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Semi-continuous Cultivation of *Chlorella vulgaris* for Treating Undigested and Digested Dairy Manures

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Abstract The present study, based on a previous batch-wise experiment, investigated a lab-scale semi-continuous cultivation of green microalgae *Chlorella vulgaris* (UTEX 2714), as a useful means for nutrient reduction as well as production of algal biomass which can be used as potential feedstock for the production of biofuel and other commodities, on 20× diluted dairy manures. Both undigested and digested samples were applied in parallel experiments for comparison regarding the requirements of hydraulic retention times (HRTs), removal efficiencies of nitrogen, phosphorus, and chemical oxygen demand (COD), biomass productivities, and CO_2 sequestration abilities. It was demonstrated that algae grown in undigested dairy manure achieved removal rates of 99.7%, 89.5%, 92.0%, and 75.5% for NH_4^+–N, TN, TP, and COD, respectively, under a 5-day HRT, while the HRT had to extend to 20 days in order to achieve 100.0% removal of NH_4^+–N in digested one with simultaneous removals of 93.6% of TN, 89.2% of TP, and 55.4% of COD. The higher organic carbon contained in undigested dairy manure helped boost the growth of mixotrophic *Chlorella*, thus resulting in a much shorter HRT needed for complete removal of NH_4^+–N. Moreover, algae grown in digested dairy manure provided more penitential than those grown in undigested one in CO_2 sequestration per milligram of harvested dried biomass (1.68 mg CO_2/mg dry weight (DW) vs 0.99 mg CO_2/mg DW), but did not surpass in total the amount of CO_2 sequestered on a 15-day period basis because of the better productivity gained in undigested dairy manure.

Keywords Dairy manure · Nutrients removal · CO_2 sequestration · *Chlorella*

Introduction

Energy-saving and environment-friendly methods for treating dairy manures are in urgent demand because of the deterioration to freshwaters by the land application of high nutrients containing manures. In previous batch-wise experiments [1, 2], it was demonstrated that

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algae strain *Chlorella* sp. could assimilate organic carbon, nitrogen, and phosphorus directly, not necessarily through the synergy with bacteria, which was proposed by a lot of researchers before [3, 4]. Design of a scalable algae bioreactor for treating manure wastewater requires the prerequisite investigation of several important parameters and conditions. Hydraulic retention time (HRT), the period that sewage was retained in the system, determined by the daily volume of sewage applied [5], is one of the most important parameters in the operation of wastewater treatment. Therefore, as an extension of the batch-wise investigation done previously [1], the objective of this study was to compare algal growth and how well they removed nitrogen, phosphorus, chemical oxygen demand (COD) in both undigested and digested dairy manures by manipulating the requirement of HRTs, under semi-continuous operations, aiming at complete NH_4^+ removals.

Materials and Methods

Algae Strain and Culture Condition

Algae strain *Chlorella vulgaris* (UTEX 2714, Austin, TX) was used in this experiment. It was preserved in Tris–acetate–phosphorus media [6] before inoculation to diluted dairy manures. Two 4-L flasks (Kimax, Capitol Scientific Inc., USA) with 1-L working volume were used as semi-continuous reactors. The culture flasks were incubated under stationary condition at 25±2°C and 120 μmol $m^{-2}s^{-1}$ continuous cool-white fluorescent light illumination on a shaker with 150-rpm rotation speed. CO_2-enriched air (2%) was supplied to stabilize the culture pH.

Dairy Manure Sources

Undigested and digested dairy manures were collected from Haubenschild Farm, Princeton, Minnesota, where a plug-flow anaerobic digester has been in operation since 1999. According to a previous study [1], 20× dilution was applied to both undigested and digested dairy manures in order to mitigate the potential inhibition on algal growth caused by the high turbidity and ammonium from the raw manures.

System Operation

HRT was controlled by the daily withdrawal and feeding volume as indicated by its definition. Evaporation was made up of deionized water accordingly. A 5-day HRT (one fifth of the reactor contents was replaced every day) was initially applied to both algae bioreactors, one of which was for undigested dairy manure and the other for the digested one. Until steady state was reached or buildup of nutrients was perceived, HRTs were altered to trail test if improved nutrient reduction efficiencies could be achieved or not. Results from different days in a steady-running period were treated as replicates.

