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Comparing continuous and perfusion cultivation of microalgae on recirculating aquaculture system effluent water

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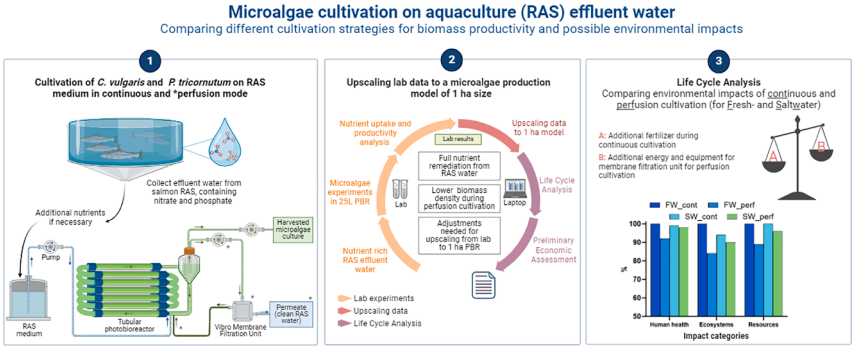
HIGHLIGHTS

- Stable perfusion cultivation through Vibro membrane filtration (up to 3.59 g L^{-1}).
- Bioremediating low nutrient concentrations through perfusion cultivation.
- Life Cycle Assessment reveals lower environmental impact of perfusion cultivation.
- Photobioreactor materials and cultivation energy impact sustainability the most.
- Membrane filtration leads to significantly higher expenses.

GRAPHICAL ABSTRACT



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ABSTRACT

Effluent water from recirculating aquaculture systems (RAS) contains nutrients from fish excrements and leftover feed. This study investigated the nutrient remediation potential from RAS effluent water through microalgae cultivation in 25 L tubular reactors. We compared nutrient uptake and biomass productivity in continuous and perfusion cultivation modes for freshwater, brackish water and saltwater. Stable high biomass densities were achieved with additional nitrate during continuous cultivation (up to 3.88 g L^{-1}) or by membrane filtration during perfusion cultivation (up to 3.59 g L^{-1}). A life cycle assessment (LCA) compared the two different cultivation modes in terms of environmental sustainability on a 1 ha scale. The LCA and preliminary economic assessment showed that perfusion cultivation appears to have a lower environmental impact for relatively low nutrient concentrations, but additional equipment and higher energy demands are leading to increased operational (+6 %) and capital expenses (up to +60 %).

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1. Introduction

One of the main principles in a circular economy is to ensure that the existing value chains are utilised to their full capacity, including reducing side- and waste streams by considering them as a resource for further valorisation (Muscat et al., 2021; Velenturf and Purnell, 2021). Norway's seafood industry is the second biggest contributor to the country's gross domestic product (GDP) and offers a high potential for better utilisation of residual raw materials (Johansen et al., 2019). Technology advancements in salmon production, such as closed containment systems or land-based recirculating aquaculture systems (RAS), facilitate the possibility of capturing the waste products from fish production. Side streams from RAS include nutrient-rich effluent water that needs to be partially removed continuously due to nitrogen accumulation in the system (Van Rijn, 2013). According to Schneider et al. (2005), only 20–50 % of the nitrogen contained in the fish feed is converted into fish biomass, the remaining 50–80 % of the nitrogen load is discharged as either solid waste in the sludge or dissolved in the RAS water. Nutrient concentrations of this effluent water vary between systems, fish species and feeding regimes, and are reported between 100 and 200 mg L^{-1} nitrate (NO_3^-) and 1–20 mg L^{-1} phosphate (PO_4^{3-}). This effluent water is often released directly into nearby water bodies, depending on regional/national regulations and required discharge permits (Bregnballe, 2022). Although nutrient concentrations of the RAS effluent water are often within the legal limits for discharge, the large water volumes emitted by RAS lead up to a substantial amount of nutrients released within a year. For a common sized RAS producing 1000 tons of fish per year, this would result in the release of 21 tons of nitrogen in the effluent water (assuming a feed conversion rate of 1.1 (Bregnballe, 2022) and no additional biological N and P removal).

One strategy to recover nutrients from RAS effluent water is biological remediation by microalgae cultivation (Ramli et al., 2020). Established cultivation systems such as tubular photobioreactors have been proven to provide a stable and controlled production environment for a variety of algae and cultivation conditions (Acién Fernández et al., 2013). Microalgae water treatment of municipal and sewage wastewater has been studied thoroughly (Mohsenpour et al., 2021), although nutrient concentrations in wastewater treatment plants are usually higher than in RAS effluent water. The nitrogen and phosphorus concentrations of the RAS effluent water change over time and are highly dependent on the production system (Qiu et al., 2022), but are usually lower than what is used in industrial microalgae cultivation. Such lower nutrient concentrations lead to lower biomass densities and productivity as long as the microalgae culture is not light-inhibited (Richmond and Hu, 2013). However, for industrial production high algal biomass densities are needed to maximize the cost-benefit of the cultivation infrastructure. Microalgae cultivation is often presented as an "environmentally friendly" water treatment (Plöhn et al., 2021). Yet, it is not guaranteed that closing the nutrient cycle of aquaculture systems is more environmentally sustainable than the traditional treatment where nitrate and phosphorus are released with the discharged water. We need to quantify the effect of the waste treatment scenarios, as increased energy consumption and necessary infrastructure and equipment might cancel out any positive effect on resulting environmental impacts.

This manuscript explores the possibilities of a cultivation system that is adaptable to different water retention times through perfusion cultivation. This could allow for full nutrient remediation of a relatively low nutrient concentrated medium while obtaining high microalgae biomass productivity without additional fertilizer. Perfusion cultivation by means of membrane filtration is not a novel technology, but there is hardly any literature reporting this type of cultivation for low-nutrient water treatment.

The experiments presented in this manuscript compare two different microalgae cultivation modes on RAS effluent water from salmon production. We compared nutrient uptake and biomass productivity in continuous and perfusion cultivation modes for freshwater with *Chlorella vulgaris* and brackish water and saltwater with *Phaeodactylum tricornutum*. During continuous cultivation mode, RAS effluent water-based medium is constantly added, while the culture is harvested at the same rate. In perfusion cultivation mode, the RAS effluent water-based medium is added according to the microalgae's nutrient uptake, while the harvesting rate is the same as in continuous cultivation. The implementation of a membrane filtration step during perfusion cultivation allows uncoupling water retention times from biomass density in the reactor to cover the microalgae culture's nutrient requirements (Bilad et al., 2014). To achieve equally high biomass productivity in both scenarios, extra nitrate is added to the RAS effluent water during the continuous phase to ensure a stable cultivation with a high biomass density. The different cultivation methods require different equipment, have different energy requirements, as well as additional fertilizer needs. Based on the experimental results gathered in this study, the possible upscaled cultivation on a 1 ha scale is modelled, and four different scenarios are compared using a life cycle assessment (LCA) and a preliminary economic assessment. These projections are important for decision-making for further research and choosing relevant production methods for nutrient remediation of low-nutrient water streams.

2. Materials and methods

2.1. Cultivation experiments

2.1.1. Aquaculture effluent water

The aquaculture effluent water used in the experiments was obtained from Marineholmen RASlab AS (Bergen, Norway). The effluent was sourced from 3 m^3 RAS modules (Alpha Aqua A/S, Esbjerg, Denmark) containing a fish tank (0.8 m3), swirl separator, mechanical filter and biofilter, as described more detailed in (Böpple et al., 2024). The water was collected daily during the experimental phase and was immediately filtered through four household water filtration units (VF-1, A-collection) with replaceable in-line filter cartridges with subsequent pore sizes of 50 μm, 20 μm, 10 μm, and 0.2 μm. Nutrient concentrations (nitrate, phosphate) of the collected RAS effluent water were analysed and are presented in Table 1, Section 2.1.4.

