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ORIGINAL RESEARCH PAPER

Characterization of the biodegradation of synthetic and organic wastewater in an anaerobic tank reactor using microalgae

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ABSTRACT

The anaerobic digestion is a well-known method in waste management of biodegradable wastes to transform waste to energy. Proper digestion requires optimal fermentation conditions to improve the quality and yield of biogas.

The objective of this study was to characterize the biodegradation process of synthetic and organic wastewater. Microalgae (*Chlorella vulgaris*) were utilized as a bioindicator for anaerobic digestion and monitoring of the fermentation process. Besides bioindication, the viability of the microalgae and the chlorophyll concentration were also assessed in such fermentation processes, since microalgae can be a potential source for biofuel production and a plant nutrient.

The biodegradation process was studied for a month in an anaerobic tank reactor. The fermentation processes and lengths of the fermentation stages were successfully monitored and separately identified based on the pH and gas development. Furthermore, the amount and dynamics of the biogas yield also revealed that the fermentation process was about 510 hours in both cases. In contrast, increased temperature in thermophilic range (45°C) accelerates the degradation processes and resulted in shorter hydrolysis (60 hours), acetogenesis (24 hours) and longer methanogenesis (81 hours) stages, where higher biogas yield was also achieved (59.3%). During the process, the concentration of nutrients showed logarithmical tendencies and COD showed power tendency in time. The extent and the direction of the changes were in correspondence with microalgae activity. In thermophilic circumstances, living microalgae biomass dropped significantly without recovery therefore such an environment is not a viable option for microalgae growth. Moreover, dead microalgae biomass seems to act as a substrate for fermentation slightly increasing the concentration of some nutrients in the wastewater.

KEYWORDS

anaerobic tank reactor, degradation stages, synthetic and organic wastewater, microalgae, nutrients, biogas

1. INTRODUCTION

From a climate change point of view the need for potential renewable energy resources is emerging globally [1]. In this context, anaerobic digestion of organic materials for biogas production appears as an alternative solution for a waste-to-energy (WtE) process. Therefore, one of the renewable resources that has been excelled as an alternative to fossil fuels is biomass. Biomass for potential biogas fermentation comes from several sectors [2]:

1. waste disposal sites, landfills (solid waste) ⇒ landfill gas;
2. sewage works (communal sewage, sewage sludge) ⇒ biogas;
3. agricultural, food industry by-products, wastes (mixed raw material) ⇒ biogas.

The highest economic benefit from biogas production can be achieved when it is based on the secondary products and wastes that are locally available [3]. Currently, the anaerobic

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treatment process is favourable to treat different types of effluents (dairy effluents [4], rice industry wastewater [5], winery and distillery wastewater [6], slaughterhouse wastewater [7], sugar and distillery waste [8], fish wastewater [9]) with a high organic material content, to decrease the organic material content and to produce energy.

However, the degradation is a complex process and depends on a balanced action of several microbial groups. Due to the performance of different groups of microorganisms, anaerobic digestion occurs in the absence of oxygen and converts organic matter into methane, carbon dioxide, water, hydrogen sulphide, ammonia and biomass [10, 11]. Anaerobic digestion is divided into four consecutive stages: hydrolysis, acidogenesis (fermentation acidification), acetogenesis and methanogenesis [12]. 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 [12, 13]. In the acidogenesis stage, 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 [10, 14]. The acetogenesis is a consecutive step in the process, where intermediate organic compounds such as propionate and butyrate are oxidized to acetate, CO_2 and H_2 [13, 15]. The final stage is the methanogenesis, which is performed by methanogenic archaea producing methane mainly from acetate [16]. The prerequisites of the process are a lack of oxygen, a pH value from 6.5 to 7.5 and a constant temperature of 35–45°C (mesophilic) or 45–55°C (thermophilic). According to Nagy et al. [17], the best C/N ratio for the effective biogas production is 25:1. On the other hand, the most common problem is the heterogeneity of available biomass, the mixed and variable composition of input materials [10, 12]. The adjustment of the appropriate quality parameters is difficult, because the amount and quality of the wastes from husbandry and plant production are seasonally different. The quality and quantity of input organic materials are often various, so it is crucial to monitor them during the biodegradation process [18].

Microalgae (e.g. *Chlorella* sp.) have a unicellular structure, with significant growth that can produce a large biomass [19, 20], therefore microalgae with a wide range of commercial applications have attracted a lot of attention from many researchers [21, 22]. Algae species, like *Chlorella,* have been widely applied for wastewater treatment and have proven abilities of removing nitrogen, phosphorus, and reducing the chemical oxygen demand (COD) [23]. Among the different biomasses studied, microalgae have also been associated with the bio-fixation of CO_2 [24] as well as in the treatment of effluents [19] and biofuel production such as biodiesel, bioethanol and biogas [25, 26]. Moreover, the use of microalgae biomass as substrate for biogas production has also been studied [27, 28].

Based on the above mentioned, in this study fermentation processes of two different effluents were carried out in an anaerobic tank reactor, integrating microalgae into the anaerobic digestion. The main goal of our study was to characterize the biodegradation process of well-defined synthetic wastewater, and seasonally changing organic wastewater using microalgae (*Chlorella vulgaris*) for bioindication of the process.

2. MATERIALS AND METHODS

2.1. Substrate - Synthetic wastewater

In the first experiment, the substrate source was composed of 15 L of synthetic wastewater and 0.15 L of microalgae (*C. vulgaris*) suspended in the bioreactor. The synthetic wastewater with approximately 5000 $mg \cdot L^{-1}$ COD was prepared based on the suggestions of Chernicharo, 2007 [11] (Table 1). The temperature was not controlled in the reactor, but it was regulated by the degradation process.

