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Recovery of Outdoor Mass Culture Bleached *Scenedesmus* sp.

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Abstract: Bleached cells of *Scenedesmus* sp. were re-incubated in polyethylene tubes containing 2000 mL of culture volume with different nutrient supplementations. Dry weight (g L^{-1}) and total chlorophyll (mg L^{-1}) were daily measured. Death and growth-rate of out door grown cells were calculated before the indoor re-cultivation, where other growth parameters including growth rate, doubling time, degree of multiplication and percentage increase were daily determined during the recovery period. Growth as dry weight and total chlorophyll was decomposed and cultures reached their minimum biomass at nearly to 12th day. Recovery period represented variable increases own to the rate of nutrient supplementation and incubation mode, where diluted and semi-diluted cultures represented the maximum. Higher growth rate of the dense cultures could be attributed to the initial biomass at zero time. Consequently, failure of growth during the sunny season could be ascribed to the effect of high light intensity as well as the generated temperature.

Key words: *Scenedesmus* sp., out door, bleaching, polyethylene, recovery

INTRODUCTION

Large-scale production systems of phototrophs are practically controlled by the major environmental factors, temperature and solar irradiation^[1]. The highest growth rate is usually obtained at the optimum temperature for growth. A rise in temperature greatly increased the saturating light intensity constant ($\text{E m}^{-2} \text{ s}^{-1}$). Also, temperature has most decisive effect maintaining energy coefficient^[2]. For instance, *Chlorella* coefficient was reduced from 15.8 kJ g^{-1} at 25°C to negligible amount at the optimum 37°C , then increasing thereafter. Thus, the positive effect of any rise in temperature up to the optimum is two-fold increasing the light and decreasing the energy spent on maintaining^[3]. The net gain of biomass productivity is directly correlated to the relation between CO_2 fixation rate and respiration, which widely depending on temperature^[4]. In spite of nutritional factors, growth failure during the high light irradiation seasons could be ascribed to the effect of light inhibitory effect and the generated temperature rise. This investigation was conducted using bleached cells of *Scenedesmus* sp., to determine the factors leading to recovery e.g., nutritional or environmental factors.

MATERIALS AND METHODS

Out-door growth: The pre-isolated green alga *Scenedesmus* sp. was used^[5,6]. Cultures were laboratory prepared using NSI macro nutrient solution and PAZ trace elements mixture^[7]. The cultures were routinely scaled-up

to 15000 L in open door ponds; as previously described^[8], during the season of 2004 (May-July). Healthy grown batches were analyzed to estimate the rate of nutrients supplementation.

Recovery experiment: Bleached batches due to high light irradiation and/or high temperature were used in this investigation. Growth containers ready-made from high-density polyethylene tubes (50 μm /6.5/70 cm for thickness, diameter and length) give 2000 mL of culture volume were used. About 10 cm of tube length from the upper part were lofted to enable bubbling. Cultures were grown under 200 μe of light intensity provided from white cool lamps and the ambient temperature was $35 \pm 1^\circ\text{C}$. Aeration was done by dried air from the lower end of culture containers at 25 bars. Buffering action as well as heterotrophic growth was performed by 6 mM sodium acetate^[9] and diluted acetic acid nearly to the neutral reaction^[5,6].

Four treatments of five replicates using the bleached cultures were exposed to the afore-mentioned conditions with four treatments as follows:

T₁ had no additional nutrient except those received from the previous open pond cultivation.

T₂ was applied as cultures were enriched by 25% of macronutrient^[8].

T₃ was applied as cultures were enriched by 50% of macro nutrient.

T₄ was applied as cultures were enriched by 100% of macro nutrient (Table 1).

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Table 1: Nutrient supplementation of recovery experiments

Treat. (g)	N* ¹	P* ²	K* ³	Dose (%)
1	0.000	0.000	0.000	0.000
2	0.075	0.025	0.0125	25.00
3	0.150	0.500	0.0250	50.00
4	0.300	0.100	0.0500	100.0

*1, Urea 46.5%, *2, 85% Phosphoric acid and *3, SoluPotass (50.0% K₂O +46.0% SO₃)

Healthy and bleached out-door produced cultures as well as their growth medium were analyzed (Table 4).

Growth measurements: Dry weight (g l⁻¹) was determined by filtering 10 mL of the culture over pre-dried membrane filter (0.45 µm) and drying the remainder over the filter. Total chlorophyll was extracted by 95% DMSO at 70°C/5 min from the obtained dry biomass^[10]. The absorbance was measured at 672 nm. Chlorophyll concentration was calculated using the formula $E_{1\%}^{1\text{cm}}=870$ ^[11]. Total nitrogen was determined using Kjeldahl method^[12]. Other macro and micro-nutrients were measured photo-metrically^[13]. All of the measurements were daily determined starting from day of running the out door cultured in the normal cells (day 1-6), then during the bleaching process (day 6-12) and also during the recovery process indoor cultures (day 12-19).