2.1.2. Microalgae inoculum production

Two microalgae species were used in this work. Freshwater experiments were conducted with the green algae *Chlorella vulgaris* (NIVA-CHL-108, NORCCA). For the brackish and saltwater experiments (16.5 ppt salinity for brackish water and 32.5 ppt in salt water), a local isolate of the diatom *Phaeodactylum tricornutum* was used, earlier described as N58 by Prestegard et al. (2009). Stock cultures were kept in 100 ml Erlenmeyer flasks. Inoculum for the 25 L experiments was produced in ten 300 ml round-bottom bubble column cultures, which are described in more detail in Böpple et al. (2024). Microalgae cultures were grown to a high density ($OD_{750} > 10$) with aeration of 1 % CO_2 enriched air and illumination of 300 μmol m^{-2} s^{-1}. For the inoculum production of *C. vulgaris* a modified 3 N Bold Basal medium with following nutrient concentrations was used: 8.82 mM NaNO3; 0.43 mM K2HPO4; 1.29 mM KH2PO4; 0.17 mM CaCl2··2H2O; 0.3 mM MgSO4··7H2O; 0.43 mM NaCl; 0.031 mM B; 0.002 mM Cu; 0.043 mM Fe; 0.017 mM Mn; 0.001 mM Mo; 0.008 mM Zn; $2.96 \cdot 10^{-4}$ mM vitamin B1; $3.69 \cdot 10^{-7}$ mM vitamin B12; $2.05 \cdot 10^{-6}$ mM Biotin. The *P. tricornutum* strain was kept on NORCE medium with concentrations of 12.5 mM $NaNO_3$; 0.88 mM KH_2PO_4; 0.031 mM B; 0.002 mM Cu; 0.043 mM Fe; 0.017 mM Mn; 0.001 mM Mo; 0.008 mM Zn. The inoculation ratio for batch and

perfusion cultivation experiments was circa 10 % (giving a start OD_{750} < 1). More details on the continuous cultivation inoculation scheme are found in the experimental setup section 2.1.4.

2.1.3. Photobioreactor setup

For the microalgae cultivation experiments, two 25 L tubular photobioreactors (LGem BV, The Netherlands) were used. The reactors were pH controlled through the automatic addition of 100 % CO_2 in the ingoing airstream, while mixing the culture by creating a wave-like movement throughout the glass tubes (resulting in a liquid working volume of 18–20 L). The reactors were placed in a temperature-controlled room at 23 °C (for *P. tricornutum*) and 25 °C (for *C. vulgaris*). Illumination was provided by dimmable LED lights (maximum of 525 µmol m^{-2} s^{-1}).

An additional pump for medium supply and continuous harvest (at the same rate) was used (Watson-Marlow 323). Medium was continuously added at the reactor's bottom and algae culture was harvested from the mixing vessel at the top. For perfusion experiments, a membrane filtration unit with Vibro® technology was added (equipped with an 800 kDA membrane; SANI Membranes, Denmark), where the filtration retentate with algae was fed back to the PBR (Watson-Marlow 530), as shown in Fig. 1.

2.1.4. Experimental setup

Initial nutrient concentration measurements of the RAS effluent water indicated deficient phosphate concentrations, lower nitrate concentrations and negligible ammonium concentrations compared to the standard growth media. No trace metal analyses were performed in the scope of this study. Additional phosphate and trace minerals were added to all experiments following standard growth media concentrations to ensure non-depletion growth conditions (Table 1). RAS effluent water with additional nutrients is called RAS medium hereafter. Firstly, batch cultivation experiments for all three RAS effluent water salinities were performed to determine maximum specific growth rates and the biomass densities achieved on the available nutrients to estimate the dilution rate and biomass density during steady state for the continuous cultivations. Batch cultivations and continuous cultivations were performed twice for each water type, once with additional nitrate (added according to NORCE medium concentrations described in 2.1.2), as well as without additional nitrate to examine the timepoint of nutrient limitation on the RAS effluent water and the maximum biomass reached. Based on those results, a dilution rate (and equal harvesting rate) of 10 L d^1 for all continuous cultivation experiments was chosen (aiming for high productivity while being able to compare results between the species). The continuous cultivation experiments were inoculated with a microalgae culture from a second 25 L reactor that was kept in growth phase through weekly dilution. The starting OD_{750} was based on the same high biomass density as determined in the batch experiments (OD_{750} ~ 10 for *C. vulgaris* and OD_{750} ~ 15 for *P. tricornutum*). Perfusion cultivation experiments were started with a low biomass density (OD_{750} < 1), and harvesting started only once the biomass concentration as determined during the steady phase of continuous experiments was achieved (OD_{750} ~ 10 for *C. vulgaris* and OD_{750} ~ 11 for *P. tricornutum*). This was to avoid the presence of leftover nutrients from an inoculum with high biomass density or inoculation with a stressed culture that was grown under nutrient-depleted conditions.

Perfusion cultivation experiments had the same harvesting rate as the continuous cultivation (10 L d^{-1}), but a higher inflow of RAS medium to provide sufficient nitrogen to cover the nitrate uptake during cultivation without additional nitrate. The RAS medium inflow (L d^{-1}) was determined based on nitrate measurements of the harvested culture to ensure the culture was not nitrogen-depleted. A technical limit of a

Table 1
Overview of experiment abbreviations, nutrient concentrations and water flows of the continuous and perfusion mode experiments. RAS effluent water with added nutrients (N, P and trace metals for continuous mode and only P and trace metals for perfusion mode) is called RAS medium. Nutrient concentration values of the RAS medium were measured with each media renewal and are averaged over the cultivation period, the standard deviation is shown as ±.

	FW_cont	FW_perf	BW_cont	SW_cont	SW_perf
Water type	Freshwater	Freshwater	Brackish	Saltwater	Saltwater
Cultivation mode	continuous	perfusion	continuous	continuous	perfusion
Temperature (°C)	25	25	23	23	23
pH-control	7.0–7.5	7.0–7.5	7.5–8	7.5–8	7.5–8
NO_3^- concentrations in RAS effluent water (mg L^{-1})	291	160	193	297	342
PO_4^{3-} concentrations in RAS effluent water (mg L^{-1})	1.6	1.6	6.4	10	10
NO_3^- concentration in RAS medium (mg L^{-1})	3172 ± 219	494 ± 192	3408 ± 81	3395 ± 205	342 ± 8
PO_4^{3-} concentration in RAS medium (mg L^{-1})	443 ± 59	110 ± 24	258 ± 40	191 ± 19	37 ± 9
RAS medium inflow (L/d)	10	40	10	10	40
Culture harvest (L/d)	10	10	10	10	10
Permeate outlet (L/d)	–	30	–	–	30

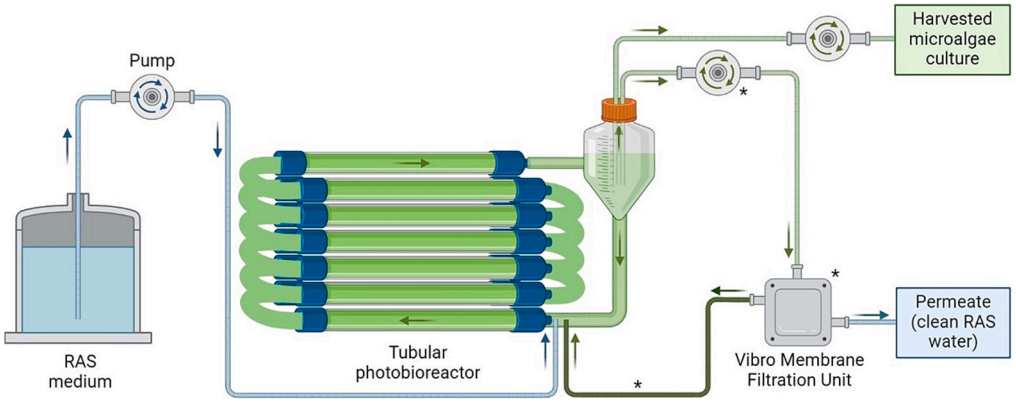

Fig. 1. Schematic drawing of the reactor setup for perfusion cultivation and continuous cultivation mode (asterisks indicate flows/equipment belonging to the membrane filtration that is only used for perfusion mode).

maximum medium inflow of 40 L d^{-1} during perfusion cultivation resulted in the necessity to add additional nitrogen to the RAS effluent water that was collected on days with unusually low nitrogen concentrations (due to a change in the cleaning process of the RAS tanks).

The medium was stored in an autoclaved 60 L closed container and refreshed when the media volume was below 10 L. Sampling was conducted at the same time points daily, collecting culture samples from the PBRs and water samples for nutrient analyses from both the medium and the outflow after membrane filtration. Cultivation duration for continuous and perfusion cultivation was aimed to be at least 3 days into steady state, during which the microalgae's specific growth rate should equal the dilution rate.

