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## Abstract

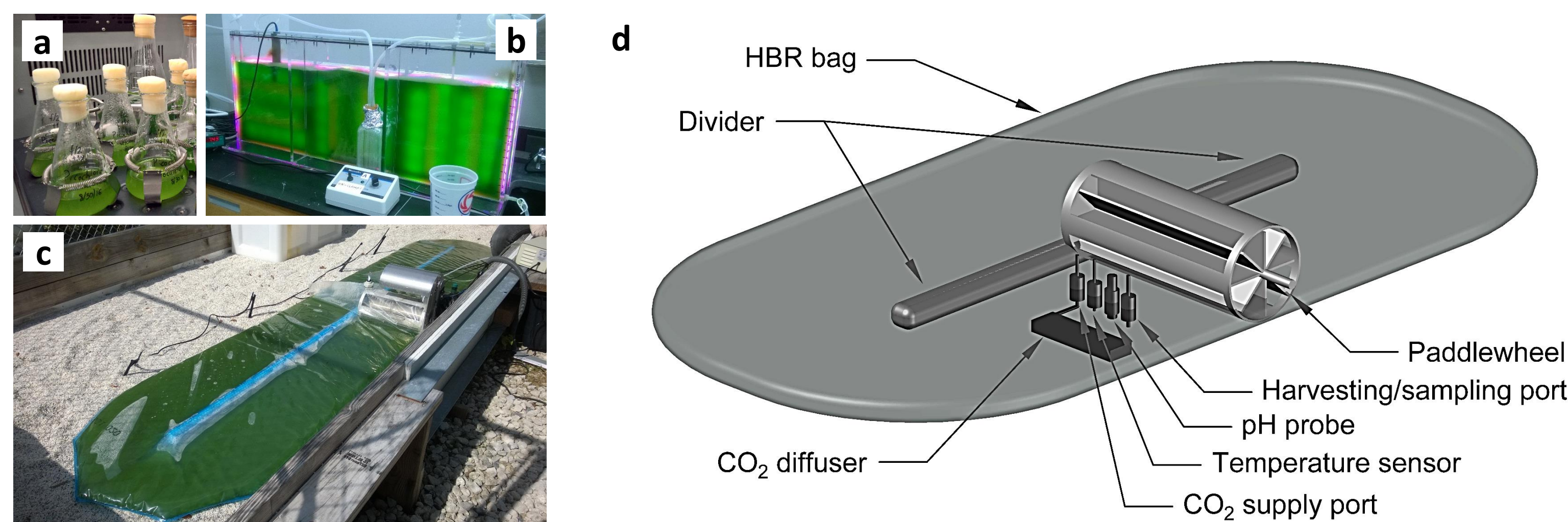
**Background.** The algae industry faces major commercialization challenges as high water and energy demands in large-scale cultivations increase the production cost of algal products, reducing their competitiveness as fossil fuel alternatives. We have designed and previously demonstrated [1,2] a novel algae cultivation system with the potential to reduce the algae cultivation cost. The horizontal bioreactor (HBR) is an inexpensive and modular cultivation system with an enclosed design that protects the culture from contamination and reduces water evaporation.

**Rationale.** In addition to the design of the cultivation unit, the type of water source utilized is a major component of the cost effectiveness and sustainability profile of algal biofuels and bioproducts. Cultivating algae in wastewater sources such as landfill leachate can accomplish a dual role of producing algal biomass and remediating the low-quality water for recycling and reuse [3].

**Objective.** We investigated the use of landfill leachate to cultivate algae in our HBR prototype (150 L working volume, 5 cm water depth, 3 m<sup>2</sup> surface area) in Central Florida under real-world conditions in semi-continuous mode for extended periods.

## Methodology

**Wastewater source.** Landfill leachate (LL) was collected from the confined deep well at the Leachate Treatment Facility at the Charlotte County Zemel Road Municipal Solid Waste Landfill, Florida. The leachate water had passed through biophysical treatment, sand filtration, and chlorination, before being injected into the well. Its low nutrient content (nitrate, phosphate) was verified using water analysis test kits (Hach).



**Fig 1.** Cultivation systems used in the present study: (a) 250-mL flasks; (b) 15-L flat-panel photobioreactor; (c) 150-L HBR; (d) schematic of the HBR.