Growth parameters: Death rate (K), maximum growth rate (μ_{max}), average growth rate (μ_{avr}), daily increment, percentage increase and doubling time of dry weight and total chlorophyll were calculated^[14].

RESULTS AND DISCUSSION

Growth measurements of out door-bleached *Scenedesmus* sp.: Growth of the bleached cells, expressed as dry weight was ultimately failure by the 12th day of incubation (Fig. 1).

However the decline in growth dry weight was slightly inhibited within the period of 7-11th days (Fig. 2). The fast decomposing followed it meaning the rise of death rate.

This rate was found to be faster when growth was expressed as total chlorophyll (8th day), whilst growth slightly prolonged might be by shifting metabolism or consumption of death component mainly organic nutrients.

Dry weight measurements and parameters during recovery: During recovery period, fast acceleration of growth rate was observed, as cells were transferred to the indoor stage. The latter effect could be attributed to the shadow effect by the type of growth container and bubbling system under the same high light irradiation.

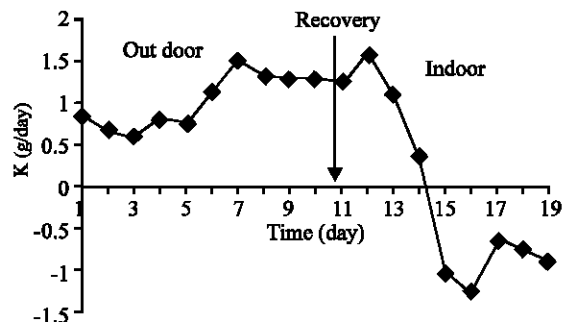


Fig. 1: Death rate (K) as dry weight of outdoor produced and recovered *Scenedesmus* sp.

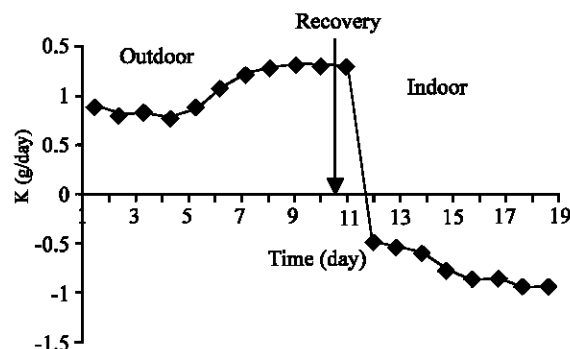


Fig. 2: Death rate (K) as total chlorophyll of outdoor produced and recovered *Scenedesmus* sp.

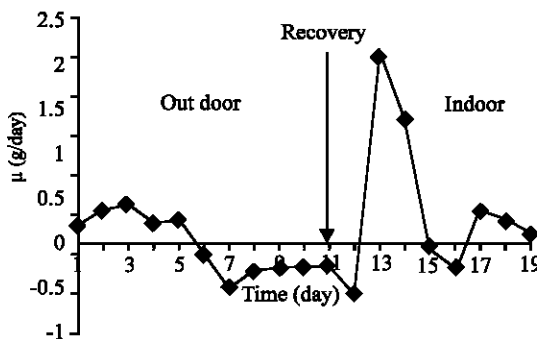


Fig. 3: Growth rate (μ) expressed as dry weight of outdoor produced and recovered *Scenedesmus* sp.

In addition, parameters during recovery resulted in varied due to the nutritional levels (Fig. 3).

Cells, which had no extra nutrient supplementation, except pre-cultivation from open pond, exhibited more response to the given experimental conditions.

The data could be supported by the calculated percentage increase as a variation between the initial and end collected biomass. The calculated increases percent were 4600, 630, 875, 206 and 1350% of the initial (Table 2).

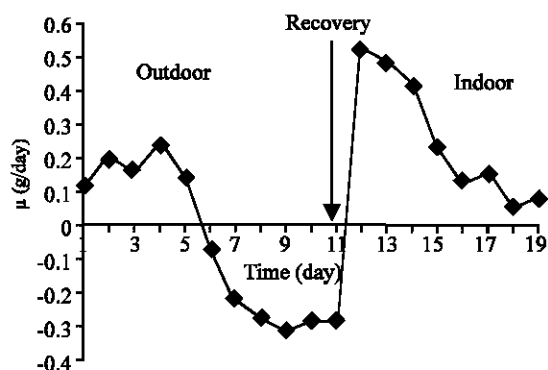


Fig. 4: Growth rate (μ) as total chlorophyll of outdoor produced and recovered *Scenedesmus* sp.

