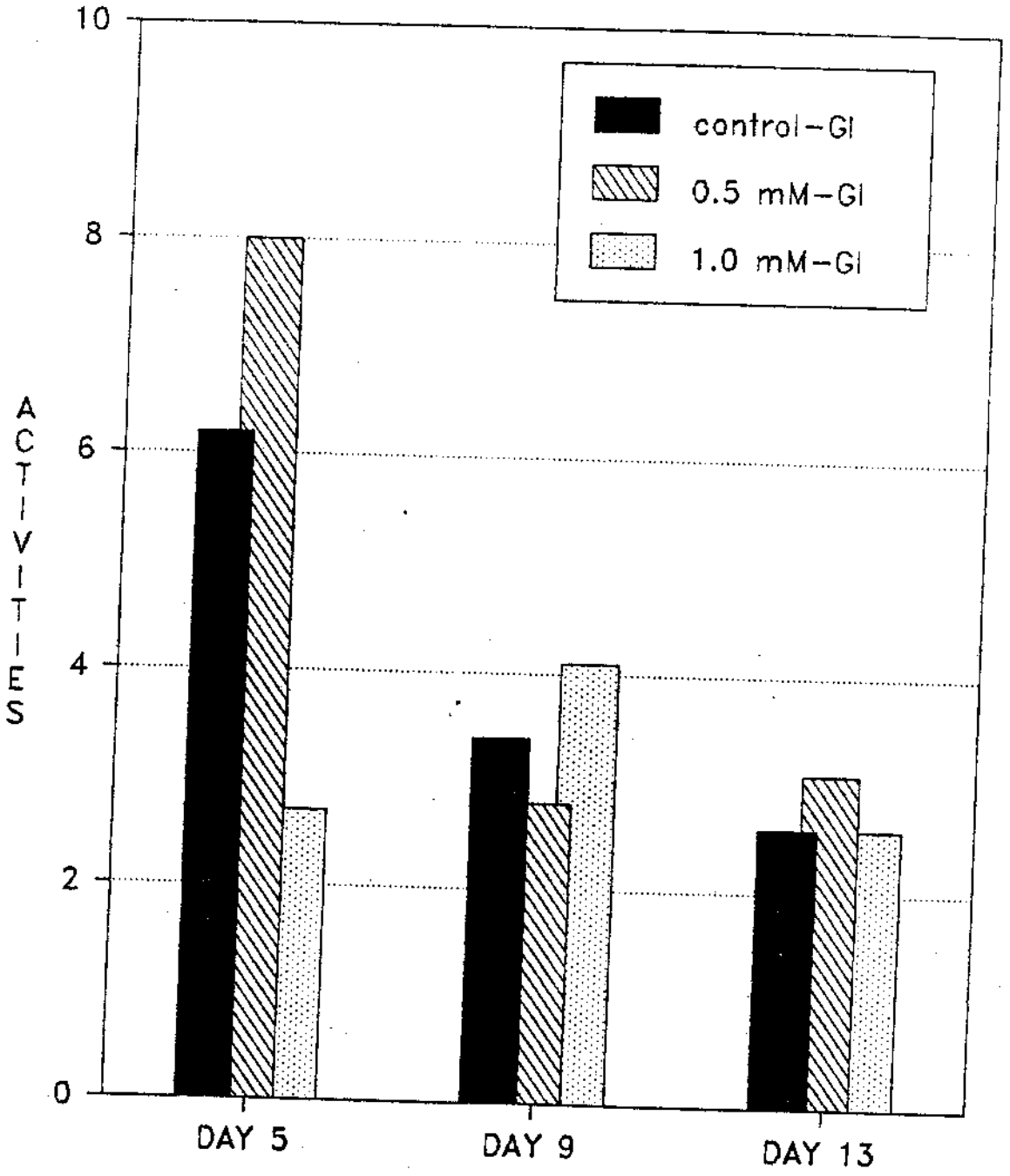

Fig. 12. Specific activity (μmole $\cdot$ min^{-1} $\cdot$ mg^{-1}) of glyoxalase I (GI) of controls and 0.5 or 1.0 mM methylglyoxal (MG) treated cultures (mean and standard deviation -> appendix C)

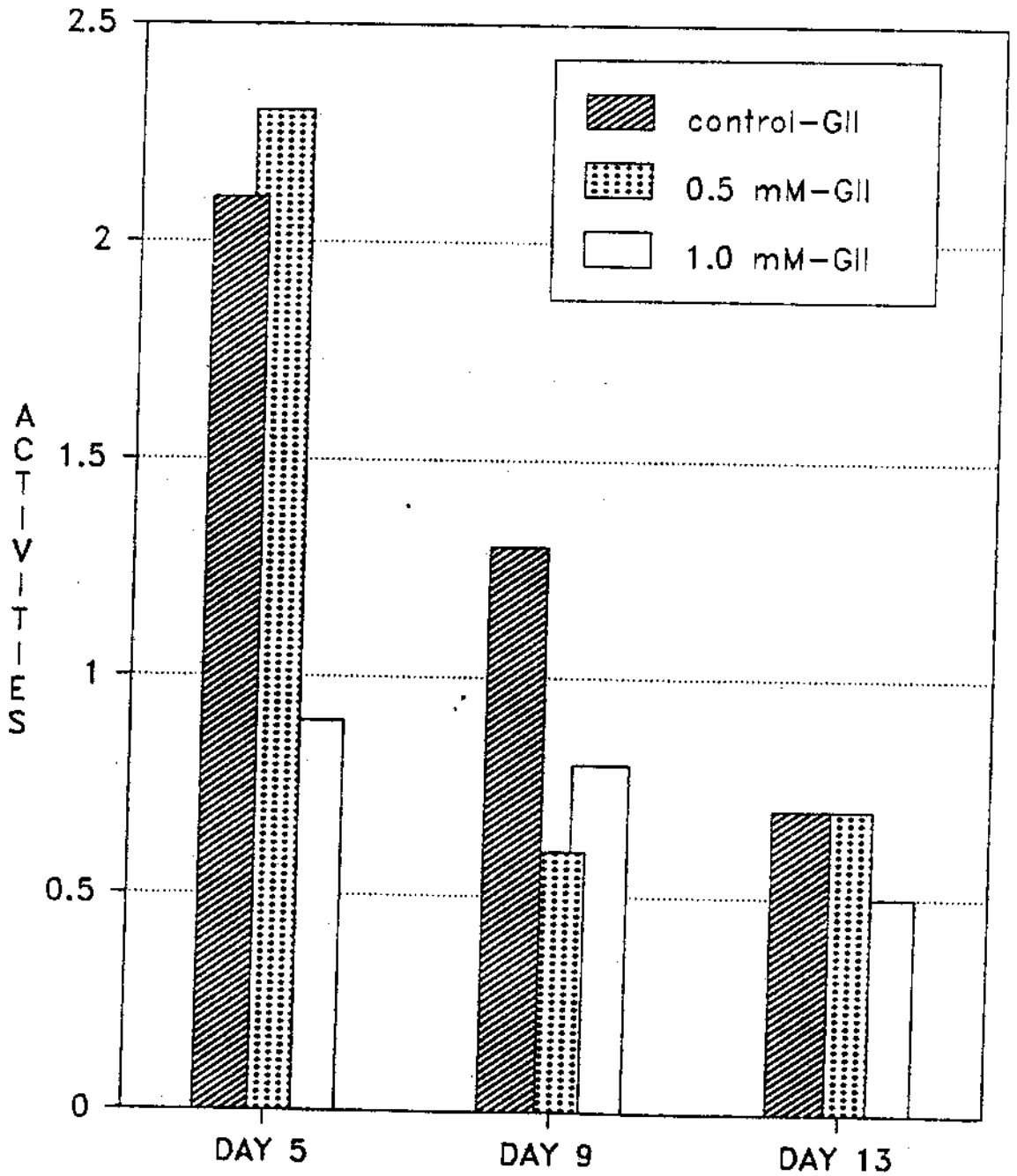

Fig. 13. Specific activity (μmole $\cdot$ min^{-1} $\cdot$ mg^{-1}) of glyoxalase II (GII) of controls and 0.5 or 1.0 mM methylglyoxal (MG) treated cultures (mean and standard deviation -> appendix C)