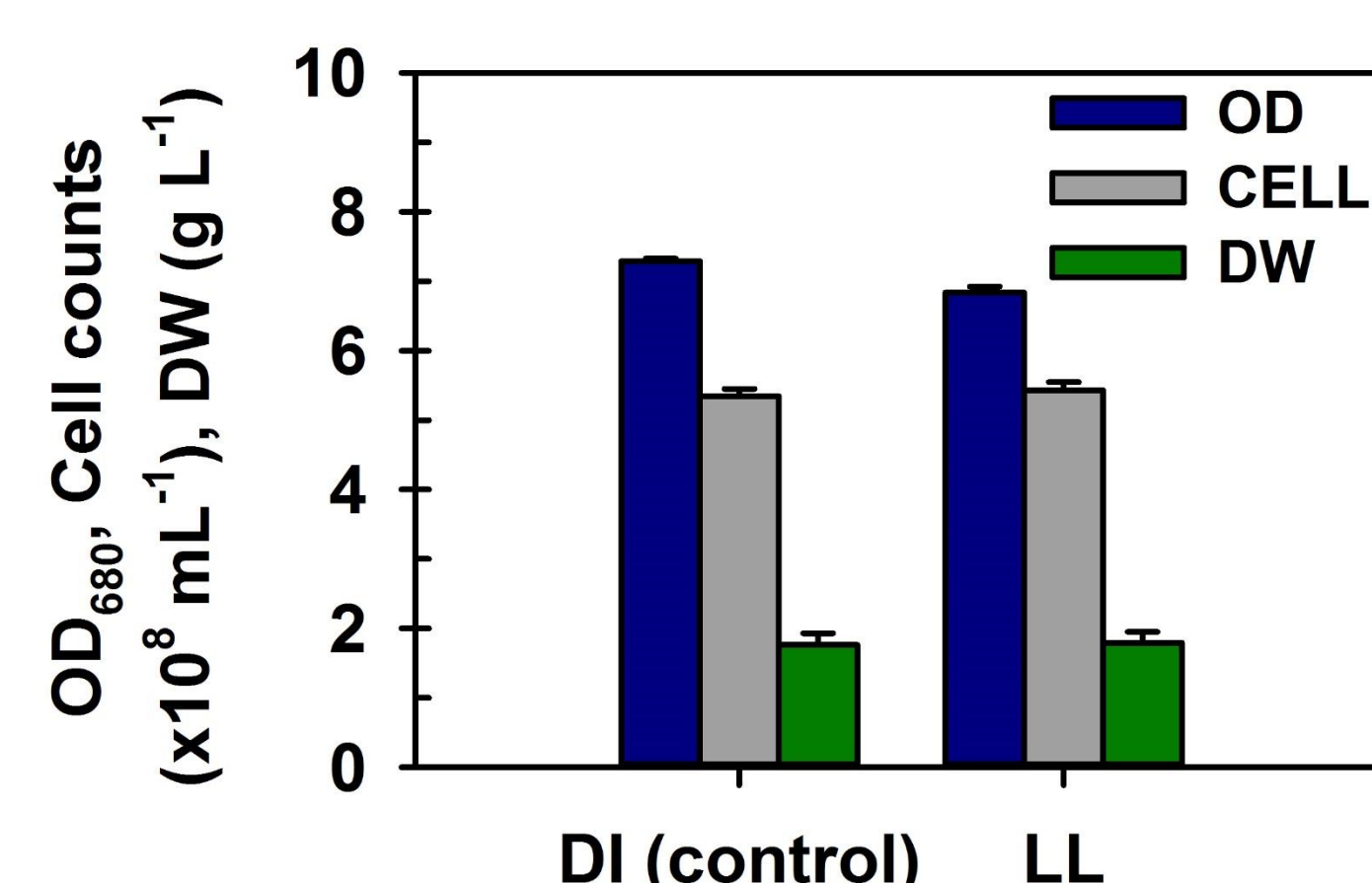
**Cultivation process.** The marine micro-algae strain *Picochlorum oculatum* UTEX LB1998 was grown in treated landfill leachate supplemented with artificial sea salts and nutrients as described previously [1, 2]. Preliminary experiments were conducted in flask cultures (Fig. 1a). Outdoor demonstration in the HBR was performed as follows:

1. *P. oculatum* stock cultures were prepared in 2-L flasks (Fig. 1a) for 2 weeks.
2. A 10% (v/v) inoculum (1.5 L) was transferred from the flask cultures to the 15-L indoor flat-panel photobioreactor (Fig. 1b) and grew for 2 weeks.
2. A 10% (v/v) inoculum (15 L) was transferred from the flat-panel bioreactor to the 150-L HBR (Fig. 1c & d) operating outdoors for 74 days in total.
3. After 37 days (1<sup>st</sup> cycle), 75 L of culture were harvested and replaced with fresh LL and nutrients, followed by a 2<sup>nd</sup> cycle (18 days) and a 3<sup>rd</sup> cultivation cycle (18 days). CO<sub>2</sub> injection in the bioreactors was controlled via pH (set at 7.5), as described [2]. Outdoor ambient temperature, solar irradiance (PAR), HBR temperature, and culture pH were monitored and recorded by a HOBO U30 logger (ONSET). Culture samples were taken regularly for growth monitoring [optical density (OD), dry cell weight (DW) and cell concentration] as described elsewhere [1,2].

## Results – Preliminary tests

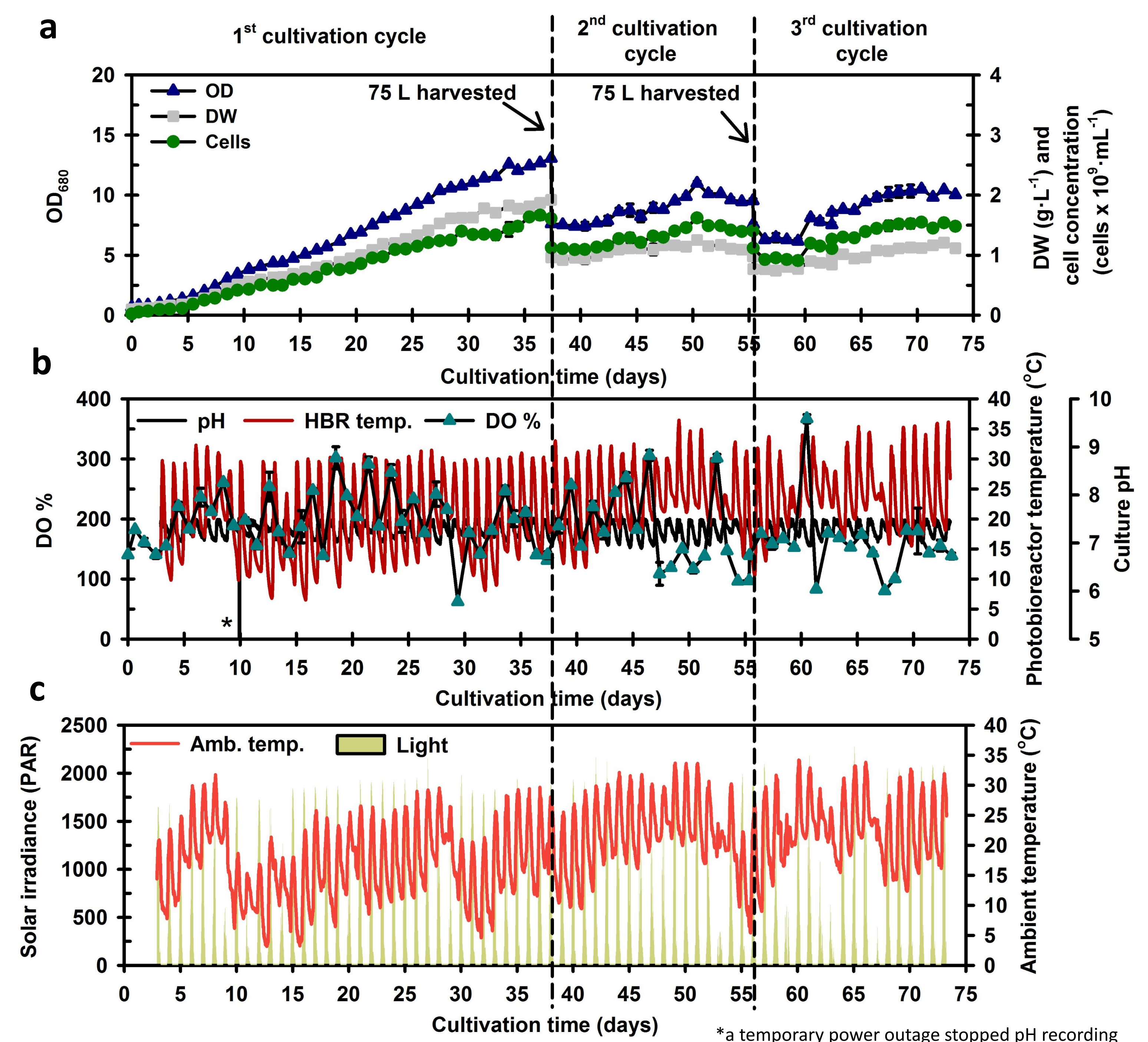
**Table 1.** Profile (macro-nutrients & salinity) of LL water as provided from the treatment facility well and as used after supplementation with nutrients and Instant Ocean for the algae cultivation experiments.