Table 2: Dry weight percentage increase during recovery period of *Scenedesmus* sp.

T ₁ (0.0%)	T ₁ (25%)	T ₁ (50%)	T ₁ (100%)
4600	630	875	1350
(100%) = 0.3 g l ⁻¹ urea 46.5% 0.1 ml l ⁻¹ Phosphoric acid and 0.05 g l ⁻¹ K ₂ SO ₄			

Table 3: Total chlorophyll percentage increase during recovery period of *Scenedesmus* sp.

T ₁ (0.0%)	T ₁ (25%)	T ₁ (50%)	T ₁ (100%)
544.4	771.4	420	371.4
(100%) = 0.3 g l ⁻¹ urea 46.5% 0.1 ml l ⁻¹ Phosphoric acid and 0.05 g l ⁻¹ K ₂ SO ₄			

Table 4: Chemical analysis of healthy and bleached out door batch cultures

		Healthy		Bleached	
Analysis		M*	A*	M*	A*
N	%	0.02	7.91	4.30	2.35
P		0.01	1.45	0.62	0.51
K		0.02	0.29	0.09	0.06
Na		0.00	0.04	0.01	0.02
Ca		0.02	0.41	0.19	0.09
Mg		0.11	0.87	0.42	0.29
Fe	ppm	29.00	229.00	98.00	104.00
Zn		9.00	62.00	31.00	29.00
Mn		4.00	53.00	28.00	23.00
Cu		0.90	6.50	1.90	2.00

M*=medium of alga growth after filtration

A*=precipitated algal bulk

However, such data represented some error because of the interface of high doses of nutrient supplementation and the differences between the initial inoculums. Also, the great variation could be ascribed to the dilution factor and self-shading of cells.

The successful growth of bleached cells when exposed to high light irradiation (200 μ e) might be back to the type of growth container which allow sufficient light saturation at short time and protection against UV irradiation. Growth parameters of the dry weight accumulation during the recovery period (Fig. 5) showed that diluted and semi-diluted cultures surpass the other examined treatments. Of the other growth parameters

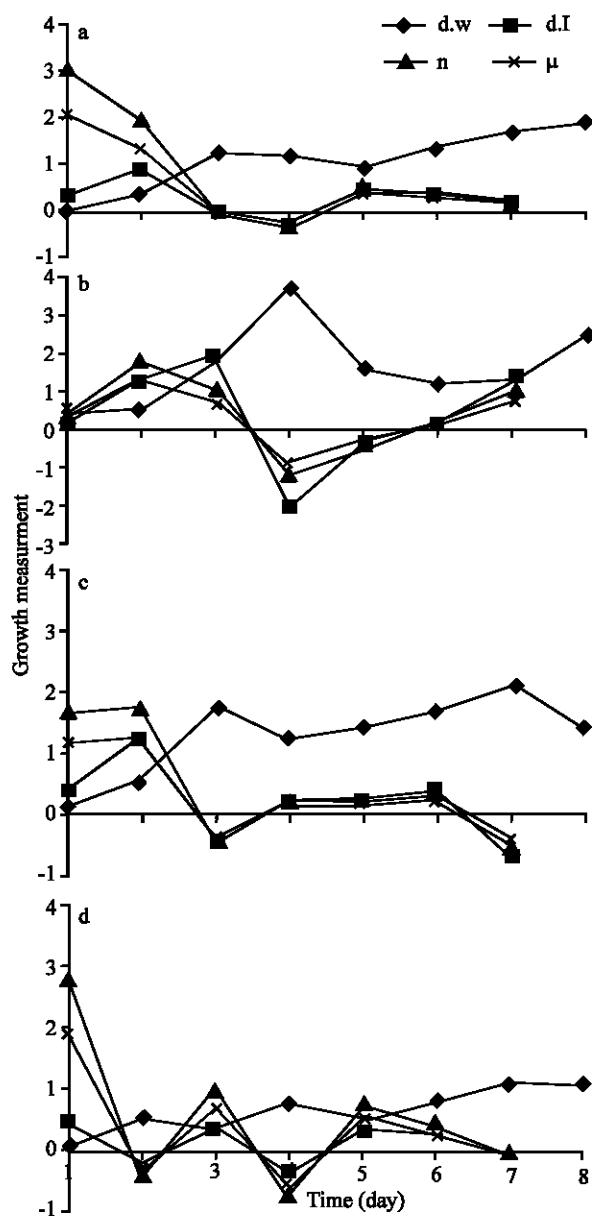


Fig. 5: Growth as dry weight measurements and parameters during recovery period (12-19 day) of *Scenedesmus* sp.