2.2. Substrate - Organic wastewater

In the second experiment, the substrate source was composed of wastewater (17.5 L) and sludge (2 L) from a fishery (fish wastewater: mechanically filtered and sludge) and microalgae (*C. vulgaris*) suspension (0.5 L) (Fig. 1). The fish wastewater was collected from the Fish Biology Laboratory of the University of Debrecen. The used fish wastewater with a low concentration of total organic content (TOC) (<5 $mg \cdot L^{-1}$) and particle size of total suspended solids (TSS) below 160 μm was first added to the reactor. In order to increase the organic content concentration to the process, fish wastewater sludge (TOC was approx. 600 $mg \cdot L^{-1}$) was added with 4.02 $g \cdot L^{-1}$ of dry material content and with particle size higher than 160 μm TSS. The total volume of the mixture was 20 L with a final 0.404 $g \cdot L^{-1}$ dry material content. The amounts of macronutrients and COD are shown in Table 2. Moreover, the jacket heating was applied and set to 45°C in this experiment (optimized temperature to the thermophilic range).

Table 1. Composition of synthetic wastewater

Chemical/Reagent parameters	Concentration
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
$MgSO_4 \cdot 7H_2O$, [$g \cdot L^{-1}$]	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

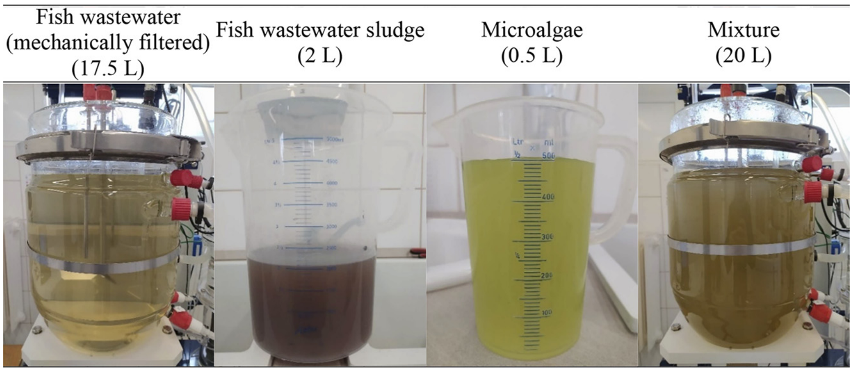

Fig. 1. Experiment procedure using organic wastewater

Table 2. Composition of fish wastewater mixture (mechanically filtered and sludge)

Chemical parameters	Concentration
COD, [$mg \cdot L^{-1}$]	127
Ammonium, [$mg \cdot L^{-1}$]	4.2
Potassium, [$mg \cdot L^{-1}$]	7
Phosphate, [$mg \cdot L^{-1}$]	15
Nitrate, [$mg \cdot L^{-1}$]	190.5

2.3. Microalgae

For both experiments, the microalgae *C. vulgaris* were used due to their versatility and good growth rate in wastewaters [29]. Algae Toximeter II were used to evaluate the effect of the synthetic wastewater fermentation on biological activities and microalgae cultivation rate. The microalgae suspension contained 59.67 and 115.2 $\mu g \cdot L^{-1}$ total chlorophyll concentration (Table 3). The microalgae activity rate using synthetic wastewater and organic wastewater was about 60 and 22%, respectively.

2.4. Experimental device - BE4 anaerobic tank reactor and operating conditions

A BE4 anaerobic tank reactor (Armfield Ltd.) was used to control the fermentation in the experiments. The reactor has a programmable logic controller (PLC) that can be used for temperature, pH and gas volume control. It has a jacket heating system with pump and hot water vessel, an automated pH adjusting system and its own software for data acquisition. Compared to the other laboratory-scale anaerobic reactors, the BE4 has great versatility because it has the advantage of being multi-configurable and can be operated in a Continuous Agitated Tank Reactor (CSTR), Upflow Active Sludge Bed Reactor (UASB) and Packaged Bed Reactor (PBR) [30]. In the case of both experiments, the BE4 reactor was used in a CSTR configuration with constant stirring (Fig. 2).

Table 3. Chlorophyll concentrations of microalgae classes for synthetic and organic wastewater

Type of wastewater	Microalgae class	Chlorophyll concentration, [$\mu g \cdot L^{-1}$]
Synthetic wastewater	Green	54.02
	Blueg	-
	Diato	-
	Crypt	5.65
		59.67
Organic wastewater	Green	115.18
	Blueg	-
	Diato	0.02
	Crypt	-
		115.2

2.5. Measurements and statistics

During the experiments, samples were collected once a week and evaluated by different analytical methods. The pH and temperature were analysed by built in probes of the BE4 anaerobic reactor, the data were collected hourly by the data acquisition system. The nutrient concentrations (ammonium, nitrate, phosphate and potassium) were analysed by photometric methods used PF-12 Plus photometer (Visocolor ECO reagents). The chemical oxygen demand (COD) was also measured photometrically, using the Nanocolor CSB 1500 method. A block digestor was used for sample preparation (148°C and 2 hours). The chlorophyll concentration of microalgae solution and the activity rate were measured by the Algae Toximeter II.

In order to analyse the temporal dynamics of the measured parameters, the curve estimation procedure was used based on the data of the whole fermentation process. The Grapher 17 software was used for the estimation of regression models.

