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EXPERIMENTAL INVESTIGATION OF BIODEGRADATION OF SYNTHETIC WASTEWATER IN AN ANAEROBIC TANK REACTOR USING MICROALGAE

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Abstract

The objective of this study was to investigate the viability of microalgae biomass (Chlorella vulgaris) as a substrate for anaerobic digestion using synthetic wastewater as well as evaluate the biogas production improvement and the microalgae cultivation rate.

The experimental procedure lasted for 700 hours, where a mixture of synthetic wastewater and microalgae were used in the BE4 anaerobic tank reactor. Based on the process parameters (pH and temperature), the degradation phases (hydrolysis, acidogenesis (fermentation acidification), acetogenesis, methanogenesis) were successfully separated from each other. A drop in pH was detected from 7 to 4.5 in the acidogenesis phase, which resulted in a delayed decrease in the total chlorophyll concentration and the microalgae activity rate. In the methanogenesis phase, the pH slightly returned to neutral and caused a relatively low biogas yield (0.15 L in total), which can be explained by the unbalanced carbon/nitrogen rate (C : N) in relation to the substrate. The increase in total chlorophyll concentration was observed by the end of the process as a result of the increasing pH.

It was found, that the concentration of major chemical constituents and the COD can be described well by logarithmical curves according to the nutrient consumption of microalgae. The COD decreased from the initial value of 5077 mg·L^{-1} O_2 to 1592 mg·L^{-1} O_2, which refers to an intensive anaerobic degradation process.

Key words: anaerobic degradation, wastewater, microalgae, tank reactor, water quality, biogas.

INTRODUCTION

The concern regarding the environmental issues has become increasingly evident due to the fast technological development associated with the high rate of non-natural resources use as an energy source, which have been leading to a high pollution potential (Hosseini Koupaie et al., 2019). The global warming current scenario and the social, environmental and economic losses has required an increase in the research demand related to technologies for mitigating greenhouse gases (GHG) and the renewable resources use as an alternative source to the fossil fuels (Zhang et al., 2019).

According to the Emission Gab Report, 2019, in order to avoid intensifying environmental problems and rising temperatures related to global warming in the near future, emissions are expected to fall to 25 Gt by 2030. Thus, policies and actions have been taken around the world, increasing the exploring for energy alternative sources and CO_2

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sequestration technologies, which currently has been growing (Gunes et al., 2019).

Due to the high productivity of biomass and ability to grow in wastewater, microalgae can bring together these themes through the mitigation of CO_2 used for the photosynthetic biomass growth, and the biomass production with nutritional value and with an appropriate composition for biofuels production such as biodiesel, bioethanol and biogas (Zabed et al., 2020).

Microalgae are one of the most efficient biological systems for transforming solar energy into organic compounds, through photosynthesis (Vonshak, 1997). These microorganisms can be used in the bio-fixation of CO_2 (Thi Nguyen et al., 2019) as well as in the treatment of effluents (Solé-Bundó et al., 2019) and biofuels production (Zabed et al., 2019). Microalgae biofuels, compared to first and second-generation fuels, have the advantage of not contributing to deforestation, and excessive water consumption. In addition, they do not compete with crop areas that could be used for food production (Li et al., 2018).

Therefore, among renewable resources explored as an alternative to fossil fuels, biomass energy has been excelled. Biomass sources can include food wastes, agricultural residues, animal manure (Kiss et al., 2020), forestry residues, energy crops, microalgae, organic-rich wastewaters, organic fraction of municipal solid waste, and industrial organic waste (Cucchiella, D'Adamo, 2016; Jankowska et al., 2017). All of them can be used as a substrate in the anaerobic digestion process for the biogas production, which currently is an energetic valorization practice of substrates (Bedoić et al., 2019).

Anaerobic digestion is a biological process that occurs in the absence of oxygen and converts organic matter mostly into methane (Kunz et al., 2019). Due to the performance of different groups of microorganisms, this process is highly complex and can be defined as symbiotic, since the products formed by a certain group must be readily consumed by the next group (Chernicharo et al., 2007; Ometto et al., 2019). According to Kleinsteuber, 2018, anaerobic digestion can be divided into four consecutive stages: hydrolysis, acidogenesis (fermentation acidification), acetogenesis and methanogenesis as is shown in the Figure 1.

