

Novel Stacked Modular Open Raceway Ponds for Microalgae Biomass Cultivation in Biogas Plants: Preliminary Design and Modelling

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Abstract – Microalgae hold great potential as a source for renewable energy due to their high photosynthetic efficiency, high growth rates and independence from fertile agricultural lands. However, large-scale cultivation systems of microalgae biomass are still not economically viable mainly due to the difficulties with maintaining optimum growth conditions of microalgae in open pond systems and high costs of biomass cultivation and harvesting. Here we propose the Novel Stacked Modular Open Raceway Ponds (SMORPs) system for microalgae biomass cultivation to be integrated in biogas production plant. The proposed technological solution will eliminate the drawbacks of current microalgae cultivation technologies, mainly, will reduce the land use, improve lighting conditions and reduce the cost of cultivation as a result of the application of waste products from biogas production, i.e. anaerobic digestion effluent and flue gas. In this study we propose the initial design of the SMORP concept and a microalgae biomass kinetic model as a simple approach to screen microalgae strains potentially applicable for large-scale ponds. The developed tool is also useful to evaluate the potential benefit of additional artificial LED light sources and to assess the maximum biomass growth rate with minimal light intensity.

Keywords – Biogas; Chlorella spp.; effect of light intensity; kinetic model; microalgae; open raceway pond

1. INTRODUCTION

The use of microalgae as a promising renewable energy source has been growing within the last decade due to the specific quality and characteristics of microalgae [1], [2] and the capability to cope with climate change from CO_2 anthropogenic emissions.

Microalgae are photosynthetic organisms able to fix solar energy and carbon dioxide into biomass and oxygen production, one of their main characteristics is good adaptability to new growing conditions [3], [4]. Due to their high CO_2 fixation rate, microalgae can grow well under high level of CO_2 making them a beneficial interface acting like a bio-filter for the treatment of exhaust gases and flue gas emissions from thermal and industrial plants [1]. The photosynthetic process of microalgae is higher in efficiency than in terrestrial plants [5], [6], moreover, in comparison with land-based feedstock, microalgae present several other key

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advantages compared to the terrestrial biomass like 5–10 faster growing time and higher biomass production rate [7], [8]. In addition, microalgae cultivation can be placed in unproductive and/or remote areas avoiding competition with food crops [9].

Microalgae farming is also providing an overall environmental benefit to remove macropollutants and nutrients (e.g. N and P) [10]–[12] in different environments. In fact, different outputs from several wastewater treatment systems can be used as nutrient sources such as: domestic water, industrial water, municipal water [13] and the liquid fraction of a biogas digestate (i.e. centrate) [10], [14], [15]. Within the use of these nutrient streams there is the possibility to have even a double-fold advantage in terms of formation of algae/bacteria consortia [16].

The nutrient supply is a key aspect in microalgae farming. Various species of microalgae vary in their need for nutrients. However, the requirements for essential nutrients are similar for most microalgae species and include macro nutrients (i.e. C, N and P), as well as K and Fe. Large amounts of nutrients are required for large scale cultivation of microalgae. It has been estimated that for a production of 100 t of microalgae biomass approximately 200 t of CO₂, 5 t of nitrogen and 1 t of phosphorus are needed [17].

As mentioned, biogas centrate can be used as a nutrient feedstock; however, the centrate use can present relevant disadvantages. In fact, the liquid phase of digestate is characterized by high turbidity and ammonia content [18]. Turbidity caused by dissolved and suspended material has been considered as a major drawback of digestate [19]. This suspended matter causes light scattering and absorbance limiting the availability of light to microalgal cultures. Ammonia inhibition is another major drawback of digestate as a nutrient source. Ammonium concentration in digestate from agricultural waste typically ranges between 500 and 1500 mg NH₄⁺ L⁻¹ [20]. High ammonium concentrations of more than 1000 mg L⁻¹ can lead to inhibitory effects of microalgae growth [21]. Ammonia content can be reduced by diluting the digestate. Adaptation of microalgae to high ammonium concentrations is likely to occur [20].

A large number of microalgae species present a higher lipid production than conventional crops [20]. This is addressing the use of the microalgal biomass to the production of biofuel and in particularly biodiesel [1] strengthening the role of microalgae as a potential substrate to reduce the food-versus-fuel dilemma [22], [23].

The biomass transformation processes can also involve other types of transformation pathways such as thermochemical, biochemical and photosynthetic microbial fuel cell thereby creating an opportunity for a flexible and viable biorefinery concept with a large fuel portfolio (i.e. syngas, bio-oil, bioethanol, biogas/biomethane and biohydrogen) and energy final transformation [24], [25].

However, there are several concerns about the overall feasibility and viability of a full-scale-based microalgae farming system both from technical and economic perspectives for several reasons. One of the main obstacles is the difficulty to achieve proper regulation and optimization of the microalgae cultivation system, particularly in relation to several interrelated input parameters and [1] potential limiting factors such as light and temperature [10].

Specifically for biodiesel, several studies report that despite the efforts made, for the industrial production it is not yet economic viable, especially due to the high cost of biomass cultivation and harvesting [26]. The study from Husesemann et al. [27] identifies the minimal productivity of 30 g/m²-day as an economically viable threshold for open pond cultivation.

Looking towards biogas production through wet anaerobic digestion of microalgae biomass into methane [28], the recovered energy makes the overall process more viable if the potential use of the digestate as a fertilizer or biostimulant is considered [10], [29].

The potential mass transfer of CO_2 excess from the industrial process to the algae ponds through a simple sparging system using porous material is a beneficial aspect related to the implementation of a microalgae-based system, nevertheless it should be considered that the CO_2 absorption in an open pond only has an efficiency of 10-20 % [30].

Several studies show that rising CO₂ concentration in algal growth medium have enhanced algal productivity, however, too high CO₂ concentrations inhibit algae growth [31]. It has been noted that carbon supply is a major factor limiting the biomass production in raceway ponds [32]. Flue gases with CO₂ concentrations ranging from 5 % to 15 % (v/v) have been successfully introduced directly into ponds [33]. Although SO_x and NO_x are known as toxic compounds for microalgae [34], it has also been observed that SO_x and NO_x impurities in flue gases have no negative effect on microalgae cultures [35]. It has been speculated that high-rate algal ponds need a supply of at least 5 % (v/v) CO₂ to maintain high growth rates [30]. It has been estimated that the cost of pure CO₂ constitutes from 8 to 27 % of the total biomass production costs [36].