3. RESULTS AND DISCUSSION

3.1. Experiment using synthetic wastewater

3.1.1. Evaluation of the process parameters. During the hydrolysis and fermentation acidification stages both the process and the laboratory temperature varied between 31°C and 35°C (Fig. 3), and then decreased after 180 hours within the acetogenesis stage. The shape of the temperature curves

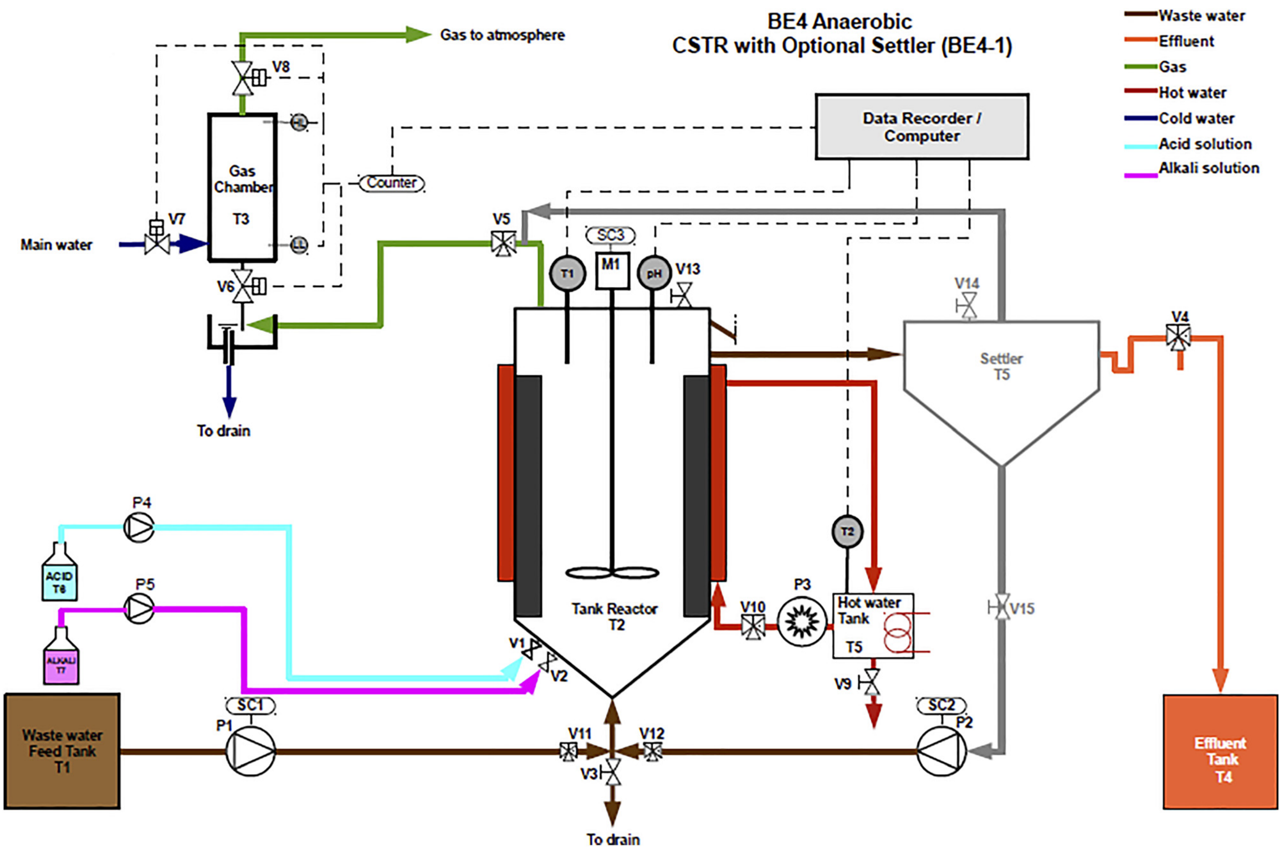

Fig. 2. The schematic of the BE4 anaerobic tank reactor (CSTR configuration) [30]

