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REVIEW
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MAB2.0 project: Integrating algae production into wastewater treatment

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Abstract

Different species of microalgae are highly efficient in removing nutrients from wastewater streams and are able to grow using flue gas as a CO_2 source. These features indicate that application of microalgae has a promising outlook in wastewater treatment. However, practical aspects and process of integration of algae cultivation into an existing wastewater treatment line have not been investigated. The Climate-KIC co-funded Microalgae Biorefinery 2.0 project developed and demonstrated this integration process through a case study. The purpose of this paper is to introduce this process by phases and protocols, as well as report on the challenges and bottlenecks identified in the case study. These standardized technical protocols detailed in the paper help to assess different aspects of integration including biological aspects such as strain selection, as well as economic and environmental impacts. This process is necessary to guide wastewater treatment plants through the integration of algae cultivation, as unfavourable parameters of the different wastewater related feedstock streams need specific attention and management. In order to obtain compelling designs, more emphasis needs to be put on the engineering aspects of integration. Well-designed integration can lead to operational cost saving and proper feedstock treatment enabling algae growth.

Keywords: wastewater treatment, microalgae, bioresource

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Background

In recent years, microalgae have received more attention in applied biotechnological studies in various aspects of energy (1), water (2) and high added-value bioproducts (3). Considering the economic aspects of integrating algae technology into a municipal wastewater treatment plant (WWTP), use of Life Cycle Assessment (LCA) and Technoeconomic Analysis could help to find a viable market position for the technology (4-7). At the moment large-scale wastewater-integrated algae facilities have not emerged in spite of the promising opportunities. Cultivation of microalgae in wastewater or related substrates is a prominent field inspiring the scientific community (8-10), because they can be used as nutrient sources for microalgae due to high nitrogen and phosphorus content (11), thereby reducing the load on the WWTP, as well as algae support microbial oxidative activities by producing oxygen during photosynthesis (12), and also accumulate heavy metals (13, 14).

The conventional mechanism of treating municipal wastewater is a sequenced process. The primary treatment aims to mechanically remove solid materials, followed by the secondary treatment to reduce organic matter and nutrients. Tertiary treatment may be also applied to polish the effluent. These steps are referred as the water line, while the usual aerated activated sludge mechanism as secondary treatment results in sludge to be managed in the sludge line.

WWTPs are focusing on the water line as that delivers clean water which is the main performance and legal indicator; improvement of the sludge line by means of energy and cost efficiency, as well as its environmental performance is lagging behind. While anaer-

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obic digestion reduces the volume of the sludge, the recovery of macro- and micronutrients is not solved despite its priority in circular economy. The liquid fraction after dewatering the digested sludge (called anaerobic digestion (AD) effluent) is a promising substrate to grow microalgae (15). Usual solution to manage this fraction is to return it to the start of the water line (referred as return flow), as nutrient content is above legal threshold. However, this return flow can be responsible for up to 30% nitrogen and phosphorus load on the biological treatment due to the significantly higher concentrations than incoming raw wastewater. As microalgae can remove this excess amount of nutrients from the AD effluent, its application leads to saving on operational cost and capacity while producing biomass as added value product. Furthermore, anaerobic digestion derived biogas is often used on-site to generate power in gas engines with flue gas emission, of which CO_2 content is a potential carbon source for microalgae (16, 17).

Valorization of waste streams can have a positive impact on the long-term sustainability of the industry. The transition to adopt new technologies, however, is a challenge, as they are outside the core business of wastewater treatment and require capital-intensive investments. However, experiments have begun in many places. Usually tertiary stage benefits from microalgae cultures, but attempts have been made to involve microalgae into the secondary stage too (18).

There are many successful experimental results in controlled, sterile, laboratory conditions using synthetic media and photobioreactors (PBRs) (19, 20), but the quality of AD effluent presents some problematic aspects, such as suboptimal composition for microalgae, seasonal changes and suspended materials difficult to settle, as well as contains a microflora that may compete with microalgae in the presence of organic carbon.

Purpose and scope

The purpose of this work is to present and discuss the steps and challenges to carry out the integration process of microalgae cultivation into wastewater treatment from zero to the actual operation of the pilot design. This step-wise and consequential process can guide the sector to design microalgae integration from the sole intention to the pilot technology. The process is composed of the next following phases:

1. Preliminary evaluation
2. Design objectives
3. Pilot operation and tests
4. Microalgae quality assessment

The necessary competences and approaches to fulfil and evaluate a phase, are described in form of protocols. These protocols, developed during the work, include all aspects of the integration in its complexity. Next to technological aspects, protocols also help to draw up economically sound scale-up scenarios and business models, as well as evaluate the legal framework and environmental impacts.

The development of the phases is based on a case study and its experiences that aimed to provide a compelling narrative and process for the wastewater sector to make this happen in other settings too. This case study was developed in the Climate-KIC co-funded Microalgae Biorefinery 2.0 (MAB2.0) project whose main goal was turning waste and emissions into a biological resource. The project built on involvement of European partners mobilizing complementary skills which was necessary to define this multidisciplinary process. Hence, the discussion is not only from a scientific point of view, but also presenting the practical and operational aspects which are important to include industry stakeholders and spread the cultivation of microalgae. In this sense, the authors aim to give a comprehensive review about how the process was established and tested in order to integrate microalgae production technology into wastewater treatment systems, and produce biomass for algae-based products. The phases and the included protocols are detailed in the next chapters.

Case study

The process was tested in a case study to demonstrate the operational process at the North Budapest WWTP (Hungary) operated by the Budapest Sewage Works Ltd. The Budapest Sewage Works Ltd. is a wastewater engineering company with the core activity of wastewater collection and treatment for the large Budapest area with a population of around 2 million inhabitants. It is the operator of two WWTPs both equipped with biogas production.

The main indicator of the North Budapest WWTP with a capacity of 600 000 population equivalent (up to 200 000 m^3 input a day) is to meet the legal threshold for the treated effluent water. The treatment process of the North Budapest WWTP is a usual aerated activated sludge technology where two distinct lines can be differentiated (**Fig. 1**). The water line operates with the expected results and provides clean water with parameters below the legal threshold for return to nature.

The sludge line produces the AD effluent from digested sludge dewatering that cannot be discharged to nature because its nutrient content is above the legal limit. **Table 1**. shows the composition of different streams from the North Budapest WWTP and the applicable legal threshold value. Even if compared to raw wastewater, the AD effluent contains more nutrients and also suspended solids due to its origin from digested sludge dewatering.