Physicochemical Analysis

Algal growth was monitored daily and reported on a dry weight (DW, g/L) basis. DW measurements were made by filtering 5-mL samples through pre-weighed glass microfiber filters (Grade GF/C, Whatman, USA). The filters were then dried at 105°C overnight and

weighed on a balance (ED124S, Sartorius, Germany). Liquid samples for nutrient consumption analysis were collected for the influent and effluent every day. Samples were centrifuged at 5,000 rpm for 15 min and supernatants were collected for the analyses of ammonium (NH_4^+–N), total nitrogen (TN), total phosphorus (TP), and COD. Measurements of NH_4^+, TN, TP, and COD were performed following the Hach DR 5000 Spectrophotometer Manual [7]. Total solids (TS) and total volatile solids (TVS) were performed for raw manure samples following the standard methods [8]. Removal rates were calculated by dividing the difference between the influent and effluent concentrations by influent concentration and then multiplying by 100. The carbon, hydrogen, and nitrogen contents of dried algae samples were measured using an elementary analyzer (CE-440, Exeter Analytical, Inc. USA).

Results and Discussion

Characteristics of Undigested and Digested Dairy Manures

Table 1 shows the characteristics of undigested and digested dairy manures used in this study.

Semi-continuous Operation for Undigested Dairy Manure

Figure 1 presents the daily profiles of inflow and outflow concentrations for NH_4^+–N, TN, TP, and COD using 20× undigested diary manure as the feedstock. November 9 to Nov. 13 was the system start-up period, and algal biomass continued to build up from 520 mg/L to around 1,000 mg/L. The NH_4^+–N removal efficiency also increased from 96.2% to 99.7%. TN, TP, and COD removal efficiencies stayed in the ranges of 84.9–89.5%, 90.4–92.9%, and 70.7–83.9% in this period, respectively. After the steady run of another 2 days (Nov. 14 and Nov.15), HRT was reduced to 3.3 days in order to seek the system's potential to handle more pollutants on a daily basis. However, the elevation of NH_4^+–N was observed immediately following the day when HRT was adjusted and the buildup continued the day after, indicating that the nitrogen loading at 3.3-day HRT exceeded the system's capacity. Based on this, HRT was changed back to 5 days and maintained the same during the rest of the test period. The biomass concentration during the operation is between 1,000 and 1,380 mg/L.

Table 1 Characteristics of undigested and digested dairy manures.

	Undigested manure	Digested manure
NH_4^+–N (mg N/L)	1,008	1,554
TN (mg N/L)	1,074	1,722
TP (mg P/L)	180	111.6
COD (mg/L)	17,820	10,320
COD/TN/TP	99:06:01	92:15:01
pH (10× dilution)	8.05	8.67
TS (%)	9.00	6.80
TVS (%)	7.40	5.30

