Starch-rich *Chlorella vulgaris* production strategies and costs at semi-industrial scale in different photobioreactors

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INTRODUCTION and AIMS

The freshwater microalga *Chlorella* genus is known to have high starch-producing strains[1], one of which being *Chlorella vulgaris* CCALA924[2]. At our laboratory, up to 70% (w/w) of starch has successfully been achieved in this strain under nitrogen starvation and high light[3], with the starch representing a promising feedstock for bioplastics production[4]. One of the aims of the Nenu2PHar project is to scale-up starch-rich *Chlorella* production to take the proof of concept closer to industrialisation. Thus, outdoor growth trials in two 180 L flat panel airlift reactors with static mixers (FP_mixers) were performed from Nov 2021 to Aug 2022 at our R&D pilot platform to establish the ease of culturing of the CCALA924 strain and the range of biomass and starch productivities achievable at pilot-scale. In parallel, the same strain was grown in three other photobioreactors (PBRs) of different design to collect data on biomass productivity and operational expenses (OPEX). The aim was to determine the most economically-attractive PBR design for large-scale implementation.

METHODS

Chlorella vulgaris CCALA924 was grown in an adapted Beijerinck medium in four different photobioreactors whose geometry, dimensions and locations are detailed in Table 1. For starch production, two FP_mixers PBRs were inoculated with fresh medium and the cultures were left to become nitrogen-starved naturally over two weeks of growth. Starch quantification was performed on lyophilised biomass using the Dubois[5] method. The PBRs were cleaned with 0,03%(v/v) NaOH solution, disinfected with 0,05% (v/v) peracetic acid and rinsed with water after each harvest.

Table 1. Environmental, growth conditions and photobioreactor design descriptions

Variables	Raceway	Tubular-PBR	FP-PBR	FP-Bag
Culture depth (m)	0,15	0,08	0,03	0,1
Volume (L)	1300	900	180	180
Dimensions (mxm)	5 x 2	4,5 x 9	2,5 x 1,8	2 x 0,75
Light environment	Greenhouse	Greenhouse	Greenhouse	Outside
Temperature	Unregulated	Cooling (25-30°C)	Cooling (25-30°C)	Cooling (25-30°C)
CO2	pH stat @ 6,8	Continuous	pH stat @ 6,8	pH stat @ 6,8
Mixing	Depression column	Pump	Bubbling	Bubbling

The PBR comparison study was carried out between March and July 2022 in a 1300 L open raceway pond equipped with a culture mixing depression column (Fig. 1A), a 900 L tubular PBR (Fig. 1B), the FP_mixers (Fig. 1C) and disposable LDPE bags (FP_bag) (Fig. 1D). Data on consumables (water, fertilisers, cleaning products), labour hours and energy consumed for cultivation and harvesting were collected for techno-economic analyses.

