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Toxicity of Trichloroethylene (TCE) on Some Algae and Cyanobacteria

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Abstract The toxicity of trichloroethylene was tested in both glass enclosures and in polystyrene immunological plates, and resulted in insignificant correlation of EC_{50} (evaluated as OD 750 nm). In enclosures, EC_{50} evaluated as O_2 evolution and as pH increment was highly significant. The optimal time for the assay of TCE in enclosures was 48–72 h; and in plates ca 200 h. After a longer time (e.g. 300 h) TCE diffused from the plates and growth was revived. TCE in low concentrations (ca. 0.1–0.2 g/L) stimulated the growth of some tested organisms, both in plates and in enclosures. Toxicity occurred from ca. 0.35–0.6 g/L (EC_{50}). The testing procedure in enclosures was a suitable method for some volatile substances, giving rapid results.

Keywords Algae · Cyanobacteria · Toxicity · Trichloroethylene

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Trichloroethylene (TCE) is a chlorinated aliphatic hydrocarbon, and one of the most widely produced organochlorine compounds, with annual world production of 10^6 tons (Pearson and McConnell 1975). It is primarily used for vapour-phase degreasing in metals fabrication, cold-metal cleaning, in the manufacture of organic chemicals, dry cleaning, and as a solvent (Horst 1985; Ward et al. 1986). It has been detected in soil, the aquatic environment, in organisms, and in drinking water (Pearson and McConnell 1975; Bringmann and Kühn 1980; Horst in Cairns 1985). The half-life of TCE in water is 2.6–6 years, and in soil 2–18 months; which demonstrates that is poorly degraded in water and soil (Inderjit and Kakuta 2003). Because of the slow biodegradation in soil and water, TCE may be responsible for long-term ecotoxic effects; it is classified as a carcinogen and mutagen of classes 2 and 3 for vertebrates (US EPA 1980; Reith et al. 2009). TCE is very volatile, its vapour pressure is 100 mm Hg at 20°C (Verschueren 2001).

TCE was found in the Czech Republic, e.g. in the water-basin of both the Tichá and Divoká Orlice rivers (Dittrt and Chvátil 1993) with concentrations up to 1.09 mg/L. The US EPA recommended max. concentration should not exceed 0.005 mg/L, in drinking water (Reith et al. 2009), and 6.7–0.67 mg/L as PEC (probable no-effect concentration) for river case (Euro Chlor).

Although TCE is widely distributed, only limited data have thus far been published on TCE toxicity and its influence on algae growth (Pearson and McConnell 1975; Bringmann and Kühn 1978, 1980; Biggs et al. 1979; Ward et al. 1986; Tadros et al. 1994; El-Jay 1996; Håkanson 1999; Ando et al. 2003).

The aim of the study was to test the effects of TCE on the growth of several cyanobacteria and algae strains grown in the laboratory and to compare a rapid exposure

assay tested in enclosures to that of a growth assay tested in polystyrene immunological plates (Wells et al. 1998).

Materials and Methods

Cultures of green algae *Chlamydomonas reinhardtii* strain UTEX 2246 137c mt^+, *Chlorella kessleri* strain LARG/1, *Desmodesmus subspicatus* (syn. *Scenedesmus subspicatus*) strain 1953/SAG 86, *Desmodesmus quadricauda* (syn. *Scenedesmus quadricauda*) strain GREIFSWALD/15, *Raphidocelis subcapitata* (syn. *Pseudokirchneriella subcapitata*, *Selenastrum capricornutum*) strain SKULBERG 1959/1, and the cyanobacteria *Synechococcus elongatus* f. *thermalis* strain KOVROV 1972/8, *Synechococcus leopoliensis* strain KRATZ-ALLEN/UTEX 625 (syn. *Anacystis nidulans*), and *Microcystis aeruginosa* strain ZAPOMELOVÁ 2006/2 were supplied from the Culture Collection of Autotrophic Organisms at Trebon, CZ. Starting concentrations of inocula in wells were 0.02–0.04 g/L of dry weight. Nutrient solution "Z", after Zehnder in Staub (1961), was spiked with TCE: 100 mg was dissolved in 2 mL of methanol (Lachema, CZ), and then added to 100 mL of sterile Z medium. Controls received an equal volume of methanol. This solution was diluted to the desired concentrations by an identical nutrient solution, but without either TCE or methanol.

