Growth of Algae and Microbiome Cultures on Anaerobic Digester Centrat*e*

M.B Benitez[1], G. Leite[1], P.Champagne[1]

[1]Department of Civil Engineering, Queen's University, Kingston, Ontario, K7L 3N6, Canada
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Presenting author email: pascale.champagne@queensu.ca

Extended Abstract

Microalgae is considered a promising feedstock as a third generation biofuel feedstock due to its high photosynthetic efficiency and high biomass productivity, CO_2 bio-sequestration, high lipid/carbohydrate accumulation capacity, tolerance to fluctuating environmental conditions and low cost per yield (Hu *et al.*, 2015; Pittman *et al.*, 2011; Cheah *et al.*, 2016). They have a reported high oil yield range of 58.7 to 136.9 k L oil ha^{-1} $year^{-1}$ and small land area requirement of 0.1-0.2 m^2 year kg $biodiesel^{-1}$ (Milano *et al.*, 2016). Despite its high potential, microalgae cultivation still presents some challenges like intensive energy consumption processes and the high costs of biomass production with respect to water consumption, fertilizer and CO_2 requirements (Ledda *et al.*, 2015); where 1.8 kg CO_2 is required to produce 1 kg of microalgal biomass and 3.8 kg of water, 0.33 kg of nitrogen and 0.71 kg of phosphate is required to produce 1 kg of algal biodiesel (Ledda *et al.*, 2015). In wastewater treatment plants one of the richest streams of nitrogen and phosphorus can be obtained from the liquid stream of the anaerobic digester process (Morales-Amaral *et al.*, 2015). A number focus of studies have focused on individual microalgal species (monocultures); but these are unstable and vulnerable to perturbation; furthermore the genetic uniformity encourages pathogens and invaders proliferation (Kazamia *et al*., 2014). In wastewater algal systems, contamination is inevitable considering the use of centrate and the prohibitive cost of large scale sterilization. The aim of this research was to cultivate two different microbiomes (unfiltered microbiome (MVA), filtered (0.45 μm) MVB) and a monoculture of *Chlorella vulgaris* using centrate under batch and fed-batch operational modes. Experiments were performed at laboratory scale with a mixture of 35% centrate as a culture medium to compare biomass productivity, nutrient removal efficiency and lipid yield.

MVA, MVB and *C. vulgaris* were cultivated in 1 L PBR surrounded by a RGB-LED light platform with a luminous intensity of 43,200 mcd under 24 h light cycle, at room temperature (23.0±0.5 ℃) and 7.84±0.00, aerated with non-filtered ambient air (0.039% CO_2) at a rate of 200 mL min^{-1}. The batch mode was performed until the culture reached the stationary growth phase (10-12 days). After this, the PBRs were operated in fed-batch mode by feeding them every 3 days for a total of 3 cycles (12-25 days). The consortia showed shorter lag phases than the monoculture. Final biomass concentrations during batch mode were 0.78±0.04, 0.99±0.11 and 0.83±0.17 g L^{-1}, for MVA, MVB and *C. vulgaris*, respectively. The MVA consortia presented higher growth rates and biomass productivities under batch and fed batch operational mode, consistent with other reports that the combination of heterotrophic microorganisms in algal cultures exhibit higher algal growth than cultures of algae alone (Subashchandrabose *et al*., 2011). The ammonium and phosphate removal rates and efficiencies under batch and fed batch modes are shown in Tables 1 and 2. Batch mode exhibited better removal with time than fed batch mode as illustrated in Figure 1.