2.1.5. Biomass and nutrient measurements

Biomass densities during the experiment were measured daily through optical density (OD_{680} nm and OD_{750} nm, V–1200 spectrophotometer, VWR) and dry weight measurements. Samples for dry weight determination were filtered through glass microfiber filters (GF/F, 47 mm, pore size 0.7 µm, Whatman International Ltd). Brackish and seawater cultures were then washed three times with ammonium formate (0.5 mM) according to Zhu and Lee (1997), freshwater samples were washed with osmosis water accordingly. The filters were then dried in an oven at 95 °C for 24 h and weighed (microbalance MT5, Mettler Toledo, Switzerland) after cooling off for two hours in a desiccator. The GF/F filters were prewashed, dried (>24 h at 95 °C) and weighed. The maximum quantum yield (QY) was measured with an AquaPen-C AP100 (Photon Systems Instruments, Czech Republic). Phosphate and nitrate concentrations of the RAS effluent water and medium were determined by colourimetric test kits with a compact photometer (PF-12Plus, Macherey-Nagel). Nitrate was measured as NO_3^- with the VISOCOLOR ECO Nitrate kit (Macherey-Nagel). Phosphate was measured as PO_4^{3-}, with the VISOCOLOR ECO Phosphate kit (Macherey-Nagel). Analyses were performed according to the kits' manual, and samples were diluted when needed to keep concentrations within the measuring range (1 – 60 mg L^{-1} NO_3^- and 0.6 – 15.0 mg L^{-1} PO_4^{3-}).

2.1.6. Calculations

Average daily biomass productivity ($P_{C,X}$ in g_{DW} L^{-1} d^{-1}) was calculated based on the dryweight of the microalgae biomass (B_x in g_{DW} L^{-1}) multiplied with the dilution rate (D in d^{-1}).

$$P_{C,X} = Bx^{*}D \tag{1}$$

Nutrient uptake per day (mg L^{-1} d^{-1}) was calculated as the difference in nutrient concentration in the RAS medium and the nutrient concentration in the harvested culture every 24 h. For the perfusion cultivation, the difference in ingoing RAS medium (Med) and outgoing harvested culture had to be taken into account, as shown in Equation (3) and (5).

$$NO_{3\ uptake} = NO_{3,\ \mathrm{Med}} - NO_{3,\ \mathrm{Harvest}} \tag{2}$$

$$NO_{3\ uptake,perf} = (V_{Med}/V_{Harvest})^{*}(NO_{3,\ \mathrm{Med}} - NO_{3,\ \mathrm{Harvest}}) \tag{3}$$

$$PO_{4\ uptake} = PO_{4,\ \mathrm{Med}} - PO_{4,\ \mathrm{Harvest}} \tag{4}$$

$$PO_{4\ uptake,perf} = (V_{Med}/V_{Harvest})^{*}(PO_{4,\ \mathrm{Med}} - PO_{4,\ \mathrm{Harvest}}) \tag{5}$$

Biomass yield on light (Y_x in g_{DW} $mol^{-1}_{\ PAR\ photon}$) was calculated by division of the average biomass productivity by the average daily light intensity (Photosynthetically active radiation: PAR) per reactor volume. For 25 L reactors: effective volume V = 18 L, illuminated reactor surface area A = 2.25 m^2, average daily light intensity I = 48.34 $mol_{PAR\ photons}$ m^{-2} d^{-1}. For 18 m^3 reactor in 1 ha model: effective volume V = 13,730 L, illuminated reactor surface area A = 480 m^2, average daily light intensity: I = 44.23 $mol_{PAR\ photons}$ m^{-2} d^{-1}.

$$Y_X = P_{C,X}/\frac{I^{*}A}{V} \tag{6}$$

2.1.7. Statistical analysis

A one-way Brown-Forsythe ANOVA was applied to investigate significant differences between the cultivation methods and water types for the experimental results. Variances from the mean values are based on measured deviations during the steady state of microalgae cultivation.

2.2. Life cycle assessment

A life cycle analysis (LCA) was performed to identify and compare hotspots of the cultivation modes. The potential environmental impact of four microalgae cultivation scenarios was assessed, following the ISO14040 and ISO 14044 guidelines.

2.2.1. Goal and scope

The purpose of microalgae cultivation on RAS effluent water is primarily nutrient remediation of the RAS effluent water as a wastewater treatment (WWT) service, with a secondary purpose of producing algae biomass. In the LCA the environmental impact of microalgae production on RAS effluent water on a 1 ha scale is assessed. A 1 ha production scale was chosen based on estimations of nutrient remediation potential of the available effluent water flows from commercial RAS facilities. Two operating regimes and two water types were considered, i.e. continuous cultivation with additional nitrate or perfusion cultivation with an additional membrane filtration step (and no extra nitrate added), for either freshwater (*C. vulgaris)* or saltwater (*P. tricornutum)*. Brackish water cultivation with *P. tricornutum* was not included in the LCA, since no perfusion cultivation experiment was performed for that water type. The system boundaries start at the pretreatment of the nutrient-rich RAS effluent water and end with the harvested microalgae biomass (paste with 22.5 % dry weight after centrifugation). The functional unit was chosen to be one year of microalgae production in a 1 ha greenhouse located in Norway. To ensure comparability between continuous and perfusion cultivation on RAS effluent water, the continuous cultivation takes into account the difference in volume of RAS effluent water treated between the cultivation modes. The additional RAS effluent water volume treated in the perfusion cultivation mode is considered in the continuous cultivation case as a direct emission into water. This liquid emission contained the same concentrations of nitrate and phosphate as measured in the experiments.

2.2.2. Life cycle inventory

The life cycle inventory was based on data partly taken from experimental results obtained in this study, as well as from literature describing 1 ha microalgae cultivation in Norway based on extrapolated data generated at the National Algaepilot Mongstad (NAM, located near Bergen, Norway) (Vázquez-Romero et al., 2022). Dilution rates, average biomass densities and biomass productivities were adjusted according to the results of this study's experiments. Additionally, the membrane filtration technology was added. Details and necessary assumptions about the upscaled model and all included processes are described in the system description 2.2.4. Life spans of the infrastructure and equipment were assumed to be 15 years, with the exception of the UV-sterilisation lamp (1 year), the LED lights (7.5 years) and the membranes of the membrane filtration unit (1 year). The life cycle inventory database ecoinvent v3 was used when possible, with some additional inputs from the Agri-footprint database. Specific location data (Norway/Europe) was used when possible. A detailed overview of the Life Cycle Inventory data is shown in the supplementary material.

2.2.3. Impact assessment

The software SimaPro (PhD plan, version 9.5.0.2, Pré Sustainability, the Netherlands) was used for LCA modelling and impact assessment.

The ReCiPe Midpoint (H) method was chosen for impact classification and characterisation.

2.2.4. System descriptions

1 ha microalgae plant model

The life cycle inventory was based on an existing techno-economic analysis (TEA) by Vázquez-Romero et al. (2022), which describes a 1 ha microalgae plant based on extrapolated data, production parameters and infrastructure of the National Algaepilot Mongstad. The TEA includes media pretreatment, the cultivation phase, harvesting via centrifuge and regular cleaning of the system.

To be able to compare the differences between the two cultivation modes, the experimental data of this study were integrated into the model (specifically dilution rate and biomass density during steady state, resulting in overall productivity). As the photobioreactors in the 1 ha models differ not only in volume but also slightly in the design from the 25 L reactors used in our lab experiments, adjustments had to be made for the upscaling of algae growth data. This is further described in the cultivation phase description below. Furthermore, the existing model was extended with the Vibro® membrane filtration equipment for the perfusion cultivation. The equipment requirements for 1 ha scale were provided by the manufacturer based on the lab-scale experimental results.

Pre-treatment

The ingoing RAS effluent water was modelled to include particle removal through ultrafiltration and a sterilisation step via UV-light. This was done according to the pre-treatment described as by Vázquez-Romero et al. (2022). Additional nutrients were added after the RAS effluent water filtration and before UV–sterilisation. Phosphate and trace minerals were added for all scenarios, nitrate only for the continuous cultivation scenarios. Nutrient addition was calculated as a function of each scenario's nutrient uptake based on experimental data and average RAS effluent water nutrient concentrations measured during the experimental steady phase for both fresh and saltwater. This process also included the electricity consumption for pumping required for filtration, nutrient addition, and further flow into the photobioreactor.