Assay in immunological plates was evaluated according to Lukavský (1992): immunological plates, polystyrene, 9 × 12 cm with 96 wells, FB of 0.25 mL (Novogen, CZ); sealed with the polyethylene foil (ALU-FIX Austria, for food wrapping); and also covered with lids; irradiated with fluorescent tubes F33 cool white 36 W (Tungsram, H) by PhAR (Photosynthetically Active Radiation, 370–720 nm) 40 W/m^2 (=200 $\mu M/m^2/s$); at 30°C temperature; and CO_2 2% (v/v). OD in plates were evaluated under an iEMS reader (Labscale, SF) at wavelength 750 nm, and the optical density was converted to dry weight after individual conversion curves for each species. EC_{50} (the concentration of a tested substance at which the cell density, biomass, O_2 production or pH increment is 50% of that of the untreated algae) was determined graphically according to standard ISO (2004) omitting the stimulation peaks. In addition, growth curves plotted as time—dry weight or OD 750, can indicate EC_{50} as concentration when cell number is constant, and stabilized. Six replicates were measured, for OD evaluation, error bars are plotted as $\pm s_x = \sqrt{(n\Sigma x^2 - (\Sigma x)^2/n^2)}$.

Assay in glass enclosures were performed according to ISO/DIS 14442 (1998) the glass enclosures (volume of 1.5 mL), were sealed with silicone stoppers coated with Teflon on the inner side (screw top vials, Supelco, USA). An identical nutrient solution was used, as described above, but also spiked with 3 g/L $KHCO_3$ (source of inorganic carbon). The inoculum of 0.04–0.06 g/L of dry weight was higher because of a shorter exposition time. The enclosures were exposed horizontally under the same light intensity and temperature as the plates and shaken 10 x/day. Every day, 0.2 mL was measured into 6 replicate wells in an immunological FB plate, and the OD 750 nm was measured identically as in the previous protocol. O_2 concentrations were measured by a polarographic Clark-type oxygen sensor, and pH by a miniaturized combined electrode, both joined to a MEM 102 multimeter (Chemoprojekt Satalice, CZ), the measurements were performed with no replicates.

Results and Discussion

In enclosures, *Chlorella kessleri*, without TCE, reached a stationary phase at 72 h. At longer times, we expected the depletion of carbon, and the inhibition of photosynthesis by an excess of O_2. Toxicity curves from 48 and 72 h were similar in the majority of graphs (Fig. 1). The optimal times, 48–72 h agreed with the 72 h, set in ISO/DIS 14442 (1998). During this time period, we observed ca. 1 division of the cells (OD750 started as 0.14 and increased to 0.22 for *Chlorella kessleri*). In plates, the cultivation times were longer and after approximately 1, 000 h a stationary phase (where population is stable) was reached in which the increments of all counts and dry weight is constant. During this time period, we observed that 3 generations (OD750 of control increased from 0.1 to 1.0 for *Chlorella kessleri*) of algae were produced, and the 3 complete life cycles were running satisfactorily under the treatment with TCE.

Enclosures showed, for some algae, a stimulation of growth in TCE at concentrations of about 0.1 g/L (Fig. 1a, c) except *Chlamydomonas reinhardtii* where the stimulation was ca. 0.3–0.4 g/L (data not presented). After 0.1 g/L, in the majority of response curves, there was a sharp decrease, but never to zero. OD values were measured up to a concentration 1 g/L of TCE, which is near its saturation limit in water (1.36 g/L at 25°C). Photosynthesis, evaluated as both the alkalization of nutrient solution (pH increment) and O_2 production (Fig. 2), also showed stimulation at ca 0.1 g/L, and the end of metabolic activity at approximarely 0.5 g/L of TCE. These photosynthetic endpoints were slightly less sensitive than growth. Response curves at 48 and 72 h were almost identical.