The higher nutrient content is also related to the fact that this biogas plant processes external organic wastes too (21), as indicated in **Table 2.** While this may be unfavourable in the view of the composition of the AD effluent, it provides the WWTP the possibility to approximate energy self-sufficiency by producing annually 11 800 MWh electricity and 13 800 MWh heat from biogas. This means that more than 80% of the electricity need of the WWTP is supplied from the on-site biogas plant.

Upgrading this AD effluent and reducing the load in the return flow are the main motivations for investigating microalgae integration. Experiments in microalgae cultivation and integra-

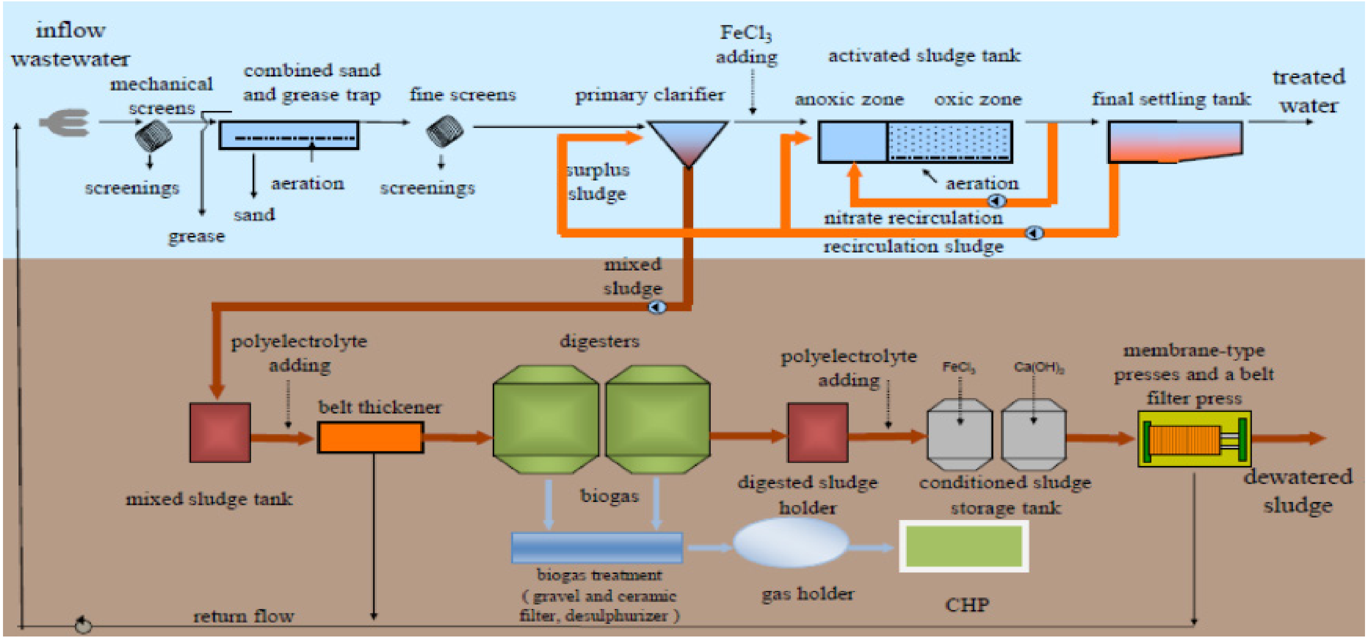

Figure 1. Process flow of the North Budapest WWTP. Besides the membrane-type presses and the belt filter press, industrial centrifuges largely complement the final dewatering process not shown on the illustration.

tion into wastewater treatment have been ongoing at the North Budapest WWTP since 2012. Considering the difficulties of AD effluent outlined earlier, the company has been seeking easy to apply processes and protocols that guide it through the process. Nutrient removal efficiency, biomass yield and reactor efficiency to reduce residence time are listed as priority factors. These factors, however, can be contrarily to each other, thus a compromised solution is needed. While short residence times can reduce the reactor volumes needed, they may not result in high nutrient removal efficiency. For example ponds can provide adequate nutrient removal, but they are not optimized for algal growth and high productivity (22).

Preliminary evaluation

This first step aims to investigate the actual wastewater treatment plant and the stream intended for algae cultivation. The aim of this step is to evaluate multiple factors including aspects of technology, biology and legislation that can determine next steps and integration options.

Wastewater streams composition

Wastewater and treatment related streams can have fluctuating and sub-optimal composition, as well as include toxic/inhibitory components. One-time sampling and measurement may lead to biased results. At the case study location, sampling of the effluent of different dewatering devices happens on a daily basis (except weekends) and 6 parameters are measured: pH, chemical oxygen demand (COD), total nitrogen and ammonium (NH_4^+), total phosphorus and phosphates (PO_4^{3-}), as reported in **Table 1**.

A protocol implying a statistical analysis was developed that can be used as a basis for assessing the historical parameters. The protocol highlights the effect of seasonality on concentration profiles and the levels of concentration and their variability in time, as well as confirms the suitability of these effluents for microalgae growth. A set of analytical tools are included in the protocol to analyse the database of historically measured data. As microalgae growth and productivity are highly dependent on a satisfactory (C:)N:P ratio, the set of data is plotted versus the general formula for microalgae composition (23) as a function of time. The data are compared to thresholds of micronutrients to highlight any imbalance in the samples. Nevertheless, considering its origin imbalances are expected, but the reason for those should be addressed for example by influencing pH to prevent phosphate precipitation (24).

Different WWTPs use different units and parameters, and statistical analysis can reveal some inconsistencies in the datasets, hence verification of the data and crosschecking the analytical methods are also advised. However, the composition of AD effluent is not measured and registered at every WWTP, as it is directed to the start of the water line as return flow, and

Table 2. Different input sources used to feed the anaerobic digesters at North Budapest WWTP (2016).