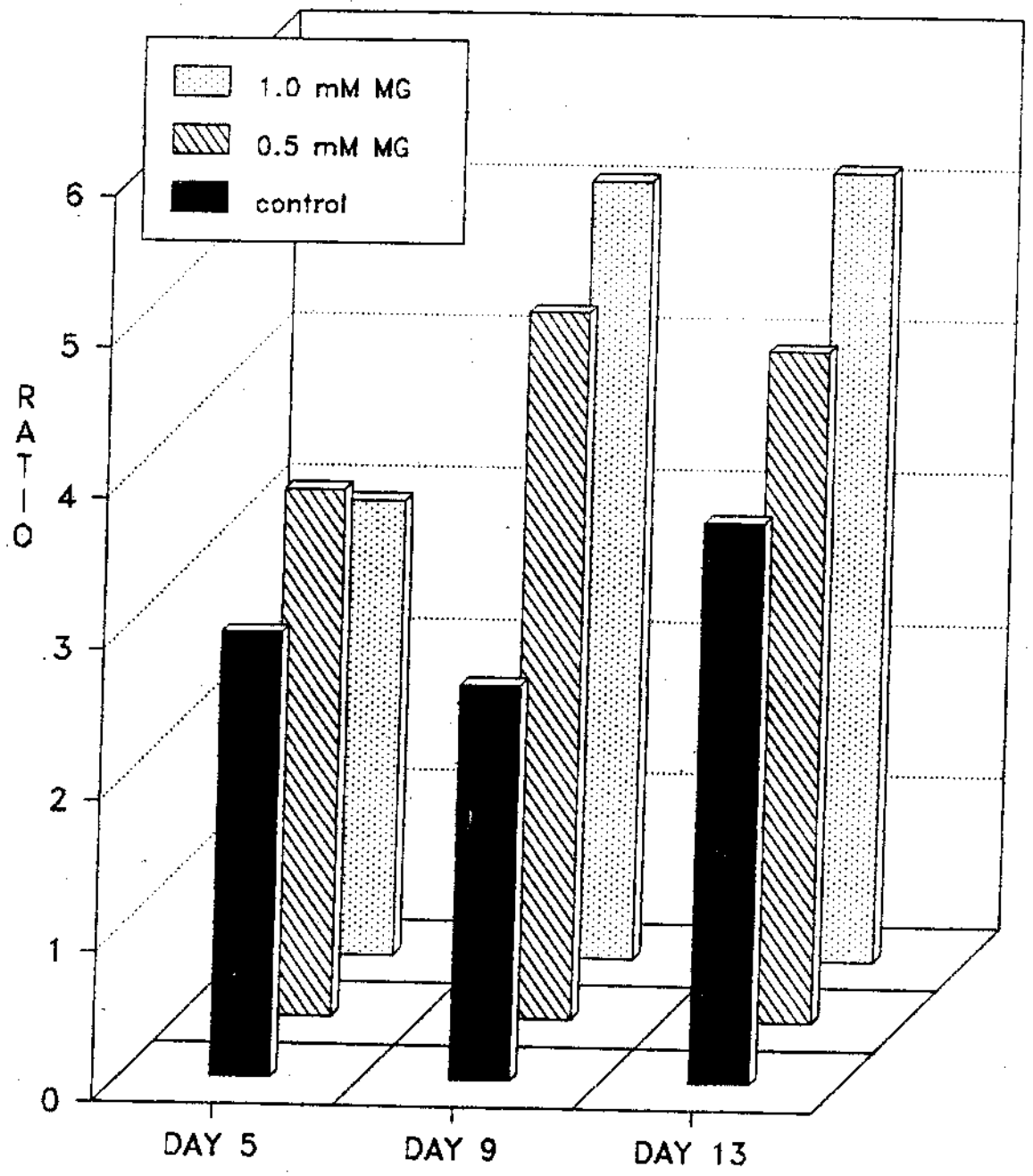

Fig. 14. Ratio of specific activity (glyoxalase I to II) of controls and MG treated cultures with 0.5 or 1.0 mM

very low throughout the growth cycle of algae, while concentration of pyruvate in MG treated cultures was increased gradually as the cell number increased after MG treatment (Fig. 15). The highest cell number was observed on day 10 in 0.5 mM MG treated (on day 4) culture, and pyruvate produced by the culture was 5 times higher than that of controls.

The glucose concentration of 0.5 mM MG treated cultures was higher than that of controls after MG treatment. However, the glucose level of MG treated cultures was decreased as the cell number increased at logarithmic phase (day 8) and maintained until end of experiment (Fig. 16).

There was no measurable amount of lactate in controls or MG treated cultures.

Photosynthetic and Respiratory Rate

Both photosynthetic and respiratory rate of 0.5 mM MG treated cultures were greater than those of controls (up to 1.60-fold of controls) which corresponded to the data for cell numbers (Fig. 17).

Cell Size

Microscopic observation revealed no difference in cell size between controls and 0.5 mM MG treated cultures (width x length; 0.010 x 0.003 μm, approx.), where cells of 1.0 mM