Nowadays microalgae cultivation technology in pilots and/or on a pre-industrial scale is focused on open or closed systems [8], [15]. The first ones are systems directly exposed to the atmosphere. The commonly used types are open raceway ponds (ORWPs) [37], [38] and High Rate Algal Pond (HRAP) used for wastewater treatment [39]. ORWPs have a relatively low cost of construction, installation and maintenance and a simpler operational system [8]. The disadvantages of ORWPs are mostly connected to system contamination with unwanted algae species, evaporation (that need to be balanced) and sometimes the lack of an automatized growing control system [40]. ORWPs also require a large land area. Moreover, the biomass concentration is relatively low [8] quantifiable in 10–25 g dry matter of algae biomass/m² [8] and the low surface to volume ratio (i.e. $5-10 \text{ m}^{-1}$) is a limiting factor for the productivity [41].

The second type of microalgae cultivation technology is based on closed systems also called photo-bioreactors (PBR). They can have different shapes: tubular reactor, flat plate reactor and pyramidal [42]. The typical most common types are in the shape of tubular, flat-tank, bubble column and serpentine [8]. The main pros of PBRs are the control of algae growth – which leads to high productivity of algal biomass – and the optimization and control of the culture system conditions, in fact avoiding the contamination with other algae species [43], [44]. The study from Jankowska *et al.* (2017) presents biomass concentration in the range of 20–100 g dry matter of algae biomass per day per m² [8]. Biomass production rates with PBRs are considered higher than ORWPs, a realistic figure can today be estimated as 60–70 tons ha⁻¹ yr⁻¹ [45].

Cultivation systems can also be classified according to the use of the artificial light sources or natural light from the outdoor environment. In contrast to open ponds, closed reactors are oriented towards mono-species algal culture and a control system for optimization of nutrients, temperature, CO_2 and pH, resulting in higher productivity per equal system volume and unit of area. PBRs can have very high concentrations due the higher surface-to-volume ratio compared with ORWPs [46]. Nevertheless, PBRs present a higher initial cost than ORWPs and are very dependent on the optimal selection of a specific microalgae strain for cultivation [46].

Looking towards the minimization of operational costs, energy consumption together with the maximization of GHG savings necessary for viable investment in microalgae production, ORWP technology has a lower energy demand compared to PBRs and a lower complexity of the optimization system and harvesting system [8].

In order to solve aspects related to economic viability (e.g. reduction of the energy cost in the plant management and operational system), a microalgae-based cultivation system can be better considered in terms of an integrated and/or side-stream process concept applicable to different wastewater systems including biogas. This can in fact more beneficially contribute to reduce the energy cost in the overall plant management and operational system [10].

Several ORWP pilot projects have already been realized [12], [15], [47] in terms of finding optimal synergies among the use of CO_2 flue gases from biogas combustion in CHP unit, the use of digestate and excess heat.

Nevertheless, none of these projects considered the possibility to develop Stacked Modular Open Raceway Ponds (SMORP) for microalgae growing as a novel hybrid technology which tries to take the best advantages from the two types of existing microalgae cultivation systems. The proposed novel technology is based on open raceway ponds (ORWPs) for the cultivation of microalgae. However, with an improved mixing system, CO₂ absorption system, lighting system, modular design and use of transparent material, the proposed technology has significant advantages over the currently available ones.

In fact, the current research and studies in the field have shown major problems related to the regulation of optimal microalgae growing conditions as well extensive land use for the ORWPs. Thanks to the combined (sunlight and artificial) lighting system with LEDs, it would be possible to optimize the diurnal and annual lighting cycle. Moreover, having the proper light wavelength (e.g. research has shown that using LEDs with red and blue light ratio 50:50 has a beneficial effect on the microalgae growth) would increase biomass production by 16 %. Modular and stacked cultivation pond design gives growth media a proper area-to-volume ratio (and micro-algae concentration) and reduces the amount of used land space by 40 %.

Thus, there is a key research question if it is possible to improve ORWPs systems to higher productivity while keeping the low cost of investment as a main advantage. The main challenge is the development of mass microalgae cultivation with lower energy requirements, thus further improving the GHGs balance and the whole LCA of the system [41].

Two levels of investigation are required for a successful cultivation of microalgae in outdoor raceway ponds. It is necessary (1) to perform the screening of algae strains and estimate the optimal cultivation conditions at laboratory scale to determine the potential strains and (2) to assess their performance in outdoor cultivation ponds. Productivity rates in open ponds are commonly lower compared to productivity achieved at a laboratory scale. Therefore, it is important to validate the performance of selected strains in outdoor pilot-scale conditions depending on specific identified variable of optimization like temperature [48]–[51], light [52]–[55], nutrients, and CO₂ supply.

The overall focus of this research is the finalization of an integrated microalgal culturing pilot system coupled with a biogas plant. The novelty of the present study consists of the presentation of the preliminary design of an ORWPs system using the proposed SMORP concept namely Stacked Modular Open Raceway Ponds. The overall research aims to evaluate the feasibility of applying a sort of microalgal-based biofilter process as a treatment and management method for the liquid digestate and flue gases from the CHP unit in biogas plant in Latvian climate conditions. A biomass growth model capable of assessing the effects of the light intensity on the specific growing rate and biomass concentration is also proposed. Based on laboratory tests the provided model is applied to a specifically selected microalgae stream under constant light and temperature conditions in order to be further used as a screening method to select algae species.

The paper will explain in the section related to the applied research method the main steps related to: the design of the novel Staked Modular Open Race Pond (SMORP), the selection of the specific material for the pond, the laboratory stand and the measurements of the microalgae biomass in laboratory conditions, the initial selection of the algae strain, and the adopted kinetic model.

2. Method

The applied research method is based on three main parts:

- Cultivation pilot stand design;
- Execution of laboratory tests depending on a single factor affecting microalgae growth rate;
- Definition of a simple biomass predicting microalgae kinetic model depending on two species-specific and two physical parameters.

2.1. Pilot Design

The overall proposed technological scheme, related to the SMORP pilot project to be realized, would enable a biogas operator to produce energy and/or biomass creating benefit from the management of waste product(s) and emissions (i.e. digestate and CO_2). At the same time, the pilot concept presented in Fig. 1 would be beneficial as a solution for the issue of digestate storage and transport.