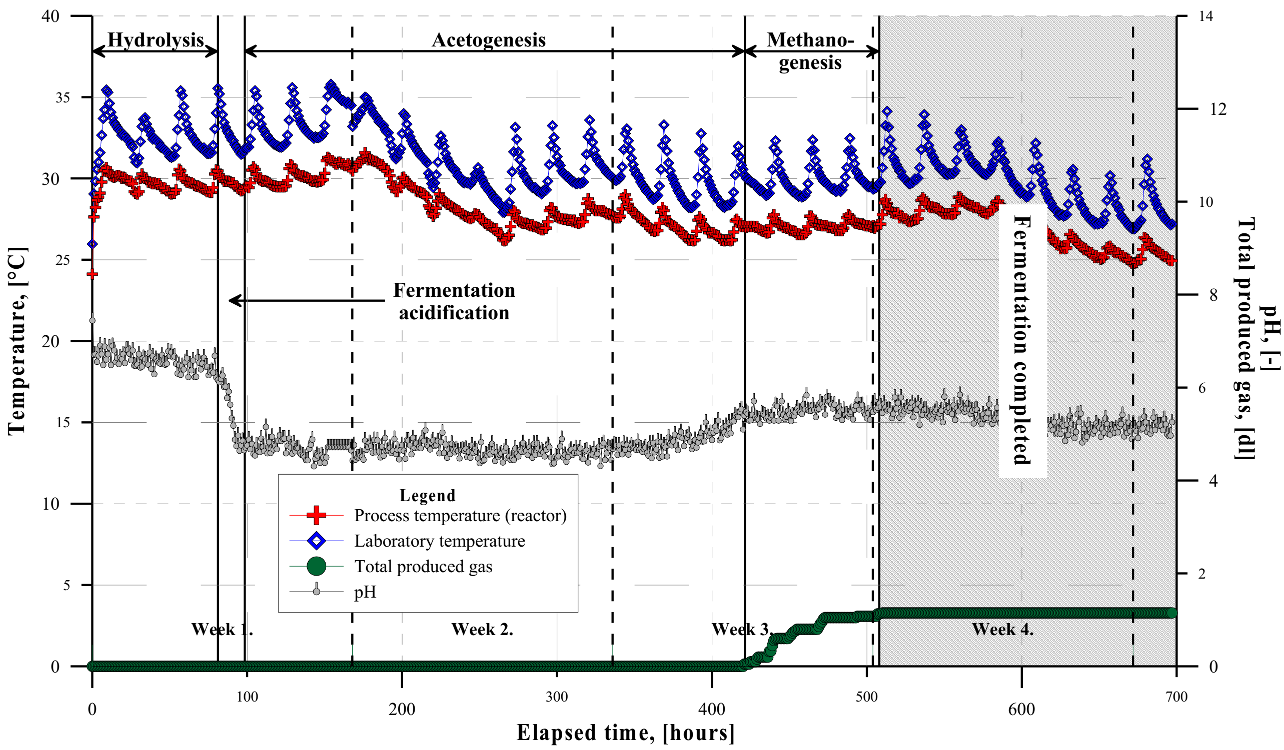

Fig. 3. Monitoring of the process and laboratory temperature, pH and total gas production during the experiment (synthetic wastewater)

was determined by the daily temperature change in the environment.

The pH dropped significantly from 7 to 4.5 in the fermentation acidification stage (84–98 hours). Decreasing the pH of the medium indicates that the anaerobic digestion process passed the hydrolysis stage (0–84 hours) and reached the acidogenesis stage (98–417 hours). In the acidogenesis stage the pH of the medium was constantly acidic (4.7 ± 0.19), below the optimal range [31], probably due to the accumulation of volatile organic acids that were produced in the previous stage. However, after 417 hours of the process, a slight increase was observed in pH and a measurable amount of biogas appeared in the gas collection tank, indicating the start of the methanogenesis stage. The biogas yield was 1.15 dl and the gas production stopped at the 506th hour, indicating the end of the fermentation process.

3.1.2. Evaluation of chemical parameters. The anaerobic degradation processes can be described by logarithmical curves of all studied nutrients (Fig. 4). Equations are shown in Table 4.

Studied nutrient concentrations were continuously decreased in the tank reactor during the experiment. The obtained results pointed out that the dominant N form was the nitrate at the beginning of the experiment. The amounts of both nitrogen forms were continuously decreased and had approached the zero value by the end of the experiment. This can be explained by the intensive nutrient consumption by the microalgae. Moreover, it can be stated that the amounts of N forms were limited in the system. This observation is in connection to the changing of other parameters (see Fig. 5). Similarly to N forms, the phosphate and potassium concentrations were also decreased during the experiment. Their consumption tendencies were similar to N forms. It was pointed out that the consumption rate and tendencies of different nutrients were similar and typical for the biodegradation process. 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, therefore it can be described by power function.

3.1.3. Evaluation of biological parameters. The total chlorophyll concentration had increased from 59.67 to

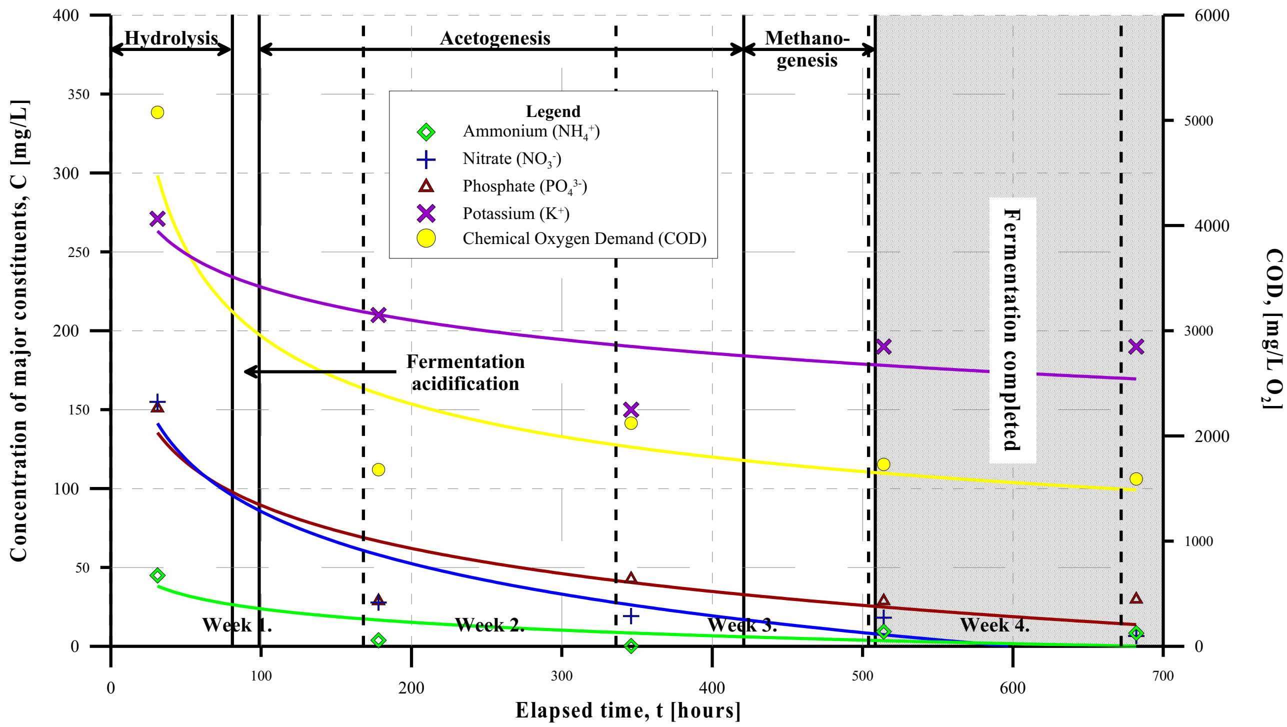

Fig. 4. The concentration of major constituents in the synthetic wastewater during the experiment