Plates responded in a similar way as enclosures, with an optimal time of approximately 200 h. However, *Chlorella kessleri* exhibited no stimulation (Fig. 3a) (also, in enclosures the stimulation was small, Fig. 1c). *Chlamydomonas reindhardtii* and *Desmodesmus quadricauda*, as well as *Chlorella kessleri* (data not presented) started new growth

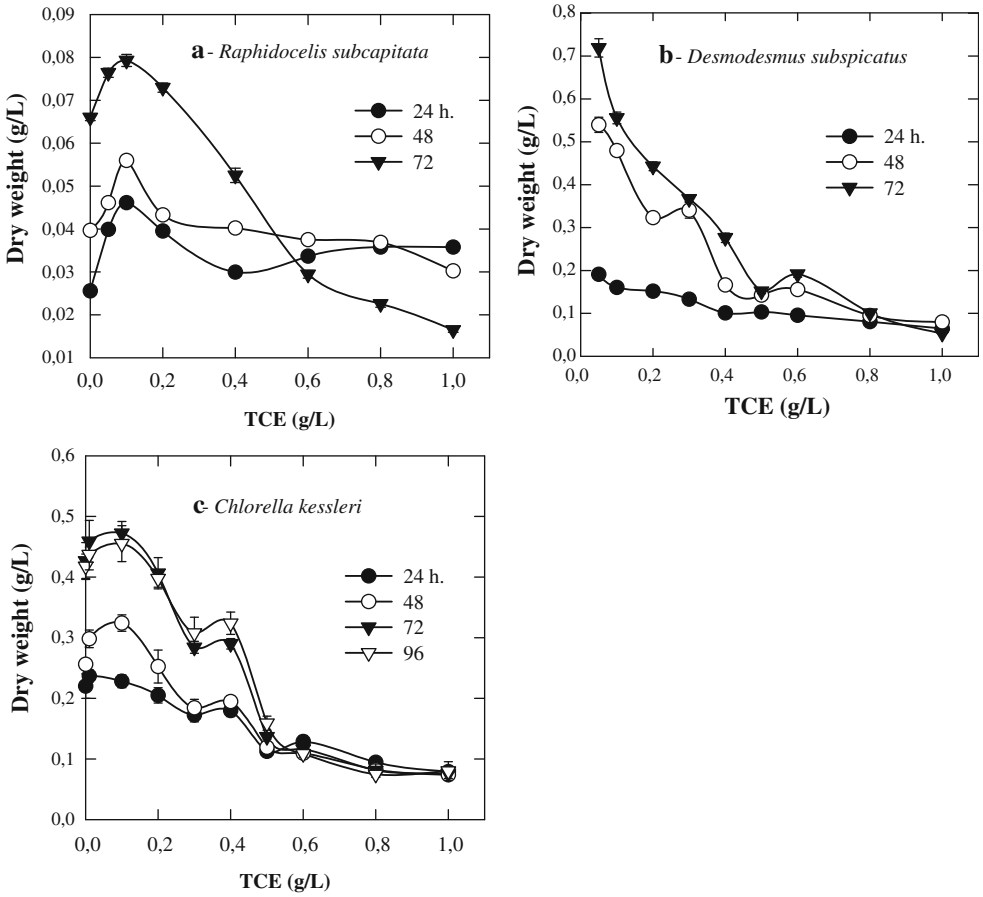

Fig. 1 Dry weights for three species of green algae exposed to TCE in glass enclosures. Data are means ± s_x., evaluated as an exposure assay in enclosures

(at 336 h of cultivation) along the entire concentration gradient of TCE. This may have been due to loss of TCE from the plates. The most sensitive (for plates) proved to be *Raphidocelis subcapitata* and *Desmodesmus subspicatus* (Fig. 3b, d). We were not able to determine if the growth over a long time period was the result of the regeneration of all cells in the population or from the overgrowth of the small proportion of naturally resistant cells.