The overall scheme should be through a system integrated into an existing biogas plant for which a microalgae-based system and its harvesting can be considered as a side-stream processing module. This solution will, in fact, create a valuable interface to transform the main environmental drawbacks from the anaerobic digestion related to the management and disposal of the digested biomass (digestate) and CO₂ reduction from the exhaust gas use (see Fig. 1) and overall a closed-loop technological system.



Fig. 1. Integrated concept of the Stacked Modular Open Raceway Ponds (SMORP) in biogas plant.

The pilot is based on a novel technological solution of Staked Modular Open Raceway Ponds (SMORP). The main aim addressed is to provide benefits towards: the reduction of land use (a drawback in all ORWP cultivations), the light limitation (due to the lack of light penetration at the bottom part and from lateral surfaces of the conventional ORWPs), and the higher investment costs of the PBR in respect to the ORWPs. The pilot is thus representing an opportunity for an "hybrid" optimized system among the state-of-art ORWPs and PBRs [56]. In fact, the typical material (i.e. acrylic) normally used for a photobioreactor would be used within an ORWP system. The proposed concept takes into account a combined sunlight and artificial lighting system with a low power consuming LEDs and a proper light wavelength in order to balance the light variation and shadow made by the upper ponds, in turn compensating with a higher biomass yield as presented in Fig. 3.

The design, the operation and monitoring of the pilot SMORP module was supported by the latest best practices for microalgae cultivation as explained within the project *EnAlgae* [45] and from the technological solutions according to Chisti [46] and Yadala [57] widely used in commercial production of algal biomass.

The main characteristics of commercial ORWPs are: elliptical shape, depth of 15–30 cm, velocity of 15–30 cm/s maintained with paddle wheels, areas among 100–1000 m² and length (L) to width (W) ratio \geq 10 [41].

For the pilot, the single modular pond presents an oblong shape shallow pond having L/W equal to 2 (i.e. L = 2 m, W = 1 m), an area of 3.6 m², a height (H) of 50 cm (considering 40 cm of culture depth) have been defined for the proposed SMORP pilot (see Fig. 2). Some studies have shown that a higher L/W ratio (L/W ≤ 11) is better in terms of flow dynamics of the system [46], [57]. However, one of the prime objectives of the proposed pilot concept is to evaluate the overall effectiveness of a "stacked modularity" of the open pond system consisting of a number of ponds with a comparatively low L/W ratio for a better mechanical resistance of the structure.



Fig. 2. SMORP single pond shape.

SMORP system is designed with a CO₂ sparging system and energy efficient LED lighting system to balance the energy requirements for the artificial light with a higher biomass production per single unit of used land.

Due to its unique configuration, a transparent material has been selected (acrylic) for construction of SMORP ponds, hence, increasing light penetration through the system.

In Fig. 3 is the proposed process flow diagram for one pond of SMORP system.

The main components of the identified technological scheme are reported below:

- Liquid Digestate as Nutrient Source: digestate discharge from the biogas plant is stored in a continuously stirred holding tank. The digestate is fed to the pond by automatically controlled peristaltic pump. Feeding volume is affiliated with the outcomes of laboratory experiments and characteristics of digestate. Critical characteristics of digestate such as pH, ORP (Oxidation Reduction Potential), Turbidity, Temperature will be continuously monitored and integrated with pump operation;
- Flue Gas as Carbon Source: flue gas emitted from biogas cogeneration unit will be used as a carbon source for growth of biomass. Gas is fed to the system through microporous tubular diffusers installed at the bottom of each pond. The effect of mixing of flue gas with ambient air on growth of biomass will also be tested by the system;
- Mixing Mechanism of Pond Culture: adequate mixing is necessary to maintain culture flow in suspension maintaining homogeneity and most importantly removing dissolved oxygen produced by photosynthesis. Mixing will be performed using a paddle wheel consisting of flat blades. Since the power consumption is greatly affected by the intensity of mixing, it is necessary to maintain the minimum turbulence required in terms of energy efficiency of the system;



Fig. 3. SMORP technological scheme.

- *Light Source*: energy efficient LED lights are installed into the pilot allowing the maximum irradiation throughout SMORP configuration;
- Monitoring of Key Parameters: sensors are planned to be installed in the pond to measure critical parameters which affect growth of microalgae such as pH, PAR (Photosynthetic Active Radiation), ORP, Temperature, DO (Dissolved Oxygen). All signals will be synchronized with a SCADA system which is remotely accessible.

Nutrient detection sensors (i.e. NH4⁺, NO3, K, Cl) will be installed in a second stage;

 Green House: the function of the greenhouse is to protect the cultivation site to external weather conditions and to reach the optimal temperature for the microalgae during the wintertime.

2.2. Laboratory Stand and Measurements of Biomass

In order to have a better optimization of the performance of the microalgae growth rate in the pilot SMORP cultivation and to better estimate the effect of several external parameters (i.e. light intensity, temperature, nutrient supply, dissolved CO_2 and O_2) [1], [55] specific laboratory tests were performed. At this stage of the research these tasks were executed by counting microalgal cells in the culture using a microscope with the Neubauer hemocytometer. Cell counting was done in the centre square of the hemocytometer following a standard procedure [58]. Cell density was calculated according to the Eq. (1):

$$cells / ml = \frac{average \ number \ of \ cell \ per \ square \ dilution - dilution \ factor}{Volume \ of \ square \ [ml]}$$
(1)

The selected microalga (i.e. *Chlorella vulgaris* strain 211-11j) obtained from the SAG Culture collection of algae at Göttingen University was maintained in a typical liquid BG-11 growth media at room temperature in low light conditions and hand mixed daily to avoid settling of cells. Sub-culturing was done approximately once per month to keep the algae culture growing and in healthy condition.

For light intensity, test algae were grown in batch cultures at +24 °C on an orbital shaker (DOS-10L, Elmi) at 150 rpm for 10 days. *C. vulgaris* cultures were cultivated in 500 ml Erlenmeyer flasks containing 200 ml BG-11 medium at pH 7.4 under a photoperiod of 16:8h (light/dark) providing no additional CO₂. Natural white LED lights were used, and light intensity was set to 50, 100, 200 or 400 µmol photons $m^{-2}s^{-1}$. The initial concentration of *C. vulgaris* cultures was ~2 × 10⁶ cells/ml. Daily growth rate was measured by counting cells with Neubauer hemocytometer.

The selected light intensity to finalize the kinetic model was 50 μ mol photons m⁻²s⁻¹ with a maximum growing rate (μ_{max}) equal to 0.25 day⁻¹. This value was selected because highest growth rate of *C.vulgaris* in light intensity test was observed under this light intensity.

