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Research article

Towards a circular economy - Repurposing side streams from the potato processing industry by *Chlorella vulgaris*

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ABSTRACT

Common wastewater treatment strategies in the food industry do not include efficient remediation strategies for nitrogen, phosphorous and organic carbon. Incorporating microalgae in water treatment plants is rising in popularity because of their high nutrient and trace element uptake driven by light. In this study, four different side streams from an Austrian potato processing company have been screened for their applicability of microalgal cultivation. The side streams were assessed for *Chlorella vulgaris* growth and their requirement of any additional pretreatment or media supplementation. One side stream specifically, called blanching water II, a stream generated by boiling the potatoes for ease of peeling, turned out very useful to cultivate *Chlorella vulgaris* and concomitantly remedy the wastewater. Compared to a state-of-the-art cultivation in BG11, cultivating *Chlorella vulgaris* in blanching water II led to a 45 % increase in specific growth rate of 1.29 day^{-1} and a 48% increase in biomass productivity to 294.6 mg/L/day, while all nitrogen and phosphate present in the side stream were metabolized. Overall, the results demonstrate that the water remediation process for blanching water II shows vast potential in regard to water purification and waste to value approaches.

1. Introduction

Sustainable energy sources as well as alternative food and feed sources are currently prospering. The climate crisis is forcing the implementation of sustainable use of resources, the remediation of industrial side streams and repurposing of water effluents. Land degradation and the change in global climate have multiple interrelated risks with feed shortages (Makkar, 2018), water scarcity and food insecurities especially in developing countries (Wheeler and von Braun, 2013). Freshwater is of tremendous importance for humans, animals and agriculture. It is the most used resource on earth with an estimated annual withdrawal quantity of about 4 trillion m^3 (Ingrao et al., 2023), mainly needed for agriculture. This number calls for action in terms of reducing and reusing water effluents.

Microalgae have the potential of tackling those challenges. They do not compete with other agricultural industries for arable land and are able to fixate atmospheric CO_2 (Markou and Elias, 2013), and remediate industrial water effluxes and industrial side streams. *Chlorella vulgaris* has been cultivated on multiple different side streams from industry, such as whey (Abreu et al., 2012; Melo et al., 2018), dairy waste (Gramegna et al., 2020), sweet sorghum bagasse (Arora and George, 2021), vinasse (Melo et al., 2018) or hydrolysates from food waste (Lau et al., 2014; Cai et al., 2022). *Chlorella vulgaris*' ability to metabolize multiple organic carbon sources, such as glucose (Barros et al., 2019), glycerol (Gougoulias et al., 2022), citrate (Marudhupandi et al., 2014), and acetate (Kriechbaum et al., 2023) proves to be beneficial in terms of remediating side streams from the food and agricultural industry. Additionally, it can metabolize multiple different nitrogen (Zhu et al., 2019) and phosphorous sources (Xing et al., 2023) as well as trace metals (El-Agawany and Kaamoush, 2022; Liu et al., 2018; Musah et al., 2022), which is crucial for wastewater remediation approaches. An additional benefit is the decrease of water and nutrient expenses for cultivation and therefore increasing the economic margin of microalgal products produced on wastewater (Yu et al., 2023).

Chlorella vulgaris is well known for its high specific protein and chlorophyll contents, reaching up to 60 % (w/w) of protein (Kholif et al., 2017) and 3.5 % (w/w) of total chlorophyll (Jeon et al., 2014) grown on defined media. Cultivated on wastewaters, protein contents up to 64.14

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% (w/w) grown on broken rice hydrolysate (Cai et al., 2022), 43.33 % (w/w) on corn steep liquor (Melo et al., 2018) and 28.49 % (w/w) on molasses (Guo et al., 2020) were reached. Applications of *Chlorella vulgaris* range from food (Barba, 2017) and feed (Kholif et al., 2017) applications, to biofuel production (Sakarika and Kornaros, 2019), and utilization as slow-release biofertilizer (Ammar et al., 2022). In commonly used biofertilization approaches, a lot of nitrogen is lost through volatilization, erosion and denitrification in bacterial processes (Chapman, 2013). Microalgae-based biofertilizers release nitrogen and phosphorous slowly into the soil, reducing the risk of water and ground eutrophication (Mulbry et al., 2007). Another advantage of microalgal-based fertilizers is the enhanced soil fertility resulting from algae created metabolites and phytohormones, like auxins or cytokinins (Ronga et al., 2019). *Chlorella vulgaris* biomass in biofertilizer applications has recently been described to promote growth and crop yields in corn, wheat and rice plants (Nosheen et al., 2021).

For a majority of countries, food wastes and wastes from food processing industries cause a severe environmental problem. Annually, about one billion tons of food waste gets disposed of in landfills (Lau et al., 2014). Utilizing waste- or side streams from food-processing industries is also aiding in reducing disposal and treatment costs of water effluents (Wang et al., 2020), and environmental impacts thereof. In Austria about 700,000 tons of potatoes get harvested each year (FAO, 2021) and approximately 50 % of those potatoes get processed further (Intelligence, 2018), resulting in vast amounts of processing side streams. During potato processing at an Austrian industrial potato processing facility, four different liquid side streams are generated, resulting in an estimated 5000–6000 L/h of volumetric flux each. In two individual processing steps, the potatoes get heated to inactivate potato-native enzymes, inhibit degradation processes and to leach out starch. Afterward the potatoes get peeled and washed resulting in a third side stream. The last side stream is generated by cutting the potatoes into fries, resulting in the starchy cutting water. These liquid potato side streams are pretreated in the operational waste water treatment system and then discarded in the municipal wastewater system. Estimations suggest that starch-processing industries produce about 6–10 m^3 wastewater per ton of starch processed (Ummalyma et al., 2023), which shows the importance of side streams remediation.

The scope of this study was to screen the four obtained side streams from a potato processing enterprise in Austria for their feasibility of mixotrophic *Chlorella vulgaris* cultivation (Fig. 1). The goal was to minimize the pretreatment prior to cultivation, and maximize biomass- and protein yields whilst maintaining the chlorophyll content of the obtained *Chlorella vulgaris* biomass, as well as decrease the environmental burden by metabolizing nitrogen and phosphorous contained in the aqueous side streams. The best performing screening run in terms of biomass growth and water remediation was upscaled into a stirred tank photobioreactor. The selected side stream was also tested for batch to batch variations in composition to determine the suitability as alternative substrate for *Chlorella vulgaris* cultivation. The reduction of total nitrogen and total phosphorous content was determined to evaluate the reduction of the environmental burden. Finally, an upscaling approach into an industrial sized tubular photobioreactor system was calculated to determine the feasibility of utilizing this side stream at industrial scale.

2. Materials and methods

2.1. Microalgae strain and inoculum

The green algae used in this study was *Chlorella vulgaris* (UTEX 2714), obtained from the Culture Collection of Algae at the University of Texas at Austin. The cultivation medium used was BG11 (Touloupakis et al., 2022) at a pH of 7.5 and 1.0 g/L $NaNO_3$. The *Chlorella vulgaris* inoculum was cultivated in 14 h light- (illuminated at 50 µmol/m^2/s) and 10 h darkness-cycles at 23 °C, with 3 % (v/v) CO_2-enriched air in a Minitron shaker (Infors HT, Basel, Switzerland) shaking at 100 rpm.

2.2. Industrial side streams

Four liquid side streams were obtained from an Austrian potato processing enterprise. In processing, the potatoes passed two stages with heated water blanching water I & blanching water II (BLI & BLII) to aid peeling and enzyme inhibition. After peeling the potatoes, the peels and water mixture was transferred to a digestion tower to produce biogas. This results in a liquid digestate rest (DR). Then the potatoes were cut and the cutting blades washed with water resulting in a starchy cutting water (ST). These four side streams were sampled and tested for carbon sources (HPLC), anions (IC), trace elements (ICP-OES), NH_3 (Cedex) and pH-values.