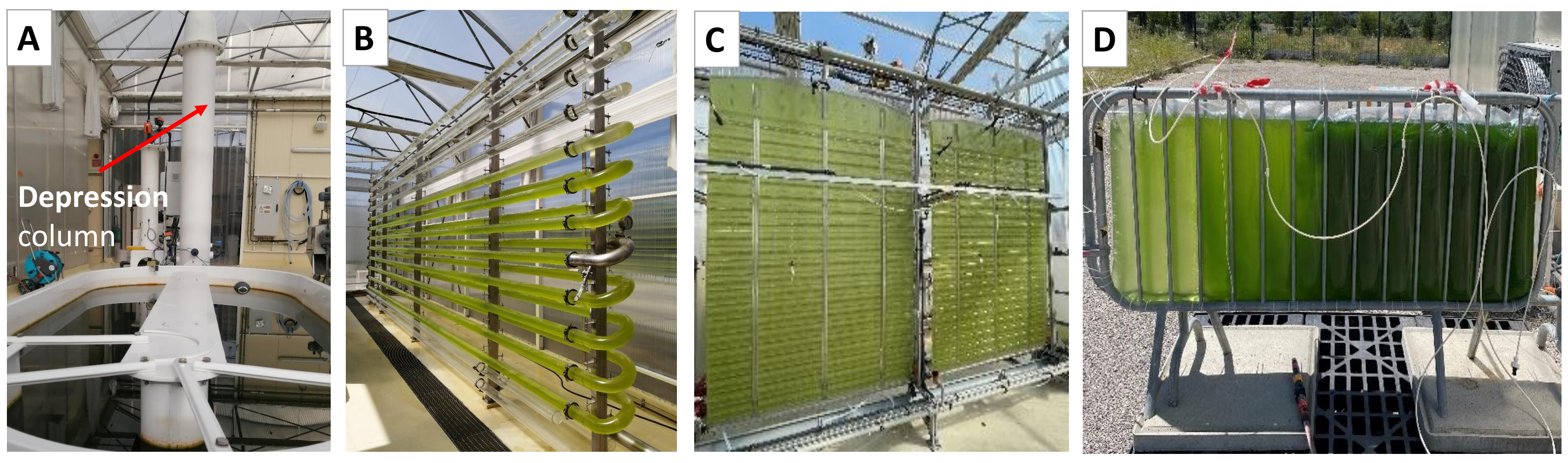

Figure 1. Types of photobioreactors used for the techno-economic analysis study

RESULTS and DISCUSSION

Starch production in FP_mixers

FP_mixers (Fig. 1C) are thin wall panels pinched at regular intervals (which create the static mixers) such that the culture and air bubbles coming from the bottom of the reactor are forced upwards through a narrow turbulent path, thereby drastically increasing gas and nutrient exchange, as well as light exposure to each algal cell. Thus, high biomass yields and sustained starch accumulation (20-60%) have repeatedly been observed in ageing but healthy cultures (e.g. Nov 21, Jan 22, May 22 and July 22) (Fig 2).