2.3. Kinetic Models

Process modelling is required as a key aspect to evaluate the performance of a microalgae cultivation technology for the explanation of growth kinetics. Several kinetic models described microalgae growth as descriptive and explanatory models. Explanatory models are mainly made to assess causal relationship or the fundamental system dynamics. Empirical model normally represents this category and are developed supported by a regression analysis of experimental data. Kinetic models can depend on single or multiple factors directly affecting the microalgae growth (i.e. light intensity, nutrient availability, dissolved CO₂ concentration, temperature, and dissolved oxygen concentration) [1].

The kinetic models are focused to evaluate the trends of the six microalgae growth phases: lag phase, exponential phase, linear phase, declining growth phase, stationary phase, and death phase [1]. In the lag phase the presence of non-available biomass defers the real growing prior the exponential phase in which cells grow according to an exponential trend [59], [60]. In this time step, light intensity and nutrients are not representing constraints for microalgae growth. In the linear growth phase, microalgae growth decreases until the rapid decline to the death phase normally explainable with lack of nutrients, uncomfortable heat, negative effect of pH, or contamination. Normally growth kinetic models are defined as directly linked with specific nutrient concentration.

In order to find favourable microalgae strains for culturing it would be useful to create a simple and flexible screening tool for testing several microalgae strains before the outdoor conditions in cultivation ponds. In this direction, it has been found that biomass growth models present two key common aspects: the assessment of the effect of the light attenuation and the evaluation of the biomass growth depending on both incident and absorbed light. Beer-Lambert's Law (see Eq. (2)) is a well-known method in which the main affecting parameter is the light intensity that declines over the depth of cultivation ponds.

Regarding the relationship between biomass growth and incident or absorbed light, most models employ multifactor regression models implemented in rather complex tools hardly usable as screening tools. Due to this criticality, a biomass growth model depending on measurable species-specific model input parameters namely: the specific growth rate function of light intensity, and the biomass light absorption coefficient is proposed in this study.

For this specific aim, the growth is assessed by the light attenuation in agreement with Beer-Lambert's Law [27]. In fact, Beer-Lambert's Law describes an exponential decrease of the light intensity, I(z), as a function of light penetration depth z.

The model takes into account two physical and two species-specific biological inputs: incident light intensity, culture depth, and the biomass light absorption coefficient and the specific growth rate as a function of light intensity.

$$I(z) = I_o \cdot e^{-k_a B z} , \qquad (2)$$

where

 I_O Incident light intensity at the bioreactor or pond surface, µmol photons m⁻²s⁻¹;

B Biomass concentration, g/L;

 k_a Biomass light absorption coefficient, g/L⁻¹m⁻¹; assumed equals to 64.7 from [27];

z Depth of light penetration, m.

Due to the increase of the microalgae concentration B with increasing pond depth, the effect of the light attenuation is reinforced over time, according to the general formula expressed in Eq. (3) [27]:

$$\mu = \mu_{\max} \cdot f(I), \qquad (3)$$

where

 μ Specific growth rate, day⁻¹;

 μ_{max} Maximum specific growth rate, day⁻¹;

f(I) Dimensionless function dependent on the light intensity species-specific and experimentally determined.

For the proposed kinetic model, the empirical model of Steele [27], [61] has been considered in terms of light-limitation and photoinhibition. This method is widely used and is able to describe the effects of light-limitation towards the ratio I/I_{opt} and photoinhibition using an exponential expression like expressed in Eq. (4):

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$$\mu = \mu_{\max} \frac{I}{I_{opt}} e^{\left(1 - \frac{I}{I_{opt}}\right)}, \qquad (4)$$

where

 μ Specific growth rate, day⁻¹;

 μ_{max} Maximum specific growth rate, day⁻¹;

I Light intensity, μ mol photons m⁻²s⁻¹;

 I_{opt} I at maximum specific growth rate μ_{max} , μ mol photons m⁻²s⁻¹.

During the exponential growing phase, the algal cells grow and divide with an exponential behaviour just before the linear growing phase occurring when growth slows down due to light limitation effect, or nutrients or inhibitors become a constraint. During this phase the specific growth rate (μ) in response to light intensity (I) will increase and the biomass concentration during time interval Δt will be accordingly adjusted to [27]:

$$B(t + \Delta t) = B(t) \cdot e^{\mu \Delta t} , \qquad (5)$$

where

B Biomass concentration, g/L;

 Δt Time step, day;

 μ Specific growth rate, day⁻¹.

Once biomass light absorption coefficient (k_a , defined in Eq. (2)) and the correlation among specific growth rate (μ) and light intensity (I; Eq. (3)) are defined for a specific microalgae species a "step-by-step" increase in biomass concentration as a function of time can be calculated using Eq. (4). The effect from temperature is not considered (i.e. constant temperature), nevertheless incident light intensity (I_0) and the culture depth (d) must be assumed. The algorithm proposed in Fig. 4 has been developed for and implemented in an excel visual basic platform.



Fig. 4. Light-limitation and photo-inhibition kinetic model algorithm readapted from [25].

The algorithm is explained as follows:

- Selection of an initial biomass concentration at an initial time t_0 ;
- Discretization of the culture volume into n equalized parallel volume layers orthogonal to I_0 , I at the midpoint of each of the n culture volume layers;
- Calculation of μ in each of the n culture volume layers;
- Calculation of the biomass concentration in each of the *n* culture volume layers during time interval Δt ;
- Calculation of the new biomass concentration $B(t+\Delta t)$ in the entire culture;
- Averaging the biomass concentrations of all *n* culture volume layers, recalculate the previous steps till the desired time set for the exponential growth (t_{esp}) .

3. RESULTS AND DISCUSSION

3.1. Selection of the Microalgae

The selection of the specific microalgae for ORWPs or PBRs is site specific. Nevertheless, from studies reporting both pilot and already industrial cultivation, the selection of microalgae strain is defined with a screening method focused on specific attributes of the cultivated microorganisms. These are: growth characteristics, lipid contents, C/N ratio or key factors like final end use of the microalgae, adaptability to the growing conditions, potential growth rate and productivity depending on abiotic effective parameters (i.e. light, pH, temperature, nutrient supply, type and composition of injected flue gases, simplicity of harvesting).