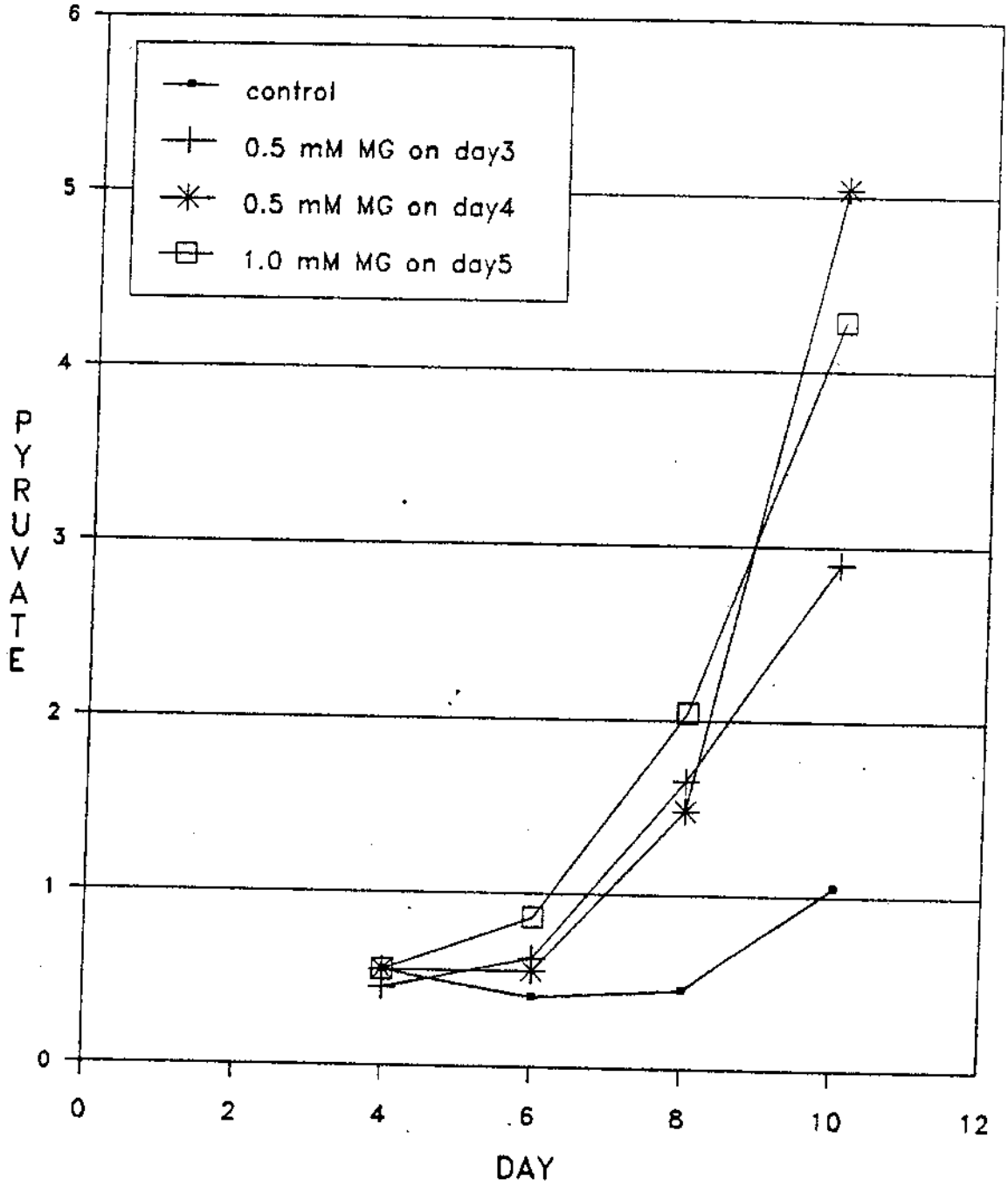

Fig. 15. Pyruvate ($\mu g \cdot ml^{-1}$) in the methylglyoxal (MG) treated Scenedesmus quadricauda cultures with 0.5 mM on day 3, 0.5 mM on day 4, or 1.0 mM on day 5 and controls (mean and standard deviation -> appendix D)

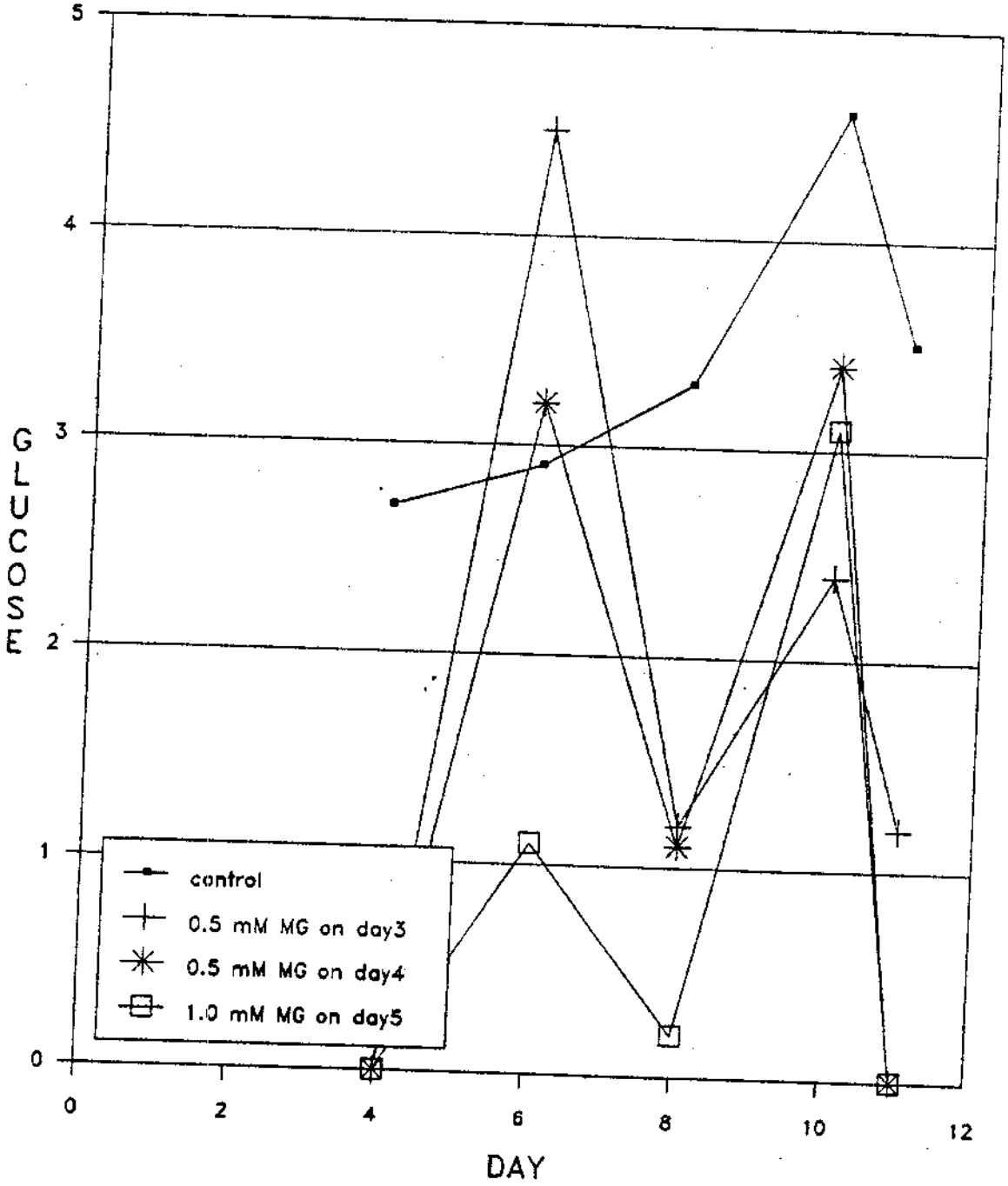

Fig. 16. Glucose ($\mu g \cdot ml^{-1}$) in the methylglyoxal (MG) treated Scenedesmus quadricauda cultures with 0.5 mM on day 3, 0.5 mM on day 4, or 1.0 mM on day 5 and controls (mean and standard deviation -> appendix E)

