SUMMARY

Algae are considered as one of the oldest life forms on Earth. They are able to reside in different aqueous environments, including fresh water, saline or brackish water. Microalgae are the algae that are only visible with the use of a microscope. In general, two major groups can be distinguished, respectively eukaryotic microalgae (chlorophyte) or prokaryotic (cyanobacteria). For both groups, their growth is based on photosynthetic reactions, where light intensity is converted intracellularly in energy that consequently can be used for growth. Inorganic carbon such as carbon dioxide or bicarbonate are in presence of nutrients (nitrogen and phosphorus) assimilated in the microalgal biomass.

The use of microalgal systems for wastewater treatment is a promising technique that has several advantages compared to conventional wastewater treatment systems. Besides removal of nitrogen and phosphorus from wastewater streams, nutrients are converted into valuable compounds that can be valorized as feedstock for biofuels, biopolymers or as feedstock for more down-streams process technology such as anaerobic digestion. Moreover microalgae have the capacity to grow on low environmental concentrations of nutrients, which makes the use of this systems for effluent polishing possible. This becomes more important with the more stringent environmental legislation.

However, the microalgal metabolism is somehow more complex compared to the metabolism of activated sludge. Different environmental conditions such as light intensity, temperature and physical- chemical composition of wastewater can have significant influence on the microalgal growth rate. Good insight in the microalgal growth kinetics is therefore essential in view of system performance, control and optimization. Hereby the development of mathematical models can be of great use, because with this technique the performance for different operational settings and water composition can be predicted. Based upon these models, different scenarios can be calculated, prior to the implementation of these systems in the real world.

Despite intensive scientific research, microalgal growth models balancing complexity with accuracy are rarely reported in literature. Next to model development, accurate determination of the microalgal growth kinetics is a prerequisite. In general, the methods that are reported in literature are based on proxy measurements such as for example organic matter or chlorophyll content. The major drawback however of such measurements is the fact that the features of such measurements can only be correlated to the microalgal growth under stable environmental

conditions. With altering environmental conditions, a certain adaption period is needed before the features can be correlated to the microalgal growth rate.

In this dissertation, a methodology to determine the microalgal autotrophic growth rate on a simple, fast but accurate way was developed. This methodology is based upon a the combined respirometric and titrimetric technique that is very well known in the scientific field to determine the growth kinetics of activated sludge. With this method, two variables are measured on-line, namely the dissolved oxygen concentration in the liquid phase and the proton addition (in order to keep the pH constant). The metabolism of microalgae differs however from the metabolism of activated sludge. Where bacteria consume oxygen by the assimilation of organic carbon, microalgae produce oxygen with abundant light intensity, inorganic carbon and nutrients. Besides, the photosynthetic activity will induce an increase of the pH. As such protons will be dosed to maintain the pH at a user defined set-point. The resulting respirometric and titrimetric profiles can be used for the determination of the microalgal growth kinetics.

At first, the combined respirometric and titrimetric methodology was developed and used to assess the microalgal growth kinetics with only one limiting factor, namely the amount of inorganic carbon. The results revealed that, next to the interpretation respirometic results, interpretation of the titrimetric profile is essential. This because the fact that the total amount of oxygen produced, calculated experimentally, did not correspond to the theoretical amount of oxygen produced, expected by the addition of inorganic carbon. The reason for this was that the amount of inorganic carbon that is not available for the assimilation due to possible stripping to the atmosphere cannot be deduced from the respirometric profile. This in contrast to the titrimetric profile, where changes in the chemical equilibrium of inorganic carbon and possible stripping of carbon dioxide to the atmosphere will influence the proton addition. As such it could be concluded that the phenomena of changes in the chemical equilibrium and possible stripping should be taken into account when interpreting the experimental results and using the data for model development. Indeed, a simple model was developed taking into account inorganic carbon limitation. By trial and error, it was then decided to consider the microalgal maximum specific growth rate (μ_{max}) and the oxygen mass transfer coefficient ($K_L a$) for model calibration. For this three separate respirometric profiles were used. Good correspondence between simulated and experimental values were noted.