Table 4. The parameters of the fitted curves on the major constituents (synthetic wastewater)

Major constituent/Chemical parameter	Type of fitting	Equation of the fitted curve	Coef. of determination (R^2)
Ammonium	Logarithmical	Y = −12.33*ln(X)+80.52	0.71
Nitrate	Logarithmical	Y = −47.70*ln(X)+305.15	0.91
Phosphate	Logarithmical	Y = −39.36*ln(X)+270.58	0.83
Potassium	Logarithmical	Y = −30.32*ln(X)+367.34	0.71
COD	Power	Y = pow(X, −0.36)*15227.84	0.83

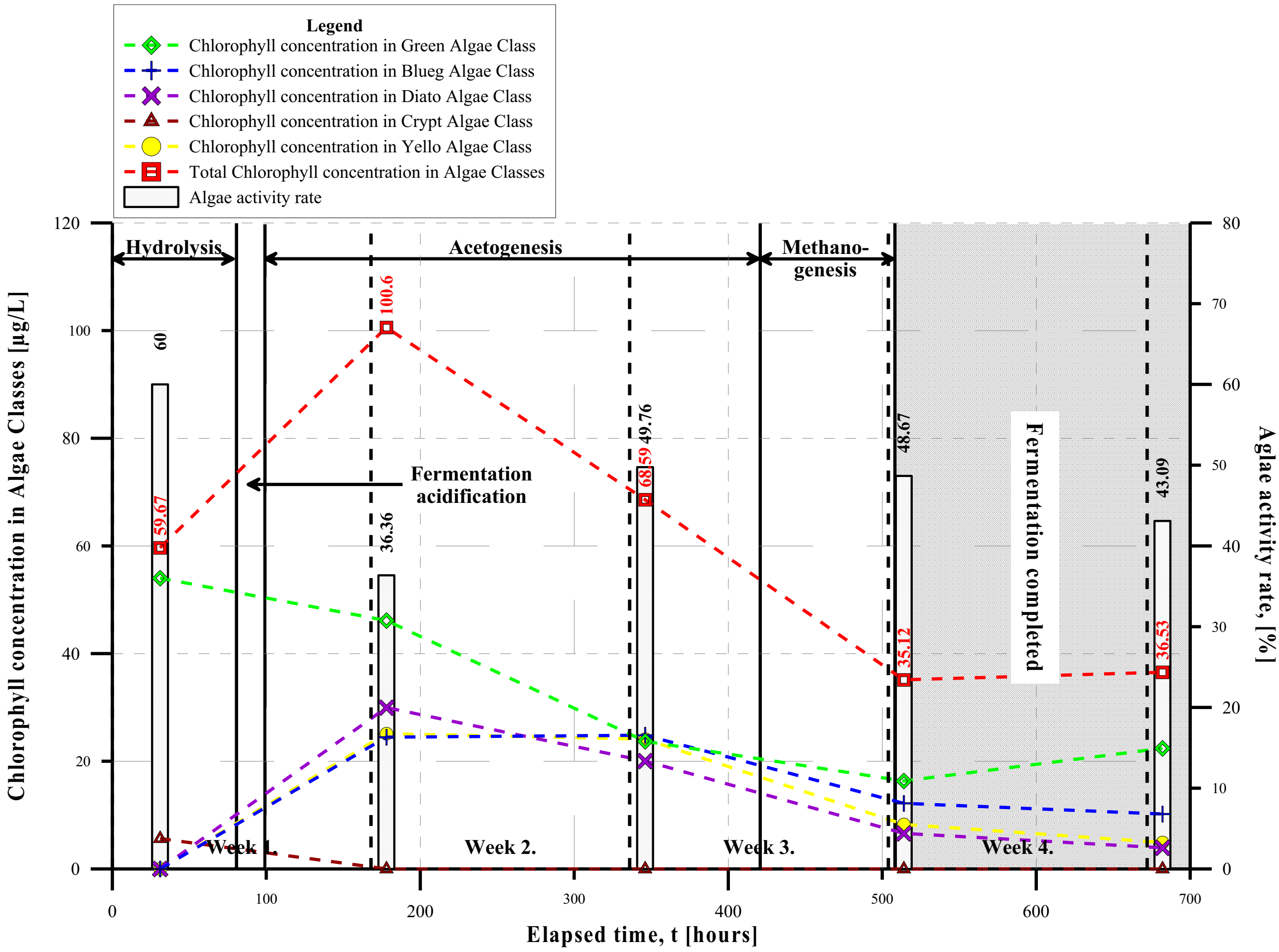

Fig. 5. Chlorophyll concentrations and microalgae activity rate during the experiment (synthetic wastewater)

100.6 μg·L^{-1} in the first 178 hours (Fig. 5), showing a greater availability of nutrients present in the medium. However, the algae activity rate decreased from 60 to 36.36% due to a drop in pH. A colour change of the wastewater was observed due to the increased chlorophyll concentration in this period (Fig. 6). The total chlorophyll concentration decreased from 100.6 to 35.12 μg·L^{-1} in the next 336 hours due to the drop in available nutrients potentially caused by the consumption of an increased amount of algae. In addition, the chlorophyll concentration of algae classes showed similar behaviour. The microalgae activity rate slightly increased (36.36–49.76%) and then remained at a constant level in this period.