Table 1: Removal efficiency (%) of NH^+_4 – N and PO^{3-}_4 – P by MVA, MVB and *C. vulgaris* under batch and fed-batch operational mode. n.r (no removal)

	MVA		MVB		C. vulgaris	
	NH^+_4-N	PO^{3-}_4-P	NH^+_4-N	PO^{3-}_4-P	NH^+_4-N	PO^{3-}_4-P
Batch	99.2±0.0	100±0.0	99.9±0.1	99.9±0.1	99.1±0.0	100.0±0.0
1st feeding	74.5±2.1	100±0.0	65.8±6.7	100±0.1	65.6±0.5	53.8±10.5
2nd feeding	42.8±2.2	n.r	55.7±8.1	95.8±3.8	33.7±5.0	5±3.6
3rd feeding	36.5±2.3	97.9±1.4	37.11±3.5	28.8±9.0	25.1±3.6	26.3±5.8

Table 2: Removal rate of NH^+_4 – N (mg $L^{-1}d^{-1}$) and PO^{3-}_4 – P (ug $L^{-1}d^{-1}$) by MVA, MVB and *C. vulgaris* under batch and fed-batch operational mode. n.r (no removal)

	MVA		MVB		C. vulgaris	
	NH^+_4-N	PO^{3-}_4-P	NH^+_4-N	PO^{3-}_4-P	NH^+_4-N	PO^{3-}_4-P
Batch	39.6±1.4	0.65±0.12	22.2±0.1	0.36±0.0	30±0.4	0.43±0.04
1st feeding	48.2±2.2	0.06±0.01	41.7±4.6	0.26±0.04	65.5±1.3	0.3±0.06
2nd feeding	50.7±2.7	nr	48.27±9.1	0.06±0.02	57.3±8.7	0.05±0.04
3rd feeding	52.4±4.3	0.44±0.05	61±7.8	0.25±0.03	37.2±4.9	0.42±0.08

The fatty acid content for MVA, MVB and *C. vulgaris* was 32.58±11.28, 41.62±10.23 and 1.10±0.33 mg g^{-1} of dry weight of algae, respectively. The microbiome presented higher lipid content than the monoculture. The fatty acid composition is depicted in Figure 2.