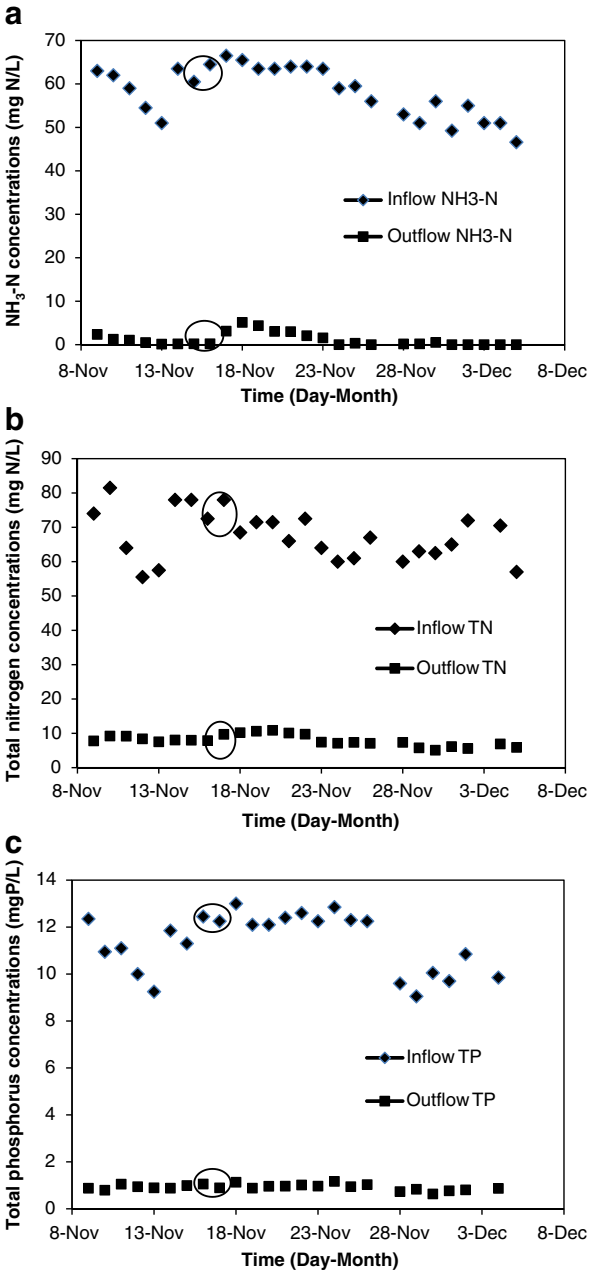

Fig. 1 Monitoring of inflow and outflow concentrations for (**a**) NH_4^+–N, (**b**) TN, (**c**) TP and (**d**) COD using undigested diary manure as the feedstock. HRT was kept at 5 days except the *circled* 2 days (HRT = 3.3 days)

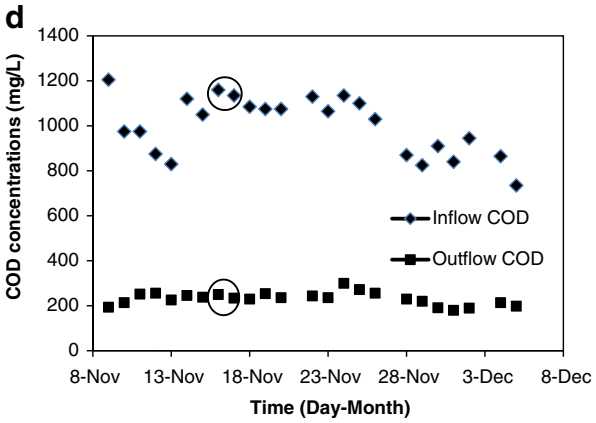

Fig. 1 (continued)

Semi-continuous Operation for Digested Dairy Manure

Figure 2 shows the daily profiles of inflow and outflow concentrations for NH_4^+–N, TN, TP, and COD using 20× digested diary manure as the feedstock. During the first period (Nov. 9–Nov. 13), the bioreactor feeding 20× diluted digested diary manure was operated under 5-day HRT; continued buildup of NH_4^+–N, TN, and COD was observed in the system, although the algal biomass climbed from 400 to 760 mg/L. In the second period (Nov. 14–Nov. 19), after HRT was increased to 10 days, the system seemed to enter into a stable running state, meaning that the removal efficiencies for all tested items remained at certain levels with little standard errors (shown in Table 2). Due to the insufficient removal of NH_4^+–N and TN, HRT was further elongated to 20 days in the third period (Nov. 20–Dec. 16), during which time ever-growing removals were obtained for NH_4^+–N, TN, and COD. The last 3 days, a steady state was finally achieved with complete NH_4^+–N removal and significant reduction of phosphorus and COD as well (shown in Table 2).