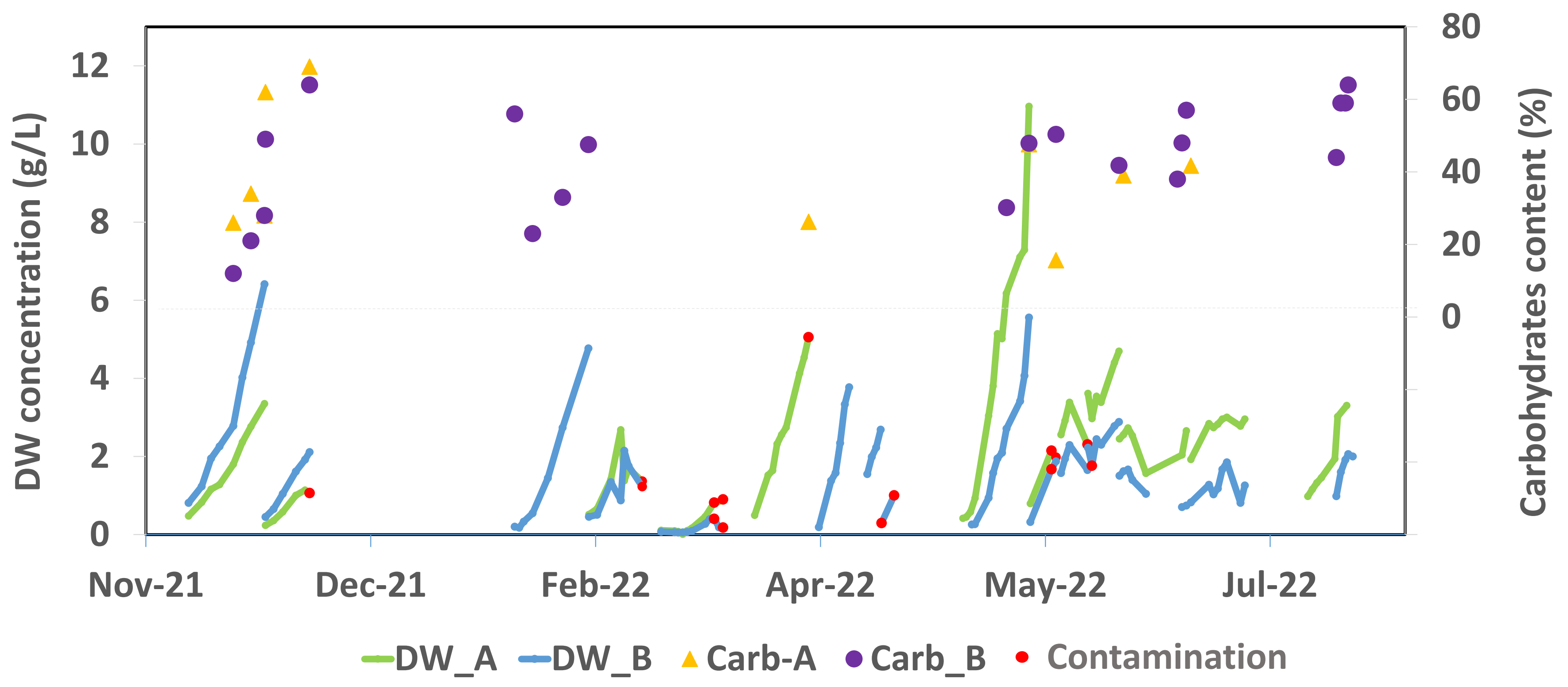

Figure 2. Dry biomass concentration and starch accumulation in the *Chlorella* cultures from Nov 2021 to Aug 2022 in flat panel reactors with static mixers.

The first signs of contamination by a predatory and mixotrophic chrysophyte, *Poterioochromonas* sp. (Fig. 3) were observed as early as Nov 21 and thereafter, it persisted in the FP_mixers throughout the growth period despite lengthy and arduous cleaning and disinfection CIP procedures.

Figure 3. *Poterioochromonas* sp. with two flagella of unequal lengths (black arrow) and with an engulfed *Chlorella* cell (red arrow).

The contamination episodes were unpredictable, hard to detect and most severe in nitrogen-deprived cultures, resulting in irregular growth and poor biomass quality and harvests, and no clear distinction in seasonal effects on biomass and starch productivity. Despite these setbacks, our results (Table 2) are in line with those obtained in outdoor starch production pilot-scale studies from *Chlorella*[2,6]

Table 2. Biomass and starch productivities in FP_mixers between Nov 21 and Jul 22

Production parameters	Mean	Max
Volumetric biomass productivity (g/L/d)	0,35	0,91
Starch content (%)	44	69
Starch productivity (g starch/L/d)	0,15	0,63

Techno-economic analysis of algal biomass production in different PBR designs

On an illuminated surface basis, the areal biomass productivities of all four PBRs in this study (Table 3) are similar to those published by the PBR manufacturers and others[7]. Unexpectedly, the cost per unit biomass of the raceway is higher than the closed PBRs (Fig. 5), which is in contradiction with the literature[8]. Likely explanations for such a high biomass cost are intensive-energy processes for the culture mixing (vacuum pump used), poor culture mixing, and relatively high labour cost (Fig. 4) for low biomass yields.

Table 3. Biomass productivities in four types of PBRs

Production parameters	Raceway	Tubular	FP_mixers	FP_Bag
Volumetric productivity (g/L/d)	0,06	0,39	0,91	0,26
Areal productivity for illuminated surface (g/m² illum./d)	7,2	8,7	36,4	31,2
Areal productivity for reactor ground surface (g/m² ground/d)	7,2	78,0	148,9	234,0
Areal productivity for reactor+ancillary equipment ground surface (g/m² Total/d)	4,0	17,1	52,8	66,9

Of the three closed PBRS, the tubular design was the least productive, which could be due to a wider light path, less vigorous mixing as compared to active bubbling in the FP PBRs and thus less efficient gas and nutrient exchange and light exposure. On the other hand, because of its self-cleaning feature, it is the most practical setup for overall maintenance and long term cultivation. Given higher biomass productivities, it could be on par to be as economical as the flat panel PBRs.