3.2. Experiment using organic wastewater

3.2.1. Evaluation of the process parameters. In this experiment, the reactor temperature was set to 45°C in order to investigate the thermophilic fermentation. It was found that the lengths of the stages throughout the process,

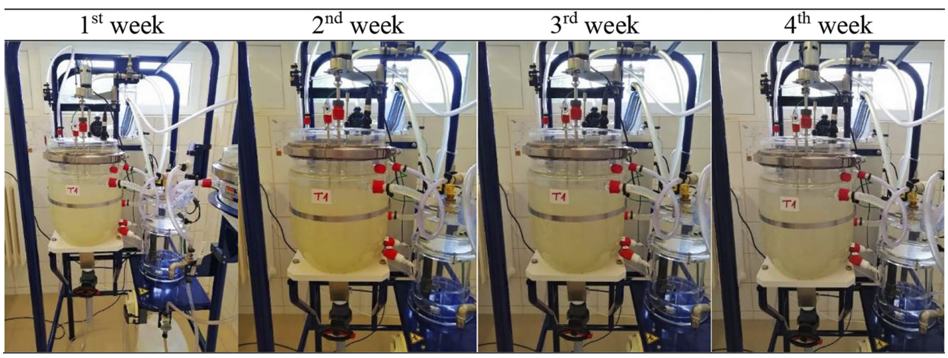

Fig. 6. Colour change indicated by chlorophyll concentration during the anaerobic digestion process using synthetic wastewater

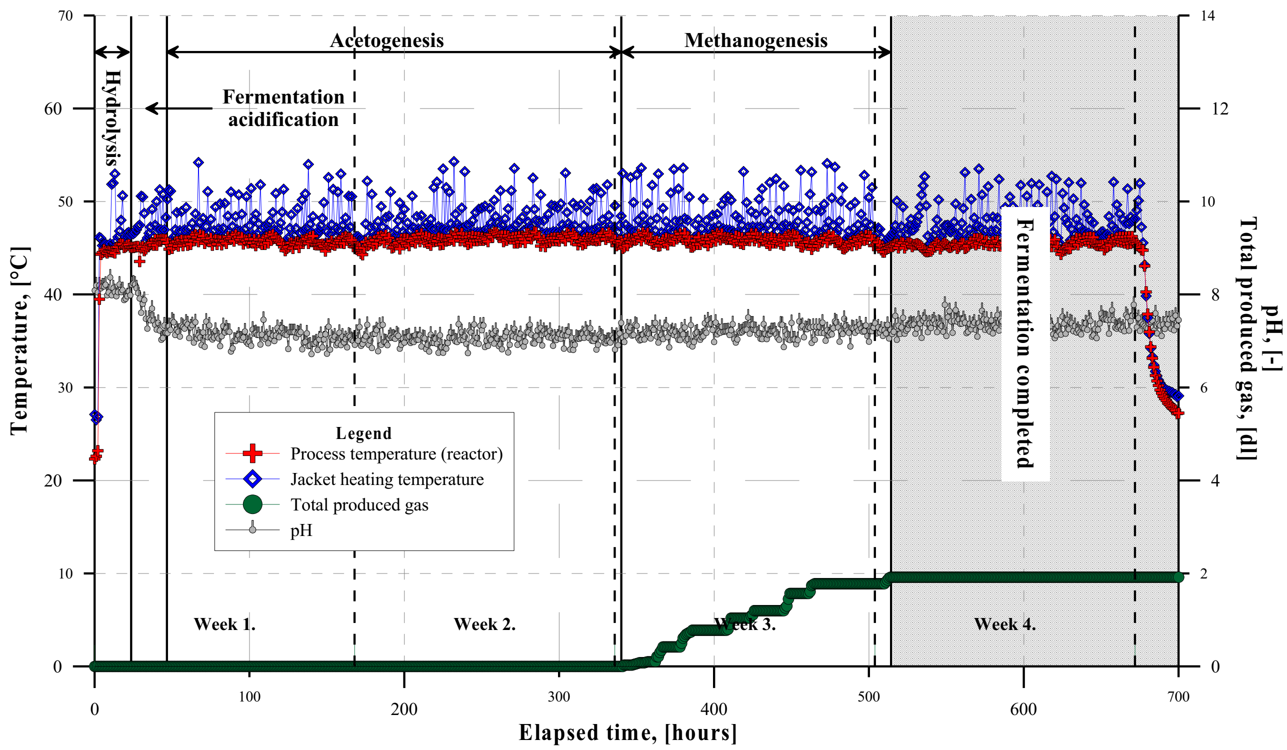

Fig. 7. Monitoring of the process and jacket heating temperature, pH and total gas production during the experiment (organic wastewater)

especially the hydrolysis stage (0–24 hours), decreased compared to the first experiment (Fig. 7). The initial pH was 8.21, which decreased to 7.18 after 46 hours by the end of the fermentation acidification stage, and then remained constant during the entire process. The start of biogas production indicated the beginning of the methanogenesis stage (341–511 hours). A higher biogas yield (59.3%) was achieved during the thermophilic fermentation. The main reason