Type	Dry matter [tonnes/a]	Organic matter w/w%
Concentrated sludge from on-site	17900	73
Slaughterhouse waste	2300	83
Dewatered sludge from other WWTPs	1000	69
External liquid waste	360	75

Table 1. Annual average composition of the incoming raw wastewater and the AD effluent from the centrifuges, as well as the discharge threshold defined by legislation for the North Budapest WWTP. COD: Chemical Oxygen Demand, TSS: total suspended solids, TNK: total Kjeldahl nitrogen, TP: total phosphorus. The legal threshold on the total nitrogen content (35 mg L^{-1}) also includes the ammonium nitrogen concentration (*). All concentrations are in mg L^{-1}.

	pH	COD	Filtered COD	TSS	$N(NH_3\text{-}NH_4)$	NO_2^-	NO_3^-	TNK	PO_4-P	TP
Incoming raw wastewater										
mean	7.7	575.8	-	313.7	55.3	0.49	1.5	71.4	15	10.4
error (±2SD)	0.55	427.9	-	301.4	30.6	1	3.4	33.5	11.4	7.8
AD effluent										
mean	8.1	7572.9	675.3	7842.4	1376.1	-	-	1688.9	53.2	263.5
error (±2SD)	0.3	7801.1	347	8568.9	415.3	-	-	521.2	48.4	241.3
Legal thresholds										
	6.5-9.0	125		35	10	35*				5

not a natural discharge with legal requirements. Thus, lack of historical data on the AD effluent composition can become an obstacle in the design process.

The stream intended as substrate for microalgae must be characterized not only by COD (and N and P) contents but also by its biological oxygen demand (BOD) and inorganic carbon (IC) content to assess its biodegradability under aerobic conditions. As AD effluent comes from the biogas plant, COD was revealed as non- or low-biodegradable. Thus, meeting the legal threshold by application of microalgae to discharge in the environment (<150 mg/L) is not a realistic expectation. Presence of biodegradable compounds can also imply possibilities for mixotrophic conditions.

Tests with samples can also indicate the need for additional treatment. For example, in the case study an extra step of settling was needed as after centrifugation, the effluent still contained suspended solid. Settling can result in up to 95% removal of total suspended solids which is most likely due to the flocculation agent added during dewatering. A filtration step could be also required to remove further particles and coarse colloids (**Fig. 2**), as they can impede light penetration and provide shelters for bacteria. The need for treatment has implications on the actual design of integration, introduced in the next chapter.

Figure 2. From left to right: untreated AD effluent, settled and filtered samples.

Strain selection approach

In order to select the most appropriate strains for a given stream, a robust, high-throughput, low cost, low labour and easy to apply strain selection protocol is needed to find the most suitable algae species. For these reasons, a microplate based protocol was developed for short-listing of strains, while further investigations for temperature optimum determination are advised in flask scale (around 0.1 L).

Literature, culture collections and own isolations are a good basis to inquire a starting set of up to 50 strains to investigate and to prepare a short list. In the case study, the initially tested strains were provided by the University of West Hungary, Mosonmagyaróvár Algal Culture Collection (MACC) and complemented with strains collected from different wastewater streams. Interestingly, those isolated strains were outperformed by the ones from the culture collection.

The strain selection protocol has two important practical aspects; to screen strains against the temperature optimum and medium strength. Considering the features of the wastewater streams, it is possible that without adaptation the strains could not survive in undiluted feedstock, thus different dilutions were tested and also the adaptive capacity of strains investigated. In the case study, the results of strain selection highlighted the importance of an adaptation period to the AD effluent.

Outdoor production of microalgae is subject to seasonal temperature fluctuations which can affect the growth of algae. Because the incident sunlight is also heating the reactors, tem-

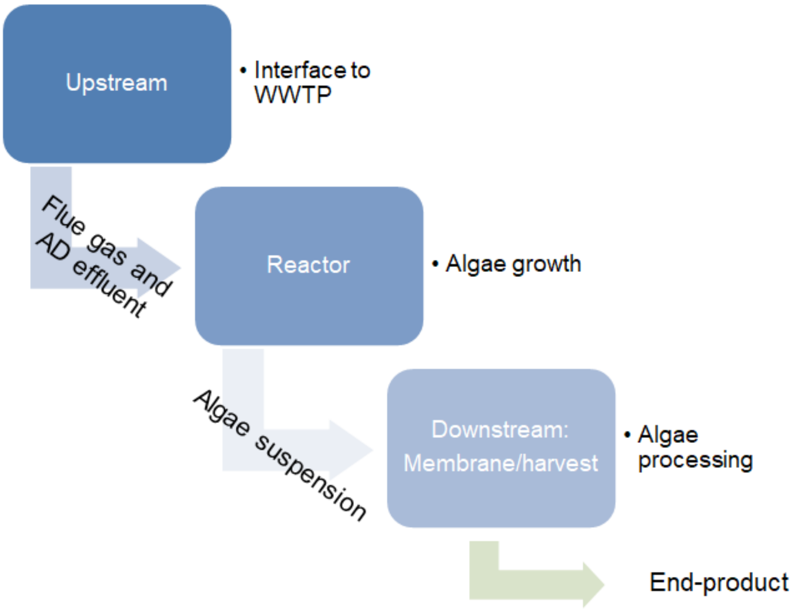

Figure 3. Flow chart of the technology integration including the three tailorable modules.

peratures over 40°C can be reached in photobioreactors. In open ponds, since evaporation contributes to cooling, the temperature is lower than 40˚C in most geographic locations. This temperature is too high for the optimal range described for most commercial algae species and at temperatures exceeding the optimum, microalgae growth rate sharply declines (25).

Cooling is often applied to keep the culture medium at a lower temperature, to optimize the efficiency and prevent crashes of algae cultures (26). However, cooling represents a major energy and cost factor. The use of a higher cultivation temperature, will not only save energy for cooling, but is a selective factor that can reduce the probability of local algae contamination making possible the maintenance of a monoculture.

The developed protocol in the case study showed a good reproducibility. Complementing the AD effluent by micronutrients resulted in a balanced growth in semi-continuous cultivation mode. In order to provide more accurate information on the conditions for optimal biomass cultivation, a lab scale photobioreactor can be used to simulate an average daily light condition at the location of the wastewater treatment unit (Budapest for the case study), operating under continuous mode. This experiment provides information needed to setup the operation protocol for algae growth at the location.

Legal aspects

Though the European policy points towards circular economy and nutrient recycling, the current legal framework on wastewaters is outside the scope of the waste legislation. The Waste Framework Directive defines 'waste' and clearly excludes sewage and wastewater from this definition (27). As a result, usually national legislation regulates wastewater and its treatment separately from waste regulations. Thus, circular economy aspects are usually not considered and implemented.

From a legal point of view, there is only wastewater and clean water that meets the threshold, so from this aspect AD effluent is considered as wastewater. As being defined as wastewater, stricter regulations apply with regards to transportation and transfer of ownership compared to regulations on waste. Moreover, there is no legal way to 'redefine' wastewater or some of its separated substances as by-product similar to what is available in case of waste with all its further possibilities of evolving into a product and commercialization.