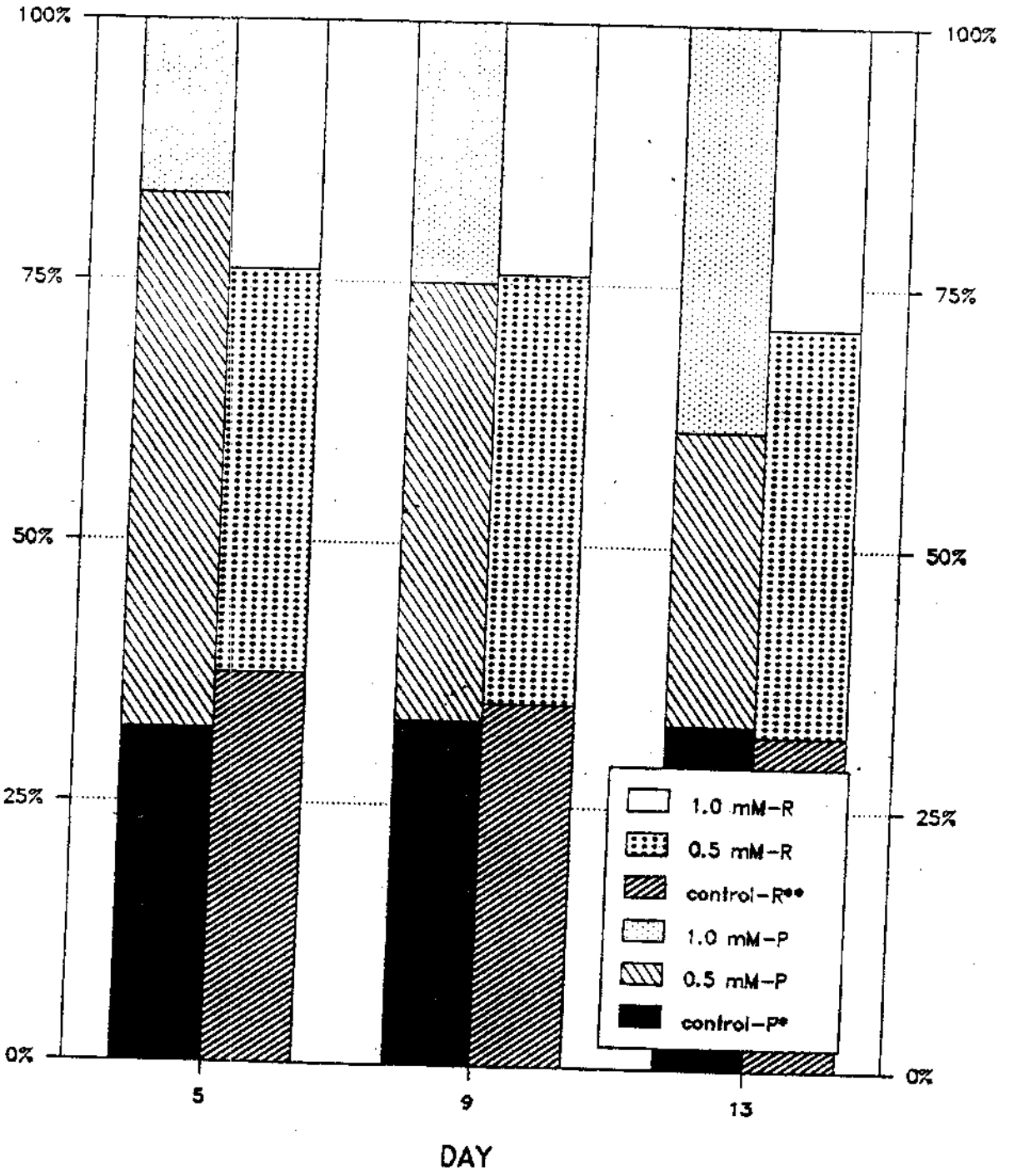

Fig. 17. Ratio of photosynthtic and respiratory rate of controls and methylglyoxal (MG) treated cultures with 0.5 or 1.0 mM (*photosynthetic rate, **respiratory rate with O_2)

MG treated cultures which were inhibitory, were smaller than those of controls (Fig. 18).

Threshold Concentration

Methylglyoxal (MG), added to the algal cultures which were different in inoculation concentration, amount of MG, and the time of MG addition was recalculated to compare the effectiveness of MG on cell division each other. To generalize the MG concentration and to decide the threshold concentration of MG whether stimulate or inhibit cell division of Scenedesmus quadricauda in the batch cultures, added MG was recalculated by the unit of mg MG per 10^6 cells.

A high concentration of MG (2.0 mM) inhibited cell division of the algae and reduced the specific growth rate (SGR) compared to those of controls or other MG treated cultures with lower than 2.0 mM (0.25, 0.5, 0.75, or 1.0 mM) concentration. The initial MG concentration in the treated cultures was 1.109 to 1.700 mg MG $\cdot$ 10^6 cells^{-1}, and the cell number was significantly lower than that of controls throughout all stages of the growth cycle of the alga.

Cell division of algae treated with 1.0 mM MG was inhibited or not inhibited, depending on the cell concentration at the time of MG treatment. When the inoculum concentration was 0.8 x 10^4 cells $\cdot$ ml^{-1} or, when

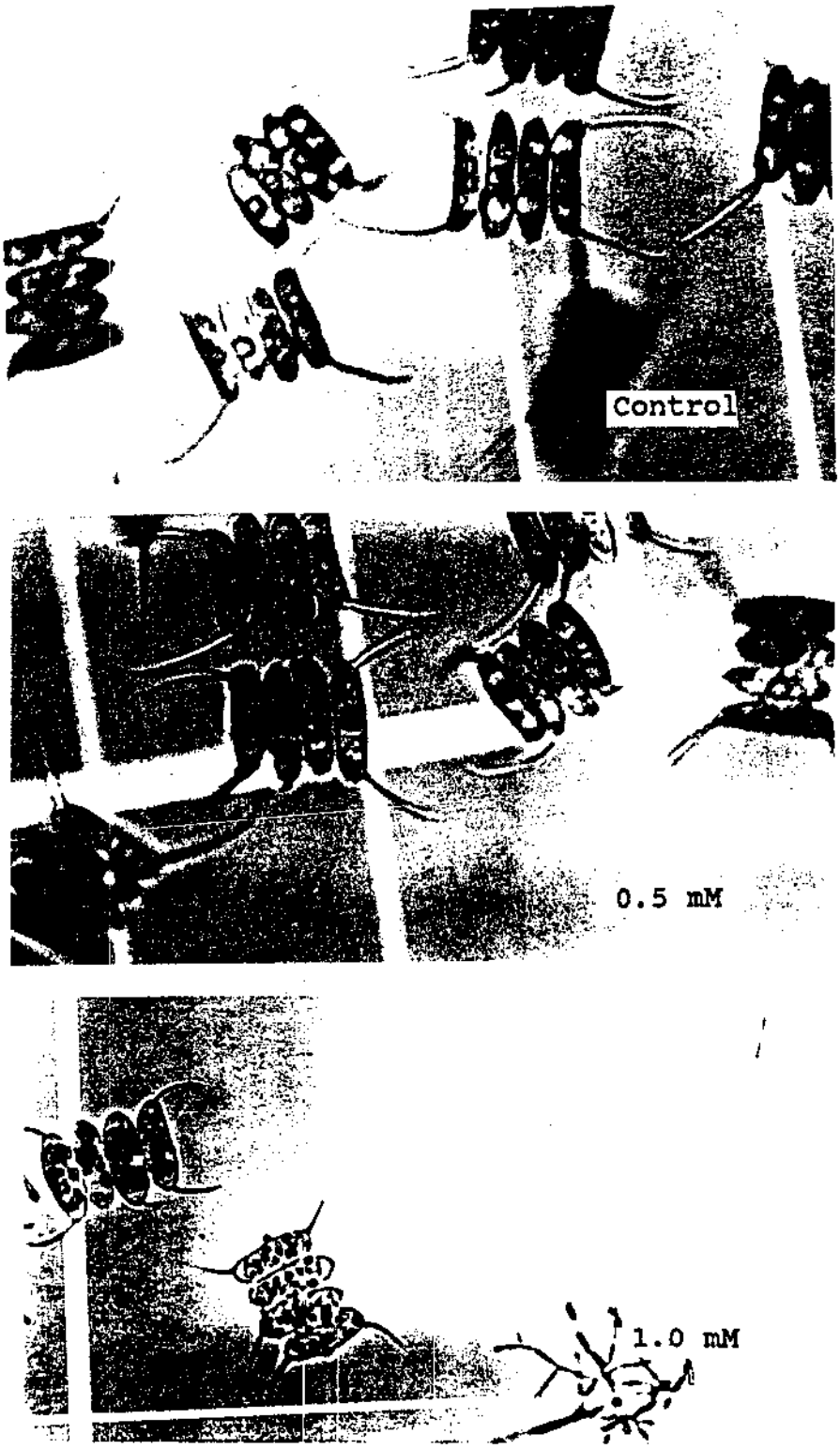

Fig. 18. Microscopic observation of Scenedesmus quadricauda cells from controls and methylglyoxal (MG) treated cultures with 0.5 mM or 1.0 mM (X 400)