The report of the *EnAlgae* project [47] presents best-case practices of pilot plants utilizing among the others: *Chlorella spp., Scendesmus spp., Nannochlopsis spp., Phaeodactylum spp., Chlamydomonas spp.* The study of Marazzi *et al.* [10] is highlighting that the most cultivated algae in ORWPs i.e. (*Dunaliella salina, Arthospira sp.* and *Clorella spp.* [10]) are those that can be grown in extreme and aggressive environments. A pilot project similar to the one proposed in this research supports a cultivation pilot plant mainly using *Chlorella spp.* and *Scendesmus spp.* Similarly, the extensive use of *Chlorella spp.* for both OPWPs and PBRs is also highlighted in Lee *et al.* [1].

Independently from the theoretical section of the algae strain, there is a need to have monoculture stocked in laboratory conditions to both have a stock culture for further tests in laboratory and to inoculate the cultivation ponds in a scaled-up system. In literature there are several findings about *Chlorella* spp. growing tests in laboratory conditions like in [1], [62].

For this reason, Chlorella vulgaris has been selected in the research described in this paper.

3.2. Study of the Material

Usually glass, fiber glass, PVC, Polyethylene (PE), Polycarbonate (PC), HDPE polymer, Plexiglas or acrylic have been used as the basic material for construction of PBRs [63]. Nowadays plastic materials are used more than glass due to characteristics of lower costs, higher light transmission, and facility of transportation, lower maintenance, and resistance to exposure to chemical compounds, durability and better mechanical properties. Among the others, Linear Low Density Polyethylene (LLDPE), High Density polyethylene (HDPE), together with fiberglass, polypropylene, polyethylene, ABS can be also an appropriate material. Nevertheless, if compared with acrylic material, the opaqueness of HDPE could be still considered an inhibiting factor for light penetration. For this reason, acrylic material has been selected for the pilot SMORP cultivation technology. Acrylic material is also used in several pilot stands [64] with the capability to be easily shaped for rounded geometry. For this reason, such materials have been selected for the SMORP concept as a promising solution. In this way, according to the proposed SMORP concept, the effect of natural light can be maximized.

3.3. Kinetic Models of the Light-Dependent Photosynthetic Activity and Biomass for the Selected Microalgae

From the application of the calculation routine according to the proposal algorithm implemented in *Microsoft Excel* visual basic and presented in the section 2.3, it has been assumed a number of layer equals to 30 with 1 cm thickness with an assumed calculation interval of $\Delta t = 0.1$ day.

Fig. 5 and Fig. 6 present the first results of the model based on the laboratory test for *Chlorella vulgaris* implementing Eqs. (2–4).

Specifically in Fig. 5 the results from the assessment of the light intensity changes taking into account the Beer-Lambert's Law are presented. For the determination of the model outputs, authors made the following specific assumptions:

- Initial biomass concentration at a time t_0 (B_0) equal to 0.1 g/L (as optical density), according to available information from literature [62], [65];
- Incident light intensity on surface equal to 50 µmoles photons/m²/sec, according to performed laboratory tests, in order to avoid photo-inhibition effects;
- Light absorption coefficient k_a equal to 64.7 (g/L)⁻¹m⁻¹, empirically found for *Chlorealla* spp. in the study of Hausemann *et al.* [27];
- Type of microalgae: Chlorella vulgaris 211-11j.

The trend presented in Fig. 5 from the implementation of the Beer-Lambert formula is showing that light is attenuated through absorption and scattering depending on light path length and cell concentration similarly like reported in the paper of Yun *at al.* [66]. According to the authors, this can be explained taking into account an average photon flux density within a volume-averaged value of the depth dependent photon flux density.

Fig. 6 shows the variation of the growth rate (μ) according to Steel's formula simulating both light-limitation and photo-inhibition over the depth of the ponds.

For determination of the graphical outputs in Fig. 7(a, b) authors made the following main assumptions:

- Maximum growing rate (μ_{max}) from the performed laboratory tests = 0.25 day⁻¹;
- Optimal incident light intensity at the maximum growth rate (μ_{max}) from the laboratory test = 50 µmoles photons/m²/sec;
- Light absorption coefficient $k_a = 64.7 \text{ (g/L)}^{-1} \text{ m}^{-1}$ (from Hausemann *et al.* [27]);
- Exponential growth $(t_{esp}) = 8$ days;
- Maximum depth if the theoretical ponds equal to 30 cm;
- Type of microalgae: Chlorella vulgaris 211-11j.







Fig. 6. Calculation of the growth rate depending on z (depth) and light intensity according to Steel's formula [58].

From the implementation of Steel formula (Eq. (4)) Fig. 6 explains well the prevalence of the effects of light-limitation (from the ratio I/I_{op}) rather than photo-inhibition.

Fig. 7(a) shows the effect of theoretical incident light intensity on the growth rate of *Chlorella vulgaris* under white LED light. It could be seen that together with the increase of the light intensity a curve-linear behaviour is followed reaching a maximum around 0.25 day⁻¹ in correspondence of an optimal saturating light of about 150 μ moles photons/m²/sec. In the model at this stage the biomass losses during the dark respiration period are not taken into account. These results are similar to those presented by Haussmann [27] specifically addressed to the valuation of the growth rate of *Chlorella* strain except for the maximum growth rate obtained (i.e. 4.7 day⁻¹) against 0.25 day⁻¹ obtained by the kinetic using the laboratory output. This obtained value is in any case more in line with results reported by the study of Daliry [62] and Lee [1] where values of growth rates for *Chlorella* spp. in the range of 0.9 and 2.9 day⁻¹ are reported. The reason of a decreased output of the maximum de with the underestimated assessment of the cell growth made with the hemocytometer.



Fig. 7. (a) Calculation of the growth rate changes for different incident light intensities; (b) biomass concentration during the exponential growth.

The output from Fig. 7(a) shows how the developed tool implementing the kinetic model can be used for finding the maximum biomass growing rate (and thus concentration) with the minimal light intensity, for this specific case equal to 150 μ moles photons/m²/sec.

Fig. 7(b) shows the concentration increase over time as output of the implemented kinetic model from Beer-Lambert and Steele empirical equation. The model is successfully predicting the overall biomass increase due to the effect of the light intensity during the exponential phase of the microalgae growth.

At this stage more attention should be addressed towards decreasing the biomass production rate caused during the dark respiration period.

It is remarkable to highlight that the idea of the model is more focused to provide a fast and consistent screening method for selecting microalgae strains in order to further assess the overall productivity in pilot or scaled-up ponds. This means that the forecasted behaviour will decline in outdoor conditions, due to counterbalancing effects such as: weather events, human errors, contamination from other microalgae species, bacteria, or predators.