2.3. Shakeflask experiments

For photoautotrophic and mixotrophic cultivations *Chlorella vulgaris* was cultivated at 23 °C in a 3.0 % (v/v) CO_2-enriched atmosphere in a Minitron shaker (Infors HT, Basel, Switzerland) at 14 h light- (illuminated at 50 µmol/m^2/s) and 10 h darkness-cycles. For the growth

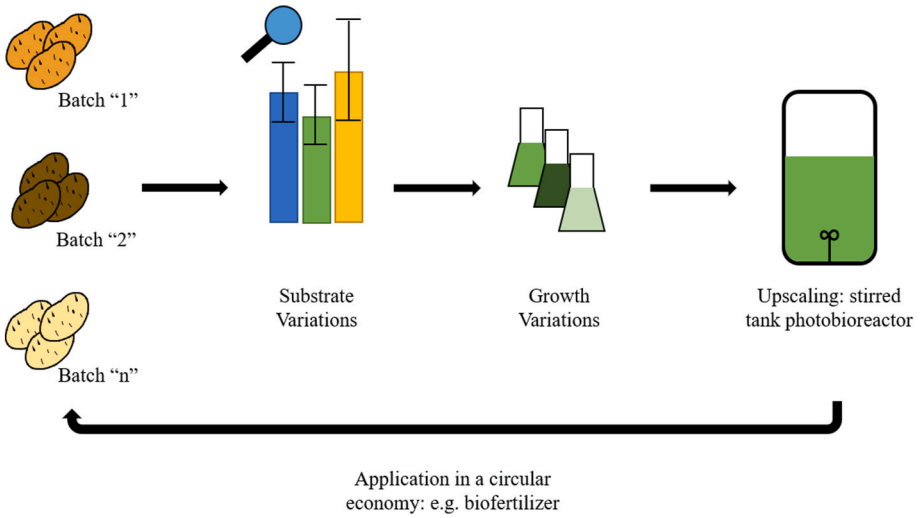

Fig. 1. Schematic representation of the study design. Different batches from four different side streams from a potato processing company have been screened for the best performing medium in terms of biomass growth and biomass composition. The best performing substrate has been upscaled into a stirred tank photobioreactor.

experiments the obtained side streams were used either (i) unsterilized as delivered (ii) sterilized without any pretreatment, (iii) unsterilized with normalized N-concentration in regards to the used BG11 medium, or (iv) sterilized with normalized N-concentration in regards to the used BG11 medium. N-normalization was done by the addition of $NaNO_3$ to match the N-concentration of BG11. All side streams were centrifuged at 10,000 g and 4 °C for 10 min to dispose of submerged particles. Fifty mL of the side stream corresponding to (ii) and (iv) were aseptically added to 250 mL Erlenmeyer flasks before inoculation to a starting optical density at 600 nm (OD_{600}) of 0.10. Experiments (i) and (iii) were carried out without any sterilization of the medium. Cultivations were performed in a 50 mL scale utilizing 250 mL shakeflasks and were monitored for any contaminations by microscopy and flow cytometry. Sampling was done in regular intervals (weekly) for 4 weeks. One mL aliquots were centrifuged at 10,000 g and 4 °C for 10 min and the supernatant was used to quantify anions by ion chromatography analysis. At the end of cultivation, the harvested biomass was lyophilized (Freezone 2.5 Benchtop Freeze Dryer, Labconco, Kansas City, MO, USA) and analyzed in terms of specific chlorophyll and protein content and productivities (specific and volumetric) over the cultivation time were calculated. The suitability of the tested side streams as cultivation medium was assessed in terms of biomass growth and composition. Depending on the results of the initial screening experiment, the best-performing side stream in terms of growth performance was chosen for additional experiments. Therefore, this side stream was sampled over a timeframe of 3 months to analyze batch to batch variations and test growth on these multiple batches. Subsequently, the cultivation was upscaled into stirred tank photobioreactor and compared to a state-of-the-art photoautotrophic cultivation.

2.4. Batch-to-batch variations

Blanching water II was sampled biweekly at eight time points in a timeframe of 3 months to test for batch-to-batch variations. The analyzed batches with the lowest and the highest acetate concentration were chosen for additional screening experiments. This was done to determine the variations in the corresponding microalgal biomass growth based on the different side stream composition. The side streams were analyzed according to chapter 2.2.

2.5. Photobioreactor cultivation

For photobioreactor cultivations (PBR), a Ralf bioreactor system (Bioengineering, Wald, Switzerland) with a 2 L working volume, was used. The illumination for the cultivation (14 h light/10 h darkness) was provided by 5 m LED stripes (Paulmann, Völksen, Germany) carrying 100 LEDs with a total luminous flux of 1920 lm wrapped around the glass vessel. Illumination inside the reactor was 100 µmol/m^2/s in BG11 pH 7.5 medium, checked with an ULM 500 Light Meter, equipped with an US-SQS/L sensor (Walz, Effeltrich, Germany). pH was measured with an in-line EasyFerm pH electrode (Hamilton, Reno, NV, USA), and controlled at 7.5 ± 0.4 (to account for the day and night shifts in CO_2 uptake and corresponding pH shifts) throughout the whole process via addition of 2 M Na_2CO_3 and 1 M HCl. Temperature was set to 23 °C and the agitation to 300 rpm in a constant manner. Aeration flow of the 3 % (v/v) CO_2-enriched air was in total 300 mL/min for PBR cultivation was separately controlled by two type 4850 mass flow controllers (Brooks Instruments, Hatfield, PA, USE) operated by the 0254 control unit (Brooks Instrument, Hatfield, PA, USA). For inoculation, the preculture was added through a septum to reach a starting OD_{600} of 0.10. Sampling was done in regular intervals (daily) for two weeks via a sampling port. The samples were analyzed for OD_{600} and afterward centrifuged at 10,000 g and 4 °C for 10 min. The supernatant was stored for ion chromatography and ICP-OES analysis at −20 °C. The harvested biomass at the end of cultivation was lyophilized and analyzed in terms of total chlorophyll- and protein content.

2.6. Determination of growth performance

Cell growth was determined by measuring OD_{600} on a Nanodrop One photometer (Thermo Fisher Scientific, Waltham, MA, USA). Ten mL of *Chlorella vulgaris* growth suspension at different OD_{600} values, were centrifuged, washed with ddH_2O and then dried in pre-weighed glass test tubes (n = 3). By weighing the dried biomass corresponding to certain OD_{600} measurements, the correlation between DCW and OD_{600} was established in the following Equation (1).

$$DCW = 0.283 \text{ x } OD_{600} \quad (1)$$

Growth parameters and productivities were calculated according to Equations (2)–(5).

$$\mu = \frac{(\ln DCW_2 - \ln DCW_1)}{t_2 - t_1} \quad (2)$$

$$q_s = \frac{1}{DCW_2} \text{ x } \frac{(S_2 - S_1)}{(t_2 - t_1)} \quad (3)$$

$$r_X = \frac{(DCW_2 - DCW_1)}{(t_2 - t_1)} \quad (4)$$

$$r_{product} = \frac{\%\ (w/w)_{product}}{DCW} \text{ x } r_X \quad (5)$$

DCW_X: dry cell weight (g/L) at timepoint t_X (day); OD_{600} optical density at 600 nm; µ: growth rate (day^{-1}); q_s: specific substrate uptake rate (g/g/day); S_x: substrate concentration (g/L) at timepoint t_x; r_X: volumetric biomass production rate (mg/L/day); $r_{product}$: volumetric product production rate of either chlorophyll or protein (mg/L/day)

2.7. Measurement of photosynthetic activity

Photosynthetic activity of the cultures was monitored using a WATER-PAM-II chlorophyll fluorometer (Walz, Effeltrich, Germany) using pulse-amplitude-modulation fluorometry. The software used was WinControl-3. The maximum photochemical efficiency of the photosystem II – PSII F_V/F_M was measured in a dark-adapted state. The samples were diluted 40-fold with 4 mL ultrapure H_2O (Milli-Q, Merck, Darmstadt, Germany) and adapted to darkness for 20 min in a dark space prior to measurement to ensure that all reaction centers of PSII were closed.