MG was added within 24 hours from the inoculation, cell division was significantly inhibited. The initial MG concentration was 0.784 to 1.639 mg MG · 10^6 $cells^{-1}$. When the inoculum concentration was higher than 1.2 x 10^4 cells · ml^{-1} and MG was added 2 days after inoculation, cell division and SGR were significantly higher than those of controls (up to 1.95 and 1.91-fold of controls, respectively). The initial MG concentration was 1.127 to 1.849 mg MG · 10^6 $cells^{-1}$. When MG was added to the cultures at 2 days after inoculation, there was no difference in cell number and SGR between controls and MG treated cultures. The initial MG concentration was 0.267 mg MG · 10^6 $cells^{-1}$,

When 0.5 mM of MG was added to the algal cultures on day 0 with initial concentration of 1.502 mg MG · 10^6 $cells^{-1}$, cell division was inhibited for 2 days after treatment. If the cell number of an algal culture was low (0.5 x 10^4 to 0.8 x 10^4 cells · ml^{-1}) and MG was added before logarithmic phase (day 0-2), the cell number and SGR of 0.5 mM MG-treated cultures were lower than those of controls for one day after treatment. However, the SGR and cell number of treated cultures increased rapidly (3 days after MG treatment) and the cell number was increased up to 1.63-fold of controls and SGR was increased up to 1.33-fold of controls by the end of the logarithmic phase (day 7-11).

When 0.25 or 0.75 mM of MG was added to the cultures

after day 2, and the initial MG concentration was lower than 0.732 mg MG · 10^6 $cells^{-1}$, there was no differences in cell number and SGR between controls and MG treated cultures.

The stimulation of cell division of S. guadricauda was most significant when cultures were treated with 0.5 mM MG, especially when MG was added at the beginning of logarithmic phase. The algal cell division was enhanced significantly throughout all the experiments when cultures were treated with 0.5 mM MG. The initial MG concentration was 0.118 to 0.924 mg MG · 10^6 $cells^{-1}$. The highest cell number (up to 2.27-fold of controls) and SGR (up to 1.89-fold of controls) were observed when 0.5 mM MG was added to algal cultures with an initial MG concentration of 0.392 to 0.924 mg MG · 10^6 $cells^{-1}$. When the algal concentration was high (2.67 x 10^5 cells · ml^{-1}), MG was less effective in increasing cell division than when algal concentration was low (9.34 x 10^4 cells · ml^{-1}) at time of treatment.

CHAPTER IV

DISCUSSION

The effectiveness of methylglyoxal (MG) on cell division of Scenedesmus quadricauda was dependent on the MG concentration in the culture, initial inoculum, cell number and growth rate at the time of treatment.

In algal cultures treated with high concentrations (2.0 mM) of MG, the inhibition of cell division was similar to that reported for Escherichia coli (Egyud and Szent-Gyorgy, 1965) and Chlamydomonas reinhardii (Morris, 1969). Morris also reported that treatment with MG at an early stage of growth prevented an increased in cell size and inhibited cell division and exponential growth. In S. quadricauda, however, 0.5 mM MG treatment at early logarithmic phase resulted in no difference in cell size. Rather, cell numbers increase exponentially and the SGR was maintained until the end of logarithmic phase.

The stimulatory effect of MG on cell division of S. quadricauda was significant when MG was added to 0.5 mM. When MG was added to the algal cultures, MG was degradaded

rapidly with increase of alagl cell number.

The SGR of the growth-inhibited cultures treated with 2.0 mM MG recovered whenever the concentration of MG was decreased to the level of 0.5 mM. Six days after the addition of 2.0 mM MG, at which time MG concentration had decreased to 0.5 mM, SGR of treated cultures was higher than that of controls.

When 1.0 mM of MG was added on day 2 to the cultures (1.2×10^4 to 1.8×10^4 cells $\cdot$ ml^{-1} inoculation), MG was rapidly reduced to the level of 0.5 mM (1 to 3 days after treatment). If the concentration of MG in the cultures was decreased to the level of 0.5 mM within 1 to 3 days, both cell number and SGR began to increase, and were higher than those of controls at the middle of logarithmic phase. When 1.0 mM of MG was added to the cultures inoculated with low cell number (0.5×10^4 to 0.8×10^4 cells $\cdot$ ml^{-1}) the degradation of MG to the level of 0.5 mM was delayed more than three days, and the cell number was not higher than those of controls.

SGR and cell number of all MG treated cultures were not significantly different from those of controls if MG concentration was decreased to the level of 0.25 mM.

The green algae, S. quadricauda detoxified MG through glyoxalase system. The growth of algae decreased the

initial amount of MG added to the cultures.

The glyoxalase system is comprised of glyoxalase I (lactoyl-glutathione lyase) and glyoxalase II (hydroxyacyl-glutathione hydrolase) and a catalytic amount of reduced glutathione. Glyoxalase I (EC 4.4.1.5) catalyses the formation of S-D-lactoylglutathione from the non-enzymatically formed hemimercaptal adduct of methylglyoxal and reduced glutathione. Several studies have shown that the detoxification of MG on cell metabolism is regulated by GI activity. The activity of GI and cell division was promoted by the addition of glutathione (GSH) in callus culture of Brassica (Sethi et al., 1988). Another study indicated that the GI regulated cell division with involvment of phosphoinositides and calmodulin in callus cultures of Amaranthus paniculatus (Das et al., 1987). Also, cell division was induced by regulating GI activity with pH control in leaf cells of coconut palm (Basu et al., 1988).

In S. quadricauda, GI activity of 0.5 mM MG treated cultures was higher than that of controls with higher cell number at the logarithmic phase. No studies were found on the regulation of GI activity by addition of MG which is growth inhibitor. In this study, GI activity was increased by addition of proper concentration of MG (0.5 mM). Cell

division of algal cell was enhanced with relation to increased GI activity by MG. This result support other studies with animal tissues and yeast, which indicated the regulation of cell division by GI activity.

The activity of glyoxalase II was lower in rapidly growing tissue than in tissue with slower growth kinetics, e.g. tumours (Jerzykowski et al., 1978) regenerating liver after hepatectomy (Principato et al., 1983). Glyoxalase II (EC 3.1.2.6) catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate and reduced glutathione.

