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Extended Abstract

Purification of Post-Fermentation Effluent Using *Chlorella vulgaris* Microalgae †

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Abstract: Waste-water rich in organic carbon, nitrogen and phosphorus may serve as a convenient source of carbon and nutrients for a year-long microalgae production. Scientific reports indicate that some single-cell microalgae such as *Chlorella* and *Scenedesmus*, are highly tolerant to waste-water environments and efficiently remove biogenic compounds. The aim of this study was to determine the possibility of using the effluent produced in the process of anaerobic degradation of whey, as a culture medium for the multiplication of *Chlorella vulgaris* algae biomass and to characterise their growth efficiency and rate. The content of nitrogen and phosphorus in waste-water was sufficient for conducting an effective culture of algae. The efficiency of nitrogen removal in the flow system was 15.61 ± 1.38 mg N/dm^3/day.

Keywords: *Chlorella vulgaris*; post-fermentation effluent; microalgae

1. Introduction

The anaerobic microbiological process, where complex organic substances are transformed into methane and carbon dioxide, is a widely applied technology for the stabilisation of waste with a concurrent generation of energy in the form of biogas [1]. Therefore, anaerobic reactors are systems which do not ensure a comprehensive removal of contamination. Waste-water treated in this way does not meet the criteria for being discharged directly to a recipient. Therefore, it requires additional technological treatment which generates further exploitation costs. For this reason, it is necessary to search for solution to improve the efficiency of anaerobic technologies and make them more universal. One such solution could be phytoremediation, which uses plants to neutralise contaminations [2]. Waste-water rich in organic carbon, nitrogen and phosphorus may serve as a convenient source of carbon and nutrients for a year-long microalgae production [3].

Different varieties of algae are able to develop in a wide variety of environments, even in degraded or contaminated areas [4]. Such cultures bring a positive effect to the natural environment because algae may be produced using communal, agricultural or industrial waste-water containing carbon dioxide which is required for their growth CO_2 [5]. Substances obtained from algae may constitute a source of nutritional value and a diet component for both humans and animals [6]. Microalgae have already been used as diet supplements, an addition to cosmetics, for waste-water treatment and as a potential biomass source in the production of biofuels [7]. The benefits of using waste-water as a culture medium for microalgae production include a reduction in water use and costs of nutritional components added to cultures and the removal of nitrogen and phosphorus from waste-water [8].

The aim of this study was to determine the possibility of using the effluent produced in the process of anaerobic degradation of whey, as a culture medium for the multiplication of *Chlorella vulgaris* algae biomass and to characterise their growth efficiency and rate.

2. Materials and Methods

2.1. Researche Station

The study used *Chlorella vulgaris* microalgae originating from a culture of the Collection of Baltic Algae of the Institute of Oceanography at the University of Gdańsk. The culture was conducted in 1.0 dm^3 (active volume) glass photobioreactors. Photobioreactors was equipped on aeration system, magnetic stirrer, fluorescent lighting. The culture temperature was maintained at 25 ± 2 °C. Effluent dosing and the receipt of inoculum were carried out using peristaltic pumps.

2.2. Post-Fermentation Effluent

The experiment tested the effluent obtained from a UASB-type model anaerobic bioreactor, supplied with waste-water prepared based on acidic whey. The anaerobic bioreactor of a labyrinth flow and active volume of 70 dm^3 worked under mesophilic conditions, at the load maintained at A = 2.6 $kgBOD_5/m^3$, hydraulic stop time of 15 days and the process temperature of 35 ± 2 °C.

2.3. Experiment

The experimental research was divided into two phases: an adaptation phase and a flow culture.

2.3.1. Adaptation Phase

The adaptation phase was carried out in a photobioreactor filled with a culture medium and an addition of inoculum (50 mg/dm^3). This adaptation culture was conducted in a stationary mode. Undiluted post-fermentation effluent was used as the culture medium. The aim of this phase was the adaptation of microalgae to the applied culture medium and biomass multiplication. This phase lasted until the maximum value of the biomass growth index was obtained. The culture was then continued in a flow culture mode (second phase). During the adaptation, the maximum daily total nitrogen removal was determined, which was the basis for determination of the daily dose of post-fermentation effluent supplied to the system. The maximum daily consumption of nitrogen compounds was from 16.55 ± 0.15 mg N/dm^3/day.

2.3.2. Flow Culture

The second experimental phase involved the operation of the culture with dosing portions of post-fermentation effluent and the removal of the culture medium.

The amount of nitrogen compounds introduced to the system with the culture medium was adjusted to be approximate to the daily nitrogen consumption in the adaptation culture (16.55 ± 0.15 mg N/dm^3/day). Considering the content of the total nitrogen in the post-fermentation effluent, which was 168.2 ± 5.5 mg N/dm^3, the daily dose was set at 96 cm^3/day (2 cm^3/0.5 h), which corresponded to the nitrogen amount at the level of 16.15 ± 0.53 mg N/dm^3/day.

2.4. Measurement of Biomass Concentration

Measurements of dry matter content in the adaptation phase were done every 24 h and every 48 h during the flow culture phase. The measurement was carried out using the gravimetric method.

2.5. Measurement of Nutrient Concentrations

To determine the biogenic compound removal efficiency in the flow culture of algae, a daily analysis was made of the total nitrogen and phosphorus at the discharge from the photobioreactors. The filtered samples were subject to analyses using LCK cuvette tests (Hach Lange, Loveland, CO, USA).