2.8. ICP-OES

For detection of the trace elements Mg, K, Zn, Co, Mn, Cu, Mo, Ni, Cr and B, induced coupled plasma optical emission spectroscopy –ICP-OES was used. The limits of detection –LODs were for Mg 0.0005 mg/L, K 0.32 mg/L, Zn 0.02 mg/L, Co 0.03 mg/L, Mn 0.0024 mg/L, Cu 0.01 mg/L, Mo 0.10 mg/L, Ni 0.113 mg/L, Cr 0.060 mg/L and B 0.03 mg/L. The analysis was carried out on an iCAP series spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with an ASX-520 auto sampler (Teledyne Cetac, Omaha, NE, USA), a standard sample introduction set consisting of a concentric nebulizer and a cyclonic spray chamber. Instrumental parameters and conditions used for determination of background corrected emission signals were previously described by Pekarsky et al. (2020). Aqueous standards with a known concentration were used for quantification via a calibration curve.

2.9. High performance liquid chromatography

The supernatant of the sampled aliquots was analyzed for carbon sources using a Vanquish Core system (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a variable wavelength diode array detector (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 210 nm and a Refractomax 521 refractive index detector (Thermo Fisher

Scientific, Waltham, MA, USA). As analytical column, an Aminex HPX-87H was used at 60 °C with 4 mM H_2SO_4 as mobile phase at a flow velocity of 0.6 mL/min running for 30 min isocratically. The carbon sources were quantified by measuring standards of pure substances (Carl Roth, Karlsruhe, Germany) and establishing a calibration curve.

2.10. Ion chromatography

The supernatant of the aliquoted samples was also analyzed for anion content during the shakeflask and photobioreactor cultivations. A Dionex Integrion HPIC System (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a conductivity detector unit (Thermo Fisher Scientific, Waltham, MA, USA), combined with an ADRS 600 suppressor (Thermo Fisher Scientific, Waltham, MA, USA) and a CR-TC continuously regenerated trap column (Thermo Fisher Scientific, Waltham, MA, USA). An IonPac AS11-HC column, including a matching guard column, was used as a stationary phase at 30 °C. The mobile phase was 100 % ultrapure water generating a gradient with an EGC 500 KOH eluent generator cartridge (Thermo Fisher Scientific, Waltham, MA, USA). The binary gradient started at 0.2 mM and was raised from 0 min to 2.4 min to 5 mM KOH, from 2.4 to 15 min to 24 mM KOH, from 15 to 32.4 min increase to 38 mM KOH, and hold until 37.5 min. After this, the initial 0.2 mM KOH was reached after 40 min and held until 50 min for equilibration before the next injection. The flow velocity was 0.3 mL/min. Nitrate, nitrate, chloride, sulfate, citrate, acetate, and phosphate standards (Carl Roth, Karlsruhe, Germany) were prepared and used for quantification. Controlling, monitoring and evaluation of the analysis was performed with Chromeleon 7.2.10 Chromatography Data System (Thermo Fisher Scientific, Waltham, MA, USA).

2.11. NH_3 quantification

Two hundred µL of each liquid side stream was analyzed using a colorimetric assay with the Cedex Bio HT Analyzer (Roche Diagnostics, Mannheim, Germany).

2.12. Chlorophyll extraction and quantification

Ten mg of lyophilized biomass were used for total chlorophyll extraction. The exact extraction was described recently by Kriechbaum et al. (2023) and quantified as described by Porra et al. (1989). The absorption of the extracts was measured at 647 nm, 664 nm and 750 nm on a Nanodrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The chlorophyll *a and b* content and the sum of those two were calculated according to the formulas described below (Equations (6)–(8)).

$$Chl_a = 13.71 \text{ x } A_{664} - 2.85 \text{ x } A_{647} \quad (6)$$

$$Chl_b = 22.39 \text{ x } A_{647} - 5.42 \text{ x } A_{664} \quad (7)$$

$$Chl_{total} = Chl_a + Chl_b \quad (8)$$

Chl_a: Chlorophyll *a* content (µg/mL); Chl_b: Chlorophyll *b* content (µg/mL); Chl_{total}: sum of chlorophyll *a* and chlorophyll *b* (µg/mL) A_{664}: Absorbance at 664 nm; A_{647}: Absorbance at 647 nm.

2.13. Protein extraction and quantification

Protein extraction was conducted according to Slocombe et al. (2013). Five mg of lyophilized biomass were weighed into a 1.5 mL Eppendorf tube. 200 µL of 24 % (w/v) TCA were added and the biomass was incubated at 95 °C for 15 min. The sample was then diluted to 6 % (w/v) TCA by adding ultrapure H_2O (Milli-Q, Merck, Darmstadt, Germany) and centrifuged at 15,000 g and 4 °C for 20 min. The supernatant got discarded and the pellet was resuspended in 500 µL of a mixture containing 48 parts 2 % (w/v) Na_2CO_3 in 0.1 M NaOH, one part 1 % (w/v) NaK tartrate*$4H_2O$ in ultrapure water and one part 0.5 % (w/v) $CuSO_4.5H_2O$. After 3 h incubation at 55 °C, the supernatant was analyzed with a BCA assay (Smith et al., 1985). By comparing to a known bovine serum albumin concentration (Sigma Aldrich, St. Louis, MO, USA), the protein concentrations were determined.

2.14. Statistical analysis

Experiments were carried out in triplicates and means and standard deviations are reported. Statistical analysis was done using one-way variance ANOVA (post hoc Tukey test) in Origin Pro (2021b) (OriginLab Corporation, Northampton, MA, USA) with a p-value of <0.05 considered significant.

3. Results and discussion

In this study, four different side streams from the potato processing industry were compared in terms of their feasibility for *Chlorella vulgaris* cultivation and concomitant water remediation. The four side streams in varying experimental setups were screened for mixotrophic *Chlorella vulgaris* growth. The obtained microalgal biomass was analyzed for composition. All of the experiments were compared to a *Chlorella vulgaris* state-of-the-art photoautotrophic cultivation in BG11 medium.

3.1. Side stream composition

In Table 1, the compositions of the 4 obtained side streams are compared to the state-of-the-art photoautotrophic cultivation medium BG11. All of the obtained side streams contained nitrogen and phosphorous s needed for microalgal cultivation. Organic carbon sources, such as acetate and citrate, can be used by *Chlorella vulgaris* in a mixotrophic cultivation (Huang et al., 2017; Marudhupandi et al., 2014).

3.2. Shakeflask screening

The screening experiments were conducted to determine the amount of pretreatment needed for the obtained side streams in terms of sterilization and N-normalization. Without sterilization prior to cultivation microbial contaminations could be seen in microscope and flow cytometer axenicity checks. DR was the only side stream resulting in moderate microalgal growth without additional sterilization, but the

Table 1
Composition of the four side streams obtained from the potato processing company; BLI - blanching water I, BLII - blanching water II, DR - liquid digestate rest, ST - starchy water; BG11 composition was calculated from the medium composition to compare with a state-of-the-art *Chlorella vulgaris* medium; LOD – limit of detection.