The importance of the proposed model is linked with the optimization of the pond design and operational phases. In fact, the physical parameters implemented in the model – like the depth of the pond – can be easily changed in order to assess the overall effect on the microalgae either concentrations or productivity.

Further improvement of the model can be focused on predicting the performance of ponds in two operative modes (i.e. semi-continuous or continuous), allowing the assessment of the optimal dilution rate for biomass productivity.

The refining of the proposed kinetic, could be further proposed taking into account testing the effect of wavelength type for *Chlorella* stream to be optimal, studies have shown that growth increases in the blue wavelengths [62]. Thus, additional experimental test validation would be needed to increase the reliability of predictions also potentially including other physical conditions like temperature and growing media type.

Nevertheless, from findings of several research studies it is highlighted that models considering multiple effective parameters deal with the complexity of the causal relationship and mechanisms affecting the modelled system sometimes making difficult to validate the model in large scale.

Further improvement for kinetic model development should be addressed to specific factors including nutrient and CO_2 supply, pH, temperature and aeration to better design the operational phase.

4. CONCLUSIONS

The presented research is providing the results of the preliminary steps for the design of a novel type of OWRP in order to provide a feasible solution to bottlenecks for the implementation of microalgae technology based on conventional open ponds and photobioreactors. The definition of Staked Modular Open Raceway Ponds (SMORP) should be beneficial towards the creation of an opportunity for "hybrid" systems taking the lower investment and ease of operability and maintenance from the OWRPs and the lower land use and improved harvest of the light from the PBRs. This aspect is reflected on a combination of the current state-of-the-art PBR technologies with the best ORWP practices. Within this idea, the proposal of a pilot concept integrating the use of transparent material (i.e. optimization of the light penetration) with novel geometry of the open ponds (i.e. a staked system is supposed to save up to 40 % of land use) integrated within an optimized artificial LED lighting system.

The pilot concept proposed is focused on the use of microalgae cultivation within wastewater management specifically, the digestate from biogas plant. The proposed pilot has been designed as a solution to the environmental drawbacks related to the management and disposal of digestate from biogas plants in fact using microalgae as an innovative type of biofilter as CO_2 sink and interface for nutrient recirculation.

At this research stage the preliminary design of the ponds and the technological system together with the selection of both the type of material for ponds and the type of microalgae have been defined. Specifically, acrylic material and *Chlorella vulgaris* have been selected from the performed literature review.

Moreover, a microalgae biomass kinetic model implemented in Excel Visual Basic platform was carried out as a simple approach to screen microalgae strains potentially applicable for open raceway ponds. The model has used Beer-Lambert's Law as growth behaviour depending on the light attenuation due to increased amount of biomass over time, and then calculating the specific growth rate in discretized culture volume slices that receive declining light intensities due to attenuation. In fact, this represents a predicting model depending on two species-specific (i.e. biomass growth rate and light absorption) and two physical (i.e. incident light intensity and culture depth light parameters) able to evaluate the effect of the light-limitation and photo-inhibition. The Steel empirical model has been selected to describe these effects using the ratio I/I_{opt} for light-limitation and an exponential expression for photoinhibition.

The preliminary outputs of the kinetic model were defined considering laboratory tests made at the Biosystem's laboratory of RTU Institute of Energy Systems and Environment using: *Chlorella vulgaris* strain 211-11j, artificial white LED of 50 µmol photons m⁻²s⁻¹, a temperature of +24 °C. The selected light intensity to finalize the kinetic model was 50 µmol photons m⁻²s⁻¹ with a maximum growth rate (μ_{max}) equal to 0.25 day⁻¹.

The developed tool is also beneficial to evaluate the potential benefit of additional artificial LED light sources and in order to achieve the maximum biomass growing rate (and thus concentration) with the minimal light intensity.

It is highlighted that the proposed model needs to be more consistently validated both at the laboratory and further at the scale of the pilot pond.

Further improvement should be addressed to: better characterization of the light absorption coefficient for the selected microalgae, validation of the model on other microalgae species prior to being used for continuous or semi-continuous cultures, refining of the kinetic model (sensitivity analysis and effect of lateral light), introduction of the effect of other variables (e.g. temperature, effects of CO₂ supply and nutrients uptake), daily or seasonal changes of light and temperature to better predict biomass productivity in outdoor conditions.