3. Results and Discussion

Cell Growth and Nutrient Concentration

The initial concentration of biomass in the adaptation phase was 50 ± 12 mg TS/dm^3. The maximum biomass productivity was obtained on day 12 of the culture and it was 413.67 ± 4.51 mg TS/dm^3/day. On day 13, the productivity remained at a similar level (409.33 ± 3.51 mg TS/dm^3/day), while on day 14, it dropped to 372 ± 4.51 mg TS/dm^3/day. At that time, the experiment passed to the second phase (flow culture). The maximum biomass concentration which was obtained in the adaptation phase was 2.915 ± 17 mg TS/dm^3. In the flow phase, the biomass concentration at the discharge from a photobioreactor remained at a similar level as in the adaptation phase, which confirms that the retention time of the effluent in the photobioreactor was well adjusted. The biomass content in the flow system ranged from 2.863–3.065 mg TS/dm^3 at an average concentration of 2.953 ± 49 mg TS/dm^3 (Figure 1).

Zhou et al. [9] studied the possibility of culturing *Chlorella zofingiensis* using communal waste-water and post-methane fermentation effluent (swine slurry), mixed in varied proportions, as a culture medium. For the culture medium consisting of only communal waste-water, the biomass productivity was 280 mg/dm^3/day. On the other hand, Sepúlveda et al. [10] used effluent from anaerobic treatment of municipal waste at different dilution degrees (0–80%). The maximum biomass growth in this study reached 400 mg/dm^3/day.

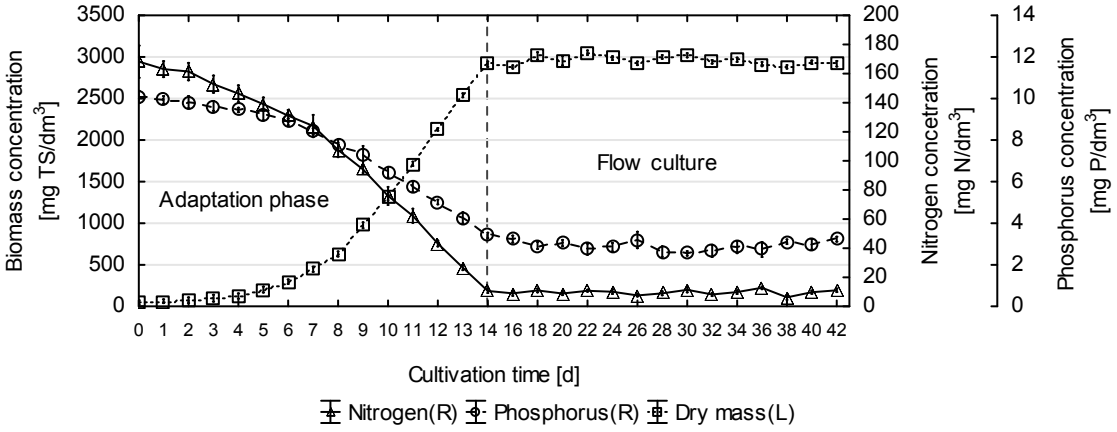

Figure 1. Biomass concentration, total nitrogen and total phosphorus concentration in the adaptation phase and flow phase.

The initial content of the total nitrogen in the culture medium in the adaptation phase was 168.2 ± 5.5 mg N/dm^3. The highest daily consumption of nitrogen compounds in the adaptation phase was 16.55 ± 0.15 mg N/dm^3/day. The dose of the supplied effluent was determined based on the daily consumption of nitrogen compounds, at the maximum biomass productivity, and was 96 cm^3/day (2 cm^3/0.5 h), which corresponded to the load of 16.15 ± 0.53 mg N/dm^3/day. The total nitrogen concentration at the transition to the flow culture phase of the experiment was 10.68 ± 0.75 mg N/dm^3. The total nitrogen concentration at the discharge from the photobioreactor during the flow phase ranged from 5.32–13.14 mg N/dm^3 at an average concentration of 9.85 ± 1.49 mg N/dm^3. The average efficiency of nitrogen compound removal in the flow culture was 15.66 ±1.15 mg N/dm^3/day (Figure 1).

The initial content of the total phosphorus in the culture medium in the adaptation phase was 10.08 ± 0.15 mg P/dm^3. The total phosphorus concentration in the transition to the flow culture was 3.42 ± 0.08 mg P/dm^3. The average efficiency of phosphorus compound removal in the flow phase was 0.84 ± 0.12 mg P/dm^3/day (Figure 1).

Zhou et al. obtained the highest degree of total nitrogen removal using only communal waste-water as the culture medium (21 mg N/dm^3/day). The removal degree of phosphorus was 4.6 mg P/dm^3/day [9]. Sepúlveda et al. in their experiment involving post-methane fermentation effluent from communal waste-water, obtained the maximum degree of nitrogen removal of 35 mg N/dm^3/day. The efficiency of phosphorus removal was 5.7 mg P/dm^3/day [10].

4. Conclusions

High efficiency in biogenic compound removal had a positive effect on the final biomass content of the tested microalgae. The application of the tested waste-water considerably reduced the necessity of using chemical reagents. The content of nitrogen and phosphorus in waste-water was sufficient for conducting an effective culture of algae. The efficiency of nitrogen removal in the flow system was 15.61 ± 1.38 mg N/dm^3/day.

Author Contributions: K.S., M.Z. conceived and designed the experiments; K.S. performed the experiments; K.S., D.S. analyzed the data; K.S. contributed reagents/materials/analysis tools; K.S., D.S. and M.Z. wrote the paper.

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