Comparison of Algal Treatment of Both Types of Dairy Manure

The phenomenon observed in this study corresponds closely to the earlier work done by Oswald et al. [5] who presented that the yield of algal cell material increases with increased BOD loading. Undigested dairy manure has a much higher organic carbon concentration than the digested one; thus, it boosted the growth of the mixotrophic *Chlorella*, resulting in a much shorter HRT needed for the complete removal of NH_4^+–N, which is the criterion to determine whether a satisfactory HRT was obtained for this study. The mechanism involved in algal treatment of high-strength manure wastewater is related to the direct nutrients' uptake and subsequent biomass separation [9]. García et al. [9] also pointed out that nutrient removal efficiency is controlled by cellular retention time, solar radiation, and temperature. In this indoor study, the radiation and temperature were kept constant; thus, by investigating cellular retention time, which was equal to HRT, different removal efficiencies were achieved. Compared to an open-pond algal research done by García et al. [10] treating urban wastewater, while the 20× diluted undigested dairy manure had similar levels of TN (around 50 mg N/L) and TP (around 9 mg P/L), the removal efficiencies achieved in this study for both of them (89.5% for TN and 92.0% for TP) were much higher than the former

ones (56.6% for TN and 31.8% for TP) under the same 5-day HRT. Several factors can affect the efficiency and cause a difference in each project. Firstly, for outdoor experiments, the seasonal variations of temperature and solar irradiation have a significant impact on productivity because they affect the rate of algal photosynthesis. Normally, a higher rate of biomass production is observed during the summer season, and thus, a shorter HRT is needed [10]. The second important factor is the loading rate of major nutrients (C, N, P), especially nitrogen. A higher loading rate corresponds to a lower retention time; if the loading rate is controlled within a certain range, the gain in productivity is directly proportional to the increase in loading rate. However, if the loading rate goes beyond a certain level, nutrients build up, which might be lethal to the algae and so result in the system collapse. There can also be a need to supplement CO_2. When the organic carbon which can be assimilated by algae in the manure is not sufficient, i.e., digested dairy manure, extra CO_2 can help boost algal growth and have a positive effect on stabilization of the culture's pH.

Co-sequestration of CO_2

A preliminary test indicated that semi-continuous feeding elevated pH to above 10 which completely inhibited algal activity for treating both undigested and digested dairy manures,

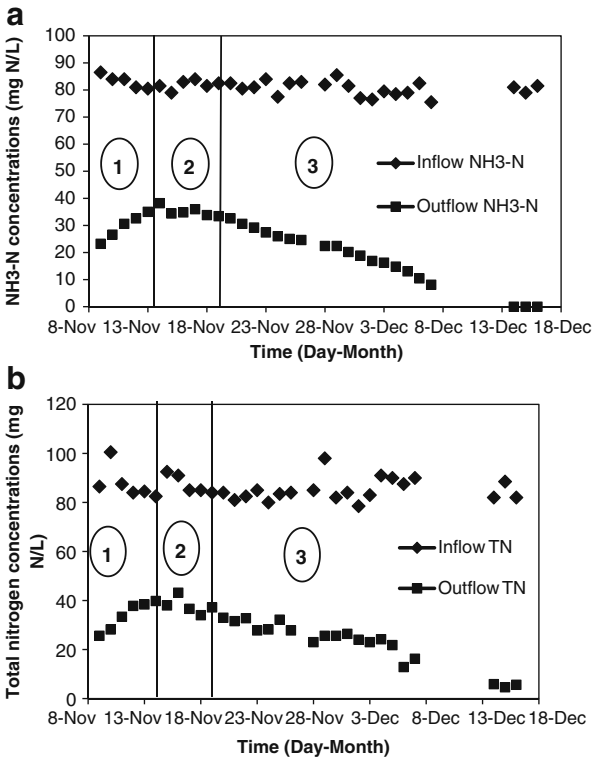

Fig. 2 Monitoring of inflow and outflow concentrations for (**a**) NH_4^+–N, (**b**) TN, (**c**) TP and (**d**) COD using digested dairy manure as the feedstock. HRT was kept at 5 days, 10 days and 20 days, for period ①, ②, ③, respectively