The first stage of anaerobic digestion is the hydrolysis of polymeric macromolecules, such as proteins, carbohydrates and lipids into smaller molecules, which are more soluble and easily assimilated by microorganisms (Kleinsteuber, 2018; Ometto et al., 2019). The next stage is the acidogenesis phase, where acidogenic bacteria intracellularly metabolize the soluble products formed during hydrolysis. The metabolism of these compounds results in the formation of alcohols, ketones, CO_2, H_2S and,

mainly, volatile organic acids, with acetic, propionic and butyric acids found in higher concentrations (Kunz et al., 2019). The acetogenesis is the consecutive step in the process, where intermediate organic compounds such as propionate and butyrate are oxidized to acetate, CO_2 and H_2 (Chernicharo et al., 2007). The final stage is called methanogenesis, which is performed by different groups of bacteria basically through two reactions. In the first reaction, the generation of methane and carbon dioxide derived from acetic acid occurs. In the second, hydrogen and carbon dioxide give rise to methane and water (Chernicharo et al., 2007). The final product is called biogas.

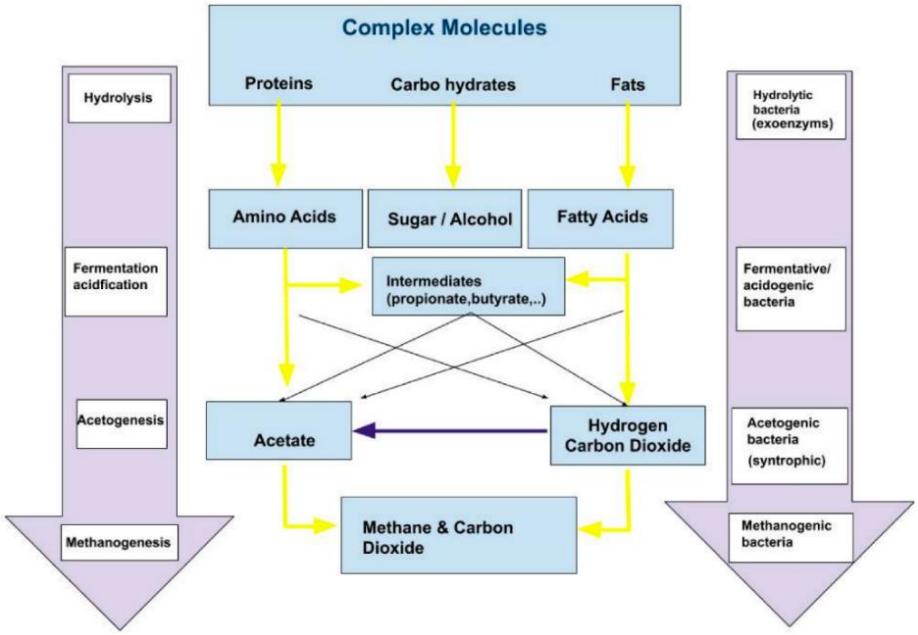

Fig. 1. Pathways of anaerobic digestion for biomethane production (Sayara, Sánchez, 2019).

Biogas is produce by a complex microbial communities during the anaerobic digestion, and is mainly composed by (volume) CH_4 (55 – 70 %) and CO_2 (30 – 45 %) and small amounts of H_2S (50 - 2000 ppm), H_2O, O_2 and some trace hydrocarbons (Zabed et al., 2020). In addition, biogas is considered an ecological fuel because it is produced through renewable sources with a potential for generating electricity, thermal energy, vehicular gas (biofuel) and organic fertilizer through the digestate (co-product generated by the end of the process) (Nagarajan et al., 2019; Stiles et al., 2018).

Furthermore, the biogas production using microalgae biomass,as a source of substrate, is further extended due to the fact that it contains a good

amount of biodegradable compounds, such as carbohydrates (4 – 57 %), lipids (2 – 40 %), and proteins (8 – 71 %) of the total solids, which can produce biomethane with the theoretical yield of 0.42, 1.01, and 0.5 LSTP CH_4/g, respectively (Frigon, Guiot, 2010).

Therefore, the objective of this study was to investigate the viability of microalgae biomass (*Chlorella vulgaris*) as a substrate for anaerobic digestion using synthetic wastewater as well as evaluate the biogas production improvement and the microalgae cultivation rate.