Component (mg/L)	BL I	BL II	DR	ST	BG11
Acetate	1142	1403	<LOD	5360	0
Citrate	860	1509	<LOD	<LOD	10
Chloride	<LOD	<LOD	<LOD	<LOD	697
Sulfate	320	377	<LOD	341	0
Phosphate	93	187	120	77	22
Nitrate	<LOD	218	<LOD	<LOD	1000
Nitrite	151	214	612	160	0
B	0.31	0.25	0.17	0.15	0.50
Ni	<LOD	<LOD	<LOD	<LOD	0
Mn	0.13	0.10	2.61	0.17	0.50
Co	1010.44	2369.28	548.92	625.69	0.01
Cu	<LOD	<LOD	<LOD	0.117	0.02
Zn	0.12	0.21	<LOD	<LOD	0.06
Mg	0.42	0.66	<LOD	0.55	15.15
K	79.47	99.60	32.83	83.28	8.98
Cr	1528.20	1949.57	1159.73	1705.28	0
Mo	<LOD	<LOD	<LOD	<LOD	0.16
pH	5.39	6.91	8.48	5.33	7.50

biomass productivity (mg/L/day) was only 25 mg/L/day being approximately 20 % of the productivity of *Chlorella vulgaris* grown on BG11. Additionally, microbial growth besides *Chlorella vulgaris* was observed and therefore sterilization was deemed necessary. N-normalization of the side streams led to increased biomass productivities of *Chlorella vulgaris* in both non-sterile and sterile screening trials. But the sterile filtered side streams showed the best results in terms of microalgal growth, with the least amount of pretreatment. Detailed results of the screening experiments regarding N-normalization and using the side streams without sterilization can be found in Supplementary Material Section 1. The maximum growth rate (day^{-1}) was 31 % higher for *Chlorella vulgaris* grown on BLII compared to BG11. Thus, we used this side steam for further investigation. The protein content of 46.43 % (w/w) in *Chlorella vulgaris* grown on sterile-filtered BLII was similar with protein contents reported in literature varying from 20 % (w/w) (Ma et al., 2016), to 43 % (w/w) (Melo et al., 2018) up to 64 % (w/w) (Cai et al., 2022) depending on the used side stream.

Maximum growth rates of mixotrophically grown *Chlorella vulgaris* on side streams are ranging from 0.12 day^{-1} obtained on cheese whey (Abreu et al., 2012), 0.32 day^{-1} obtained on BBM +1 % corn steep liquor (Melo et al., 2018), to 1.41 day^{-1} obtained on BG11-based vinasse (Candido and Lombardi, 2020). The maximum growth rate of *Chlorella vulgaris* grown in shakeflasks on sterile-filtered BLII obtained in this study was 0.51 day^{-1} indicating the suitability of this side stream for microalgal cultivation, as no further medium supplementation was required. Compared to the maximum growth rate on BG11 in shakeflasks of 0.39 day^{-1}, a 30 % increase was achieved.

BLII was then sampled biweekly at the potato processing company over a timeframe of 3 months to determine the batch variabilities in ion content, trace metal content, pH-values and NH_3-content and the influence on microalgal growth performance.

3.3. Batch to batch variations of BLII

Eight additional batches of BLII were analyzed for variabilities in composition (Table 2). Batch to batch variations were tested to estimate fluctuations and assess concentration ranges for future applications. Deviations in the processed potatoes, such as varying strains or potato skin thickness, resulted in differences in the composition of the tested side streams. Acetate concentrations ranged from 912 mg/L to 2231 mg/L in the varying batches. As acetate is knowingly a feasible carbon source for *Chlorella vulgaris* cultivation (Ms et al., 2023; Cai et al., 2022), the following growth experiments were designed using BLII with the highest and lowest acetate concentration. Other important sources of energy for microalgal cultivation include N and P. Phosphate as a phosphorous source exceeds the amount of P contained in BG11 in every single BLII batch by at least 5-fold and at most 20-fold. The nitrogen source in BLII is composed of nitrite, nitrate and ammonia, compared to only nitrate in BG11. However, the total N-concentrations in BLII is lower in all of the analyzed batches compared to BG11 (Table 2).

The presence of heavy metals (B, Mn, Co, Cu, Cr & Zn) was detected in all BLII samples being common pollutants of soil and harvested tubers thereof (Priya et al., 2023; Bedoya-Perales et al., 2023). BG11 medium contains B, Mn, Zn, Mo, Cu and Co (Touloupakis et al., 2022) as well, which are necessary trace elements for *Chlorella vulgaris* growth. B, Cu and Zn were present in all of the BLII samples, but below the recommended values of the World Health Organization for drinking water (WHO, 2022). Mn is an important trace element in soil needed for healthy plant growth (Li et al., 2021), therefore the increased manganese content in the side stream most likely stems from the potato native manganese, taken up from the fields (Pandey et al., 2023). Cr and Co and are only found in $BLII_{original}$, indicating a possible contamination in one potato field (Fig. 2b). Studies show that heavy metal recovery from soil or liquid wastes through microalgal processes, including *Chlorella vulgaris* (Ahammed et al., 2023; Musah et al., 2022; Yousefi et al., 2023), are promising solutions for heavy metal polluted areas. In agreement with literature, Cr and Co were also taken up in $BLII_{original}$ (data not shown) proving the water remediating properties of *Chlorella vulgaris*. Due to chrome reductases present in *Chlorella vulgaris*, toxic Cr(VI) is reduced to Cr(III) posing less risk to the environment (Zou et al., 2020).

Nitrite as N-source was also only present in the $BLII_{original}$ batch (Fig. 2a). This further showed the importance to analyze different batches and study the effects on microalgal growth, as nitrogen is a valuable energy source for microalgae. Ammonium is favored by microalgal species as nitrogen source, as it can readily metabolized, while other nitrogen sources have to go through a cascade of enzymatic reactions (Pozzobon et al., 2021). Nitrate gets taken up by the cell and is then converted through a nitrate reductase to nitrite, which will then be successively reduced to ammonium. Still ammonia has to be monitored carefully as it is prone to stripping, which could lead to inhibition of microalgal growth processes (Markou et al., 2014).

To test the influences of the batch variabilities on the growth of *Chlorella vulgaris*, the highest and lowest acetate concentrations were used to test the influences of acetate concentration on growth and composition. $BLII_{high}$ with the highest acetate concentration was not as suitable as substrate compared to the $BLII_{original}$, presumably due to the

Table 2
Composition of the different blanching water II - BLII batches obtained; the different batches were taken in a time frame of 3 months to account for multiple different potato batches used; $BLII_{high}$ – indicates the highest acetate concentration; $BLII_{low}$ indicates the lowest acetate concentration; LOD – limit of detection.

Component (mg/L)	A	B	C	D	E	F	G ($BLII_{high}$)	H ($BLII_{low}$)	$BLII_{original}$
Acetate	1074	2082	1517	1225	1099	1901	2231	912	1403
Citrate	1412	1819	1437	1530	1756	2709	2817	1607	1509
Chloride	318	526	341	362	455	561	577	226	< LOD
Sulfate	306	513	401	420	475	664	567	197	377
Phosphate	287	449	251	289	363	535	328	213	187
Nitrate	58	92	53	47	60	96	126	58	218
Nitrite	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	214
B	0.16	0.26	<LOD	0.06	0.19	0.51	0.52	0.22	0.251
Ni	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mn	0.41	0.58	0.40	0.46	0.62	0.97	0.70	0.32	0.01
Co	<LOD	<LOD	<LOD	<LOD	0.22	<LOD	<LOD	<LOD	2369.28
Cu	0.13	0.27	0.16	0.15	0.37	0.73	0.28	0.28	<LOD
Zn	0.70	1.27	0.62	0.80	1.27	2.08	1.58	1.11	0.21
Mg	103.36	140.07	99.90	112.65	142.62	226.91	163.12	90.85	0.66
K	1867.95	2649.14	1921.81	2046.06	2046.06	1780.64	3153.81	1780.64	99.60
Cr	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1949.56
Mo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
NH_3	42.00	67.00	49.02	34.20	34.20	124.95	162.14	124.95	55.69
pH	5.99	5.70	5.64	5.98	5.98	6.30	5.31	6.30	6.91