EC_{50} values for the tested algae ranged from 0.24 to 0.8 g/L TCE (Table 1). The arithmetic means for EC_{50} values were 0.42 for tests performed in polystyrene plates and 0.50 g/L, for tests performed in glass enclosures. These means of EC_{50} values were no significantly different ($t = 0.22$, $P5\% = 2.2$). Likewise, mean EC_{50} values as determined from measurements of oxygen evolution or pH change with TCE exposures, in enclosures, were not significantly different ($t = 0.096$, $P5\% = 2.13$).

There was not significant correlation between EC_{50} evaluated as growth assays in plates and in enclosures (evaluated as OD 750 nm, $r = 0.36$, $P\ 5\% = 0.73$, $n = 6$, Owen 1962). The correlation between assays after O_2 concentration and pH increment, in enclosures, was highly significant ($r = 0.95$, $P\ 1\% = 0.75$, $n = 9$, Tab. 1.) Additionally, O_2 and chlorophyll *a* are in a good correlation (data not presented). Coefficient of variation of an OD750 in the growth assay was 11.6% (arithmetic mean for the whole assay), and 5.9% for an OD750 from enclosures. The optimal time for the assay of TCE in enclosures was 48–72 h; and in plates ca 200 h.

Stimulation effects of TCE is prominent in some of the algae tested, in both methods of testing, enclosures and plates, (Fig. 1a, c, Fig. 2—*Desmodesmus subspicatus*, Fig. 3b–d). The exception was *Chlorella kessleri*, which was tested in plates (Fig. 3a). The stimulation is in concordance with Tadros et al. (1994); however, the mechanism of this stimulation has not been studied yet.

Assay in enclosures, and growth assay in plates each have their own merits. The shorter time for enclosures (48–72 h) vs 200 h in immunological plates, is one obvious advantage. On the other hand, when using miniaturized enclosures it is a disadvantage that it is necessary to use the whole tube for one evaluation of pH, O_2 and OD (for the