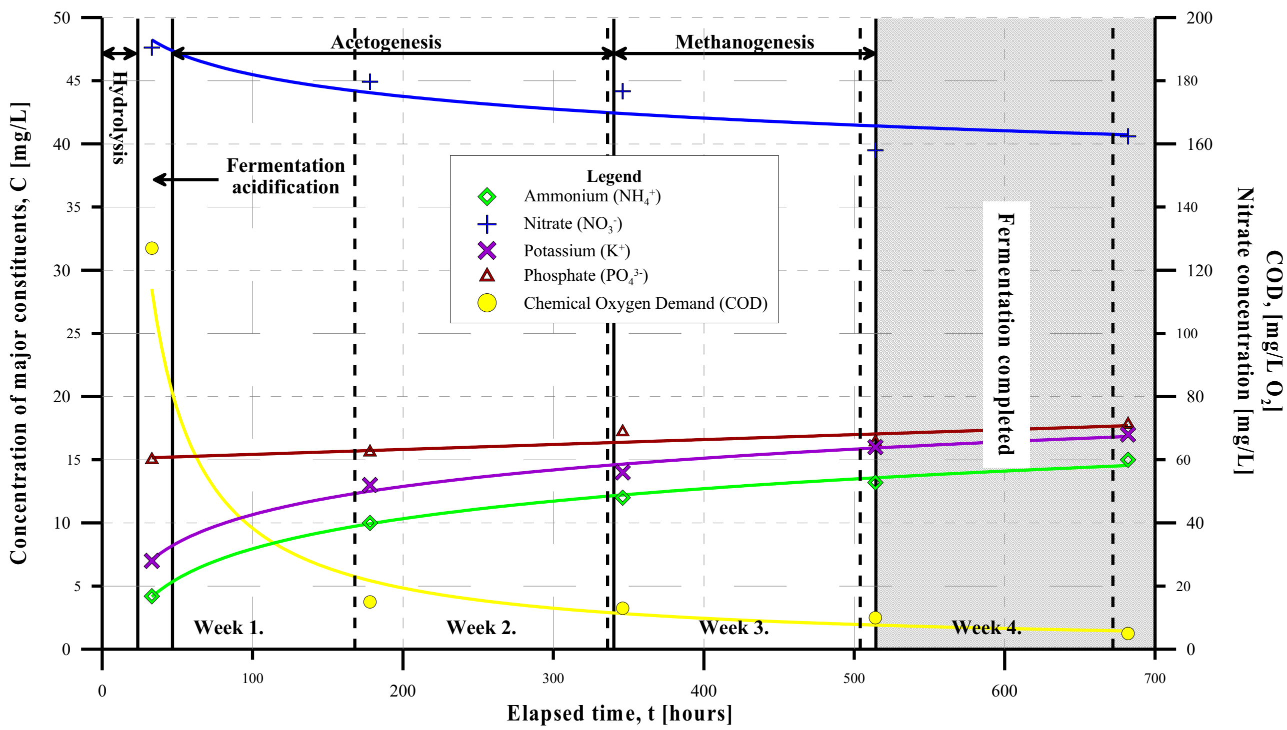

Fig. 8. The concentration of major constituents in the organic wastewater during the experiment

for higher biogas production was the more favourable temperature and the larger volume of microalgae added into the reactor. The duration of the gas production was 170 hours.

3.2.2. Evaluation of the chemical parameters. In this experiment, some of the examined major constituents showed different characteristics compared to the one in which synthetic wastewater was used (Fig. 8).

The amount of all examined nutrients was lower than in the previous experiment. The concentrations differed by one order of magnitude. The nitrate content was lower than the previous experiment and slightly decreased during the whole fermentation process. Ammonium, phosphate and potassium concentrations were increased during the experiment. Ammonium and potassium curves showed similar logarithmical tendency. Despite the different tendencies of the major constituents, they can be characterized well by logarithmical curves, except the phosphate where the tendency showed a linear characteristic (Table 5). Obtained results can be explained by the lack of available nutrients causing a sudden decrease of the microalgae population as well as their activity rate. Between the first and second sampling of the experiment, the total chlorophyll concentration was decreased by 83% (see Fig. 9), therefore it can be established that a decent proportion of the microalgae was involved in the degradation process as a substrate, increasing the ammonium, phosphate and potassium concentrations in the reactor. The COD decreased drastically from 127 $mg \cdot L^{-1}$ O_2 to 15 $mg \cdot L^{-1}$ O_2 within 145 hours,

Table 5. The parameters of the fitted curves on the major constituents (organic wastewater)

Major constituent/Chemical parameter	Type of fitting	Equation of the fitted curve	Coef. of determination (R^2)
Ammonium	Logarithmical	Y=3.43*ln(X)−7.85	0.99
Nitrate	Logarithmical	Y=−9.92*ln(X)+227.60	0.82
Potassium	Logarithmical	Y=3.22*ln(X)−4.18	0.99
COD	Power	Y=pow(X,−0.98)*3561.44	0.96
Phosphate	Linear	Y=0.03*X+15.03	0.78