MATERIAL AND METHOD

Substrate

The feedstock used as a substrate source in the bioreactor was composed of synthetic wastewater and microalgae biomass (*Chlorella vulgaris*). The synthetic wastewater was prepared dissolving weighed amounts of chemicals in distilled water and the mixing with measured volumes of trance metals solutions A and B. Trace metal solutions was prepared in advance. The Table 1 shows the chemical composition of synthetic wastewater.

Table 1

Chemical composition of synthetic wastewater

Chemical/Reagent Parameter	**Concentration**
Approximate COD ($mg \cdot L^{-1}$)	5000
Glucose ($g \cdot L^{-1}$)	4
Ammonium hydrogen carbonate ($g \cdot L^{-1}$)	0.20
Potassium dihydrogen phosphate ($g \cdot L^{-1}$)	0.20
Sodium hydrogen carbonate ($g \cdot L^{-1}$)	0.50
Potassium hydrogen carbonate ($g \cdot L^{-1}$)	0.5
Trace metal Solution A* (ml)	1
$MgSO_4 7H_2O$ ($g \cdot L^{-1}$)	5
Trace metal Solution B* (ml)	1
$FeCl_3$ ($g \cdot L^{-1}$)	5
$CaCl_2$ ($g \cdot L^{-1}$)	5
KCl ($g \cdot L^{-1}$)	5
$CoCl_2$ ($g \cdot L^{-1}$)	1
$NiCl_2$ ($g \cdot L^{-1}$)	1

The total volume of wastewater used to feed the bioreactor was 15 L. However, a volume of 150 ml of microalgae biomass (*Chlorella vulgaris*) was added in order to evaluate the improvement of the biogas production and cultivation rate.

Microalgae

For this experiment, it was used the microalgae *Chlorella vulgaris* due to its versatility and good growth rate in wastewater, as well as when degraded it can present a good yield for biogas production.

The microalgae was grown in a synthetic medium and the initial volume used to feed the bioreactor was 150 ml at a total chlorophyll concentration of 59.65 $\mu g \cdot L^{-1}$. The initial chlorophyll concentrations of microalgae classes are shown in Table 2. The microalgae activity rate was 60 %.

Table 2

Chlorophyll concentrations of microalgae classes

Microalgae class	**Chlorophyll concentration, [$\mu g \cdot L^{-1}$]**
Green	54.02
Blueg	0
Diato	0
Crypt	5.63

Experimental device - BE4 anaerobic tank reactor and operating conditions

In order to performance this experiment, was used the BE4 anaerobic tank reactor. This reactor has been designed exclusively for teaching and research development, its system presents a programmable logic controller (PLC) which provides temperature control, pH control and gas collection (rate and totalisation) calculations. It has a jacket heating system with pump and hot water vessel; an automated volumetric gas collection system measures; an automated pH dosing system to maintain the vessel pH within a pre-determined range (user programmable) and a data logger and software as standard (data acquisition) (BE4 Anaerobic Column Reactor - Armfield).

Moreover, BE4 reactor has greater versatility compared to other anaerobic reactors because it has the advantage of being multi-configurable and can be operated in Continuous Stirred Tank Reactor (CSTR), Upflow Active Sludge Bed Reactor (UASB), and Packed Bed Reactor (PBR). The main parts of the BE4 anaerobic tank reactor are shown in the Figure 2.

In this experiment, the BE4 reactor was configured as a CSTR reactor and operated with 15 L of the total capacity of 20 L, in constant stirring. The samples were collected once a week and evaluated using analytical instruments.

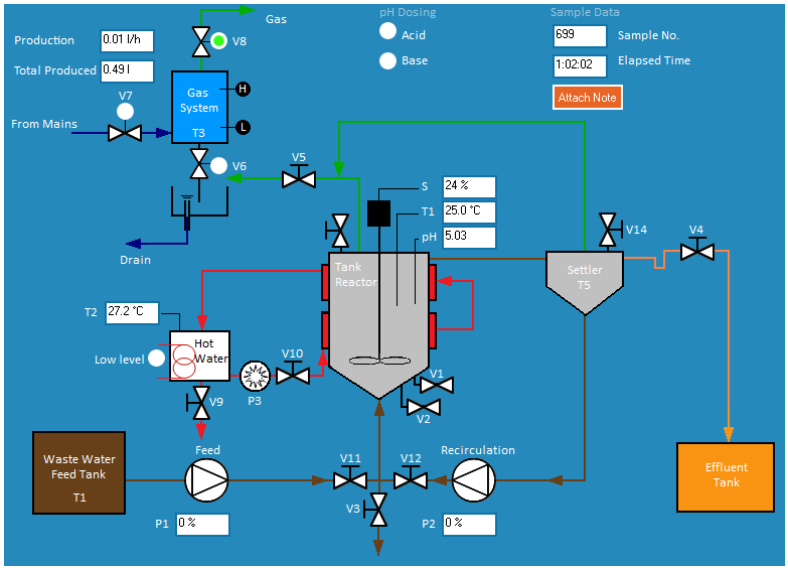

Fig. 2. The schematic of the BE4 anaerobic tank reactor.

