Municipal Wastewater Selection for Microbial Biodiesel Production

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ABSTRACT: This research compared the effects of municipal wastewaters (i.e., primary and secondary treated) from the Christchurch Wastewater Treatment Plant (CWTP) in Christchurch, New Zealand (NZ) on microbial (microalgal-bacterial) biomass production, settleability, and quality as biodiesel feedstock. Inoculums consisted of native, mixed cultures from an oxidation pond and an activated sludge process. Growth of settleable biomass was encouraged by recycling settleable solids within laboratory-scale sequencing batch reactors (SBRs) operated using a 24-hr cycle, 8-day hydraulic residence time (HRT), and controlled climate conditions. Generally, biomass concentrations of reactors fed with primary wastewater (i.e., 200/400 mg/L final mean for Cold/Warm conditions) were at least double those of secondary wastewater reactors (i.e., 70/210 mg/L final mean for Cold/Warm conditions) due to greater nutrient loading and microbial growth. Furthermore, primary wastewater reactors demonstrated much greater settling (i.e., 76 vs. 22% on average) indicating more efficient biomass harvesting. Lipid contents and types were comparable for all microbial cultures. The benefits of high carbon and bacterial concentrations in primary wastewater appeared to outweigh any disadvantage of reduced light penetration to microalgae from shading.

Keywords: Microalgal-bacterial biomass, activated sludge, wastewater treatment, biodiesel

1. INTRODUCTION

Microalgal biomass grown on wastewater has potential for sustainable biofuel production [1, 2]. Although this field has been researched since the 1950s [e.g., 3, 4], this is the first known study to compare genuine municipal wastewaters as a substrate for native microbial (microalgal-bacterial) biomass production. The productivity, settleability, and quality of biomass grown on CWTP wastewaters were examined.

2. MATERIALS AND METHODS

2.1 Experimental Setup

Four SBRs were inoculated with a 21 L solution of indigenous microalgae and other microbes sourced from Oxidation Pond 6 (P6) wastewater collected from CWTP. One SBR (designated as AP) was also inoculated with 1.5 g (as total suspended solids [TSS]) of activated sludge as previous studies showed that it improved biomass settleability [5]. Four feed water conditions were examined for each climate regime: tap water control, secondary treated wastewater

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(SE), and primary treated wastewater without (PE) and with an activated sludge inoculum (AP). Primary wastewater (Table I) was comparable to, but secondary wastewater had slightly higher values than generalised by others [5]. Wastewaters were collected fresh weekly from CWTP, stored at 4 ºC, and dosed to achieve an 8-day HRT. The SBRs were operated as shown in Figure 1 with volume and depth ranging from 21 to 24 L and 30 to 34 cm, respectively.

Table I: Wastewater Feed Characteristics for Microbial Reactors.

Parameter	Sample Count[(a)]	Primary Wastewater	Secondary Wastewater
Total COD	67	358 ± 91	88 ± 46
Total N	23	41.0 ± 7.5	37.9 ± 6.3
NH_4-N	28	26.8 ± 8.2	28.8 ± 5.1
Total P	21	6.3 ± 1.9	6.3 ± 4.3
TSS	58	128 ± 55	30 ± 16

(a) Data provided by CWTP.
Note: Values are mean ± SD in mg/L. COD = chemical oxygen demand; N = nitrogen; NH_4 = ammonia; P = phosphorus.

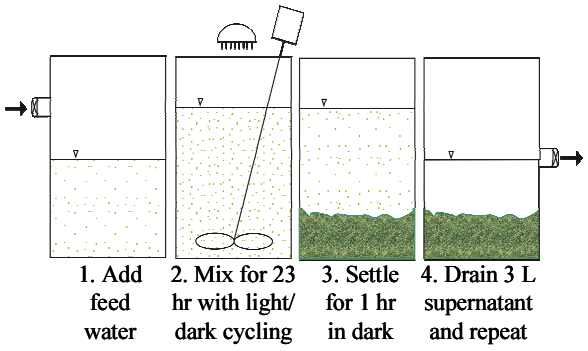

Figure 1: Operating Sequence and Microbial Accumulation of Reactors.

Using a controlled climate laboratory, two Cold Studies replicates (i.e., 410 µmol/m^2/s photosynthetically active radiation [PAR] for 9.6 hr/day at 13 °C mean water temperature) and three Warm Studies replicates (i.e., 925 µmol/m^2/s PAR for 14.7 hr/day at 21 °C mean water temperature) were conducted. (The third Cold Studies replicate was deemed unrepresentative due to operational difficulties causing substantial downtime.) Illumination was provided by Philips 400W incandescent bulbs.