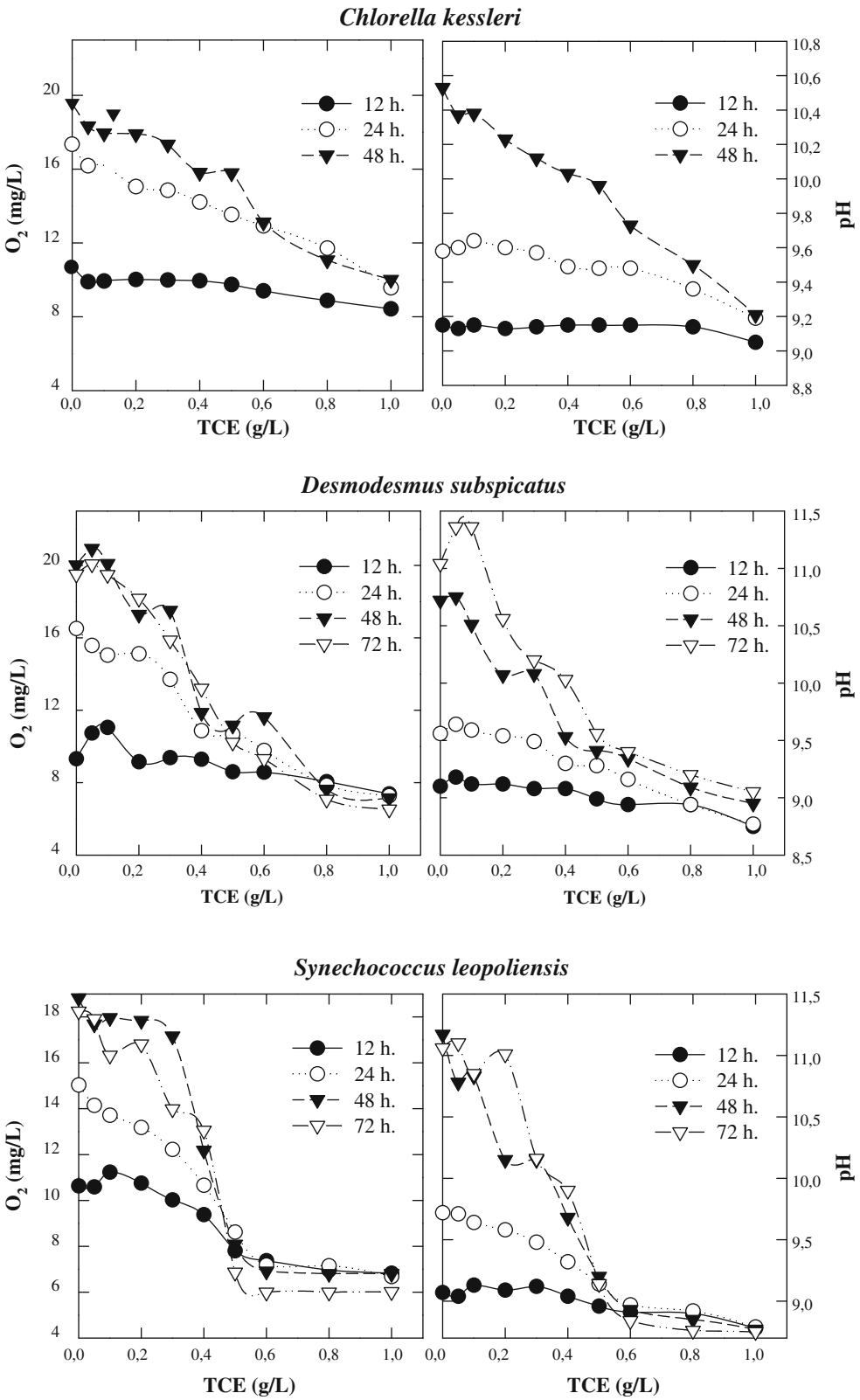

Fig. 2 O_2 evolution and pH level for two species of green algae and one cyanobacterial species to TCE in glass enclosures

latter it is necessary to transfer the suspension into the cell of spectrophotometer). Growth is slower on immunological plates, but it is possible to read the OD directly in the wells repeatedly. It is a non-destructive and very rapid measurement. Miniaturized sensors and electrodes exist, which are capable of measuring pH and O_2 directly in wells.