Cultivation phase

The cultivation phase included the growth of microalgae in photobioreactors on the respective RAS media, as well as the cleaning processes for both membrane filtration and PBRs. Tubular glass photobioreactors were placed in a temperature-regulated greenhouse, where illumination is provided by a combination of daylight and LED panels. All necessary greenhouse infrastructure was included. The upscaling of the experimental data from 25 L PBRs to the modelled 18 m^3 PBRs required the following adaptations. The differences in the

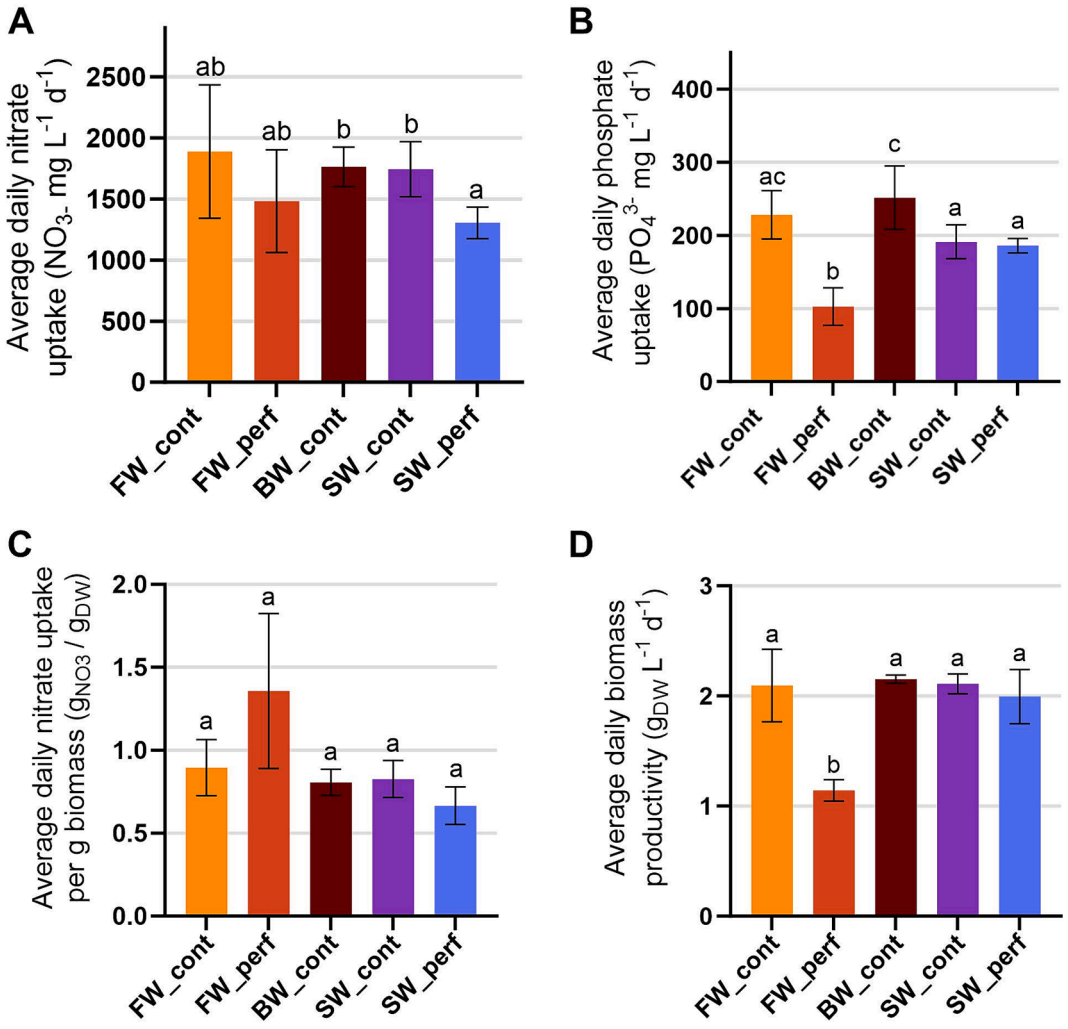

Fig. 2. Average daily nutrient uptake (mg L^{-1} d^{-1}) of nitrate (A) and phosphate (B) during microalgae cultivations. C: Average daily nitrate uptake per g biomass. D: Average daily biomass productivity (gW L^{-1} d^{-1}) during the steady phase of microalgae cultivations. Error bars show the standard deviation during the steady state phase of cultivation. Significant differences are denoted as letters on top of the bars, for all variables with the same letter, the difference between the means is not statistically significant. FW: freshwater; BW: brackish water; SW: salt water; cont: continuous cultivation; perf: perfusion cultivation.

reactor design of 25 L PBRs and 18 m^3 PBRs (larger diameter of the glass tubes, thus longer light path in the 18 m^3 reactors) are leading to different ratios of available light per reactor volume (a 4-fold difference; 25 L reactors: 6.04 $mol_{photons}$ L^{-1} d^{-1}, 18 m^3 reactors: 1.55 $mol_{photons}$ L^{-1} d^{-1}). To accommodate equal irradiation per volume of microalgae culture, the biomass density achievable on a 1 ha scale was reduced 4-fold compared to the obtained experimental biomass density in the 25 L reactors (incident light intensities were comparable, 500 μmol m^{-2} s^{-1} averaged daylight and LED combined at 1 ha scale and 525 μmol m^{-2} s^{-1} in the 25 L reactors). Another possibility to overcome this would be to increase the illumination via LED lights 7–fold at the 1 ha scale plant to reach a total illumination of 2000 μmol m^{-2} s^{-1}. This alternative was disregarded due to the high additional electricity requirements necessary. In the upscaled 1 ha system, illumination is provided via natural sunlight and additional LED lights (light intensity of 250 μmol m^{-2} s^{-1}) that are turned on when the daylight irradiance sinks under 100 μmol m^{-2} s^{-1}.

The PBR design included air pumps and water pumps that circulated the algae culture through the glass tubes into the mixing vessel, and 100 % CO_2 was sparged into the air stream for pH control. Heating was provided via a heating coil for temperature control, in addition to a heat exchanger in the greenhouse.

Three thorough cleaning cycles were assumed necessary per year. To factor in downtime caused by maintenance, photobioreactor cleaning and culture crashes, the productive cultivation period was assumed to be 300 days per year. In the continuous cultivation scenarios, microalgae culture is continuously harvested according to the dilution rate established during lab experiments (0.56 d^{-1}) and pumped from the PBRs to the centrifuge. The water volume necessary to cover nutrient uptake during the cultivation phase in perfusion mode was calculated based on the average RAS effluent water nutrient concentrations and the nutrient uptake rate of the respective scenario. The culture was harvested continuously in all scenarios according to the experimentally derived dilution rate of 0.56 d^{-1} and a total reactor volume of 220 m^3 per hectare, resulting in 36613 m^3 harvested culture per year. The difference between ingoing RAS medium and harvested culture during perfusion cultivation was discharged as permeate after the membrane filtration process.

Harvesting

Harvesting of the microalgae culture was done by dewatering through centrifugation with an EVODOS 50 disc stack centrifuge. A harvesting efficiency of 95 % was assumed, leaving 5 % of the microalgae culture as a supernatant. Depending on local regulations, a filtration step to clean out any residual microalgae in the emitted water stream could be necessary. This possibly necessary filtration was not included in the LCI as this was outside the system boundary, and the water volume to be treated was equal in all scenarios. This water discharge was assumed to be a direct emission to nature (water) and included leftover nutrients of the medium (3 % nutrient concentrations of the original media, for detailed composition see supplementary material).

2.3. Preliminary economic assessment

An economic assessment was performed to estimate the cost difference between perfusion cultivation with membrane filtration technology and continuous cultivation with the addition of nitrate fertilizer. The calculations were based on the 1 ha microalgae cultivation plant model as described in 2.2.4 and the techno-economic analysis (TEA) by Vázquez-Romero et al. (2022). Three scenarios were compared; 1: conventional continuous microalgae cultivation on chemical fertilizer without nutrients from RAS effluent water, assuming the same biomass productivity as the continuous cultivation on RAS medium, total costs as reported by Vázquez-Romero et al. (2022) for 1 ha production: OpEx: 2,228,066 €/year and 3,935,849 € major equipment cost (MEC); 2: continuous microalgae cultivation on RAS effluent water with additional nitrate fertilizer as described in the experiments of this study; 3: perfusion cultivation on RAS effluent water without additional nitrate fertilizer, taking into account costs for membrane filtration equipment and electricity needed for the membrane filtration and cleaning.

A cleaning cycle of each membrane filtration unit once a week with a commercial alkaline membrane cleaning product was assumed (2 % v/v dilution), with chemical costs of 7.29 €/L (Ultrasil™ 110, Ecolab Inc., USA). The membrane filtration equipment was estimated at 120,000 € per Vibro-I 80 m^2 unit, leading to an initial investment cost of 1,080,000 € for 9 units as needed for 1 ha (preliminary quote from SANI Membranes A/S, Denmark), and an additional cost of 504,000 € yearly for replacing the membranes. The costs for additional fertilizer were only calculated for sodium nitrate (3.30 €/kg, HjelleKjemi AS, Norway), since additional phosphate had to be added to all scenarios. An average electricity price of 0.007 €/kWh was assumed (Eurostat, 2024).