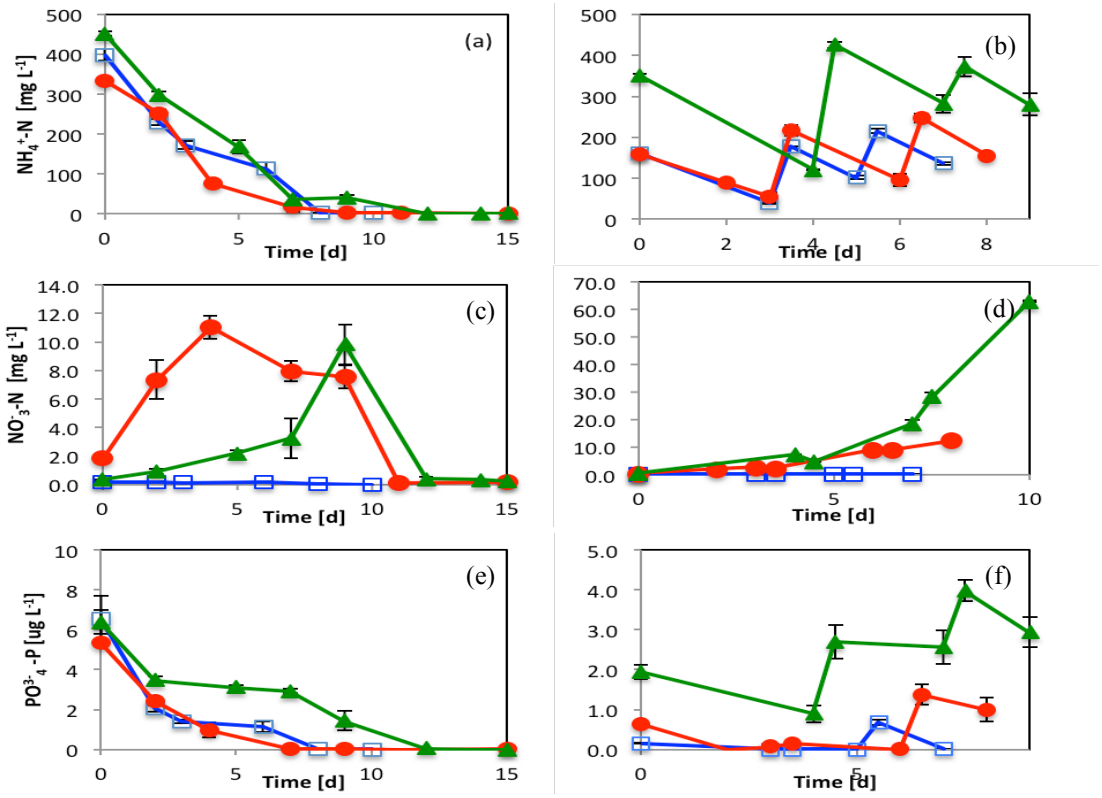

Figure 1. Changes in NH^{+}_{4} – N (mg L^{-1}), NO^{-}_{3}-N (mg L^{-1}) PO^{3-}_{4} – P (ug L^{-1}) concentrations in batch (a, c, e) and fed-batch (b, d, f) operational mode

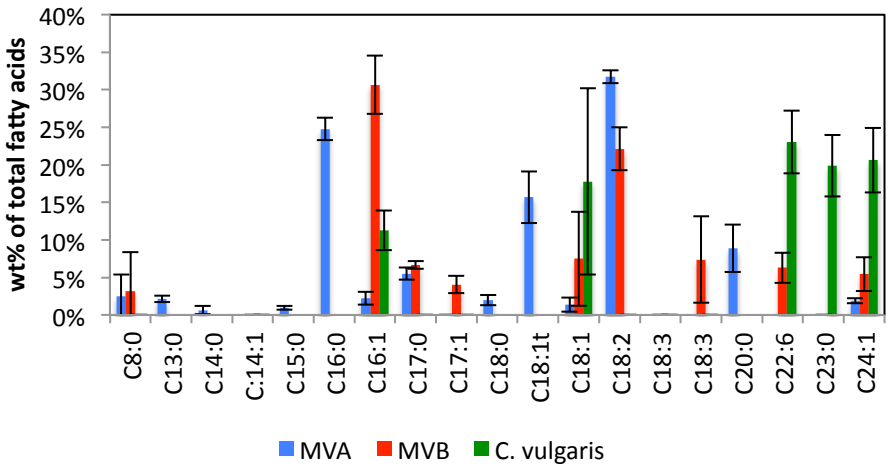

Figure 1. Fatty acid composition of lipid extracted from the species MVA, MVB and C. vulgaris at the end of fed batch operational mode. The letter t after the fatty acid name (C18:1t) denotes trans-isomerism, when no letter t appears, fatty acid is of cis-isomerism

The microbiome presented higher fatty acid content of C16:0 (24.8%), C16: 1 (30.6%), C18:1 (15.7%) and C18:2 (31.7%), while the monoculture presented more content of C 22:6 (23.06%), C 23:0 (19.89%) and C 24:1 (20.64%). High-quality biodiesel comes from a fatty acid profile where C16:0, C18:1 and C18:2 are the principal fatty acids. Microalgal lipids are mainly composed by unsaturated fatty acids (50-65%) (Halim et al, 2012; Gouveia and Oliveira, 2009), especially cis-isomers (Halim et al, 2012). The single algal strain of *C. vulgaris* and the microbiome MVB showed a low saturated fatty acid content of 20.6% and 8.6% when compared to the total cis-unsaturated fatty acid content of 61.5% and 52.8%, respectively. The presence of fatty acids such as palmitoleic (C16:1) and oleic (C18:1) can provide biodiesel with high CN values (Zendejas *et al.*, 2012). As such, the microbiome contain a fatty acid profile that could produce high quality biodiesel, while providing relatively high nutrient removal when cultivated in 35% centrate.

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