During the physiological differentiation process, for example, the maturation of embryo, the activity of GI was decreased and the activity of GII was increased (Principato et al., 1982). In human leukaemia cells, a differentiation is accompanied by a decrease in the GI to GII activity ratio (Hooper et al., 1987). In particular the substrate of GI inhibits GII, a feed-forword inhibition (Carrington and Douglas, 1986). It was suggested that this feature might give rise to a transient elevation of cellular S-D-lactoylglutathione levels (Oray and Norton, 1980). A high ratio of GI to GII activity (high GI activity and low GII activity) was measured in intensely proliferating tissue during embryonic development of chicken liver (Principato et al., 1982). By inhibiting GII activity, which catalyses S-

D-lactoylgltathione to D-lactate, certain levels of S-D-lactoylglutathione were maintained and microtuble assembly for active mitotic division was maintained (Gillespie, 1979; Carrington and Douglas, 1986; Thornally, 1987).

When the algal cultures were treated with 0.5 mM MG, a high ratio of GI to GII activity was measured at the logarithmic phase and a low ratio of GI to GII activity was measured at the stationary phase. The significance of the difference in the ratio of GI to GII activity between dividing and resting animal tissue was supported by this study with green algae, S. quadricauda. This ratio may be more important to study physiological responses rather than absolute enzyme activity because of the expected effects of the changes in cellular maturation include cell division and differentiation. In algal batch culture, the changes of GI to GII activity ratio was distinctive with the changes of the growth stages of algae.

When the level of MG in the treated cultures was decreased to less than 0.25 mM which was not an effective concentration on cell division of algae, there were no differences from the controls in activities of GI and GII and the ratio of specific activity (GI to GII). This data also indicates that there is a proper concentration of MG (threshold concentration) to affect cell division.

In animals, the reported metabolic product of methylglyoxal is glucose *via* the shunt:

dihydroxyacetone phosphate ---> methylglyoxal ---> pyruvate ---> glucose

in glycolysis and gluconeogenesis. The formation of glucose from methylglyoxal proceeds *via* the α-ketoaldehyde dehydrogenase system which yields pyruvate, with an active metabolic process rate of 70% pyruvate to 30% L-lactate (Saez *et al.*, 1985). Formation of pyruvate from MG *via* MG reductase or α-ketoaldehyde dehydrogenase was reported in animal tissue (Ray and Ray, 1984) and in yeast (Murata *et al.*, 1986). The final product of MG degradation *via* methylglyoxal system is D-lactate which is poorly metabolized, and normally excreared to urine in animal tissue (Thornally *et al.*, 1987).

In *Scenedesmus quadricauda*, a high activity of glyoxalase system was measured than that of other enzymes which metabolize MG. While D-lactate, the end product of MG metabolism through glyoxalase system in animal tissue was not detected or was not a significant level. The conversion MG to D-lactate *via* glyoxalase system in microorganism was considered a glycolytic by-pass sequence, and possible conversion of D-lactate to glucose was reported in the study of MG metabolism in *Pseudomonas saccharophila* (Cooper, 1974). Biotransformation of D-lactate to L-

lactate by lactate racemase was also reported (Dennis, 1962), and D-lactate or L-lactate converted to pyruvate by D-lactate dehydrogenase and L-lactate dehydrogenase, respectively (Brandt, 1982; Byers, 1982).

The activity of lactate dehydrogenase was not measured in S. quadricauda while the activities of glyoxalase system was similar to those of animal tissues and yeast (2.5 - 8.0 μmloe $\cdot$ min^{-1} $\cdot$ mg^{-1} for GI and 0.5 - 2.3 μmloe $\cdot$ min^{-1} $\cdot$ mg^{-1} for GII). Possibly, in algal system, D-lactate, the terminal product of MG metabolism via glyoxalase system, is not excreated unlike animal system. MG metabolism in algae may be a bypath of glycolysis or gluconeogenesis for the efficient conversion of D-lactate to glucose via pyruvate. The conversion of MG to pyruvate in the culture of S. quadricauda was positively correlated to the amount of treated MG, especially after growth recovery of treated cultures. The rate of biotransformation from MG to pyruvate was 10 % in the MG treated cultures. In Scenedesmus quadricauda, the glucose level of 0.5 mM MG treated cultures was lower than that of controls. Possibly, an active cell growth stimulated by proper concentration of MG needed more carbon source than that of controls

A cell from the MG-stimulated cultures was not

different from a cell of controls in its photosynthetic and respiratory rate, or its size. The activity of α-ketoaldehyde dehydrogenase and MG reductase were not measured. The disruption of the cell wall is a difficulty encountered in plant enzyme studies. In this study, the undetectable activity of α-ketoaldehyde dehydrogenase, MG reductase, and lactate dehydrogenases may be due to the difficulty of breaking the cell wall, or relatively low activity compare to the glyoxalase system. The glyoxalase system was the main pathway of MG metabolism in S. quadricauda with high activity.

A literature review did not indicate any reports on the growth dynamics of algae with MG as a growth or cell division stimulator. Also, studies to determine the threshold concentrations of MG to stimulate cell division in any system were not found. It is apparent that the stimulation of cell division in S. quadricauda by MG depends upon certain threshold concentrations of MG at the time of treatment (0.4 to 0.9 mg MG · 10^6 $cells^{-1}$). The function of MG and its metabolites or intermediates are not fully understood. The increase of GI activity, the high ratio of GI to GII activity, and possible regulation of S-D-lactoylglutathione level in the 0.5 mM MG treated algal cultures can be one of the keys to explain the stimulatory

effect of MG on algal cell division. The stimulatory effect of MG on algal cell division may also be due, in part at least, to an effect on microtubule assembly.

APPENDIX A

CELL NUMBER OF METHYLGLYOXAL TREATED AND UNTREATED *SCENEDESMUS QUADRICAUDA* CULTURES

A 1. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.5, 1.0, or 2.0 mM MG) and untreated Scenedesmus quadricauda cultures with initial inoculation of 2.4 x 10^4 cells · ml^{-1}

	Culture			
Day	control	0.5 mM	1.0 mM	2.0 mM
1	$^{a\ 2)\ 1)}$ 0.023 (0.002)	^{b} 0.016 (0.004)	^{a} 0.022 (0.001)	^{a} 0.022 (0.002)
2	^{b} 0.043 (0.002)	^{b} 0.034 (0.003)	$^{b\ 3)}$ 0.036 (0.006)	^{a} 0.043 (0.002)
3	^{a} 0.102 (0.005)	^{a} 0.113 (0.012)	^{b} 0.051 (0.015)	$^{a\ 3)}$ 0.102 (0.022)
5	^{b} 0.270 (0.030)	^{a} 0.340 (0.061)	^{c} 0.113 (0.012)	^{b} 0.207 (0.050)
7	^{b} 0.560 (0.046)	^{a} 0.670 (0.062)	^{c} 0.307 (0.071)	^{c} 0.300 (0.030)
9	^{a} 0.880 (0.072)	^{a} 1.010 (0.089)	^{b} 0.463 (0.072)	^{b} 0.430 (0.135)
11	^{a} 1.030 (0.070)	^{a} 1.110 (0.096)	^{b} 0.630 (0.070)	^{b} 0.553 (0.087)
13	^{ab} 1.050 (0.070)	^{a} 1.123 (0.102)	^{bc} 0.920 (0.072)	^{c} 0.817 (0.075)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) one day after MG treatment

A 2. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.5, 1.0, or 2.0 mM MG) and untreated Scenedesmus quadricauda cultures with initial inoculation of 4.8 x 10^4 cells · ml^{-1}