	LL from treatment facility	LL after supplemented w/nutrients & Instant Ocean
Nitrate (mg/L)	5.6	1400
Phosphate (mg/L)	0.75	90
Salinity (%)	0.19	2.77



**Fig 1.** Cultivation of *P. oculatum* in flask cultures for LL toxicity assessment. Growth metrics compared after 31 days (max). Deionized water (DI) served as control growth

## Results – Outdoor demonstration



**Fig 2.** Cultivation of the marine microalgae *P. oculatum* in the 150-L HBR using landfill leachate. (a) Growth metrics [optical density (OD), dry cell weight (DW) and cell concentration]; (b) HBR conditions [pH, temperature, and dissolved oxygen (DO)]; (c) ambient conditions [temperature and solar irradiance (PAR)].

**Table 2.** Algae biomass concentration and productivities achieved in the HBR system during outdoor cultivations, using treated landfill leachate and clean water.

HBR cultivation in	Landfill leachate			Clean water
	1 <sup>st</sup> cycle	2 <sup>nd</sup> cycle	3 <sup>rd</sup> cycle	
<b>Total cultivation time (days)</b>	34	18	18	68 (2x 34)
<b>Areal productivity (g·m<sup>-2</sup>·d<sup>-1</sup>)</b>	average 2.8 (up to 7.9)	average 2.4 (up to 8.2)	average 1.8 (up to 5.3)	average 4.5
<b>Volumetric productivity (g·L<sup>-1</sup>·d<sup>-1</sup>)</b>	average 0.06	average 0.05	0.04	average 0.09
<b>Max biomass concentration (g·L<sup>-1</sup>)</b>	1.9	1.6	1.5	3.5
<b>Max cell density (cells·mL<sup>-1</sup>)</b>	1.67 · 10 <sup>9</sup>	1.25 · 10 <sup>9</sup>	1.21 · 10 <sup>9</sup>	1.75 · 10 <sup>9</sup>
<b>Reference</b>	This study	This study	This study	[2]

## Discussion

- Since N and P levels in leachate were low, the cultures were supplemented with exogenous nutrients (Table 1).
- Preliminary experiments verified the ability of *P. oculatum* to grow in landfill leachate without apparent inhibition compared to clean water (Fig. 1).
- The lag phase in the HBR was shorter after each harvest-dilution step, possibly due to acclimation of the algal cells to the landfill leachate and/or the reduction of low morning temperatures (Fig. 2c).
- High-density growth of *P. oculatum* without contamination issues for long periods of time was achieved, although biomass productivity was less than previous cultivations of *P. oculatum* in clean water and in the same HBR system and location (Table 2).

## Conclusions

- The marine *Picochlorum oculatum* species was able to grow well in landfill leachate in the outdoor HBR, despite harsh semitropical ambient conditions.
- Demonstration of the use of landfill leachate for commercial algae cultivation is underway in our scale-up 2,000-L HBR system.
- **Future research.** Cultivation of other types of algae, including freshwater species, in landfill leachate in the HBR will expand its applicability for sustainable production of algal biomass for biofuels and bioproducts.

## References

- [1] Dogaris I, Welch M, Meiser A, Walmsley L, Philippidis G. A novel floating horizontal photobioreactor for high-density cultivation of microalgae. *Bioresour Technol* 2015;198:316-324.
- [2] Dogaris I., Brown T.R., Loya B., Philippidis G. Cultivation study of the marine microalgae *Picochlorum oculatum* and outdoor deployment in a novel bioreactor for high-density production of algal cell mass. *Biomass Bioenergy* 2016;89:11-23 (\*contributed equally)
- [3] Edmundson S.J., Wilkie A.C. Landfill leachate – a water and nutrient resource for algae-based biofuel. *Environ Technol* 2013;34(13-14):1849-1857.

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