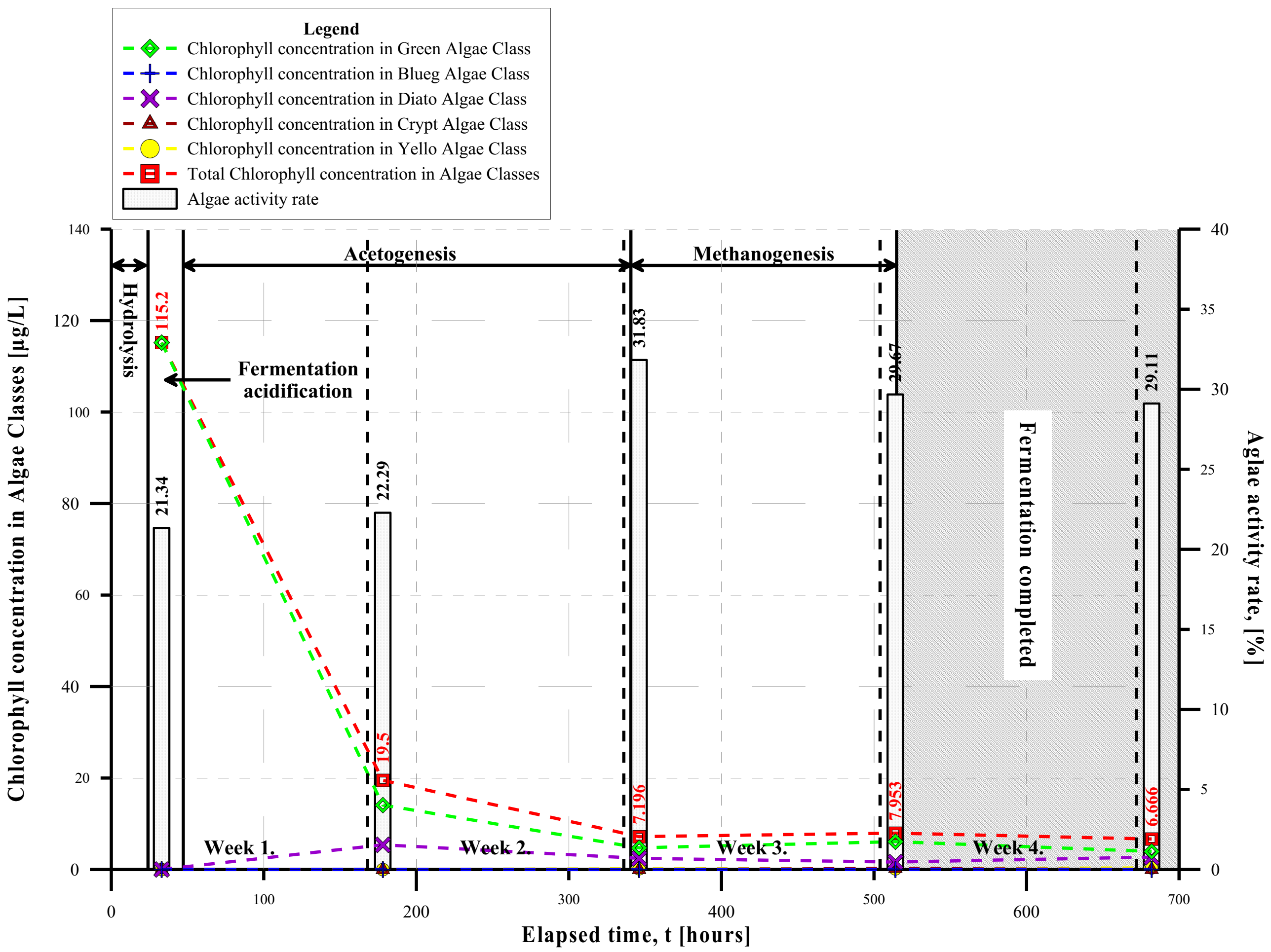

Fig. 9. Chlorophyll concentrations and microalgae activity rate during the experiment (organic wastewater)

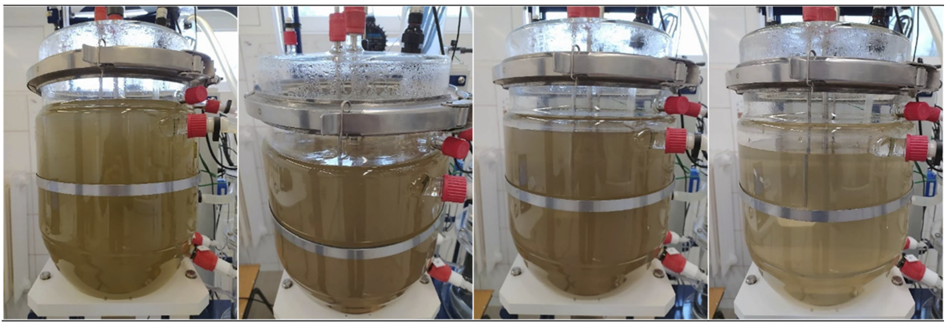

Fig. 10. Colour change indicated by chlorophyll concentration during the anaerobic digestion process using organic wastewater

and remained nearly constant during the rest of the experiment. These results refer to an intensive anaerobic degradation process, therefore it can also be described by power function.

3.2.3. Evaluation of biological parameters. The highest chlorophyll concentration was observed in the acidic fermentation stage that significantly decreased from 115 to 19.5 $\mu g \cdot L^{-1}$, while the activity rate was stable in this period (Fig. 9). The low concentration of nutrients and the high concentration of microalgae added to the reactor resulted in drastically decreased chlorophyll concentrations. After 346 hours, each chlorophyll concentration reached a minimum value and remained constant. However, after the rapid reduction in chlorophyll content, at the beginning of the methanogenesis stage the activity rate of microalgae increased indicating the tolerance of the remaining microalgae community. As a positive effect of the necrosis, microalgae biomass served as a substrate for degradation, contributing to the increase of the total biogas volume produced at the end of the process.

With the decrease in chlorophyll concentration during the process, the suspension in the tank will become lighter in colour over the duration of the experiment (Fig. 10).

4. CONCLUSION

Based on the experiments performed by using synthetic and organic wastewater, the following conclusions can be drawn:

- The anaerobic degradation stages can be separated based on the pH and the gas development.
- Increasing the temperature to the thermophilic range (45°C) accelerated the degradation processes and resulted in shorter hydrolysis (60 hours), acetogenesis (24 hours) and longer methanogenesis (81 hours) stage. Moreover, higher biogas yield (59.3%) was observed at the organic wastewater experiment.
- Despite the different circumstances, the duration of the entire fermentation process was similar in both cases.
- The changing of amounts of nutrients was characterized by logarithmical curves, except the phosphate, where linear characterization was observed at the organic wastewater experiment. However, the changing of concentrations of nutrients showed opposite tendency.
- The decreasing tendency of the COD that followed from the intensive fermentation was characterized statistically by power function.
- pH had a significant effect on the microalgae cultivation (chlorophyll concentration and microalgae activity rate). The *C. vulgaris* were applicable to track the biodegradation processes as a bioindicator.
- A strong correlation was found between the concentration of nutrients and the microalgae activity rate, if appropriate amounts of nutrients were available for their growth. Otherwise, the microalgae was involved in the fermentation process as a substrate slightly increasing the concentration of some nutrients in the wastewater.
- More experiments are required to get a more comprehensive view on the optimal circumstances of the anaerobic degradation process and the microalgae cultivation.
- The recycling possibilities of organic wastes and by-products especially from the agricultural sector can be a potential interest in the near future.

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