Day	Culture control	0.5 mM	1.0 mM	2.0 mM
1	$^{b\ 2)\ 1)}$ 0.035 (0.002)	^{a} 0.040 (0.002)	^{b} 0.035 (0.002)	^{b} 0.035 (0.001)
2	^{c} 0.065 (0.005)	^{a} 0.092 (0.002)	$^{b\ 3)}$ 0.074 (0.007)	^{c} 0.065 (0.001)
3	^{b} 0.125 (0.018)	^{a} 0.204 (0.023)	^{b} 0.140 (0.034)	$^{b\ 3)}$ 0.111 (0.004)
5	^{b} 0.503 (0.064)	^{a} 0.630 (0.079)	^{c} 0.237 (0.065)	^{c} 0.237 (0.035)
7	^{a} 1.150 (0.114)	^{a} 1.200 (0.161)	^{b} 0.900 (0.070)	^{c} 0.460 (0.053)
9	^{a} 1.277 (0.172)	^{a} 1.350 (0.130)	^{a} 1.117 (0.211)	^{b} 0.737 (0.217)
11	^{a} 1.327 (0.110)	^{a} 1.357 (0.067)	^{a} 1.173 (0.188)	^{b} 0.907 (0.107)
13	^{a} 1.147 (0.031)	^{ab} 1.057 (0.050)	^{a} 1.140 (0.096)	^{b} 0.937 (0.102)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) one day after MG treatment

A 3. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.25, 0.5, or 0.75 mM MG) on day 3 and untreated Scenedesmus quadricauda cultures with initial inoculation of 0.5 x 10^4 cells · ml^{-1}

Day	Culture control	0.25 mM	0.5 mM	0.75 mM
5	0.077 (0.006) [1]	0.087 (0.012)	0.083 (0.015)	0.057 (0.021)
7	0.213 (0.006) [b 2]	0.263 (0.021) [a]	0.193 (0.015) [b]	0.137 (0.023) [c]
9	0.520 (0.070) [a]	0.493 (0.102) [a]	0.413 (0.051) [ab]	0.303 (0.051) [b]
11	0.837 (0.060) [a]	0.767 (0.076) [a]	0.823 (0.117) [a]	0.603 (0.060) [b]
13	0.840 (0.070)	0.860 (0.061)	0.847 (0.046)	0.720 (0.113)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

A 4. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.5, 0.75, or 1.0 mM MG) on day 4 and untreated Scenedesmus quadricauda cultures with initial inoculation of 0.8 x 10^4 cells · ml^{-1}

Day	Culture control	0.5 mM	0.75 mM	1.0 mM
5	[b 2) 1)] 0.123 (0.021)	[a] 0.223 (0.021)	[b] 0.137 (0.042)	[b] 0.103 (0.042)
7	[bc] 0.327 (0.045)	[a] 0.590 (0.095)	[b] 0.423 (0.049)	[c] 0.267 (0.049)
9	[c] 0.600 (0.027)	[a] 1.013 (0.095)	[b] 0.817 (0.035)	[d] 0.427 (0.051)
11	0.917 (0.038)	1.183 (0.085)	1.157 (0.163)	1.110 (0.130)
13	1.120 (0.060)	1.213 (0.087)	1.157 (0.182)	1.167 (0.117)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

A 5. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.5 or 1.0 mM MG) on day 4 and untreated Scenedesmus quadricauda cultures with initial inoculation of 2.4 x 10^4 cells · ml^{-1}

	Culture		
Day	control	0.5 mM	1.0 mM
5	[a 2) 1)] 0.300 (0.027)	[ab] 0.263 (0.015)	[b] 0.233 (0.012)
7	[a] 0.760 (0.079)	[b] 0.657 (0.032)	[b] 0.607 (0.015)
9	1.103 (0.070)	1.120 (0.070)	1.047 (0.035)
11	1.210 (0.092)	1.297 (0.045)	1.247 (0.059)
13	1.207 (0.070)	1.330 (0.046)	1.270 (0.131)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

A 6. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.25, 0.5, or 1.0 mM MG) on day 2 and untreated Scenedesmus quadricauda cultures with initial inoculation of 0.8 x 10^4 cells · ml^{-1}

	Culture			
Day	control	0.25 mM	0.5 mM	1.0 mM
3	^{ab} 0.033 (0.005)	^{a} 0.036 (0.003)	^{b} 0.027 (0.006)	^{c} 0.014 (0.002)
4	^{a} 0.101 (0.018)	^{a} 0.097 (0.006)	^{a} 0.082 (0.012)	^{b} 0.055 (0.007)
5	^{a} 0.207 (0.031)	^{a} 0.187 (0.031)	^{a} 0.197 (0.006)	^{b} 0.130 (0.002)
7	^{b} 0.433 (0.025)	^{b} 0.433 (0.031)	^{a} 0.547 (0.070)	^{c} 0.303 (0.015)
9	^{b} 0.717 (0.085)	^{b} 0.730 (0.020)	^{a} 1.010 (0.148)	^{c} 0.510 (0.036)
11	^{ab} 0.927 (0.067)	^{ab} 0.960 (0.061)	^{a} 1.117 (0.141)	^{b} 0.797 (0.107)
13	0.983 (0.107)	1.020 (0.100)	1.160 (0.151)	0.910 (0.115)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

A 7. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.25, 0.5, or 1.0 mM MG) on day 2 and untreated Scenedesmus quadricauda cultures with initial inoculation of 1.2 x 10^4 cells · ml^{-1}

Day	Culture control	0.25 mM	0.5 mM	1.0 mM
3	$^{c\ 2)\ 1)}$ 0.041 (0.006)	^{b} 0.047 (0.001)	^{a} 0.061 (0.002)	^{a} 0.061 (0.002)
4	^{c} 0.083 (0.006)	^{a} 0.205 (0.023)	^{ab} 0.187 (0.036)	^{b} 0.161 (0.009)
5	0.207 (0.051)	0.287 (0.080)	0.350 (0.100)	0.293 (0.065)
7	^{b} 0.517 (0.058)	^{a} 0.683 (0.076)	^{a} 0.750 (0.044)	^{a} 0.653 (0.042)
9	0.830 (0.060)	0.917 (0.067)	1.170 (0.234)	1.200 (0.195)
11	^{b} 1.060 (0.020)	^{ab} 1.183 (0.033)	^{a} 1.370 (0.079)	^{ab} 1.247 (0.155)
13	1.177 (0.093)	1.243 (0.169)	1.403 (0.182)	1.257 (0.127)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

A 8. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.25, 0.5, or 1.0 mM MG) on day 2 and untreated Scenedesmus quadricauda cultures with initial inoculation of 1.8 x 10^4 cells · ml^{-1}

	Culture			
Day	control	0.25 mM	0.5 mM	1.0 mM
3	[c 2) 1)] 0.065 (0.004)	[b] 0.091 (0.008)	[ab] 0.108 (0.016)	[a] 0.124 (0.002)
4	[b] 0.129 (0.005)	[a] 0.210 (0.027)	[a] 0.244 (0.026)	[a] 0.251 (0.026)
5	[b] 0.210 (0.027)	[a] 0.333 (0.068)	[a] 0.357 (0.051)	[a] 0.407 (0.049)
7	0.577 (0.012)	0.703 (0.131)	0.750 (0.115)	0.770 (0.005)
9	0.933 (0.076)	1.043 (0.129)	1.170 (0.085)	1.207 (0.182)
11	1.067 (0.035)	1.210 (0.205)	1.317 (0.126)	1.300 (0.123)
13	1.163 (0.160)	1.233 (0.137)	1.330 (0.115)	1.290 (0.104)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