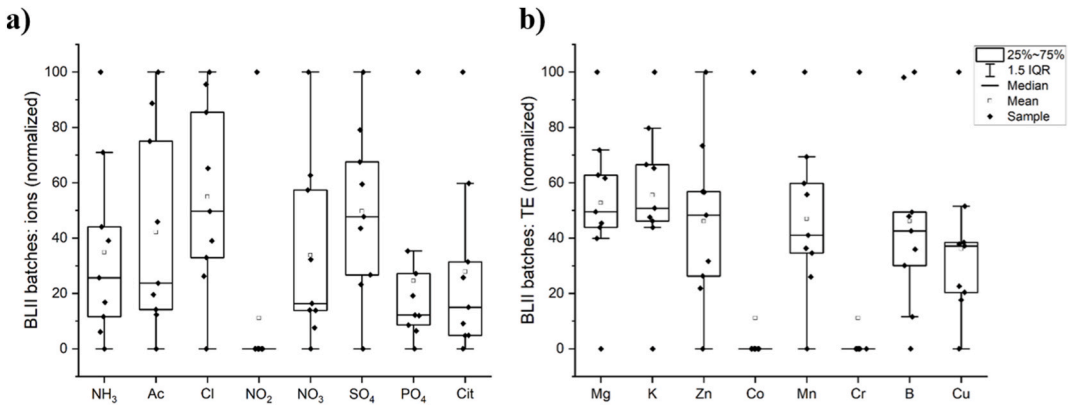

Fig. 2. a) Comparison of ion composition in 9 different batches of blanching water II – BLII, normalized, **b)** comparison of trace element - TE composition in 9 different batches of BLII; the batches were collected over the course of 3 months; Data was normalized to 1 to visualize the variations in the different batches analyzed; IQR – interquartile range; Ac – Acetate; Cit - Citrate.

lower starting pH value. On the contrary, $BLII_{low}$ resulted in 2-fold the final DCW (g/L) (Fig. 3a).

Specific protein contents were not significantly influenced by variations in BLII composition (Fig. 3b). However, specific chlorophyll contents were significantly influenced, as the acetate concentration differed. Increasing acetate concentrations in *Chlorella vulgaris* medium has shown to positively influence chlorophyll (Huang et al., 2017; Kriechbaum et al., 2023) and pigment biosynthesis in general (Cai et al., 2021), which is congruent with this study. Acetate is transported into the cells, where it is metabolized to acetyl-CoA, a crucial cofactor in chlorophyll biosynthesis. This may explain the positive effect of acetate on chlorophyll production. Roach et al. (2013) suggest that adding acetate to the medium protects microalgal cells from photoinhibition, which could reduce chlorophyll molecule degradation. Therefore, acetate addition can both enhance chlorophyll production and protect cells from chlorophyll degradation. A total chlorophyll content of 3.50 % (w/w) was obtained by cultivating *Chlorella vulgaris* in $BLII_{high}$, a net increase of 30 % compared to *C. vulgaris* cultivated in $BLII_{low}$. Generally, photoautotrophic cultivation of microalgal species tend to lead to higher chlorophyll production compared to mixotrophic cultivation (Patel et al., 2022), matching this study with the highest obtained chlorophyll content of 3.96 % (w/w) of *Chlorella vulgaris* grown on BG11.

Maximum growth rates of 0.52 day^{-1} were achieved in $BLII_{low}$ with the lowest acetate concentration (Table 3). The increase in acetate and

Table 3
Comparison of the growth performance in terms of maximal growth rate (t = 0–7 days) μ_{max} (day^{-1}), volumetric biomass productivity (t = 0–42 days) r_x (mg/L/day), protein productivity – r_P (mg/L/day) and Chlorophyll productivity – r_{Chl} (mg/L/day) in 3 different BLII batches and a state-of-the-art cultivation in BG11 in shakeflasks - SF and photobioreactor - PBR cultivation; BLII – blanching water II, $BLII_{original}$ – original BLII from first batch, $BLIII_{ow}$ – BLII with the lowest acetate concentration, $BLII_{high}$ – BLII with the highest acetate concentration; Errors indicate standard deviation (n = 3) and letter code indicates statistical significance, where different letters indicate significant difference ($p < 0.05$ – ANOVA).

Sample	Cultivation Vessel	r_X (mg/L/day)	μ_{max} (day^{-1})	r_P (mg/L/day)	r_{Chl} (mg/L/day)
BG11	SF	105.11 ± 4.40^{a}	0.39 ± 0.02^{a}	39.87 ± 1.67^{ab}	4.17 ± 0.17^{a}
$BLII_{original}$	SF	92.00 ± 2.04^{b}	0.51 ± 0.01^{b}	42.72 ± 0.95^{a}	2.83 ± 0.06^{b}
$BLII_{low}$	SF	156.50 ± 9.69^{b}	0.52 ± 0.00^{b}	71.32 ± 4.42^{c}	3.85 ± 0.24^{a}
$BLII_{high}$	SF	62.53 ± 1.34^{c}	0.47 ± 0.00^{c}	33.47 ± 0.72^{b}	2.18 ± 0.05^{c}
BG11	PBR	198.77	0.89	90.84	6.03
$BLII_{original}$	PBR	294.64	1.29	136.80	9.09

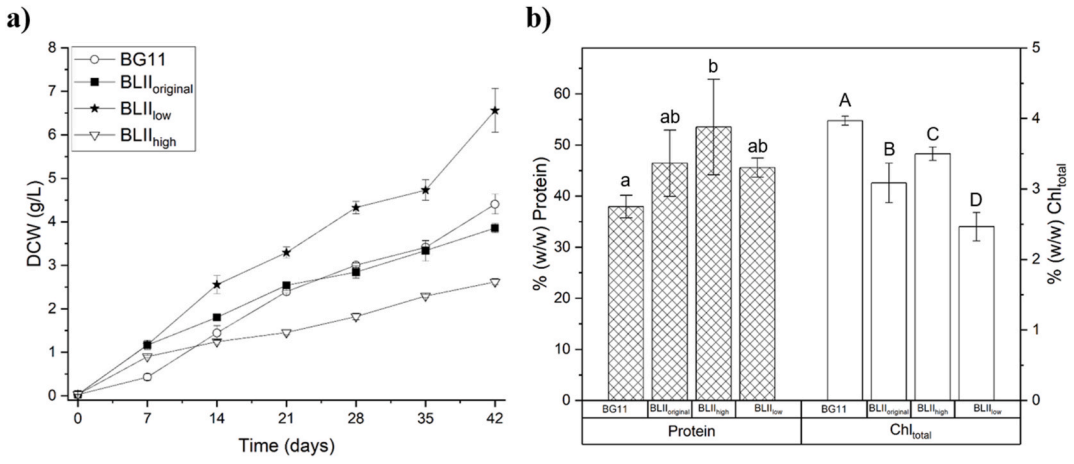

Fig. 3. a) Comparison of obtained *Chlorella vulgaris* dry cell weight (DCW) in g/L in 3 different batches from shakeflask cultivations with blanching water II – BLII as substrate **b)** Specific protein content % (w/w) and total chlorophyll content % (w/w) of *Chlorella vulgaris* obtained from these cultivations; $BLII_{low}$ – BLII with lowest acetate concentration, $BLII_{high}$ – BLII with highest acetate concentration. $BLII_{original}$ – original BLII from the first substrate screening; Error bars indicate standard deviation (n = 3) and letter code indicate statistical significance, where different letters indicate significant difference ($p < 0.05$ – ANOVA).

citrate content leads to a decrease in pH-value and therefore limiting growth of *Chlorella vulgaris* having its pH optimum at about 7.50–8.50. Even though cultivation on $BLII_{high}$ exhibited a higher specific growth rate than *Chlorella vulgaris* grown on BG11, the biomass productivity is almost halved, as the acidic pH retarded the microalgal growth (Rachlin and Albania, 1991). Growth rates were calculated during the first 7 days of cultivation, where a lot of organic C-sources are still present in $BLII_{original}$, $BLII_{low}$ and $BLII_{high}$. This explains the higher growth rates in all mixotrophic cultivation, compared to the photoautotrophic cultivation in BG11.