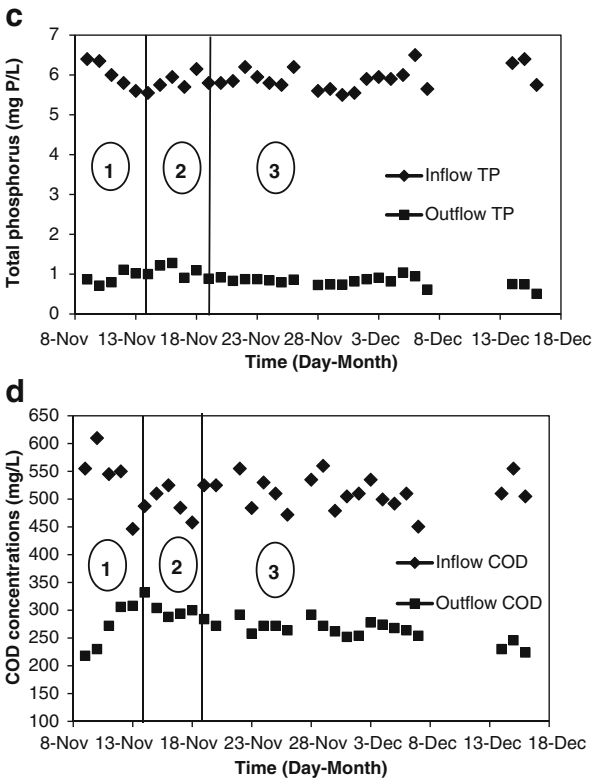

Fig. 2 (continued)

while CO_2 supplementation stabilized pH to a range of 7.6–8.0 for both systems. Based on the C elemental analysis of harvested algal biomass and mass balance calculation, it is encouraging to find that CO_2 was also consumed by algae as a carbon source besides the organic one in dairy manures, thus resulting in the co-sequestration of this greenhouse gas (Table 3). Moreover, algae grown in digested dairy manure provided more penitential than those grown in the undigested one in CO_2 sequestration per milligram of harvested dried biomass (1.68 mg CO_2/mg DW vs 0.99 mg CO_2/mg DW), but did not surpass those grown in undigested dairy manure in the total amount of CO_2 sequestered on a 15-day period basis because of the better productivity gained in the undigested dairy manure.

Table 2 Average removal efficiencies for NH_4^+–N, TN, TP, and COD under three different conditions.

	NH_4^+–N	TN	TP	COD
Undigested dairy manure[a] (HRT = 5 days)	0.997±0.004	0.895±0.013	0.920±0.010	0.755±0.024
Digested dairy manure[b] (HRT = 10 days)	0.583±0.010	0.563±0.031	0.824±0.028	0.412±0.054
Digested dairy manure[c] (HRT = 20 days)	1.000±0.000	0.936±0.010	0.892±0.017	0.554±0.004

[a] Seven samples taken in steady running period

[b] Four samples taken in steady running period

[c] Three samples taken in steady running period

Table 3 Calculation of CO_2 sequestered (unit is in mg).

	Biomass produced[a]	Total C in biomass[b]	Total COD removed	COD–C equivalent	CO_2–C uptake	CO_2 equivalent
Undigested dairy manure	3,768	1,857.6	2,245.4	842	1,015.6	3,723.9
Digested dairy manure	1,352	697.5	204.7	76.8	620.7	2,275.9

[a] Biomass produced is based on a 15-day sampling period. For undigested dairy manure, the period is Nov.20–Dec.5, except Nov.27. For the digested one, the period is Nov.25–Dec.7, except Nov.27 and Dec.14–16

[b] C ratio in algae harvested in undigested dairy manure is 49.3%, while those harvested in the digested one is 51.6%

Conclusions

This study provides valuable information on different HRTs required for undigested and digested manures sampled from the same dairy farm, which could also be a reference for other dairy farms that have similar manure compositions. HRT is important and specific to different types of wastewater and should always be pre-investigated before larger scale application can be implemented. Undigested dairy manure, containing a higher usable organic carbon concentration, could significantly boost algal growth; thus, a shorter HRT can be applied and less capital investment for high-rate algal ponds required. Based on the results from this research, undigested dairy manure might be the preferable feedstock when using algal treatment in dairy farm for the dual purpose of wastewater treatment and biofuel feedstock production compared to the digested one, which requires a much longer HRT to attain complete NH_4^+–N removal and less biomass generated in the same time frame. The harvested algae biomass could be processed to biodiesel or green diesel. Although it may not be economically viable for large-scale application with the price of petroleum at current levels, the additional advantages of waste remediation and carbon sequestration will surely make this an attractive technology in the future.

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