Subsequently the methodology was extended to different settings of environmental factors, respectively light intensity, temperature, nitrogen, phosphorus and microalgal biomass concentration. For this an experimental statistic design was implemented to assess the main effects of these degrees of freedom on several responses that are related to the photosynthetic activity. Only significant influence of nitrogen and biomass concentation could be observed. This could be explained by the fact that the experimental statistic design focusses on the main effect of degrees of freedom. As such no interaction is taking into account. Apparently this is a reason why no main effect of temperature and light intensity could be observed. Also the range of these degrees of freedom should be broadened in future experiments. Based upon the experimental results, it was decided at this stage of the research to expand the earlier developed simple model with kinetics for nitrogen species (NH_4^+ and NO_3^-) and phosphorus (PO_4^{3-}). The latter because phosphorus is an essential element for the microalgal growth.

In a next stage, the parameter included in this expanded model were assessed for identifiability. The results of this illustrated that the two parameters that where at first chosen by trial and error for model calibration, were the only parameters that were uniquely identifiable to the combined respirometric and titrimetric data. Consequently these two parameters, respectively the maximum specific growth rate (μ_{max}) and the oxygen mass transfer coefficient ($K_L a$) were calibrated to seven separate experiments. With optimized parameter settings, good visual correspondence between experimental and predicted profiles was noted. Further the model performance was evaluated by using the Theil's Inequality Criterium (TIC). For all calibration experiments, the threshold value of 0.3 was not exceeded. Additional model validation with two other experiments also illustrated good correspondence between model prediction and measured profiles. For the simulations, the μ_{max} was taken as the mean value of the different separate optimized values, namely μ_{max} = 0.261 d^{-1}. For the $K_L a$ an empirical relation was defined as function of the microalgal biomass. As such a value of $K_L a$ = 11.31d^{-1} for the first validation experiment and $K_L a$ = 5.71 d^{-1} for the second validation experiment was used. Also TIC did not exceed the threshold value for these experiments, respectively TIC = 0.05 and TIC = 0.08.

In the final part of the research, the experimental methodology was used to assess the growth kinetics of microalgal species that were isolated from a waste stabilization pond (WSP) situated in the Andes in Ecuador. This is an open pond system, where the combination of bacteria and microalgae is used to treat the domestic wastewater of a nearby city (Cuenca). The main goal

of the research was to investigate whether there is a difference in growth kinetics between both microalgal species. For this an experimental set-up analogues to the set-up developed earlier was built. Further in this specific stage, it was decided to investigate if the influence of light intensity and temperature is significant for the microalgal growth rate. This was done, because although expected, it was not observed in the preceding research. As such a broader range of light intensity and temperature was used. It was observed by the experimental results, that the effect of light intensity and temperature is significant in the ranges that were applied. Also the interaction between light intensity and temperature was significant. Consequently the microalgal growth kinetics were expanded with a function that describes this interdependent relation. Further the model was calibrated with two additional separate experiments for both microalgal species. Also the maximum specific growth rate (μ_{max}) and the oxygen mass transfer coefficient ($K_L a$) were used for model calibration. The results showed again good correspondence between simulated and experimental dissolved oxygen evolution and proton addition for both microalgal species. This for the visual aspect as based on the TIC. Also a difference between the optimized maximum specific growth rate between species was noted. This could be explained by the fact that different pigments are synthesized between species. Influence of height and as such by decreased atmospheric pressure was not noted. This could possibly be explained by the fact that microalgae are able to adapt to this elevated situation.

Throughout this dissertation a specific experimental methodology was developed and used to assess the identification of the autotrophic microalgal growth kinetics, even under varying conditions of environmental factors, such as nutrients, light intensity, biomass concentration and temperature. Also a mathematical model was expanded from a very simple model to a more mechanistic model taking into account all these environmental factors. This experimental methodology and the developed model are a solid base for future research involving wastewater treatment systems with microalgal biomass. It could be used for the identification of the growth kinetics with heterotrophic and mixotrophic conditions. Also the combination of microalgae and bacteria would be interesting to investigate. Such combination would occur in the treatment

of wastewater in open pond systems, which aim for less complex compared and less costly waste water treatment.