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REFERENCES

- Lee E., Jalalizadeh M., Zhang Q. Growth kinetic models for microalgae cultivation: A review. Algal Research 2015:12:497–512. <u>https://doi.org/10.1016/j.algal.2015.10.004</u>
- [2] Caporgno M. P., et al. Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane. *Algal Research* 2015:10:232–239. <u>https://doi.org/10.1016/j.algal.2015.05.011</u>
- [3] De Morais M. G., Costa J. A. V. Biofixation of carbon dioxide by *Spirulina sp.* And *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *Journal of Biotechnology* 2007:129(3):439–445. https://doi.org/10.1016/j.jbiotec.2007.01.009
- [4] Hsueh H., Li W., Chen H., Chu H. Carbon bio-fixation by photosynthesis of *Thermosynechococcus sp.* CL-1 and *Nannochloropsis occulta. Journal of Photochemistry and Photobiology B: Biology* 2009:95(1):33–39. https://doi.org/10.1016/j.jphotobiol.2008.11.010
- [5] Lam M. K., Lee K. T. Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnology Advances* 2012:30:673–90. <u>https://doi.org/10.1016/j.biotechadv.2011.11.008</u>
- [6] McKendry P. Energy production from biomass (Part 1): Overview of biomass. Bioresource Technology 2002:83(1):37-46. <u>https://doi.org/10.1016/S0960-8524(01)00118-3</u>
- [7] Kröger M., Müller-Langer F. Review on possible algal-biofuel production processes. *Biofuels* 2012:3(3):333–49. https://doi.org/10.4155/bfs.12.14
- [8] Jankowska E., Sahu A. K., Oleskowicz-Popiel P. Biogas from microalgae: Review on microalgae's cultivation, harvesting and pretreatment for anaerobic digestion. *Renewable and Sustainable Energy Reviews* 2017:75:692–709. https://doi.org/10.1016/j.rser.2016.11.045
- [9] Saharan B. S., et al. Towards algal biofuel production: a concept of green bioenergy development. *Innov Rom Food Biotechnol* 2013:12:1–21.
- [10] Marazzi F., et al. Outdoor pilot trial integrating a sidestream microalgae process for the treatment of centrate under non optimal climate conditions. *Algal Research* 2019:39:101430. <u>https://doi.org/10.1016/j.algal.2019.101430</u>
- [11] Hernández D., et al. Microalgae cultivation in high rate algal ponds using slaughterhouse wastewater for biofuel applications. *Chemical Engineering Journal* 2016:285:449–458. <u>https://doi.org/10.1016/j.cej.2015.09.072</u>
- [12] Sahua A. K., Siljudalen J., Trydal T., Rusten B. Utilisation of wastewater nutrients for microalgae growth for anaerobic co-digestion. Journal of Environmental Management 2013:122:113–120. https://doi.org/10.1016/j.jenvman.2013.02.038
- [13] Sepùlveda C., et al. Utilization of centrate for the production of the marine microalgae Nannochloropsis gaditana. Algal Research 2015:9:107–116. <u>https://doi.org/10.1016/j.algal.2015.03.004</u>
- [14] Ramanna L., Guldhe A., Rawat I., Bux F. The optimization of biomass and lipid yields of *Chlorella sorokiniana* when using wastewater supplemented with different nitrogen sources. *Bioresource Technology* 2014:168:127–135. <u>https://doi.org/10.1016/j.biortech.2014.03.064</u>
- [15] Romero-Villegas G. I., et al. Fiamengo M., Acién-Fernández F. G., Molina-Grima E. Utilization of centrate for the outdoor production of marine microalgae at the pilot-scale in raceway photobioreactors. *Journal of Environmental Management* 2018:228:506–516. <u>https://doi.org/10.1016/j.jenvman.2018.08.020</u>
- [16] Arcila J. S., Buitrón G. Microalgae-bacteria aggregates: effect of the hydraulic retention time on the municipal wastewater treatment, biomass settleability and methane potential. *Journal of Chemical Technology and Biotechnology* 2016:91:2862–2870. <u>https://doi.org/10.1002/jctb.4901</u>
- [17] del Mar Morales-Amaral M., et al. Production of microalgae using centrate from anaerobic digestion as the nutrient source. *Algal Research* 2015:9:297–305. <u>https://doi.org/10.1016/j.algal.2015.03.018</u>
- [18] Cai T., Park S. Y., Racharaks R., Li Y. Cultivation of *Nannochloropsis salina* using anaerobic digestion effluent as a nutrient source for biofuel production. *Applied Energy* 2013:108:486–492. <u>https://doi.org/10.1016/j.apenergy.2013.03.056</u>
- [19] Cai T., Park S. Y., Racharaks R., Li Y. Cultivation of *Nannochloropsis salina* using anaerobic digestion effluent as a nutrient source for biofuel production. *Applied Energy* 2013:108:486–492. <u>https://doi.org/10.1016/j.apenergy.2013.03.056</u>
- [20] Monlau F., et al. New opportunities for agricultural digestate valorization: Current situation and perspectives. *Energy and Environmental Science* 2015:8(9):2600–2621. <u>https://doi.org/10.1039/C5EE01633A</u>
- [21] Abeliovich A., Azov Y. Toxicity of ammonia to algae in sewage oxidation ponds. Applied and Environmental Microbiology 1976:31(6):801–6. <u>https://doi.org/10.1128/AEM.31.6.801-806.1976</u>
- [22] Caporgno M. P., et al. Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane. *Algal Research* 2015:10:232–239. <u>https://doi.org/10.1016/j.algal.2015.05.011</u>
- [23] Rawat I., Kumar R. R., Mutanda T., Bux F. Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. *Applied Energy* 2013:103:444–467. <u>https://doi.org/10.1016/j.apenergy.2012.10.004</u>
- [24] Chew K. W., et al. Microalgae biorefinery: High value products perspectives. *Bioresource Technology* 2017:229:53–62. <u>https://doi.org/10.1016/j.biortech.2017.01.006</u>