Measurements of photosynthetic activity showed no significant decreases of *Chlorella vulgaris* cultivated in $BLII_{high}$ compared to $BLII_{low}$ and $BLII_{original}$ (Supplementary Materials Section 2). These results are similar to *Chlorella vulgaris* grown mixotrophically on different side streams, such as 120 mg/L/day on whey (Melo et al., 2018), 80 mg/L/day on molasses (Guo et al., 2020) or water from a wastewater treatment plant 250 mg/L/day (Mendez et al., 2016).

In terms of protein productivities (Table 3), *C. vulgaris* grown on $BLII_{original}$ and $BLII_{low}$ outperformed the cultivation on BG11 by 1.3-fold and 2.2-fold, respectively, while $BLII_{high}$ produced equal amounts of protein per day. In terms of volumetric chlorophyll productivity, *Chlorella vulgaris* grown on BG11 showed the highest productivity with 4.17 mg/L/day, while $BLII_{original}$ produced 2.83 mg/L/day, $BLII_{low}$ 3.85 mg/L/day and $BLII_{high}$ 2.18 mg/L/day.

For biofertilization approaches, microalgal biomass should be high in N, P, K, and Mn (Ng et al., 2024; Cao et al., 2023; Mariotti et al., 2008). Therefore, *Chlorella vulgaris* protein content produced in all BLII batches meets the requirement of at least 2.5 % (w/w) nitrogen in bio-based fertilizers (European ParliamentCouncil of the European Union, 1991). The biomass composition of *Chlorella vulgaris* cultivated in $BLII_{high}$, $BLII_{low}$ and $BLII_{original}$ is given in Supplementary Material Section 5. Maximization of protein content % (w/w) and volumetric productivity (mg/L/day) during the microalgal process is key to produce the most valuable biofertilizer. Pigments from bacteria in combination with vitamins have been shown to benefit plants' resistance to environmental stress factors when used in fertilization approaches (Orlandi et al., 2022). Increased chlorophyll contents might also aid plant growth and crop yield, but there needs to be additional research addressing the impacts of microalgal pigments on plants.

3.4. Photobioreactor cultivation

After shakeflask experiments, *Chlorella vulgaris* cultivation in a stirred tank photobioreactor in BG11 medium was compared to a cultivation using $BLII_{original}$ as substrate. $BLII_{original}$ was autoclaved prior to cultivation in the PBR, but not centrifuged as BLII batches contained barely any submerged particles ($OD_{600} = 0.05$). Autoclavation did not have an effect on the $BLII_{original}$ composition, verified by HPLC, IC, Cedex and ICP-OES measurements.

In Fig. 3a *Chlorella vulgaris* growth in $BLII_{original}$ and BG11 is compared over the timeframe of an 18-day long cultivation. The maximum growth rate in $BLII_{original}$ was 32 % higher than the growth rate in BG11 (Table 3). This resulted in a final DCW content of 4.75 g/L in $BLII_{original}$ compared to 3.22 g/L DCW in BG11. Due to increased CO_2 and O_2 diffusion and more homogenous illumination in stirred tank photobioreactor systems compared to shakeflask cultivations (Doppler and Oliver, 2021), the increased growth rates and final DCWs of *Chlorella vulgaris* cultivated in a PBR system can be explained in both $BLII_{original}$ and BG11. In the illuminated shaker the light source is on top, which leads to less homogenous illumination within the cultivation flask (Nedbal et al., 2020), compared to the photobioreactor which is illuminated from all directions. During wastewater treatment, dissolved oxygen content might be critical for organic carbon respiration (Flores-Salgado et al., 2021).

The specific protein and chlorophyll content were not significantly different compared to the photoautotrophic state-of-the-art cultivation in BG11 (Fig. 3b). The specific protein contents were 45.70 % (w/w) in BG11 and 46.43 % (w/w) in $BLII_{original}$. The specific total chlorophyll contents were 3.03 % (w/w) in BG11 and 3.07 % (w/w) in $BLII_{original}$.

Compared to shakeflask cultivations, PBR cultivations of *Chlorella vulgaris* in BG11 led to a 20 % increase in protein content % (w/w) of the harvested biomass. In photoautotrophic cultivation, homogenous lighting is crucial for biomass composition and growth, whereas mixotrophic cultivation is not as light-dependent. At harvest, the DCW of *C. vulgaris* in the PBR cultivation was 36 % higher, resulting in increased self-shading and decreased light availability (Fig. 4a). Decreased light availability during cultivation has been shown to negatively influence protein production (Seyfabadi et al., 2010). A factor influencing pigment production is phosphorous limitation, which lasted much longer in the shakeflask cultivation compared to the PBR cultivation. These depletions have been shown to increase chlorophyll production in microalgae (Ahmad et al., 2022). *Chlorella vulgaris* grown on $BLII_{original}$ had phosphorous reserves from the 10-fold higher starting concentration, sustaining its composition.

Biomass productivities were 48 % higher in *Chlorella vulgaris* cultivated in the PBR using BLII as a substrate compared to BG11 (Table 3). The volumetric productivities r_P (mg/L/day) and r_{Chl} (mg/L/day) of *Chlorella vulgaris* grown on BLII were therefore also about 50 % higher than for *Chlorella vulgaris* grown on BG11. This is due to the higher biomass production in mixotrophic cultivation compared to photoautotrophic cultivation (Patel et al., 2021), but similar biomass compositions (Fig. 4b).

Overall, the substitution of BG11 with BLII in the stirred thank photobioreactor showed promising results in terms of competing with BG11 as a microalgae medium, as the side stream used did not alter the biomass composition significantly in regard to protein and chlorophyll content. This shows that microalgal cultivation in side streams resulting in biomass with similar economical value is possible. Therefore, potato processing side streams could lead to reduction of fresh water consumption for microalgal medium preparation.

3.5. Water remediation

Microalgal nutrient retrieval from wastewaters has been the scope of extensive research in the last years, due to the substantial capacity in N and P uptake from side streams (Yu et al., 2023). The uptake of total available N and total available P was calculated to determine the wastewater remediation effect of *Chlorella vulgaris* on the tested side streams. N and P are two main compounds responsible for water eutrophication (Abdelfattah et al., 2023), hence the removal of those two compounds is of utmost importance. The reduction of N and P has been calculated in percentage of total removal from different starting concentrations (g/L) of the varying BLII batches in shakeflask and photobioreactor cultivations (Table 4). The N and P uptake of the screening experiments can be seen in the Supplementary Materials Section 3.

As in the photobioreactor cultivations, all of the available phosphorous was consumed during the experiment, even in $BLII_{high}$, where phosphorous content was 0.187 g/L and thus about 10-fold the concentration of phosphate in BG11. In a mechanism called "luxury uptake", microalgae are able to store excess phosphorous from a medium as polyphosphates within the cells. These polyphosphate storage granules may then be metabolized in phosphorous limited environments (Powell et al., 2009). The nitrogen contents were also decreased by at least 90 % during the cultivation, showing the potential of wastewater remediation by *Chlorella vulgaris*. These results show the enormous added value of adding a microalgal wastewater cleaning facility to the potato processing plant, as the nitrogen and phosphorous sources could be eliminated from the industrial side streams almost completely.