This regulatory framework considerably narrows the commercial viability of an otherwise microbiologically sound and widely researched method of producing microalgae and cleaning the AD effluent. According to current laws wastewater flows in a one-way direction. Wastewater (including AD effluent by legal means) can only leave the point of origin by direct transport (pipeline or collecting truck) to the treatment plant. Also, whenever it has reached the treatment plant there is no legal way to transfer any part of that to any third party (to set up algae production outside the WWTP). This way the only two places where algae producing investments can take place are the point of origin or the wastewater treatment plant which is the same for AD effluent. This considerably limits the feasibility of any similar investment given certain limitations including available area at the WWTP for own microalgae production. This has to be considered in the planning process.

Design objectives

The results and observations derived from the preliminary evaluation are used as input for the design phase. For proper design, technologies and scales, the results of the preliminary evaluation are taken into account. These approaches and protocols are described in this chapter.

Basic process design

The proposed integration design has three tailorable technology modules (**Fig. 3**).

Table 3. Volume of the AD effluent in 2017 (first 11 months) processed by different techniques at the North Budapest WWTP.

	Thickening table (m^3)	Centrifuge (m^3)	Press (m^3)	Belt press (m^3)	Total (m^3)
Monthly average volume (m^3)	19 886.61	25 965.42	3 029.19	8 032.57	52 381.40
error (±2SD)	±6795.47	±9019.76	±1665.19	±11984.39	±9290.88
Annual total volume (m^3)	218 752.73	285 619.61	33 321.04	32 130.29	576 195.38

The upstream part, referred as interface, aims to integrate and condition incoming streams of AD effluent and flue gas to make them appropriate for microalgae growth. The interface is responding the need for treatment as concluded by the preliminary evaluation. It is designed to reduce exposure to parameter fluctuation, decrease suspended solid content and toxicity, as well as mix flue gas with air. Prerequisite for the interface is that the WWTP has sludge dewatering and flue gas (as CO_2 source and heating medium) available. Technology elements that may be part of the interface include settling tanks, sand filters, storage tanks and flue gas heat exchanger and blower. The parameters and features of the interface depend significantly on the composition of the AD effluent, the hydraulic endowments of the hosting plant, the existing infrastructure (i.e. pipelines and wells), as well as the composition and temperature of the flue gas. Hence, some of these elements are optional in the interface design, the decision on exact design needs to be taken based on the preliminary evaluations. In the case study location, the AD effluent comes from dewatering centrifuges (**Table 3**) and still contains large amount of suspended solids removable by settling and filtering as indicated during the preliminary evaluation.

The next step is the reactor that secures the appropriate conditions and habitat for microalgae, thus a set of technical criteria was defined. The microalgal culture needs to be in optimum environment to successfully outcompete the bacteria present in the feedstock (whether it is AD effluent, raw wastewater or any related stream). The presence of bacteria, and organic matter can promote the formation of biofilm on surfaces, thus leading to biofouling and wall growth that can block light penetration. In order to minimize it, the selected reactor needs to have an optimal mixing and an easy cleaning procedure. As the AD effluent has a higher nutrient content than raw wastewater or other usual algae media, in order to significantly reduce nitrogen and phosphorus concentration, a reactor with high volumetric productivity is required. From a biological point of view, this means that the light intensity per unit of volume needs to be maximized. Nevertheless, other factors may compromise the biological performance such as limited area available, economic factors, tailorable design, as well as easy maintenance, cleaning and accessibility of internal surfaces.

The downstream step aims to concentrate the microalgae, for which technology options and their relation to the final products need to be accessed. The selection of the microalgal harvesting method is crucial (28) since harvesting costs can reach up to 20-30% of the total production costs (29). This selection is dependent on many factors including cell type, density and size, downstream processing requirements and the value of the end product (30). Therefore, developing an efficient harvesting strategy is a major challenge in the commercialization of products from microalgae. Davis et al. point at the strong economy of scale sensitivities for the downstream processing operations, which should be taken into account when extrapolating results (31).

In view of the algae end product criteria and targeted market, a protocol to access harvesting alternatives and process parameters was developed. The application fields of the produced algae determine the wanted final concentration in the algal paste, and also the state of the microalgae (if destructive methods can be applied or cells need to stay intact). The value of the end product is crucial in the final economic analysis, allowing or banning more expensive harvesting and drying methods.

This protocol also relies on findings of the preliminary evaluation phase. The selection of a strain best adapted to the given wastewater stream limits the harvesting options; certain species of algae are much easier to harvest than others. For example, *Spirulina*, which is a long microalga (20-100 µm) can be harvested by microscreening. This technique cannot be applied to smaller cells like *Chlorella sp.* which performed best in the case study strain selection process.

The protocol also investigates membrane unit inclusion scenarios. According to Bilad et al initial dewatering via membrane filtration followed by a further dewatering step via centrifuges could most probably be optimal (32) since membranes offer a cost-effective strategy for a dewatering step before centrifugation. Application of membrane unit decreases the flow that needs to be set into rotation, thus reducing energy costs and increasing the solids content in the input and output of the centrifuge. An advantage of membrane dewatering is that its efficacy has been proven at large scale over sustained operations, although fouling – and, in general, system performance – will depend on the strain, the state of the culture and the water matrix. The protocol contains specific experiments to assess membrane filtration performance and determine the optimal working conditions. The case study includes a membrane filtration unit as a harvesting or pre-harvesting step, without any recirculation of the biomass into the reactor.

After harvesting, with further drying processes it is possible to obtain a dry biomass with water content as low as 3%. How-