3. Results and discussion

3.1. Microalgae growth experiments

Microalgae cultivation on RAS medium has been investigated for wastewater treatment potential in terms of nutrient remediation and microalgae biomass growth. Two cultivation modes were compared: continuous cultivation mode (on freshwater, brackish water and saltwater) and perfusion cultivation mode (on freshwater and saltwater). Fig. 2 shows the average daily nutrient uptake and biomass productivity during continuous and perfusion cultivations.

Average daily NO_3 uptake rates (mg L^{-1} d $^{-1}$) were measured for FW_cont: 1888 ± 546; FW_perf: 1482 ± 421; BW_cont: 1762 ± 162; SW_cont: 1744 ± 224; SW_perf: 1305 ± 128, as shown in Fig. 2A. The only significant difference in nitrate uptake was between *P. tricornutum* continuous cultivations (both brackish and saltwater) and *P. tricornutum* perfusion cultivation (Welch's ANOVA, $P < 0.05$). Rather high variations in daily NO_3^- uptake were observed during freshwater cultivation of *C. vulgaris*. When calculating the nitrate uptake as consumed mg NO_3^- per g biomass (Fig. 2C), no significant difference between water types or algae during continuous cultivation was observed (in g_{NO3-} g_{DW} d^{-1} for FW_cont: 0.92; FW_perf: 1.30; BW_cont: 0.81, SW_cont: 0.83; SW_perf: 0.66).

The additional phosphate provided in the medium was consumed each day entirely and was not dependent on biomass growth but on phosphate concentrations in the medium (PO_4^{3-} uptake in mg L^{-1} d^{-1} for FW_cont: 228 ± 33.0; FW_perf: 103 ± 25.7; BW_cont: 252 ± 43.4; SW_cont: 191 ± 23; SW_perf: 186 ± 10, as shown in Fig. 2B). This effect is well described in literature as "luxury uptake" of phosphate, where amongst other microalgae, both *C. vulgaris* and *P. tricornutum* can absorb and store more phosphorus than necessary for cell growth and maintenance (Dell'Aquila et al., 2020; Powell et al., 2009; Solovchenko et al., 2019). The experiments were designed to maintain the microalgae cultures at a high specific growth rate to achieve optimal biomass productivity and therefore never run out of nutrients entirely. In an industrial production scenario, real-time measurement of nitrate concentrations in the microalgae culture could allow for automated steering of in- and outgoing flowrates. This would facilitate as high as possible nutrient remediation while keeping the culture in nutrient repletion. As both the continuous cultivation on RAS effluent water with additional nitrogen and phosphate, as well as the perfusion cultivation with only additional phosphate can be adapted as a "feed on demand" system, almost complete nutrient remediation would be possible. The continuous culture harvests of 10 L d^{-1} led to biomass productivities from 1.14 ± 0.10 g L^{-1} d^{-1} (perfusion cultivation on freshwater with *C. vulgaris*) to 2.15 ± 0.04 g L^{-1} d^{-1} (continuous cultivation on brackish water with *P. tricornutum*) as shown in Fig. 2D. The most significant difference between cultivation modes was observed during freshwater cultivation with *C. vulgaris,* where the biomass productivity dropped from 2.10 ± 0.33 g L^{-1} d^{-1} during continuous cultivation to 1.14 ± 0.10 g L^{-1} d^{-1}

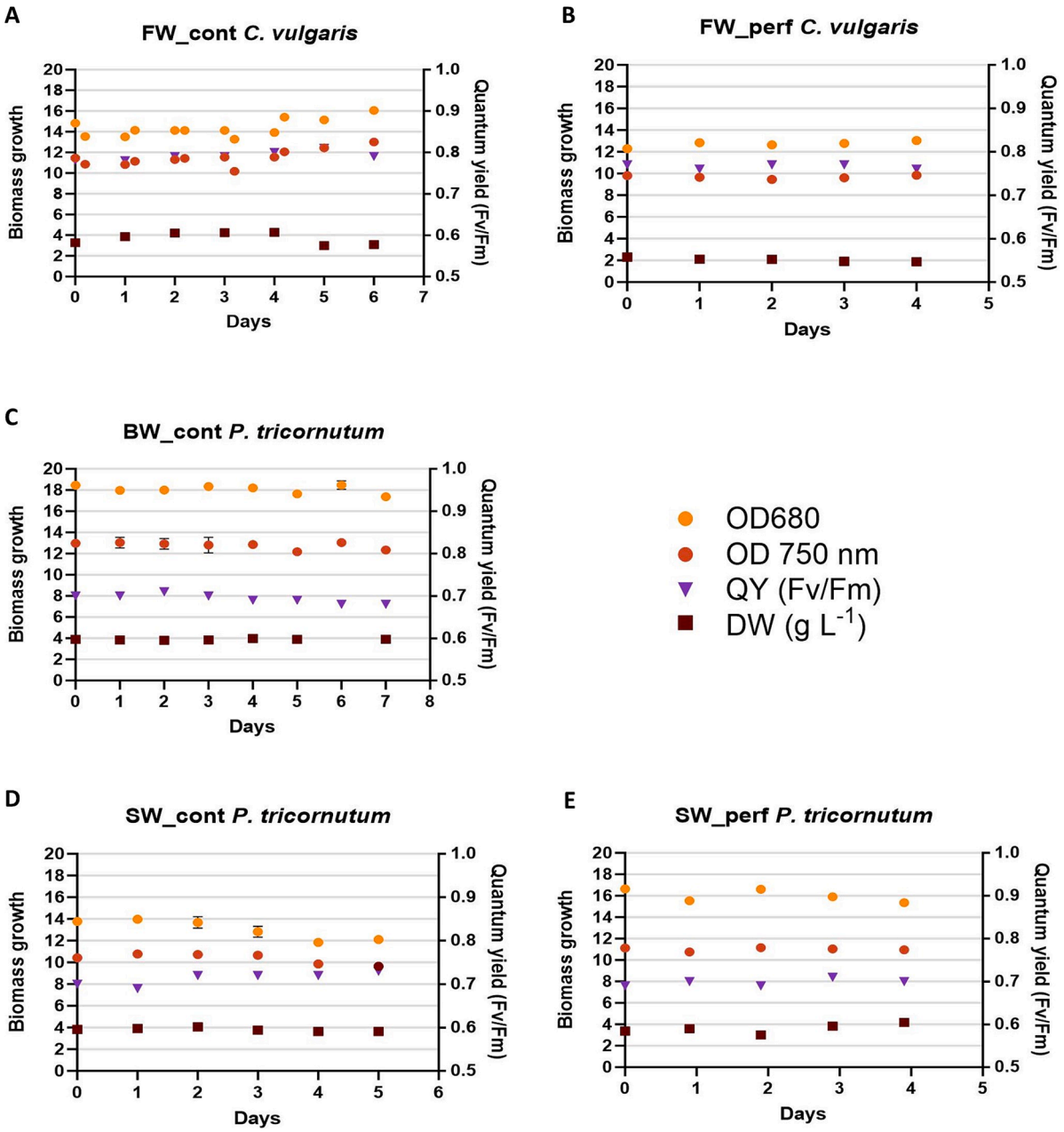

Fig. 3. Biomass growth in dryweight and OD, as well as maximum quantum yield during the steady phase of microalgae growth on RAS effluent water-based medium during for A) continuous cultivation of *C. vulgaris,* freshwater; B) perfusion cultivation of *C. vulgaris*, freshwater; C) continuous cultivation of *P. tricornutum,* brackish water; D) continuous cultivation of *P. tricornutum,* salt water; E) perfusion cultivation of P. tricornutum, salt water. Optical density depicted in orange (680 nm) and red circles (750 nm); dry weight (g L^{-1}) is shown in brown cubes and the Quantum yield in purple triangles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during perfusion cultivation. A lower biomass productivity for perfusion cultivation compared to continuous cultivation was as well observed for *P. tricornutum* saltwater cultivation, although the difference was not significant (continuous mode: 2.11 ± 0.09 g L^{-1} d^{-1}, perfusion mode: 2.00 ± 0.25 g L^{-1} d^{-1}). It is reported that membrane filtration technology requires a certain shear rate at the membrane surface to avoid membrane fouling (Bilad et al., 2014). Although the shear forces of the Vibro® Technology in our setup are reduced due to the vibration of the membrane module rather than cross-flow pressure, an additional shear force is introduced compared to the continuous cultivation. Excessive shear stress can cause cell damage and decreased growth. Growth inhibitions are highly species-dependent (Wang and Lan, 2018) but are reportedly observed for both *P. tricornutum* (Sánchez Mirón et al., 2003) and *C. vulgaris* (Leupold et al., 2013). We have not measured shear rates of the membrane filtration setup, but we hypothesize additional pumping and the vibration of the membrane module will add shear stress in comparison to continuous cultivation.