- [25] Balat H. Prospects of biofuels for a sustainable energy future: a critical assessment. Energy Educ Sci Tech Part A 2009:24:85–111.
- [26] Kwietniewska E., Tys J. Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus onmicroalgal biomass fermentation. *Renewable and Sustainable Energy Reviews* 2014:34:491– 500. <u>https://doi.org/10.1016/j.rser.2014.03.041</u>
- [27] Huesemann M. H., et al. A Screening Model to Predict Microalgae Biomass Growth in Photobioreactors and Raceway Ponds. *Biotechnology and Bioengineering* 2013:110(6):1583–1594. <u>https://doi.org/10.1002/bit.24814</u>
- [28] Appels L., Baeyens J., Degrève J., Dewil R. Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science 2008:34:755–781. <u>https://doi.org/10.1016/j.pecs.2008.06.002</u>
- [29] Arias D. M., et al. Integrating microalgae tertiary treatment into activated sludge systems for energy and nutrients recovery from wastewater. *Bioresource Technology* 2018:247:513–519. https://doi.org/10.1016/j.biortech.2017.09.123
- [30] Putt R., Singh M., Chinnasamy S., Das K. C. An efficient system for carbonation of high-rate algae pond water to enhance CO₂ mass transfer. *Bioresource Technology* 2011:102:3240–3245. https://doi.org/10.1016/j.biortech.2010.11.029
- [31] Bhola V.,et al. Overview of the potential of microalgae for CO2 sequestration. International Journal of Environmental Science and Technology 2014:11(7):2103–2118. <u>https://doi.org/10.1007/s13762-013-0487-6</u>
- [32] de Godos I., et al. Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. *Bioresource Technology* 2014:153:307–314. <u>https://doi.org/10.1016/j.biortech.2013.11.087</u>
- [33] Kumar A., et al. Enhanced CO₂ fixation and biofuel production via microalgae: Recent developments and future directions. *Trends in Biotechnology* 2010:28(7):371–380. <u>https://doi.org/10.1016/j.tibtech.2010.04.004</u>
- [34] Brown L. M. Uptake of carbon dioxide from flue gas by microalgae. Energy Conversion and Management 1996:37(6–8):1363–1367. <u>https://doi.org/10.1016/0196-8904(95)00347-9</u>
- [35] Sheehan J., Dunahay T., Benemann J., Roessler P. A look back at the U.S. Department of Energy's aquatic species program: biodiesel from algae. NREL/TP-580-24190. National Renewable Energy Laboratory, USA, 1998.
- [36] Li S., Luo S., Guo R. Efficiency of CO₂ fixation by microalgae in a closed raceway pond. *Bioresource Technology* 2013:136:267–272. <u>https://doi.org/10.1016/j.biortech.2013.03.025</u>
- [37] Harun R., Singh M., Forde G. M., Danquah M. K. Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews* 2010:14:1037–47. https://doi.org/10.1016/j.rser.2009.11.004
- [38] Zhao B., et al. Efficient anaerobic digestion of whole microalgae and lipid-extracted microalgae residues for methane energy production. *Bioresource Technology* 2014:161:423–30. <u>https://doi.org/10.1016/j.biortech.2014.03.079</u>
- [39] Passos F., Ferrer I. Microalgae conversion to biogas: thermal pretreatment contribution on net energy production. Environ Sci Technol 2014:48:7171–8. <u>https://doi.org/10.1021/es500982v</u>
- [40] Koller M., et al. Characteristics and potential of microalgal cultivation strategies: a review. J Clean Prod 2012:37:377-88. <u>https://doi.org/10.1016/j.jclepro.2012.07.044</u>
- [41] Chiaramonti D., et al. Review of energy balance in raceway ponds for microalgae cultivation: Re-thinking a traditional system is possible. *Applied Energy* 2013:102:101–111. <u>https://doi.org/10.1016/j.apenergy.2012.07.040</u>
- [42] Placzek M., Patyna A., Witczak S. Technical evaluation of photobioreactors for microalgae cultivation. E3S Web of Conferences 2017:19:02032.
- [43] Saharan B. S., et al. Towards algal biofuel production: a concept of green bioenergy development. Innov Rom Food Biotechnol 2013:12:1–21.
- [44] Schenk P. M., et al. Second generation biofuels: high efficiency microalgae for biodiesel production. *Bioenergy Research* 2008:1(1):20–43. <u>https://doi.org/10.1007/s12155-008-9008-8</u>
- [45] Tredici M. Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. *Biofuels* 2010:1(1):143–62. <u>https://doi.org/10.4155/bfs.09.10</u>
- [46] Chisti Y. Biodiesel from microalgae. Biotechnology Advancements 2007:25:294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001
- [47] EnAlgae project [Online]. [Accessed 23.05.2019]. Available: http://www.enalgae.eu/
- [48] Singh S. P., Singh P. Effect of temperature and light on the growth of algae species: A review. *Renew. Sustain. Energy Rev.* 2015:50:431–444. <u>https://doi.org/10.1016/j.rser.2015.05.024</u>
- [49] Park J. B. K., Craggs R. J. Algal production in wastewater treatment high rate algal ponds for potential biofuel use. Water Science and Technology 2011:63(10):2403–2410. <u>https://doi.org/10.2166/wst.2011.200</u>
- [50] Peter K. H., Sommer U. Phytoplankton Cell Size Reduction in Response to Warming Mediated by Nutrient Limitation. PLoS One 2013:8(9):1–6. https://doi.org/10.1371/journal.pone.0071528
- [51] Skau L. F., Andersen T., Thrane J.-E., Hessen D. O. Growth, stoichiometry and cell size; temperature and nutrient responses in haptophytes. *PeerJ* 2017:5(C):e3743. <u>https://doi.org/10.7717/peerj.3743</u>
- [52] Park J. B. K., Craggs R. J. Algal production in wastewater treatment high rate algal ponds for potential biofuel use. Water Science and Technology 2011:63(10):2403–2410. <u>https://doi.org/10.2166/wst.2011.200</u>

- [53] Lee C. G. Calculation of light penetration depth in photobioreactors. *Biotechnology and Bioprocess Engineering* 1999:4(1):78–81. <u>https://doi.org/10.1007/BF02931920</u>
- [54] Gonçalves A. L., Pires J. C. M., Simões M. The effects of light and temperature on microalgal growth and 2 nutrients removal: an experimental and mathematical approach. RSC Advances 2016:27:22896–22907. https://doi.org/10.1039/C5RA26117A
- [55] Jasimone F., et al. Effect of light intensity and nutrients supply on microalgae cultivated in urban wastewater: Biomass production, lipids accumulation and settleability characteristics. *Journal of Environmental Management* 2018:223:1078–1085. <u>https://doi.org/10.1016/j.jenvman.2018.07.024</u>
- [56] Narala R. R., et al. Comparison of Microalgae Cultivation in Photobioreactor, Open Raceway Pond, and a Two-Stage Hybrid System. Frontiers in Energy Research 2016:4:29. <u>https://doi.org/10.3389/fenrg.2016.00029</u>
- [57] Yadala S., Cremaschi S. A Dynamic Optimization Model for Designing Open-Channel Raceway Ponds for Batch Production of Algal Biomass. *Processes* 2016:4:10. <u>https://doi.org/10.3390/pr4020010</u>
- [58] Guillard R. R. L., Sieracki M. S. Counting Cells in Cultures with the Light Microscope. In Algal Culturing Techniques. Ed. Andersen R. A. New York: Elsevier Academic Press, 2005:239–252.
- [59] Richmond A., Hu Q. Handbook of Microalgal Culture: Biotechnology and Applied Phycology (2nd Ed.). Hoboken: Wiley-Blackwell, 2013.
- [60] Posten C., Feng Chen S. Microalgae Biotechnology. Switzerland: Springer International Publishing, 2016.
- [61] Steele J. Environmental control of photosynthesis in the sea. Limnology and Oceanography 1962:7(2):137–150. https://doi.org/10.4319/lo.1962.7.2.0137
- [62] Daliry S., et al. Investigation of optimal condition for Chlorella vulgaris microalgae growth. Global Journal of Environmental Science and Management 2017:3(2):217–230. <u>https://doi.org/10.22034/gjesm.2017.03.02.010</u>
- [63] Katuwal S. Designing and Development of a Photobioreactor for Optimizing the Growth of Micro Algae and Studying Its Growth Parameters, 2017.
- [64] Conti F., et al. CFD modelling of biomass mixing in anaerobic digesters of biogas plants. Journal of Climate and Environmental Technologies. In Press.
- [65] Lv J. M., et al. Enhanced lipid production of Chlorella vulgaris by adjustment of cultivation conditions. Bioresource Technology 2010:101(17):6797–6804. <u>https://doi.org/10.1016/j.biortech.2010.03.120</u>
- [66] Yun Y. S., Moon Par J. Kinetic Modeling of the Light-Dependent Photosynthetic Activity of the Green Microalga Chlorella vulgaris. *Biotechnology and Bioengineering* 2003:83(3):303–11. <u>https://doi.org/10.1002/bit.10669</u>