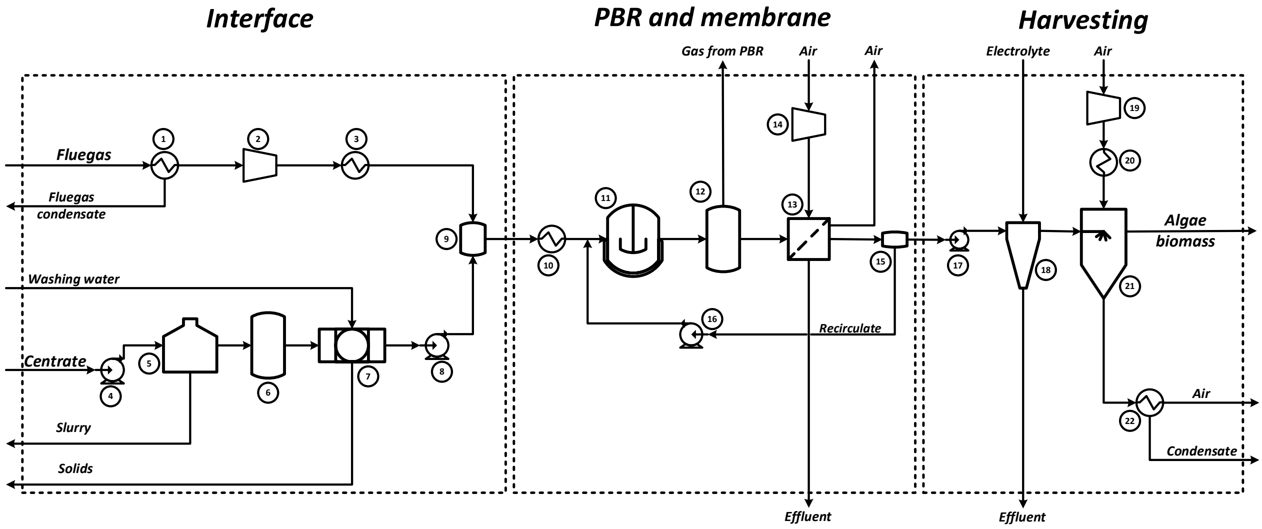

Figure 4. Simplified flowsheet diagram of the microalgae integration system. Major equipment list: 1. Heat exchanger, 2. Blower, 3. Heat Exchanger, 4. Pump, 5. Settler, 6. Buffer tank, 7. Sand filter, 8. Pump, 9. Mixer, 10. Heat exchanger, 11. Photobioreactor, 12. Degasing unit, 13. Membrane, 14. Blower, 15. Splitter, 16. Pump, 17. Pump, 18. Centrifuge, 19. Blower, 20. Heat Exchanger, 21. Dryer, 22. Heat Exchanger.

ever, these are expensive processes, which require costly equipment and have a high energy demand. The removal of 1 kg of water during drying requires more than 0.9 kWh of energy, and thus any reduction of water content by previous dewatering techniques is beneficial from energetic and cost standpoints (33). It is estimated that the drying techniques can only be applied to obtain products with a high market price (above 1 $/kg) (31). Since some of these techniques make use of steam, hot water or hot gases, the presence or absence of such utilities at WWTPs are taken into account for the economic balance of the processes, together with the heat tolerance or volatility of compounds of interest.

Impact assessment and scale-up designs

In order to assess the techno-economic features of algae integration scale-up options for WWTPs, as well as to learn about the environmental impact, standard protocols were developed. For assessing the technical performance, process models were developed in Aspen Plus v8.4 (Aspen Technology, Inc., USA), and later exported into Microsoft Excel spreadsheets. Several elements of a biorefinery system are not available in the databases of Aspen Properties, therefore an in-house property database of the National Renewable Energy Laboratory was used based on the work of Wooley and Putsche (34). The model can be easily tailored to different reactor types by changing the relevant parameters; in the case study the model was prepared to compare 3 different reactor types (raceway pond, tubular reactor and flat panel) on 2 scales (100 and 1 000 m^3 input a day).

Independently of the type of algae cultivation units, the configurations are modelled following the same processing steps as described before: an interface, a growth and pre-harvesting step,

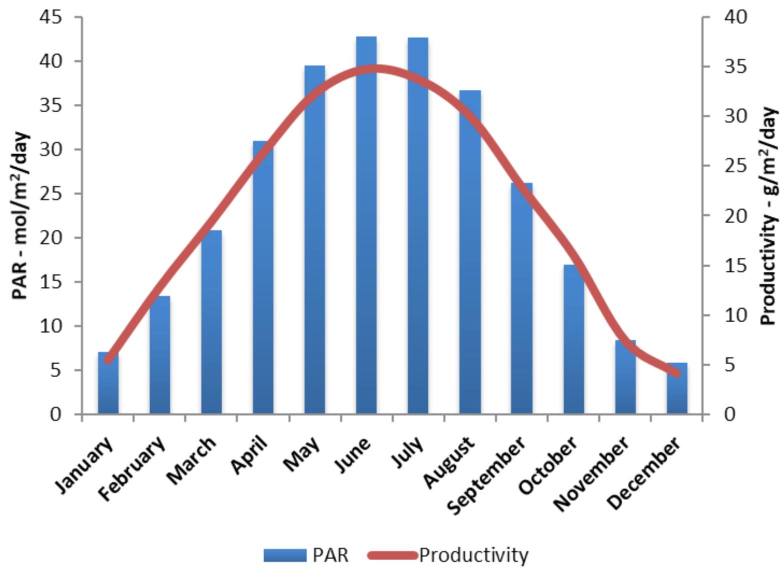

Figure 5. Average photosynthetically active radiation (PAR) data and algal productivity data for Budapest as used for the modelling.

and a harvesting step where biomass is dewatered. **Fig. 4** shows a diagram of the flowsheet used to calculate the mass and energy balances.

The protocol offers the possibility for WWTPs to tailor the model to their location and study how the climate conditions affect the algae production. To this end, a simple microalgal growth model by Blackman (35) in combination with a simple light model by Lambert-Beer (36) was included into the modelling. Calculations are based on the monthly averaged irradiance values (**Fig. 5** for the case study location).

The biological characteristics of the selected microalga strains are considered too, and based on the main aspect of the strain selection protocol the optimal temperature is assumed to be 35 °C. The model is tailorable to the specific characteristics of the selected photobioreactor such as volume to area ratio and different concentrations. Productivity can vary depending on the specific parameters of each reactor by modifying the hydraulic retention time (HRT) and thus specific microalgae concentration, and/or by modifying the number of months of operation (37). Additionally, a membrane unit is also included in the modelling attached to the reactor as pre-harvesting step.

As output, the model delivers mass and energy balances, and costs (CAPEX, OPEX) for the selected scale. The economic advantage for the WWTP operator embodies in reducing the nutrient content of the return flow which is difficult to quantify due to combined treatment of return flow with the incoming raw wastewater. Further research is needed to calculate the direct saving considering the ratio of return flow. Optimization of the design can happen through the available sensitivity analyses. In the case study, the sensitivity analyses suggest to look for ways in reducing the energy costs as being the main contributor to OPEX. Investigating the impact of different energy costs can encourage WWTP operators to understand and exploit energy integration options with biogas and other on-site sources, as well as to research options when algae production can deliver more revenues per energy unit as electricity wholesale market (which is lower than the feed-in-tariff schemes for biogas based electricity). Due to lack of actual large-scale algae integration into WWTPs there is large uncertainty in capital costs.

The model is also flexible because it is possible to switch on/off operating months and process sections (e.g. to change where to remove the microalgae from the system: as algae suspension or dried biomass).