2.2 Analytical Methods

The pH, dissolved oxygen concentration (DO), and water temperature were measured with an EDT Series 3 pH Meter, a YSI Model 57 Oxygen Meter, and a solid-state temperature sensor, respectively, and were automatically recorded at 15-min intervals. Probes were cleaned and calibrated weekly to maintain accuracy. An Apogee Model QSO-S Quantum Sensor measured PAR at the water surface.

The SBRs were sampled at the end of a light period prior to supernatant discharge (Figure 1, Step 2). The TSS (SM 2540D) and settled volume (SM 2540F) were determined according to American Public Health Association (APHA) [6]. Settleability was calculated (following SM 2540F [6]) as the mass percent of solids settled after 1 hr of sedimentation in the dark (Figure 1, Step 3).

At the end of each replicate experiment, supernatant was decanted from each SBR and settled biomass was harvested and frozen until processing.

Fats are commonly analysed to gauge feedstock potential for biodiesel. To enable sufficient sample for testing, biomass from each set of replicate experiments was combined and dried in an incubator at 60 °C. Fat analysis was then conducted by AsureQuality in Auckland, NZ. Total fats were extracted with diethyl ether and petroleum (based on IDF 5B [7] and 127A [8] and AOAC 922.06, 950.54, 948.15, 954.02, 933.05, and 945.44 [9]). Fatty acid methyl esters (FAMEs) were extracted from a separate aliquot of sample using methanolic sodium hydroxide and boron trifluoride methanol (AOAC 991.39 [9]). The individual FAMEs were then grouped as polyunsaturated, monounsaturated, and saturated fatty acids (PUFAs, MUFAs, and

SFAs) according to chemistry (i.e., >1, 1, and 0 double bonds, respectively). Unidentified fats were calculated via difference between total fats and identified FAMEs.

3. RESULTS AND DISCUSSION

Following a 2-week adaptation period, the microbial cultures were monitored and sampled twice weekly for 3 weeks to develop trends to compare the effect of the feed waters on biomass production, settleability, and quality for biofuels.

3.1 Biomass Production

The TSS concentrations of the wastewater cultures (SE, PE, and AP) increased as microbial biomass accumulated and grew (Figure 2). Greater TSS concentrations existed during the Warm Studies, indicating that the microbes were limited by light (i.e., 30% of warm climate radiation) and/or temperature (i.e., 13 vs. 21 °C mean) during the Cold Studies. By the end of the monitoring period (day 19), TSS concentrations had increased by 193, 120, and 42% on average for SE, PE, and AP, respectively, relative to the Cold Studies. The TSS of the control culture decreased over time for both climates (data not shown) presumably due to lack of nutrients in tap water for growth and the ensuing microbial washout. Variability between replicates was attributed to differences in development of the complex microbial communities.

Primary wastewater provided the best substrate for microbial growth probably because it had the greatest carbon content (i.e., 358 vs. 88 mg/L COD in SE). This condition favoured the growth of bacteria and other heterotrophs. Furthermore, microalgae and bacteria have a symbiotic relationship that exploits O_2/CO_2 exchange since bacterial respiration supplies O_2 for microalgal photosynthesis, which, in turn, supplies CO_2 for bacterial respiration [10].

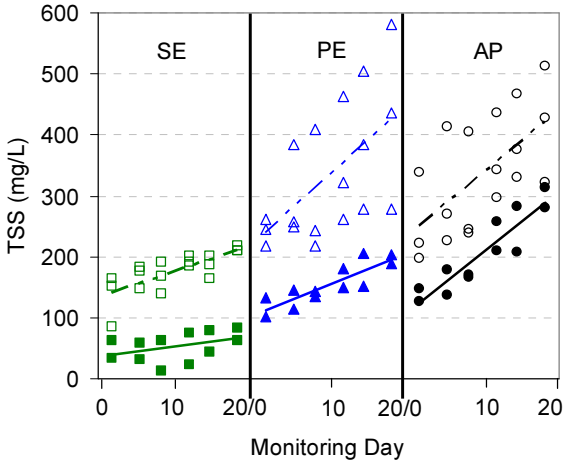

Figure 2: TSS Concentrations of Reactor Mixtures over Time. (Note: filled symbols indicate cold studies' and hollow symbols indicate warm studies' values.)