Incorporation of N and Ps into intracellular storage granules of microalgae is superior to state-of-the-art N and P capture of wastewater treatment plants (WWTP). In municipal wastewater treatment, nitrogen

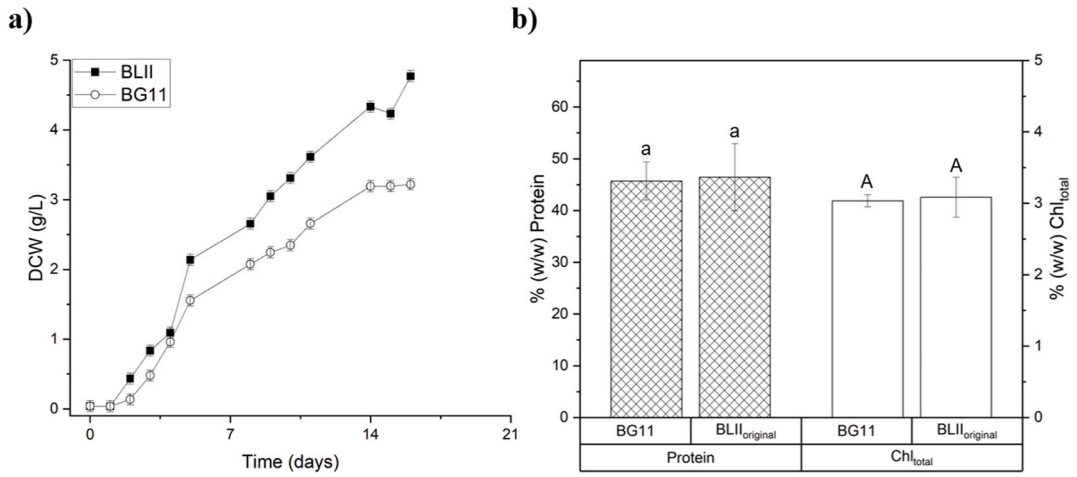

Fig. 4. a) Comparison of the dry cell weight – DCW (g/L) of *Chlorella vulgaris grown* on BG11 and *Chlorella vulgaris* grown on blanching water II in a photobioreactor cultivation. BLII was autoclaved in the used photobioreactor and was therefore sterile **b)** Specific protein content % (w/w) and total chlorophyll content % (w/w) of *Chlorella vulgaris* from photobioreactor cultivations with blanching water II – BLII and BG11 as media; Error bars indicate standard deviation (n = 3) and letter code indicate statistical significance, where different letters indicate significant difference ($p < 0.05$ – ANOVA).

Table 4
Starting concentration - c_{start} (g/L)of nitrogen and phosphorous in BG11 and blanching water II batches. The total % of metabolized nitrogen and phosphorous during shakeflask - SF and photobioreactor - PBR cultivation with different batches of blanching water II – BLII as substrate; $BLII_{original}$ – original BLII from the initial screened batch, $BLII_{low}$ – BLII with the lowest acetate concentration, $BLII_{high}$ – BLII with the highest acetate concentration.

Sample	Cultivation Vessel	N-Uptake		P-Uptake	
		c_{start} (g/L)	%	c_{start} (g/L)	%
$BLII_{original}$	SF	0.488	90.77	0.187	100
$BLII_{low}$	SF	0.242	100	0.213	100
$BLII_{high}$	SF	0.297	100	0.328	100
BG11	PBR	1.150	59.44	0.020	100
$BLII_{original}$	PBR	0.488	100	0.187	100

is going through bacterial nitrification and denitrification where high external oxygen input is required (Brar et al., 2019). This process results in the conversion of organic to molecular N which is released into the atmosphere and lost for alternative use. Capture of organic N in microalgal processes is therefore preferred as this leads to direct reuse (Ortiz Tena et al., 2024) as well as CO_2 capture. In WWTPs, phosphorous is usually chemically precipitated using Al or Fe ions resulting in removal efficiencies of about 93–99% (Di Capua et al., 2022), but these processes are chemically intensive, expensive and not sustainable. Through microalgal capture of N and phosphorous, those compounds would be stored in the biomass and the complete biomass could be used for biofertilization approaches.

Alike shakeflask cultivations, the complete phosphorous was removed during photobioreactor cultivations. In BLII PBR cultivation, the complete N (NO_3, NO_2, NH_3) was metabolized by *Chlorella vulgaris*. The increased CO_2 diffusion and light homogeneity in a stirred tank PBR led to an increased N metabolization in *Chlorella vulgaris* compared to the shakeflask cultivations. Similar N and phosphorous removal efficiencies in microalgal water remediation processes have been described in literature (Chawla et al., 2020; Rasoul-Amini et al., 2014).

In Fig. 5 the decrease of phosphorous and N content during the photobioreactor cultivation can be seen. The dotted line represents the maximum concentrations allowed in WWTP effluents concerning N and P (European ParliamentCouncil of the European Union, 1991). As all of the N and phosphorous contained in BLII was metabolized during *Chlorella vulgaris* cultivation, these threshold concentrations have been met.

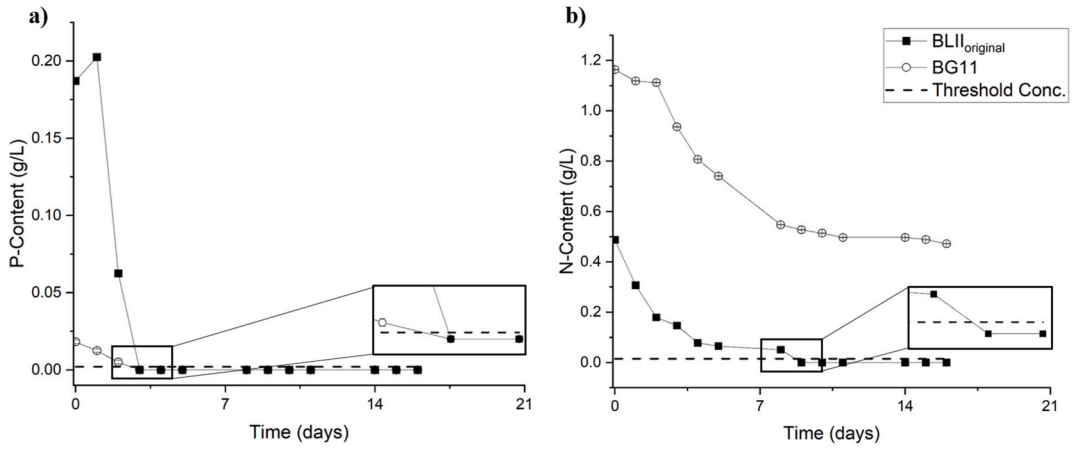

Fig. 5. Decrease of **a)** nitrogen – N-Content (g/L) and **b)** phosphorous – P-Content content (g/L) in the photobioreactor cultivation of *Chlorella vulgaris* using BG11 and blanching water II – $BLII_{original}$ as substrate over time; The dotted line represents the maximum discharge concentration in nitrogen (15 mg/L) and phosphorous (2 mg/L); Error bars represent the standard deviation of the measurement (n = 3).

4. Scale-up calculation

With the data obtained from the stirred tank photobioreactor cultivation and data available from literature a scale-up into a continuously operated tubular photobioreactor system (Fig. 6) was calculated. The critical growth rate (μ_{crit}) 0.034 h^{-1} was assumed very conservatively at 80 % of the maximal growth rate (μ_{max}) 1.29 day^{-1} estimating a dilution rate (D) avoiding washout at any chance assuming a constant biomass yield.

The volumetric flow was estimated to be 5500 L/h which is the yearly average of the blanching water II being produced at the potato processing company. To take the differences in sunlight during the seasons into account the average time of light per day (timeanddate, 2024) in Austria was calculated. Feasible concentration factors of 2-fold. 4-fold and 10-fold of BLII have been provided by the potato processing company, to reduce the volumetric influxes F_{in} (g/L) into the theoretical up-scaled photobioreactor system. Specific P uptake rates q_P 0.08 g/g/h and specific N uptake rates q_N 0.12 g/g/h were calculated (Equation (3)) to determine the maximal feedrate g/L/h, based on S_{in} 0.187 g/L P, in order to not wash out and N or P present in BLII. As feasible biomass concentration without cell retention, X_{in} 2 g/L of *Chlorella vulgaris* was chosen, based on the results of the photobioreactor cultivation.