Applied measuring methods

The pH was analyzed by the pH probe, and the process and water temperatures were analyzed by temperature probe from the BE4 anaerobic reactor, the dates were collected, hourly by the data acquisition system.

However, the nutrient concentration (nitrate, potassium, ammonium 3 and phosphate) were analyze by Visocolor ECO method. The Visocolor ECO presents a product group of colorimetric and titrimetric test kits, which allow even the determination of low limiting values with sufficient accuracy. The high sensitivity and accuracy is accomplished by single reagents which can be dosed precisely and by the possibility to compensate turbidity and color of water samples.

In addition, the COD was analyze using the Nanocolor CSB 1500 method, which is a photometric determination of decrease in chromate concentration after two hours and oxidation with potassium sulphuric acid at 148 °C. In order to reach the temperature required the equipment Nanocolor R-8 was used. Thus, all the results were evaluated photometrically, with the compact photometers PF-12Plus.

Another important analytical parameter to be evaluated is the concentration of *Chlorella vulgaris* and the microalgae activity rate present in the samples. In order to perform this procedure, it was used the Algae Toximeter II instrument (Figure 3). This instrument is capable to continuously monitor photosynthetic activity of the microalgae from the concentration of chlorophyll production, as well as measuring the precise determination of microalgae concentrations in water/sample.

Fig. 3. Algae Toximeter II equipment.

RESULTS AND DISCUSSION

Evaluation of process parameters in the fermentation tank

The pH, water and process temperature and the gas production rate were monitored for approximately 30 days (700 hours). According to the Figure 4 generated through the collected data, it is possible to realize that during the first 4 days (100 hours) both the laboratory and the process temperature varied abruptly reaching peaks around 35 and 31 °C.

The abrupt variations that occurred are due to the interference of the daily temperature, change in the environment, where, peaks of sunshine are more frequent in the middle of the day, which also can be observed during the whole process, as well as the temperature decreased after 8 days (180 hours) which is influenced by the whether season changing (summer to autumn).

However, comparing the first and the fourth day of monitoring it is possible to notice a slight increase in the temperature of the process. This intensification in the temperature is due to the increase in microbial activity, indicating a phase change in the process. This change in the phase can also be detected by the drop in pH that varied from 7 to 4.5, and by the non-production of biogas, which indicate the anaerobic digestion process went from the hydrolysis to the acidogenesis phase, increasing the microbial activity and decreasing the pH of the medium.

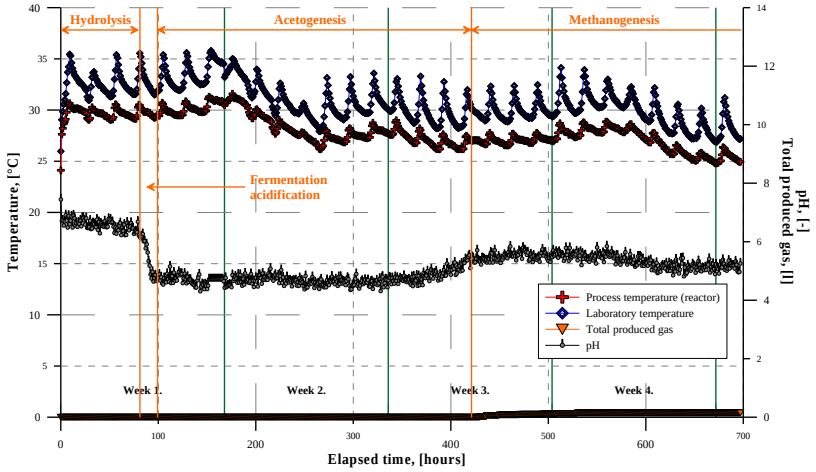

Fig. 4. Monitoring of the process and laboratory temperature, pH and total gas production during 700 hours of experiment.