The outputs of the technoeconomic analysis are linked to the LCA model. The LCA aims to assess the potential environmental impacts of the production of microalgae biomass from wastewater treatment effluents. The protocol is a prospective Cradle-to-Gate Analysis following ISO standard (38) and using ReCiPe impact characterization method (39). Assuming that the microalgae system will always be part of WWTP, inputs such as AD effluent and flue gas are burden free, as upstream environmental impacts are assumed to be allocated to treated water for consumers.

Case study analysis compared different functional units (e.g. unit bioplastics, unit fertilizer and unit wastewater treated). Similar to the techno-economic analysis, results indicate that integration within the wastewater treatment, including the use of on-site produced utilities, enhances the environmental performance of microalgae production. Case study results indicate that environmental impact depends on the selection of the functional unit, thus as hint for WWTP operators, interpretation needs to be carefully revised, as the functional unit might provide a misleading message.

Pilot testing

At the case study location, a glasshouse with a ground area of 18x12 m (216 m^2) and a maximum 5 m inside height is available to host pilot installations. The facility is also equipped with lightning protection, rainwater drainage and special shading mat system and furthermore a cooling and heating system. The interface consisting of a settling and storage tank, as described earlier, is placed outside the glasshouse. The glasshouse infrastructure allows the testing of multiple reactors parallel with junctions to connect the reactors to the flue gas, air and water supply. During the trials, experiences with different kinds of reactor have been gathered, and the WWTP is open to test further types of algae cultivation units.

The raceway pond from Zöldségcentrum Ltd. (Hungary) was operated for months continuously at the case study location in Budapest, while the tested closed reactors proved to be not feasible due to problems with cleaning after a certain period. The operating capacity of the raceway pond is up to 12 m^3 (13,5x4 m ground area) with a maximal depth of 0.25 m. The pond is equipped with automatic water level controlling system, sensors and data collection (temperature, pH, electric conductivity, oxidation reduction potential, flue gas flow as bubbled into the open pond) with embedded software for online monitoring.

A membrane filtration system was also installed in the case study and used as a pre-harvesting step prior to other harvesting or drying methods. For this, the membrane unit is installed after the cultivation system, so that the inlet to the membrane is the culture from the cultivation system. Two outlets are obtained: a solids-free stream which still contains inorganic nutrients and might contain soluble organic matter, and an microalgal suspension with a higher solids concentration than in the culture system. The concentration factor and, consequently, the final TSS achieved depend on the operation of the membrane. Additionally, the membrane filtration unit can be coupled to the cultivation system so that it is used to maintain the biomass concentration in the reactor on a set level, thus increasing its productivity.

The membrane module consists of two hollow fibre (0.03 micron) bundles with a total membrane area of 3.44 m^2 each. One bundle is for working and a spare one to use alternatively during membrane cleaning or system maintenance (nevertheless, it is possible to work with both modules in parallel). The recommended working permeate flow corresponding to the surface of 3.44 m^2 is 70 L/h (1.7 m^3/d). The bundles are in-

Table 4. Quality of pre-treated AD effluent and its difference between each batch. Data are shown in (mg L^{-1}).

	May	June	July	August
pH	7.62	7.39	8.15	8.06
COD	615	1597	750	667
TSS	30	140	150	65
total-P	97	100	61	75
dissolved-P	68	56	58	36
N(NH_4-NH_3^+)	683	622	930	872
organic-N	8	8	53	67
nitrite-N	0.010	0.036	0.041	0.023
nitrate-N	6.200	1.300	0.152	0.274
Ca	127	187	117	101
Mg	38	59	17	33
Cu	0.01	0.05	0.01	0.02
Cd	0.003	0.001	0.001	0.001
Ni	0.007	0.05	0.019	0.002
Pb	0.012	0.017	0.002	0.005
Mo	0.05	0.05	0.05	0.05
As	0.01	0.01	0.016	0.01
Cr	0.014	0.006	0.002	0.007
Fe	1.71	5.26	1.18	1.04

stalled in two columns each with a height of approximately 2.5 m and a volume of 15 L. All sensors and auxiliary equipment of the membrane unit are connected to a Programmable Logic Controller (PLC) for data logging and operation control by a SCADA system which centralizes all the signals from different sensors.

Treatment and quality of AD effluent for algae cultivation

As indicated during the preliminary evaluation, the significant amount of TSS content (**Table 1**) represented a challenge in pilot scale too, but removing it was necessary due to its black colour absorbing light. The interface module of the case study contains a 11 m^3 settling tank with conical bottom operated in batch mode. The AD effluent is sent through a Honeywell type flow-through water fine filter to separate the particles still present after settling.

The storage tanks for the settled effluent allows the balancing of the feedstock supply for algae growth in case of fluctuations, toxic content or other errors in the operation of the plant, and additionally helps polyelectrolyte decomposition which is added to dewatering to assist flocculation (40). Polyelectrolyte can be present also in the AD effluent and certain

Table 5. Settling time of the AD effluent used for growing microalgae at the case study location of the North Budapest WWTP.

Month of refilling	Settling time (day)	Recovered clean AD effluent (m^3)
April	34	3
May	23	8
June	27	5
August	13	7