Fig. 3 shows the biomass growth measured through optical density (680 nm and 750 nm) and dry weight (g L^{-1}), as well as the maximum quantum yield. A decreasing maximum quantum yield is an indicator of stress, such as nutrient depletion or light inhibition, and was used to confirm a stable photosynthetic activity during steady phase of the cultivation experiments.

A steady state with steady OD, DW and nutrient uptakes was obtained at 3–8 days for FW_cont; 5–9 days for FW_perfusion; 3–11 days for BW_cont; 6–11 for SW_cont and 8–12 days for SW_perf cultivation. After

Table 2
Input parameters for one year of microalgae cultivation on a 1 ha scale, located in Norway. Freshwater cultivation with *C. vulgaris* and saltwater cultivation with *P. tricornutum*.

FU: cultivation on 1 ha scale per year	Freshwater		Saltwater	
	cont	perf	cont	perf
Cultivation period (days)	300	300	300	300
Plant size (ha)	1	1	1	1
Reactor volume (m^3 per ha)	220	220	220	220
Dilution rate (d^{-1})	0.56	0.56	0.56	0.56
Biomass density (g L^{-1})	0.95	0.53	0.97	0.92
Productivity DW (g L^{-1} d^{-1})	0.53	0.29	0.54	0.51
NO_3 uptake (g L^{-1} d^{-1})	0.48	0.38	0.45	0.33
PO_4 uptake (g L^{-1} d^{-1})	0.06	0.03	0.05	0.05
RAS effluent water NO_3 concentration (g L^{-1})	0.23	0.23	0.32	0.32
RAS effluent water PO_4 concentration (g L^{-1})	0.002	0.002	0.010	0.010
Yield on light (g_{DW} $mol^{-1}_{photons}$ d^{-1})	0.30	0.19	0.35	0.33
Microalgae cultivation (m^3 $year^{-1}$)	36,613	36,613	36,613	36,613
RAS effluent water treated (m^3 $year^{-1}$)	36,613	61,648	36,613	38,244
Untreated RAS effluent water to WWT (m^3 $year^{-1}$)	–	25,035	–	1631
Algae paste, 22.5 % DW (t $year^{-1}$)	147	81	150	142
Biomass DW (t $year^{-1}$)	35	19	36	34

the start of the perfusion cultivations, *C. vulgaris* stayed 5 days in growth phase before reaching a stable biomass density, while *P. tricornutum* on saltwater RAS medium took 8 days to reach a stable biomass density. Continuous cultivation of *P. tricornutum* (brackish water) reached the highest average optical biomass density (OD_{750}: 12.77 ± 0.33), followed by continuous cultivation of *C. vulgaris* (11.48 ± 0.75), perfusion cultivation of *P. tricornutum* on saltwater (11.01 ± 0.16), continuous cultivation of *P. tricornutum* on saltwater (10.35 ± 0.48) and perfusion cultivation of *C. vulgaris* (9.67 ± 0.16). Similar biomass density trends were observed with dry weight measurements, although continuous cultivation of *P. tricornutum* led to a higher dry weight than perfusion cultivation (FW_cont DW: 3.70 ± 0.57; FW_perf DW: 2.06 ± 0.18; BW_cont DW: 3.88 ± 0.06; SW_cont DW: 3.80 ± 0.16; SW_perf DW: 3.59 ± 0.44). A stable maximum quantum yield and a stable OD_{680} /OD_{750} ratio indicated a steady microalgae culture that does not show any major signs of stress by photoinhibition.

P. tricornutum on brackish water showed the highest biomass density based on both OD and DW, although the difference in DW was not significant to the other *P. tricornutum* growth experiments on salt water. Previous experiments with this *P. tricornutum* strain observed the same pattern (Prestegard et al., 2014), where cultivation of *P. tricornutum* on brackish water led to higher biomass productivity than on higher salinities.

The high-density cultivation of microalgae was maintained for five to seven days in the presented experiments, and both the photobioreactors manufacturer (LGEM), as well as other authors have shown that stable long-term cultivation (up to several months) is possible in such a closed cultivation system (Lgem, 2022; Oostlander et al., 2020).

3.2. Upscale model

The results from the lab experiments shown in the previous paragraph 3.1 were used to update and expand an existing model for 1 ha microalgae cultivation (Vázquez-Romero et al., 2022) as described in paragraph 2.2.4. The upscaled cultivation data are shown in Table 2, where biomass densities and productivities, as well as nutrient uptake rates, are relative to the lab results.

The calculated biomass productivities per year are in the same range as reported for the TEA model of the National Algaepilot Mongstad (Vázquez-Romero et al., 2022) and other models of pilot/large-scale production of *P. tricornutum* in tubular reactors in the Netherlands (Slegers et al., 2013). As presented in paragraph 3.1 earlier, a significant difference (1.8-fold) of the biomass densities (dry weight) was observed between the continuous cultivation and perfusion cultivation mode during freshwater cultivation with *C. vulgaris*. This consequently resulted in a 1.8–fold difference between continuous and perfusion cultivation of *C. vulgaris* on the total modelled yearly biomass production.

The amount of annually treated RAS effluent water with perfusion cultivation depends on the nitrogen concentrations of ingoing RAS effluent water. We observed that nitrogen concentrations can fluctuate throughout the cultivation period, depending on the cleaning regime of the RAS system, fish size and feeding regime. The membrane filtration equipment in the 1 ha is scaled to treat an average amount of RAS effluent water (based on NO_3 concentration, as given in Table 2), but would be able to handle both more and less RAS effluent water without any changes to the modelled equipment. Phosphate is added in all scenarios.

Several factors play an important role when modelling upscaled production based on lab scale results. The scalability of the chosen labscale cultivation system is one of the key factors to ensure accurate modelling of biomass production, although many large-scale photobioreactors have lower yields than their equivalent lab-scale reactors (Benner et al., 2022; Grobbelaar, 2012). We assume a good scalability of the 25 L reactors used for the experiments in this manuscript, compared to the modelled performance of the 18000 m^3 large scale tubular photobioreactor, since similar Lgem reactor design, mixing and pH-control by supplying CO_2 on demand is used. However, a different light path of lab-scale and large-scale reactor influenced the yield on light (g_{DW} mol^{-1} $_{PAR\ photon}$) and therefore the overall biomass productivity (g L^{-1} d^{-1}) negatively in upscale reactors (4-fold decrease as described in paragraph 2.2.4). Furthermore, temperature control is more unforeseeable in large-scale operations, which highlights the importance of well adapted algae to the season and location (Borowitzka and Vonshak, 2017).

3.3. LCA and preliminary economic assessment

A life cycle analysis of the four scenarios was performed and the relative environmental impacts are shown in Fig. 4. In Fig. 4A, only those impact categories are shown where the difference in environmental impacts among the cultivation modes and water types was more than 10 % (5 out of 18 impact categories). The results for all impact categories are shown in the supplementary material. Results are reflecting the total impact of each scenario entailing microalgae cultivation from nutrient rich RAS effluent water to dewatered algae paste. A significant difference between continuous and perfusion modes was observed in the impact category stratospheric ozone depletion, and for freshwater cultivations also in freshwater eutrophication, marine eutrophication, as well as water consumption. Those differences were due to different nutrient uptake rates between the cultivation modes and therefore the possibility of treating more or less RAS effluent water during a year.