After the fourth day of the process, it is possible to notice that the pH of the medium was constantly acidic, presenting values below the ideal (6.6 to 7.4) during the acetogenesis phase, probably due to the accumulation of volatile acids that occurred in the previous phase.

However, it was possible to observe a slight increase in pH after 18 days of process as well as a low rate of biogas production (0.15 L) indicating the start of the methanogenic phase. The low biogas yield at the beginning of the methanogenic phase can be explained due to the unbalance of the carbon/nitrogen rate (C : N) in relation to the substrate, which can cause low methane yield. The ideal C : N ratios for the anaerobic digestion process vary from 20 : 1 to 30 : 1, but the values for microalgae have been reported from 4.65 : 1 to 1 : 17, which can cause a disturbance in the process phases (Zhang et al., 2019).

Evaluation of chemical parameters of the synthetic wastewater

During the experiment, the chemical parameters of the synthetic wastewater were also investigated. Based on Figure 5 the anaerobic degradation process can be described well by logarithmical curves in case of the examined major constituents and COD, which were fitted on the measured points (Table 3).

Nutrients concentrations like N forms, potassium and phosphate are continuously decreased in the tank reactor during the experiment. Concentration of N forms (ammonium and nitrate) reached almost the zero value 4 weeks after the start. This can be explained by the intensive nutrient consumption by the algae. Our results are pointed out that the amount of N forms were limited in the system. Similarly to N forms the phosphate and potassium concentration in the tank are, also, decreased during the

experiment. Their consuming tendency were similar to N forms consuming. It means that the shapes and equations of describing curves of nutrient consuming were quite similar and typical for biodegradation.

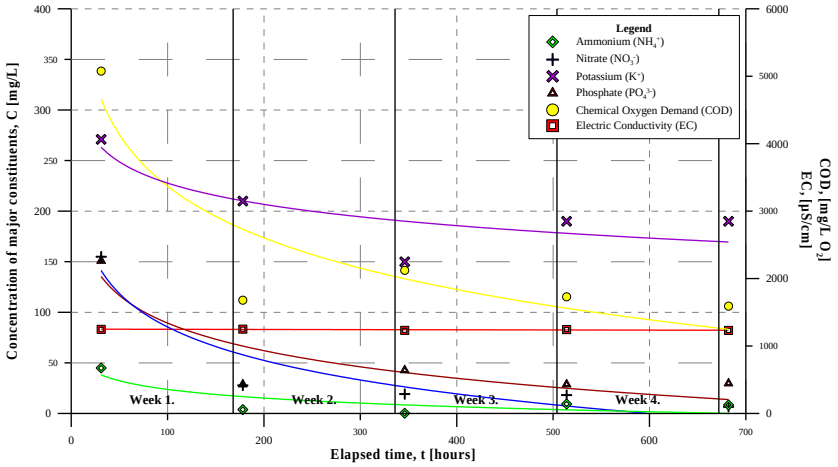

Fig. 5. The concentration of major constituents in the synthetic wastewater during 700 hours of experiment.

Table 3

The parameters of the fitted curves on the major constituents

Major constituent / Chemical parameter	**Equation of the fitted curve**	**R^2 value**
Ammonium	Y = -12.3251 * ln(X) + 80.5213	0.709
Nitrate	Y = -47.7042 * ln(X) + 305.1524	0.906
Potassium	Y = -30.3240 * ln(X) + 367.3417	0.714
Phosphate	Y = -39.3586 * ln(X) + 270.5829	0.828
COD	Y = -1104.6492 * ln(X) + 8456.0008	0.837
EC	Y = -0.0239 * X + 1249.9709	0.509

The COD decreased from the initial value of 5077 $mg \cdot L^{-1}$ O_2 to 1592 $mg \cdot L^{-1}$ O_2, which refers to an intensive anaerobic degradation process. Since the anaerobic reactor can be classified as an isolated system, the EC value remained constant during the entire process with the value of 1250 µS/cm, therefore in this case linear fitting was applied.

Evaluation of biological water quality of the synthetic wastewater

In order to monitoring the microalgae concentration and activity rate during the anaerobic digestion process, the Algae Toximeter II equipment was used to collect the dates. This way, according to Figure 6 and Figure 7 it was observed that the total chlorophyll concentration has increased from 59.67 $\mu g \cdot L^{-1}$ to 100.6 $\mu g \cdot L^{-1}$ during 7 days, showing a greater availability of nutrients present in the medium.