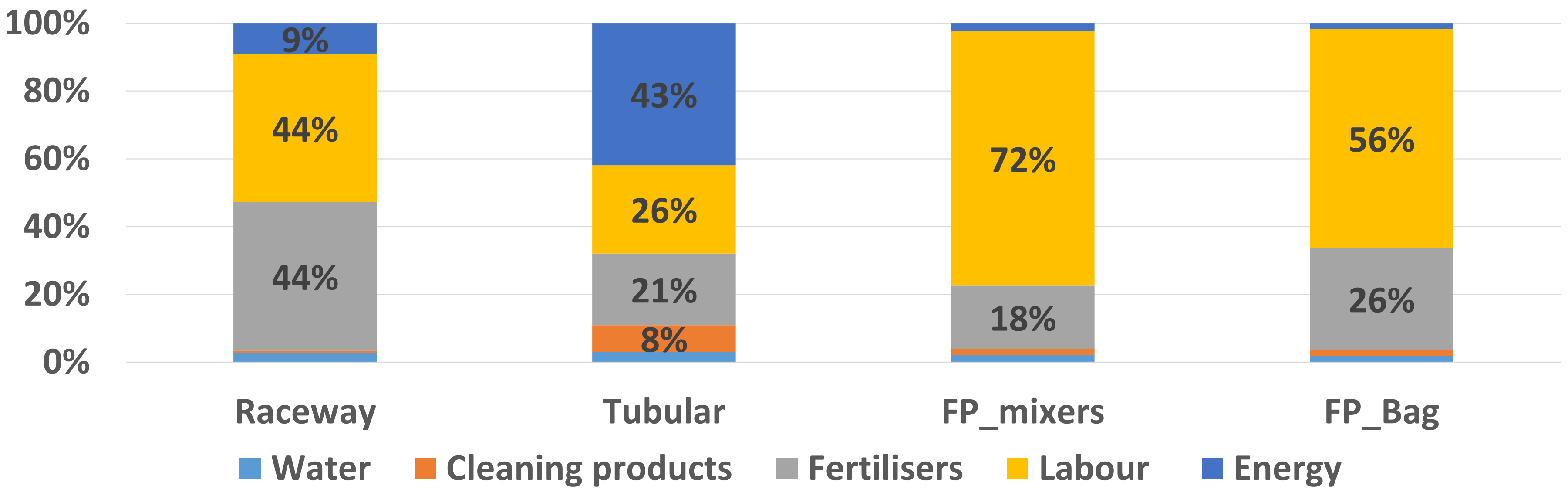

Figure 4. Cost percentages per OPEX category

The FP_mixers PBR was the most productive (Table 3) of the flat panel PBRs and the least costly per kilogram of biomass produced (Fig. 5) despite its excessive labour cost (72% of OPEX). This PBR is easily prone to biofouling and is difficult to clean, thus imposing regular and lengthy downtimes accompanied by long labour hours and large amounts of cleaning products over a year.

The FP_bag, despite its slightly higher cost (Fig. 5), seems to be a commercially attractive option on a large scale due to its very high biomass productivities relative to the land surface required for the PBR and ancillary equipment (Table 3).