Fig. 4B shows the contribution analysis for continuous cultivation and Fig. 4C of perfusion cultivation, in both cases of *C. vulgaris* on freshwater. The materials of the PBR infrastructure had the largest impact in 11 impact categories (ozone formations, fine particulate matter formation, terrestrial acidification, terrestrial ecotoxicity, freshwater ecotoxicity, marine ecotoxicity, human carcinogenic toxicity, human non-carcinogenic toxicity, mineral resource scarcity). The steel frame structure and the borosilicate glass tubes of the photobioreactors stood for most of these impacts. Electricity consumption for illumination had the largest impact on global warming, ionizing radiation, land use and water consumption and represents the second-highest contributor to environmental impacts in most of the categories apart from eutrophication. The negative impact on the water consumption category was caused by the water that is released back to a local water body after centrifugation. Both freshwater and marine eutrophication impacts were primarily caused by the wastewater treatment of RAS effluent

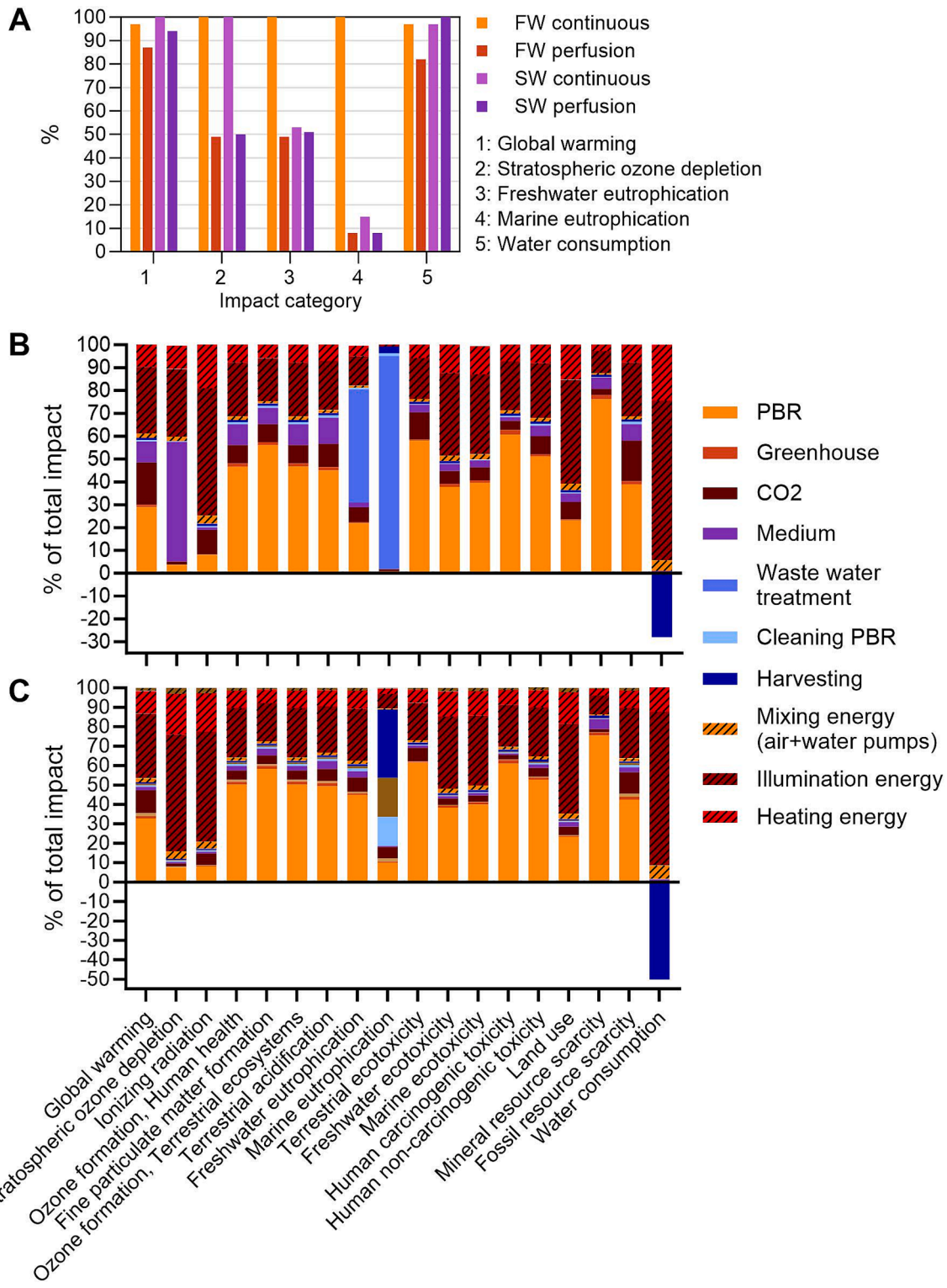

Fig. 4. Comparisons of the environmental impacts for the cultivation of *C. vulgaris* on freshwater RAS medium on a 1 ha scale (for 1 year). A: Total relative environmental impacts. FW: freshwater; SW: salt water. B: Total contributions to environmental impacts during continuous cultivation. C: Total contributions to environmental impacts during perfusion cultivation. Calculated in SimaPro with the ReCiPe Midpoint (H) method.

water that was not used for microalgae cultivation (volume equals the difference of RAS effluent water which is treated in continuous cultivation and the treated RAS effluent water volume in perfusion cultivation).

Similar to continuous cultivation, the largest impact of the perfusion cultivation of *C. vulgaris* in 11 impact categories was caused by the PBR infrastructure. Another similarity to continuous cultivation was that the electricity used for illumination had the second-highest relative impact in most of the impact categories apart from eutrophication and had the largest impact on global warming, stratospheric ozone depletion, ionizing radiation, land use and water consumption. The necessary additional membrane filtration equipment and electricity for the

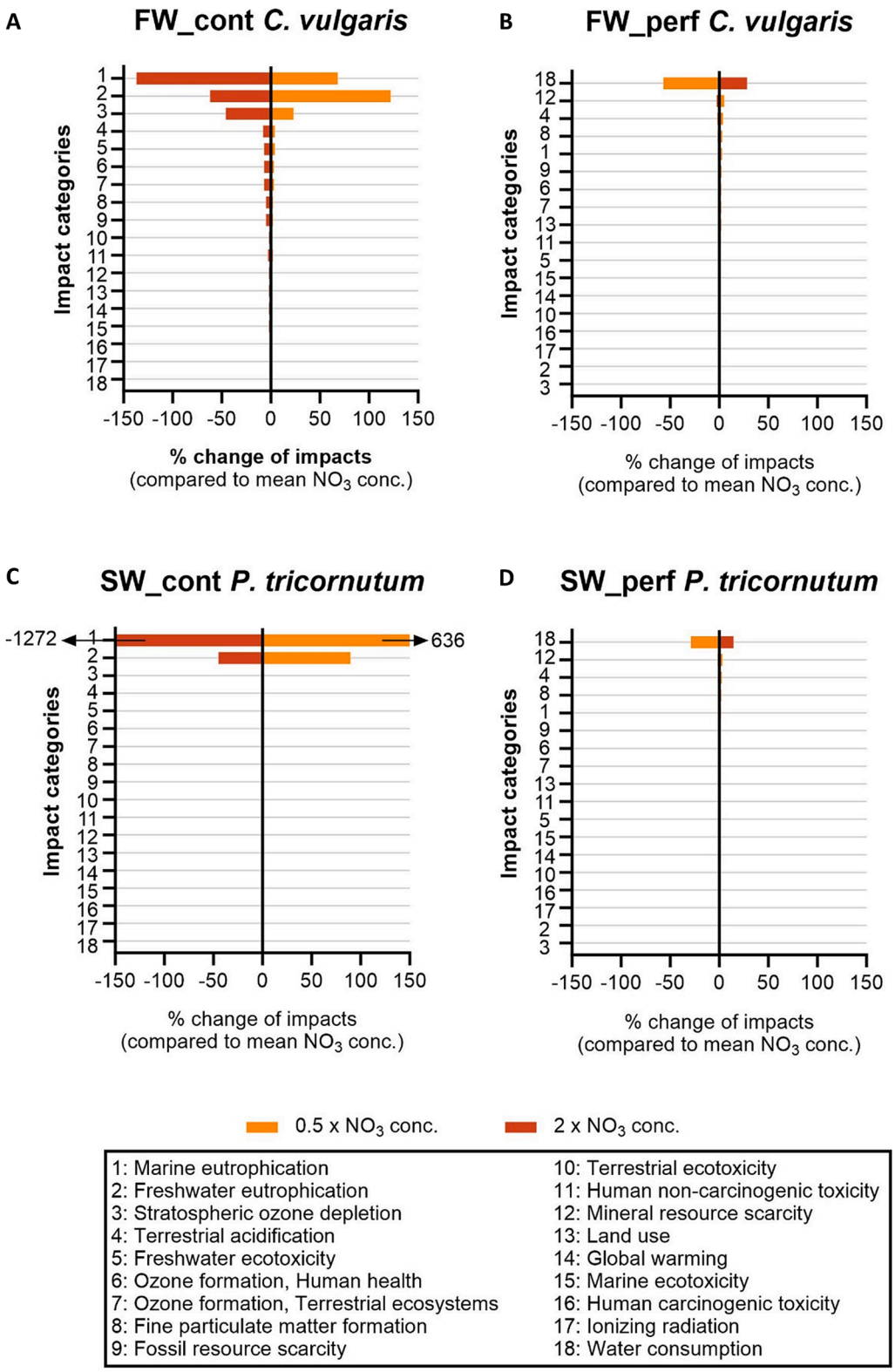

Fig. 5. Parameter analysis for NO_3^- concentrations of RAS effluent water for A) continuous cultivation of *C. vulgaris*, freshwater; B) perfusion cultivation of *C. vulgaris*, freshwater; C) continuous cultivation of *P. tricornutum,* salt water; D) perfusion cultivation of *P. tricornutum*, salt water. NO_3^- concentration in freshwater: Mean: 0.23 mg L^{-1}; 0.5x NO_3^- concentration: 0.11 mg L^{-1}, 2 x NO_3^- concentration: 0.45 mg L^{-1}. NO_3^- concentration in salt water: Mean: 0.32 mg L^{-1}, 0.5x NO_3^- concentration: 0.16 mg L^{-1}, NO_3^- concentration: 0.64 mg L^{-1}.