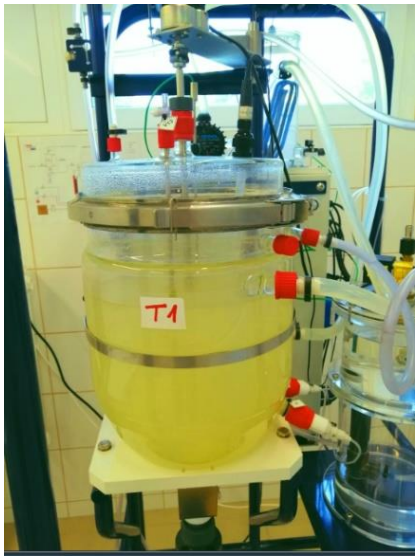

Fig. 6. The anaerobic tank reactor after 7 days of experiment.

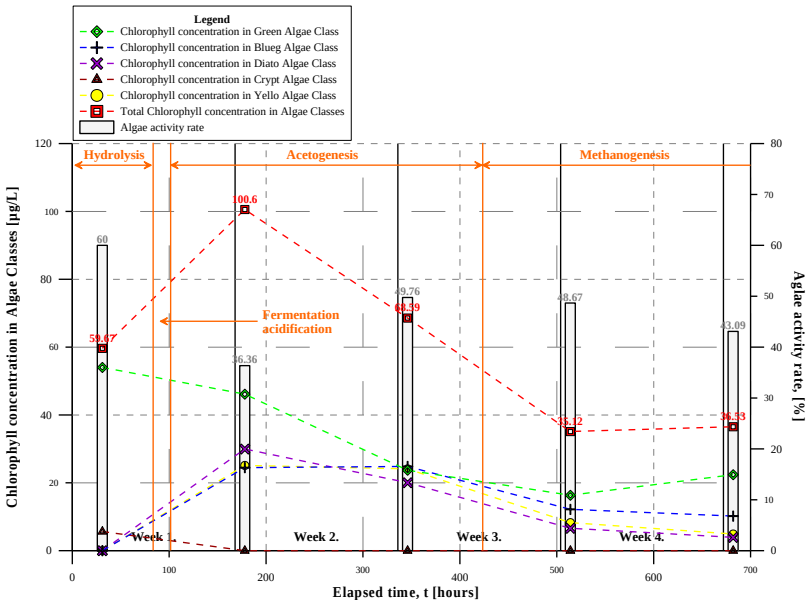

Fig. 7. Microalgae concentration and activity during 700 hours of experiment.

However, the algae activity rate has decreased from 60 to 36.36 % due to a drop in pH during the acidogenic phase. In addition, the chlorophyll concentration of algae classes show different behavior, the chlorophyll concentration in green and crypt algae classes has decreased, while the others has increased in concentration.

After 7 days, due to the drop in nutrients available for microalgae, the total chlorophyll concentration decreased from 100.6 to 68.59 $\mu g \cdot L^{-1}$.

However, with the adaptation to the acid medium, the algae activity rate of the remaining microalgae increased from 36.36 to 49.76 %. After 21 days of process, due to changes in the environment in relation to the increase in pH and the greater availability of nutrients, the total chlorophyll concentration increased.

Furthermore, even with the improvement of environment conditions, the algae activity rate decreased from 48.67 to 43.09 % probably due to environmental conditions, not yet ideal for microalgae. In addition, the same behavior of the total chlorophyll concentration can be observed in relation to chlorophyll concentration in green algae class, which is the microalgae class of interest.

CONCLUSIONS

Based on the experiment performed, the following conclusions can be drawn:

−The anaerobic degradation phases can be separated from each other based on the pH and the reactor temperature.

−The pH has a significant effect on the microalgae cultivation (chlorophyll concentration and microalgae activity rate), therefore adding the microalgae to the system is strongly suggested after the acetogenesis phase in order to achieve a higher degradation rate in the wastewater.

−The concentration of the studied nutrients decreasing continuously during the experiment according to the nutrient consumption of microalgae.

−The decreasing tendency of different nutrients was quite similar and can be described well by logarithmical curves.

−The C : N ratio should be adjusted to the optimal at the beginning of the degradation process to obtain higher biogas yield.

−More experiments should be done to get a comprehensive view on the optimal circumstances of the microalgae cultivation.

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