A 9. Cell number (2 x 10^6 cells · ml^{-1}) methylglyoxal (MG) treated (0.5 mM MG on day 2, 3, 4, or 5) cultures and untreated Scenedesmus quadricauda cultures with initial inoculation of 0.8 x 10^4 cells · ml^{-1}

	Culture				
Day	control	day 2	day 3	day 4	day 5
3	b 2) 1) 0.058 (0.002)	a 3) 0.067 (0.005)	b 0.058 (0.001)	b 0.056 (0.003)	b 0.057 (0.001)
4	b 0.153 (0.023)	ab 0.177 (0.015)	a 3) 0.210 (0.036)	b 0.157 (0.012)	b 0.157 (0.012)
5	b 0.280 (0.020)	a 0.370 (0.044)	a 0.380 (0.040)	ab 3) 0.330 (0.052)	b 0.260 (0.030)
7	d 0.597 (0.032)	c 0.717 (0.049)	b 0.863 (0.451)	a 1.137 (0.093)	b 0.910 (0.027)
9	c 0.747 (0.050)	b 0.960 (0.072)	ab 1.073 (0.042)	a 1.183 (0.091)	b 0.987 (0.119)
11	c 0.887 (0.050)	b 1.050 (0.154)	ab 1.167 (0.023)	a 1.097 (0.075)	ab 1.097 (0.075)
13	c 0.960 (0.056)	bc 1.087 (0.127)	ab 1.190 (0.027)	a 1.247 (0.025)	ab 1.143 (0.070)

1) Mean (standard deviation), n=3

2) same letter is not significantly different each other (Duncan's Multiple Range Test, $\alpha = 0.05$)

3) one day after MG treatment

APPENDIX B

BIODEGRADATION OF METHYLGLYOXAL IN *SCENEDESMUS QUADRICAUDA* CULTURES

B 1. Degradation of methylglyoxal (MG: $\mu g \cdot ml^{-1}$) in the Scenedesmus quadricauda cultures (0.8×10^4 cells $\cdot$ ml^{-1} inoculation) with 0.25, 0.5, 0.75, 1.0 (high inoculation*), 1.0 (low inoculation), or 0.5 mM (without algae#) treatment

Culture \ Day	0	2	4	6	8	10
	1)	[2)	f	d	d	
0.25 mM	18.00 (-)	14.23 (0.25)	5.57 (0.40)	0.13 (0.06)	0.00 (0.00)	0.00 (0.00)
		e	e	d	d	
0.5 mM	36.00 (-)	32.57 (0.74)	11.33 (0.58)	0.17 (0.06)	0.00 (0.00)	0.00 (0.00)
		c	d	c	d	
0.75 mM	54.00 (-)	43.17 (1.04)	16.67 (0.58)	4.73 (0.46)	0.07 (0.06)	0.00 (0.00)
		b	c	b	c	
1.0* mM	72.00 (-)	61.17 (0.76)	25.33 (0.58)	7.83 (0.29)	1.17 (0.29)	0.03 (0.06)
		a	a	a	b	
1.0**mM	72.00 (-)	67.67 (1.53)	46.33 (1.53)	33.67 (0.58)	10.67 (0.58)	1.17 (0.29)
		d	b	a	a	
0.5# mM	36.00 (-)	36.17 (0.29)	35.83 (0.29)	33.33 (0.58)	27.33 (1.16)	17.67 (0.58)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

* High inoculation (1.2×10^4 cells $\cdot$ ml^{-1})

Medium without algae

B 2. Degradation of methylglyoxal (MG: $\mu g \cdot ml^{-1}$) in the Scenedesmus quadricauda cultures (0.8×10^4 cells $\cdot$ ml^{-1} inoculation) treated with 0.5 mM MG on day 3, 5, or 7

Day / Culture	3	5	6	7	9	11
Day 3	1) 36.00 (-)	26.67 (0.57)	16.67 (0.58)	c 8.33 (0.58)	b 2) 0.03 (0.06)	0.00 (-)
Day 5	- (-)	36.00 (-)	24.33 (0.57)	b 15.67 (1.53)	a 7.67 (0.58)	0.10 (0.17)
Day 7	- (-)	- (-)	- (-)	a 36.00 (-)	a 8.33 (0.57)	0.13 (0.12)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

APPENDIX C

SPECIFIC ACTIVITY OF GLYOXALASE I AND GLYOXALASE II

C. Specific activity (umole · min^{-1} · mg^{-1}) of glyoxalase I (GI) and glyoxalase II (GII) of controls and 0.5 or 1.0 mM methylglyoxal (MG) treated cultures

Enzyme	GI			GII		
Day / Culture	5	9	13	5	9	13
control	b 1) 2) 6.188 (0.122)	b 3.322 (0.188)	b 2.526 (0.020)	a 2.108 (0.040)	a 1.319 (0.072)	a 0.674 (0.051)
0.5 mM	a 7.954 (0.029)	c 2.796 (0.061)	a 3.137 (0.064)	a 2.320 (0.068)	c 0.591 (0.029)	a 0.669 (0.029)
1.0 mM	c 2.593 (0.150)	a 4.061 (0.040)	b 2.614 (0.105)	c 0.930 (0.203)	b 0.781 (0.045)	b 0.496 (0.039)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

APPENDIX D

PRODUCTS IN THE METHYLGLYOXAL TREATED *SCENEDESMUS QUADRICAUDA* CULTURES

D 1. Pyruvate (ug · ml^{-1}) in the methylglyoxal (MG) treated Scenedesmus quadricauda cultures with 0.5 mM on day 3, 0.5 mM on day 4, or 1.0 mM on day 5 and controls

Culture \ Day	4	6	8	10
control	a 2) 1) 0.523 (0.025)	d 0.443 (0.012)	d 0.443 (0.006)	d 1.067 (0.029)
0.5 mM on day3	b 0.430 (0.017)	b 0.613 (0.012)	b 1.633 (0.029)	c 2.910 (0.017)
0.5 mM on day4	b 0.433 (0.015)	c 0.543 (0.012)	c 1.477 (0.006)	a 5.047 (0.050)
1.0 mM on day5	b 0.443 (0.006)	a 0.870 (0.010)	a 2.073 (0.058)	b 4.243 (0.040)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

D 2. Glucose (ug · ml^{-1}) in the methylglyoxal (MG) treated Scenedesmus quadricauda cultures with 0.5 mM on day 3, 0.5 mM on day 4, or 1.0 mM on day 5 and controls

Culture \ Day	4	6	8	10	11
control	a 2) 1) 2.567 (0.115)	c 2.833 (0.058)	a 3.267 (0.058)	a 4.433 (0.058)	a 3.333 (0.298)
0.5 mM on day3	b 0.000 (0.000)	a 4.533 (0.153)	b 1.167 (0.058)	d 2.433 (0.058)	b 1.133 (0.115)
0.5 mM on day4	b 0.000 (0.000)	b 3.133 (0.115)	b 1.067 (0.115)	b 3.333 (0.115)	c 0.100 (0.173)
1.0 mM on day5	b 0.000 (0.000)	d 1.100 (0.100)	c 0.167 (0.058)	c 3.067 (0.058)	c 0.067 (0.115)

1) Mean (standard deviation)

2) same letter is not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$)

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