Table 3
Cost differences between conventional microalgae cultivation based on chemical fertilizer, continuous cultivation on RAS effluent water with additional N fertilizer, and perfusion cultivation on RAS effluent water without additional N fertilizer; for both freshwater (FW) and saltwater (SW) cultivation. Changes in the operating expenses (OpEx) and capital expenditures (CapEx) were based on conventional cultivation (Fertilizer cont.) as described by Vázquez-Romero et al. (2022).

Per 1 ha per year	FW			SW		
	Fertilizer cont.	RAS cont.	RAS perf.	Fertilizer cont.	RAS cont.	RAS perf.
Chemical N fertilizer (€)	58,370	31,154	–	53,926	15,295	–
Membrane filtration equipment cost (€)	n.a.	n.a.	576,000	n.a.	n.a.	576,000
Membrane cleaning chemicals (€)	n.a.	n.a.	10,217	n.a.	n.a.	10,217
Membrane filtration electricity (€)	n.a.	n.a.	14,690	n.a.	n.a.	14,690
Change in production cost (€/kg_{dw} microalgae)	n.a.	− 1 %	+158 %	n.a.	−1 %	+ 49 %
Change of OpEx	n.a.	− 1 %	+ 6 %	n.a.	− 2 %	+ 6 %
Change of CapEx	n.a.	n.a.	+60 %	n.a.	n.a.	+60 %

perfusion cultivation mode combined to not more than 3 % of the total impact, except for the water consumption impact category (5 %).

The distribution of impacts for continuous and perfusion mode during saltwater cultivation with *P. tricornutum* showed the same trends as for *C. vulgaris* in Fig. 4B and C, see supplementary material. The only significant difference between FW and SW cultivation scenarios appeared in the eutrophication categories, where the wastewater treatment of RAS effluent water accounted for only 4 % of the freshwater eutrophication impact during saltwater continuous cultivation, compared to 50 % in freshwater continuous mode. The distribution of the marine eutrophication impact category during saltwater cultivation changed to 57 % caused by the RAS effluent wastewater treatment, compared to 93 % in freshwater continuous mode.

Nitrate and phosphate concentrations of the RAS effluent water are not only dependent on the RAS site but also on production season and can even vary daily due to differences in cleaning and feeding protocols. Higher nutrient concentrations decrease the demand for additional fertilizer and lower the difference between RAS effluent water volumes treated in continuous cultivation mode and the perfusion mode. As a result, the volume of untreated RAS effluent water that is released into nature is decreased. We hypothesized that the nutrient concentrations of the RAS effluent water are an important hotspot for the microalgae process design and necessary equipment. The impact of varying nitrate concentrations in the RAS effluent water was investigated with a parameter analysis.

Fig. 5 shows the effect on environmental impacts when nitrate concentrations of the RAS effluent water are half and double the average used in this model. The largest effect of varying nitrate concentrations appears to be on the eutrophication impact categories (both freshwater and marine), as well as stratospheric ozone depletion, which aligns with the outcomes from the contribution analysis (Fig. 4B and C). Lower nitrate concentrations in the RAS effluent water lead to a higher medium renewal rate during perfusion cultivation. Therefore, more RAS effluent water will be treated and can be released as back into nature either from membrane filtration permeate or from the final centrifugation step. We assume that the membrane filtration unit can accommodate variations in RAS effluent water nitrate concentrations, meaning that the filtration equipment remains unchanged for the different nutrient concentration scenarios.

The parameter analysis shows the same trends for freshwater and saltwater cultivation, but in a different order of magnitude. Changing nitrate concentration during continuous cultivation on saltwater RAS medium (Fig. 5C) showed the greatest change in the marine eutrophication impact category. This change differs by an order of magnitude from the maximum change observed during continuous cultivation, due to the lower average nitrate concentrations in RAS effluent freshwater than RAS effluent saltwater. The difference in RAS effluent water volume that is treated during continuous and perfusion cultivation is substantially higher during freshwater cultivation than during saltwater cultivation. Since only a small impact is caused on marine eutrophication during saltwater perfusion cultivation, this impact category is sensitive to small changes in nitrate concentrations in the RAS effluent water.

All in all, this work demonstrates that both cultivation modes are suitable for RAS effluent wastewater treatment and reach full nutrient remediation. Perfusion cultivation with membrane filtration appears to be the more sustainable method growing microalgae on relatively low nutrient concentrations, as present in the RAS effluent water described in this study's experiments. The parameter analysis shows that this also holds for even lower nutrient concentrations. However, for doubled nutrient concentrations, the difference in environmental impacts between perfusion and continuous cultivation seems to become less obvious. Photobioreactor infrastructure and electricity consumption during cultivation are the major hotspots in the environmental impact. This is in line with comparable LCAs of microalgae cultivation, showing that the choice of infrastructure and energy-efficient operation is key (Gurreri et al., 2023). Temperature-adapted microalgae are important especially during the winter months, as it is foreseen that illumination and heating will increase the environmental impact as well as the overall operating costs (Vázquez-Romero et al., 2022; Pérez-López et al.,2017).

A preliminary economic assessment shows the results of the cost estimates for continuous and perfusion cultivation scenarios, as well as a baseline scenario of conventional microalgae cultivation on chemical fertilizer (Table 3). The estimated cost-benefit from the partial substitution of chemical N fertilizer by the nitrogen content in the RAS effluent water is about – 1 % for the continuous cultivation on RAS medium. In the perfusion cultivation scenario, the nutrient benefit is cancelled out by the additional need for membrane cleaning chemicals and electricity connected to the membrane filtration, leading to a 6 % increase in the operational costs. Moreover, capital expenditures are estimated to be 60 % higher for perfusion cultivation than continuous cultivation due to the additional membrane filtration equipment. Note that the estimations of the membrane filtration capacity and lifespan of the membranes have not yet been proven on this scale.

The increased costs for perfusion cultivation at a potential industrial scale make production less feasible. One way to compensate for this could be the utilization of the membrane filtration equipment for multiple purposes. Membrane technology can be more cost-efficient than traditional harvesting methods, due to its low electricity consumption. This could make a two-step harvesting approach beneficial: initial up-concentration of algae with membrane filtration, final dewatering through centrifugation (Fasaei et al., 2018; Zhao et al., 2023). The membrane technology could also be utilized to recycle the medium, which would allow for an increased medium retention time that is necessary for full nutrient recovery. This could be an advantage when there are higher nutrient concentrations available in the RAS medium, for example after incorporating side streams from dewatering fish sludge.

4. Conclusion

We demonstrated nutrient remediation from RAS effluent water during both continuous and perfusion microalgae cultivation. A stable, high-density biomass production was achieved (up to 2.15 g L^{-1} d^{-1}) either through additional nitrate fertilization during continuous cultivation or by integration of a membrane filtration step that allows a

flexible water retention time in perfusion mode. The LCA revealed that perfusion cultivation is the more environmentally sustainable method for RAS effluent water with low nutrient concentrations, although leading to higher operational and investment costs. The photobioreactor infrastructure and electricity requirements during cultivation have the highest contribution to the environmental impacts.

CRediT authorship contribution statement

Hanna Böpple: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Petronella Margaretha Slegers:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Peter Breuhaus:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Dorinde M.M. Kleinegris:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The authors report no commercial or proprietary interest in any product or concept discussed in this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.131881.

Data availability

Data will be made available on request.

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