As P and N uptake mostly occurs during the day, night phases have to be taken into account when calculating the theoretical upscaling into a tubular photobioreactor system. In dark phases microalgal N and P uptake is usually decreased compared to photosynthesis driven nutrient uptake in light conditions (Ortiz Tena et al., 2024). Hence a light factor – sunlight/day – was added to the calculation of the dilution rate (day^{-1}; h^{-1}). This factor was calculated based on the average hours of sunlight in Austria during the seasons obtained from timeanddate (2024). The envisioned process was assumed to be dependent on the light phases of the day, on the season and the side stream influx being fed into the semi-continuous system (Equation (12)). During night time, feed and bleed is assumed to stop and the system will be maintained only with aeration. This would allow organic C-sources and excess nutrients to be metabolized. In Supplementary Materials Section 4, estimated dilution rates for that process are shown. During spring and summer time, about 60 % of the day is in light conditions, whereas during autumn and winter time, only about 40 % of the day is bright on average. In Table 5 are the calculated dilution rates (h^{-1}) for the hypothetical upscaling into a tubular reactor system, to remediate the side stream, in the different seasons with different amounts of light during the day and the corresponding N and P uptake during that season.

Equations (9)–(13) describe the calculation of the new estimated seasonal dilution rate D (h^{-1}) and the yearly Uptake of N and P

$$\mu = D \tag{9}$$

$$D = \frac{F}{V} \tag{10}$$

$$\mu_{crit} = \mu_{max} \text{x } 80\% \tag{11}$$

$$D_{season} = \mu_{crit} \text{ x sunlight/day} \tag{12}$$

$$\frac{\text{Uptake (P, N)}}{\text{Year}} = V \text{ x } S_{in} \text{ x } D_{season} \text{ x } 8760 \text{ h} \tag{13}$$

μ: growth rate (day^{-1}); D: dilution rate (day^{-1}; h^{-1}); F: volumetric flow rate of substrate into (F_{in}) and out (F_{out}) of photobioreactor system (L/h); V: volume of photobioreactor system (L).

A vessel size of 156.7 m^3 was calculated for the theoretical photobioreactor system, as this was the calculated volume required for a system running at the estimated dilution rate (D_{season}) 0.0175 h^{-1} at maximum capacity in wintertime with a 2-fold concentration of the side stream. The dilution rates for spring, summer and autumn have been adapted accordingly, to run in the same sized PBR system, with decreased dilution rates, to maintain a feasible water remediation throughout the year. Theoretically, higher dilution rates could be implemented in spring or summer season, as the increased light availability during the day, opens the possibility for increased dilution rates (D_{season}). At the potato processing facility heat is generated through biogas combustion, providing heat throughout the complete production area and all processing steps. The process heat would then be supplied to the tubular photobioreactor system via heat exchangers, during the colder seasons of autumn and winter to counteract the drops in temperature and concurrently prevent washout of biomass and nutrients.

The estimated area needed for facilitating such a process is calculated based on the size of an Austrian microalgae producer (Jongerius-Ecoduna, 2023) and would approximately be 2000 m^2, as Jongerius-Ecoduna (2023)states their areal need as 10,000 m^2 for 800 m^3 tubular photobioreactor. During each 3-month long season, working with a 2-fold concentrated side stream as feed, in a 156.7 m^3 vessel, would result in the capture of 1.51 tons of P and 3.94 tons of N, highlighting the microalgal capability of wastewater recovery of potato processing water.

5. Conclusion

In this study, we showed that wastewater or side stream remediation through microalgal processes is feasible for an Austrian potato processing plant, but knowing the composition and variation is crucial in process design. *Chlorella vulgaris* was able to metabolize at least 90 % of the provided nitrogen and all of the phosphate contained within several batches of the obtained side streams. Not only was the potential of

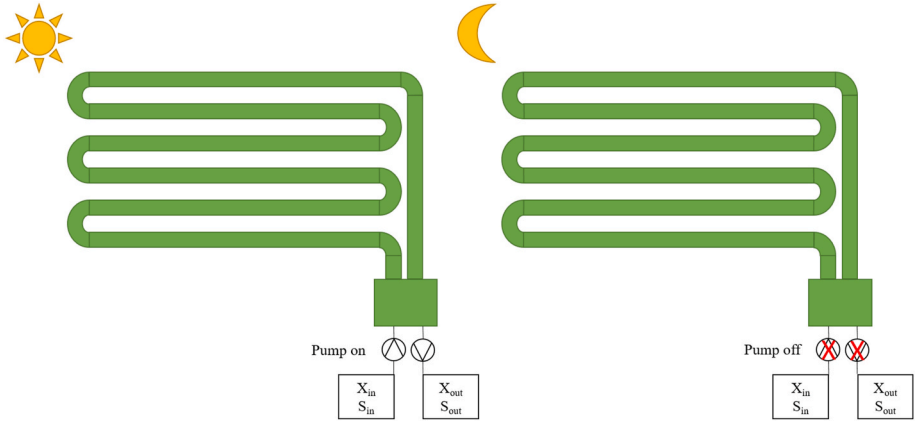

Fig. 6. Schematic representation of a continuously operated tubular photobioreactor during daytime (light) and nighttime (dark). During the nighttime the volumetric influx will be stopped, to not wash out the biomass due to limited growth in the darkness; X – biomass concentration (g/L), S – substrate concentration (g/L).

Table 5
Calculated nitrogen - N and phosphorous - P (t) uptake during each season of the year, in an estimated vessel size of 156.7 m^3. Sunlight/Day was calculated based on average hours sunlight/day (timeanddate 2024). The concentration factor of 2-fold was supplied as technically feasible by the Austrian potato processing enterprise; F_{in} (L/h) – volumetric influx of side stream; S_{in} – P concentration in 2-fold concentrated blanching water II influx; D_{season} (h^{-1}) is the maximum dilution rate feasible in each season, but must be at least 0.0175 h^{-1} due to seasonal light variations. In one year, 3.94 t of N and 1.51 t phosphate would be remediated by this hypothetical microalgae facility.

Season	Sunlight/Day	Conc. Factor	F_{in} (L/h)	S_{in} (g/L)	D_{season} (h^{-1})	N-Uptake/ season (t)	P-Uptake/ season (t)
Spring	60.69 %	2 x	2750	0.374	0.0261	3.94	1.51
Summer	60.16 %				0.0259		
Autumn	40.83 %				0.0176		
Winter	40.81 %				0.0175		

wastewater remediation shown, but *Chlorella vulgaris* generated in cultivation processes with side streams, showed similar biomass compositions compared to a state-of-the-art cultivation with BG11 medium. This biomass should be further tested for its biofertilization purposes as microalgae have been proven to be sustainable alternatives to state-of-the-art fertilization, to close the loop to the potato plant. An initial upscale design was calculated and would result in approximately 16 t N and 6 t phosphate remediation from the processing plant throughout the year.

However, further studies are required to include hydrodynamic properties and temperature fluctuations within the proposed upscaling design of the process into a tubular photobioreactor. As mixing, aeration and temperature consistency are very different in stirred tank photobioreactors with tubular flow, compared to a mostly laminar flow driven tubular reactor system. To conclude, it has been shown, that the side stream blanching water II of an Austrian potato processing company can readily be remediated through microalgal processes, in addition to generating a valuable product, namely *Chlorella vulgaris* biomass.

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Availability of data and material

The data obtained in this study is available on request from the corresponding author.

Code availability

Not applicable in this study.

CRediT authorship contribution statement

Ricarda Kriechbaum: Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Laura Kronlachner:** Writing – original draft, Methodology. **Andreas Limbeck:** Writing – review & editing, Supervision, Methodology. **Julian Kopp:** Writing – original draft, Supervision, Investigation. **Oliver Spadiut:** Writing – review & editing, Supervision, Resources, 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.

Data availability

Data will be made available on request.

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The graphical abstract was created with Biorender.com.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.121796.

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