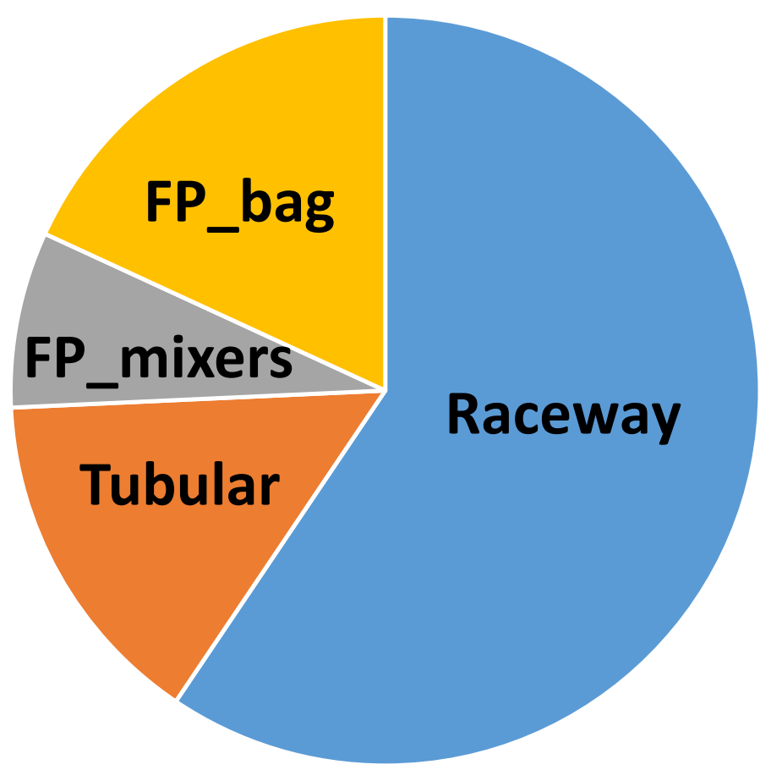

Figure 5. Relative cost per Kg of biomass between four PBR types

CONCLUSIONS

This is the first report of our pilot-scale starch and biomass production capacity at our R&D platform. Our baseline data and the invaluable experience gathered during the experiments show that it is indeed possible to scale up starch production and that flat panel reactors would probably be the most economical option for large-scale implementation. Contamination by the *Poterioochromonas* sp. poses a persistent challenge and should be addressed with urgency. Simulating production with industrial operational cost inputs will certainly bring productivity and the techno-economic outcomes closer to reality than those obtained in this study.

REFERENCES and ACKNOWLEDGEMENTS

[1] Takeshita T. *et al*. (2014) Starch and lipid accumulation in eight strains of six Chlorella species under comparatively high light intensity and aeration culture conditions. Bioresourc. Technol. 158:127-134. doi.org/10.1016/j.biortech.2014.01.135
[2] Brányiková I. *et al*. (2011), Microalgae—novel highly efficient starch producers. Biotechnol. Bioeng., 108: 766-776. doi:10.1002/bit.23016
[3] Compadre A. *et al*. (2021) *Chlorella vulgaris*, a promising feedstock for starch-based bioplastics, Algae Europe 2021, Oral presentation
[4] Alvarez P. *et al*. (2021) *Chlorella Vulgaris* High-glucose syrup as carbon feedstock for PHA-producing bacteria, Algae Europe 2021, Oral presentation
[5] Dubois M. *et al*. (1956) Colorimetric method for determination of sugars and related substances. Anal. Chem. 28(3):350-356. doi.org/10.1021/ac60111a017
[6] Carnovale G. *et al*. (2021) Starch Rich *Chlorella vulgaris*: High-Throughput Screening and Up-Scale for Tailored Biomass Production, Appl. Sci. 11 (19):9025. doi.org/10.3390/app11199025
[7] Zittelli G. C. *et al*. (2013) Photobioreactors for mass production of microalgae. In Richmond A. and Hu Q. (Eds.) *Handbook of microalgal culture: Applied Phycology and Biotechnology* (pp.225-266), UK: Blackwell Publishing Ltd
[8] Clippinger J. and Ryan D. (2019) Techno-Economic Analysis for the Production of Algal Biomass via Closed Photobioreactors: Future Cost Potential Evaluated Across a Range of Cultivation System Designs. Golden, CO: NREL/TP-5100-72716. https://www.nrel.gov/docs/fy19osti/72716.pdf

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