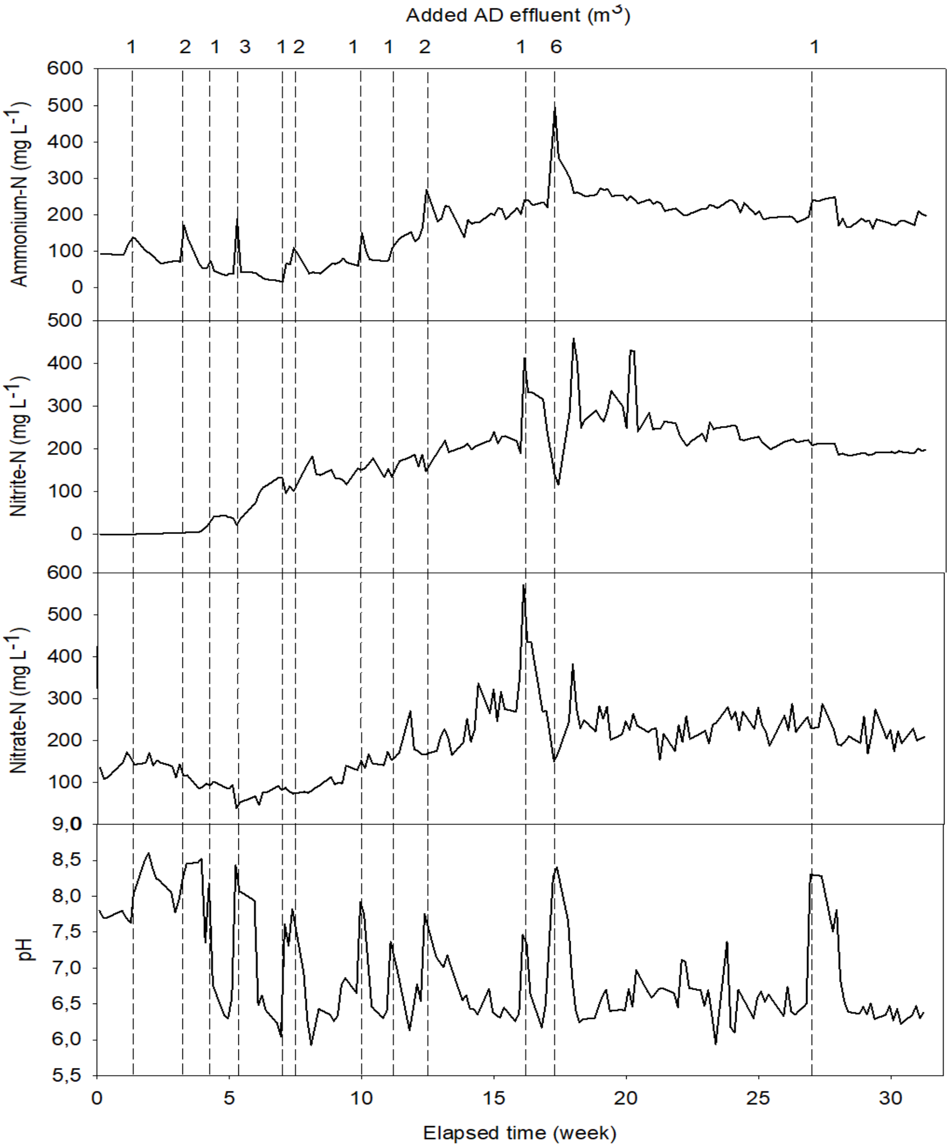

Figure 6. Changes of pH and nitrogen forms during operating of the open pond. Peaks in pH indicate the feeding of treated AD effluent (dashed vertical lines) which quickly falls due to ammonium loss and conversion. Peaks not marked are due to evaporation replenishing with tap water.

types can, if above a concentration (which depends on dosing and dewatering), aggregate also algae cells. Nevertheless, after 72 hours (or less) these compounds decompose.

In line with the caution to assess the statistical parameters of datasets, the averaged composition of AD effluent (**Table 4**) shows large difference between each batch indicating the changing quality and different settling properties. This fluctuation may be caused by the integrated rain water sewage system in Budapest influencing sludge quality and external organic material added to the biogas plant (**Table 2**). As expected during the preliminary evaluation phase, imbalances in the medium conditions exist leading to suboptimal composition for algae growth. Precipitation of phosphate salts due to the high pH also contributes to the unavailability of micronutrients.

The fluctuations in the AD effluent volume and quality made the settling process unpredictable, thus the retention time in the settler was varied for each batch (**Table 5**). Additionally, during settling the AD effluent separates to three phases indicating that the clean AD effluent must be taken from the middle phase. In order to improve the treatment of the AD effluent, further investigations are needed on the large-scale design of the interface module. The optimisation needs to reduce the long residence time in the settling tank that may influence the composition of the AD effluent too. These experiences highlight the importance of proper integration with the process of wastewater treatment for scale-up and continuous operation.

Microalgae production experiences

An open cultivation algal system, such as the used raceway pond, is highly exposed to predation and contamination by undesired microorganisms due to unsterilized wastewater media (41). In order to reach a sufficient background concentration of

Table 6. Chemical analysis of the unknown precipitation, data in mg kg^{-1}. TC: total carbon, TN: total nitrogen, TP: total phosphorus, TS: total sulfur.

Precipitation										
Na	B	Mn	TC	Zn	Ca	Cu	TN	TP	Mo	Hg
1100	825	649	194800	142	208000	60.5	27550	103877	22.5	0.5
Se	TS	K	Pb	Fe	Mg	As	Co	Ni	Cd	
9.02	3880	0.36	4.6	6.06	4830	1	0.54	0.79	0.2	

Table 7. Mass balance table. The results are calculated from the added AD effluent composition, current nutrient concentrations and in form of microalgae biomass (both harvested and remaining in the pond) as remaining biomass composition.

	Input in AD effluent	Current nutrient concentrations	In form of microalgae	Loss
Nitrogen	100%	41%	4%	55%
Phosphorus	100%	22%	40%	38%

microalgae able to compete and grow in this medium, inoculation of the system was performed with a batch of selected microalgal culture in high cell density. This resulted in a starting TSS concentration of 400 mg L^{-1}. The inoculum was prepared under sterile heterotrophic conditions to have the inoculum ready in a short time, as *Chlorella* is able to consume organic carbon too (42). The two-stage inoculation strategy was an appropriate method to inoculate large-scale algal ponds (43).

The pond had operated in semi-continuous way for 32 weeks and treated AD effluent was fed manually. Usually, 1-2 m^3 effluent was added into the pond each week, but on occasions, no AD effluent was added for two-three weeks. Operation of the pond was tracked by daily sampling for determination of TSS, nitrogen and phosphorus forms, and microscopic analysis. The aims of the experiments were to investigate the impact of varying AD effluent qualities, validate findings in protocol developments and conclude operational strategy for scale-up due to WWTP specific factors. For these reasons, the long run operation allowed to obtain findings that a WWTP operator should be aware of when scaling-up algae cultivation.

Main findings include that i) maximal dry matter concentration was 800 mg L^{-1}, ii) predation and competition influenced algae production largely, iii) thick foam layer formed in months with lower temperature and illumination, iv) the creation and implementation of a pH control strategy is necessary, v) phosphorus precipitation caused challenges in harvesting, vi) most of the ammonium was stripped out, vii) presence of nitrifying bacteria can lead to nitrate and nitrite accumulation. Impact of those findings are detailed herein.