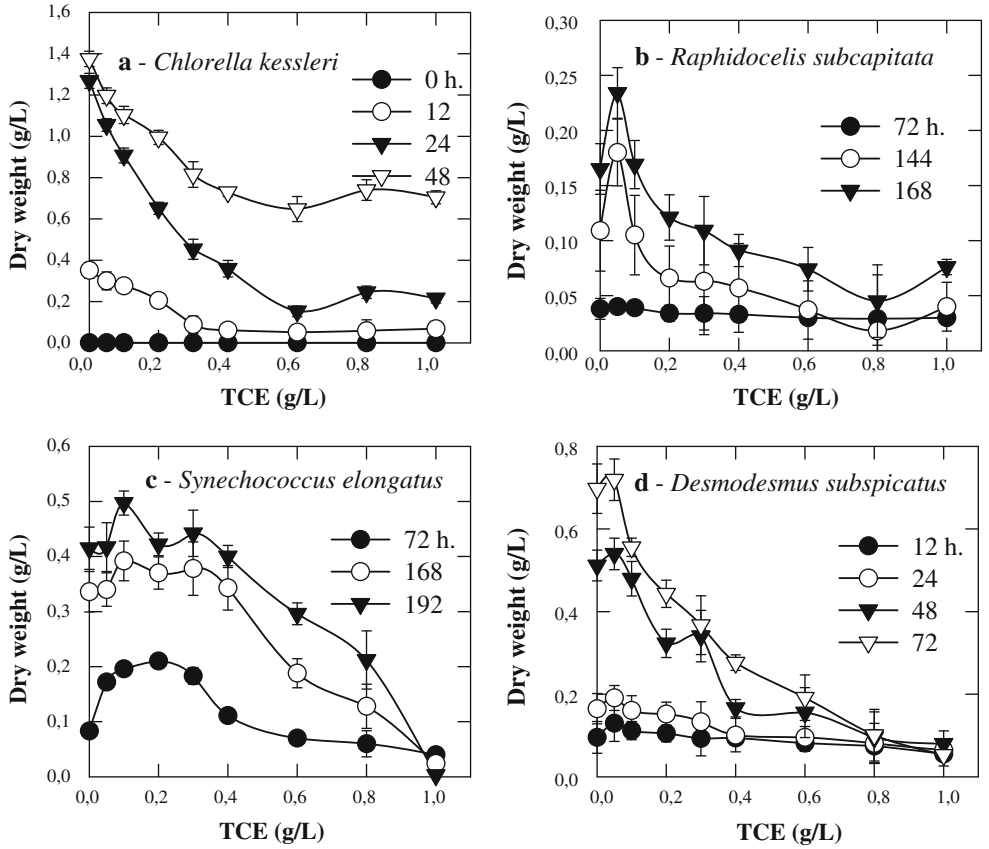

Fig. 3 Dry weights for four species of green algae and one cyanobacterial species exposed to TCE in polystyrene immunological plates. Data are means ± s_x.

Table 1 EC_{50} values for TCE from growth assays (dry weight, DW) in polystyrene plates or glass enclosures, and from measurements of O_2 evolution and pH change in glass enclosures

	EC_{50} of TCE (g/L)			
	Plates	Enclosures		
	DW	DW	O_2	pH
Chlamydomonas reinhardtii	–[a]	0.52	0.70	0.70
Chlorella kessleri	0.24	0.43	0.70	0.70
Raphidocelis subcapitata	0.45	0.22	0.70	0.70
Desmodesmus quadricauda	0.40	0.50	0.50	0.60
Desmodesmus subspicatus	0.35	0.82	0.40	0.40
Synechococcus elongatus	0.80	0.80	0.60	0.70
Synechococcus leopoliensis	0.30	0.60	0.48	0.45
Microcystis aeruginosa	–[a]	0.13	0.10	0.25
Arithmetic mean	0.42	0.50	0.53	0.56
Student test	0.52		0.13	
Correlation	0.36		0.95	

–[a] Not clearly expressed, and for that reason an EC_{50} value was not evaluated

The EC_{50} of TCE was reported to be 8 mg/L for the unicellular marine diatom *Phaeodactylum tricornutum* (Pearson and McConnell 1975). Later Biggs et al. (1979) showed that 0.05–0.1 mg/L of TCE caused no detectable effects upon algal growth or size of mixed cultures containing a marine diatom, *Thalassiosira pseudonana* and a green brackish flagellate, *Dunaliella tertiolecta*. A total lethal concentration equal to 1,000 mg/L TCE for the green alga, *Desmodesmus quadricauda* was reported by Bringmann and Kühn (1980); but Ward et al. (1986) reported an EC_{50} of 95 mg/L TCE for a marine diatom, *Skeletonema costatum*. Tadros et al. (1994) examined the effect of selected solvents on unicellular green algae species to determine whether they differ in their response to these chemicals. They concluded that TCE stimulated the green alga, *Gleocystis ampla* to almost twice the growth compared to controls, at a concentration of 65.7 mg/L. Increasing the concentration of TCE (130 and 260 mg/L) lowered the growth response, but it was still positive. *Nannochloris* sp. and *Tetraselmis* sp. tolerated TCE at all concentrations, while *Scenedesmus obliquus, Chlorococcum* sp. and *Chlorella ellipsoidea* were inhibited with increasing TCE concentrations. Ando et al. (2003) found a great difference in the toxicity of TCE, since the growth of *Chlorella vulgaris* and *Raphidocelis subcapitata* was not influenced up to 3 mg/L, but *Volvulina steinii* was completely inhibited from 0.003 mg/L of TCE.

These data cited above, showed wide variations in responses to TCE, from 0.008 to 1 g/L, among individual species of cyanobacteria, green algae, and diatoms. In our experiments, different species exposed under identical conditions, showed a much smaller range (0.24–0.8 g/L), which highlights the importance of standardizing both the testing protocol and cultivation conditions.

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