◆ = dry weight; ■ = daily increment; ▲ = degree of multiplication; × = growth rate

(Fig. 5) the same pattern was observed and the semi-diluted cultures (25%) represented the maximum, especially at the end of incubation period.

Total chlorophyll measurements and parameters during recovery period: As for total chlorophyll, percentage increase reached its maximum when bleached cultures were fed by 25% (T₁) of the recommended NPK (Table 3)

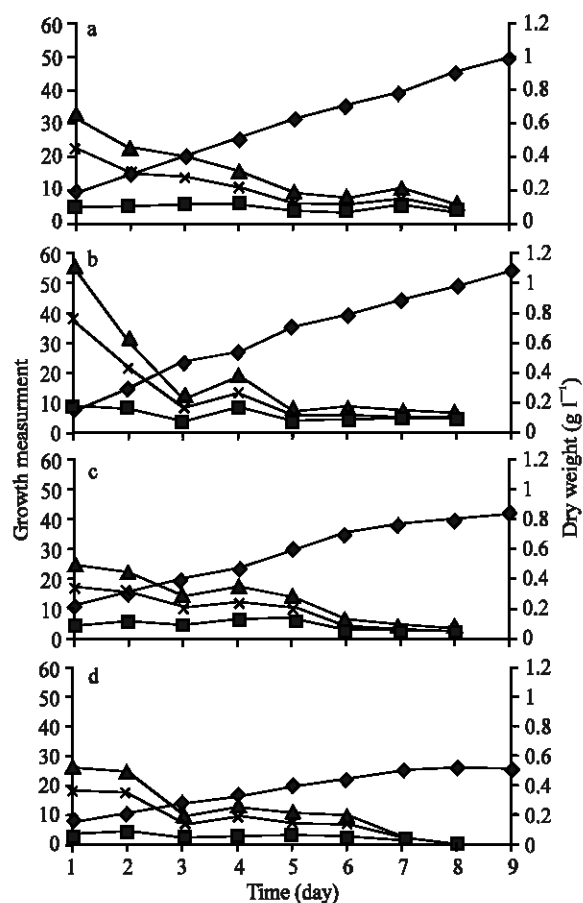


Fig. 6: Growth as dry weight measurements and parameters during recovery period (12-19 day) of *Scenedesmus* sp.
a = 0.0%; b= 25%; c=50% and d=100% of NPK
◆ = dry weight; ■ = daily increment;
▲ = degree of multiplication, × = growth rate

suggesting that the failure of growth or chlorophyll decomposition can be attributed to the effect of temperature proportion to high light irradiation. It may be concluded that this dose seems to be sufficient for chlorophyll re-formation by daughter cells.

When data were subjected to viable total chlorophyll and their parameters, it was found that 25% of additional nutrients dose surpass the others followed by 0.0%, which they yielded about 50 mg l⁻¹ (Fig. 6).

As for other parameters, daily increment was ultimately stopped at the end of recovery period with 100% of supplementary dose and inhibited by 50%, while increment took place with 0.0 and 25%. Both of degree of multiplication and growth rate exhibited the same trend.

In general, the conclusions^[4-15], could support the obtained results. They explain the relationship between

high regime and the population density; mainly mixing and depth of the culture. Furthermore, decreasing the culture depth shifts the optimal population density (i.e.; that density which yields the highest aerial output) to higher value of cells concentration^[16]. The present cultivation unit (enclosed system) like tubular reactor, in which they had a very small aerial volume, useful in elevating the temperature and eliminate insects. Hence, cultures tend to increase the population densities, which in turn will reduce the cost of production and increase the efficiency of harvesting. Increasing of lipid content; due to stress factors; is closely associated with the decreasing of both total nitrogen and total chlorophyll, parallel with the increasing of carotenoid pigments^[17].

Here, growth failure could be attributed to the effect of light and the associated rise in temperature.

Chemical composition of bleached and healthy cultures:

As shown in Table 2, macro and micro-nutrients were accumulated within the healthy cells as a major constituent of their structure. It was early observed that minor part of these nutrients was detected on the surrounded medium after they entered cells may be goes back to the exchangeable action, osmosis regulation or both^[18]. The opposite manner was observed with bleached cells, where most of the macro and micro-nutrients were detected on the filtrate solution (growth medium) due to the rise of death rate.

Successful mass production of algal biomass, under different nutritional and environmental factors; widely depends on the availability of the required inoculums, in which they represented the rise of running costs. Thus it is easily to overcome the failure of growth by enhancing the recovery of bleached cells without any addition of nutritional supplementary based on the shift of cultivation type. Also, full light frequency (24 h) allow the exposed cells to keep their night temperature as possible to reduce the extent of night biomass loss throughout respiration. Here, use of such low-priced containers seems to be more advanced technique.

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