Based on agar plate culture assays, microscopic and Denaturing Gradient Gel Electrophoresis (DGGE) examinations, the main microorganism was the selected *Chlorella vulgaris*, but cyanobacteria, fungi, ammonium oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and other eukaryotic algae were detected too. The presence of AOB was also confirmed from the conversion of ammonium in the pond. Due to the high pH and presence of AOB, ammonium in the added AD effluent got almost completely stripped out and turned into nitrite and nitrate within a few days after feeding. The pH profile also indicates those processes, the feeding of AD effluent results in peaks of around pH 8 which sharply falls to around 6.5 where it stabilizes (**Fig. 6**). This acidification is a result of consumption of ammonium by microalgae and the nitrification process by nitrifying bacteria. A lower pH helped to reduce the phosphorous precipitation (44) and ammonia stripping (45), but it also harmed the culture. Main experience puts an emphasis on the pH control, as it is needed to find the balance between the pH level and the solubility of phosphorus and ammonium in the presence of competitive microorganisms. The initial pH control through flue gas that was set to a threshold pH value of 7.5 was never activated due to the quick drop to 6-6.5 by ammonium conversion.

During the months of operation, a quickly settling and coarse sand like solid matter content had appeared in the pond. Analysis confirmed (**Table 6**), that this solid matter is made of calcium, magnesium and phosphorus indicating precipitation of salts either formed in the pond or previously. Presence of carbon indicates organic matter, probably cell debris and external biomass. As consequence, determination of the amount of algae biomass by dry matter content may lead to biased results, and those particles can cause problems in harvesting technologies. Regular shut down and cleaning of the open pond, as well as adding extra filtration both in sampling and harvesting process can help to reduce errors.

The above experiences are also reflected in the mass balances for nitrogen and phosphorus (**Table 7**) at the end of the run. Based on the input as form of AD effluent feeding (aggregated), into the pond, current nutrient concentrations in the pond and

Table 8. Heavy metal content analysis from two different batches of the harvested microalgae biomass, and food contact material limit. The symbol (*) shows the concentration value is close to the limit and the symbol (**) means the value exceeds the limit.

	As µg kg^{-1}	Cd µg kg^{-1}	Co µg kg^{-1}	Cr µg kg^{-1}	Cu mg kg^{-1}	Mo µg kg^{-1}
Batch 2	2674*	52.1	567	4833	84.6**	650*
Batch 3	1848	80.8	631	6072	38.5*	1195**
Limit	5000	500	-	50000	50	1000
	Ni µg kg^{-1}	**Pb µg kg^{-1}**	**Zn mg kg^{-1}**	**F mg kg^{-1}**	**Se mg kg^{-1}**	**Hg mg kg^{-1}**
Batch 2	24986*	2638	145*	56.04*	1.53**	1.02**
Batch 3	2166	4306	255**	184.4**	1.02**	1.02**
Limit	25000	50000	150	100	0.75	0.5

output as microalgae (harvests and in the pond at the end of the experiments) the balances were defined. The loss in case of both elements is a significant portion of the balance which may be due to ammonium stripping and phosphate precipitation.

Algae quality assessment

The aim of the last phase was to set up and implement protocol to verify the quality of algae in different potential application fields and determine the composition of produced microalgae. These protocols help to understand the requirement for microalgae on certain markets with the notion and limitations of wastewater derived biomass. In this sense, applications were sought to valorize the protein content (as whole algae), as well as use the algae suspension directly as soil fertilizer for non-food chains (such as public parks and energy plantations). With the case study delivered algae, trials were carried out for bioplastics and wood panel (plywood) production. However, as being novel applications for biomass from wastewater origin there could be gaps in legislation resulting in a time-consuming permitting process.

The protocol development revealed hot spots. Presence of precipitation and extraneous fibre like materials in the algae biomass may lead to difficulties in cell disruption necessary to release microalgal components. More importantly, as grown in wastewater, heavy metal content can limit applications in certain fields. In line with the heavy metal accumulation properties of microalgae, concentration of some metals was lower or around the detection limit in the open pond but clearly accumulated in the biomass. This is clearly a challenge, as measurements of heavy metals from the feedstock stream cannot indicate which metal and to what extent will accumulate in the cells. **Table 8** shows the measured heavy metal contents compared against the food contact material legislation, a potential target for algae containing bioplastics. The figures indicate that the algae can be applied only as additive to bioplastics if concentrations are below the requested values.

Conclusion

Integration of algae into wastewater treatment can have benefits for the wastewater sector by the utilization of CO_2 emission and excess nutrients reducing load and costs, as well as producing algae for different uses. In order to facilitate the spread of the technology, hereby the paper proposed a guide for WWTP operators to evaluate, design and test algae cultivation.

Stakeholder interactions confirmed the general interest of the wastewater sector towards algae integration, but a series of challenges underpin the necessity of this protocol based process. The standardized way of the process makes it simple to tailor what is necessary as WWTPs can differ in actual design, data availability, management aspects and legislative framework.

This process and the associated protocols assess the different aspects of algae cultivation integration into existing wastewater treatment processes. Through the case study that demonstrated, the operational process, the phases and protocols were evaluated, as well as practical experiences collected.

Findings highlight that, in order to obtain compelling, as well as economically and technically sound designs, more emphasis needs to be put on the scientific and engineering aspects of integration. Already in the preliminary evaluation phase, datasets recorded by WWTPs must be harmonized and studied from the aspects of algae growth. Parameters and supply of wastewater streams (AD effluent specifically in the case study) make it more difficult to sustain algae growth than artificial media, thus the developed preliminary evaluation phase allows to conclude design aspects for seamless integration. Main conclusion is the need of an interface module able to condition the effluent to enable better algae growth.

The protocols within the design objectives phase underpin the necessity for an integrative design both by means of energy and feedstock streams. Building on the existing infrastructures to use on-site produced energy can reduce operational costs of algae production, as well as proper treatment of the income feedstock is necessary for balanced algae growth, nutrient removal and productivity. As savings for the WWTP result through integration, a method to quantify the financial saving by reduction of nutrients in the return flow needs to be developed.

Well-designed integration and the implementation of specific operational strategy (with main focus on pH) harmonized with the changing parameters of the feedstock are crucial to meet the expectations of the wastewater treatment sector. Successful integration needs close cooperation of the WWTP operators and algae professionals not solely from technological point of view but considering management and operational factors too. This cooperation is also key on pH control with having a close look on biological processes influencing pH already in the interface module.

Produced algae biomass was tested in different applications. Wastewater origin and regulations can limit the use of algae in some sectors, but advent of circular economy policies may help to position products better and support permitting processes.

Integration approach including the practical design and financial considerations are key for successful algae cultivation integrated into wastewater treatment. For this, cooperation of wastewater sector and algae professionals is needed in practice oriented way to further study bottlenecks and propose technology and engineering measures.

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