Greater carbon in primary wastewater and activated sludge over secondary wastewater sustained more active bacterial populations and facilitated development of a more balanced microbial community able to maximise the metabolic exchange.

The pH and DO concentrations of the cultures (Table II) also impacted biomass production. More dissolved CO_2 was available for photosynthesis at lower pH [11] of PE and AP cultures (i.e., 7.5/8.5 and 7.2/8.0 standard pH units [S.U.] for Cold/Warm Studies, respectively) compared to SE cultures (i.e., 8.3/10.2 S.U. for Cold/Warm Studies) due to carbonate chemistry. In addition to carbon limitation, secondary wastewater probably restricted microbial growth of SE cultures since bacterial inhibition at pH greater than 9 S.U. [12] and photooxidative damage to microalgae at DO levels above air-saturation (i.e., 8.9 and 10.5 mg/L at 13 and 21 °C, respectively) [13] can occur.

Table II: Mean pH and DO Values of Microbial Cultures for Cold/Warm Studies.

	SE	PE	AP
pH (S.U.)	8.3 /10.2	7.5 /8.5	7.2 /8.0
DO (mg/L)	9.8 /12.5	4.6 /8.0	2.9 /5.6

3.2 Biomass Settleability

Biomass settleability affects the economics of harvesting by natural settling. Settleability was not affected by climate (Figure 3a). Typically, settleability was much greater for primary wastewater (PE and AP) than secondary wastewater (SE) fed cultures (Figure 3b). The AP culture demonstrated greatest settleability for Cold Studies, but was similar to PE for Warm Studies (data not shown).

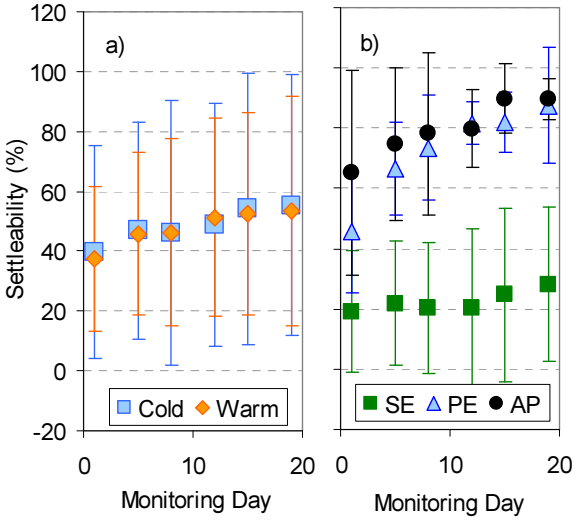

Figure 3: Settleability of Microbial Biomass over Time by a) Climate Regime and b) Reactor Mixture.

Settleability of cultures generally increased over time as microbes aged and culture density increased. Previous studies have shown that settleability improves with decreasing growth rate and increasing cell age [14] attributable to reduced negative cell surface charge allowing bioflocculation during quiescent periods [15]. Bioflocculation was easily induced daily in the SBRs by temporarily suspending mixing (Figure 1, Step 3), but its efficiency was variable among replicates (Figure 3b). In addition to bioflocculation, development of permanent microbial flocs affected settleability due to increased size and density of the aggregated microbes. Floc sizes were larger at greater organic loading from ≤500 µm for SE up to 1,000 µm for AP. Flocs also grew in size over the duration of the studies which increased settleability over time since settling velocity is proportional to particle size according to Stokes' Law [16].

3.3 Lipid Quality for Biodiesel

Lipid quantity and type have a significant influence on biodiesel quality [17]. Shorter SFAs have greater storage stability and are less likely to polymerize during combustion [18], but they have poorer cold temperature properties (e.g., cloud point) [19]. Longer PUFAs can oxidise and gel during storage [18]. Often, blending oil from various feedstocks offers advantages in fuel performance over the neat form.

The microbial biomass contained 8.5 to 12.6% lipids (Figure 4). These values are relatively low compared to pure microalgal cultures, which can attain up to 70% lipids, but they are comparable to those obtained from transesterification of municipal sludges. Lipid content of the SBR cultures was lower than that reported for primary sludge (i.e., up to 14.5%), but greater than that of secondary (or activated) (i.e., 2.5-6.2%) sludges [20, 21].

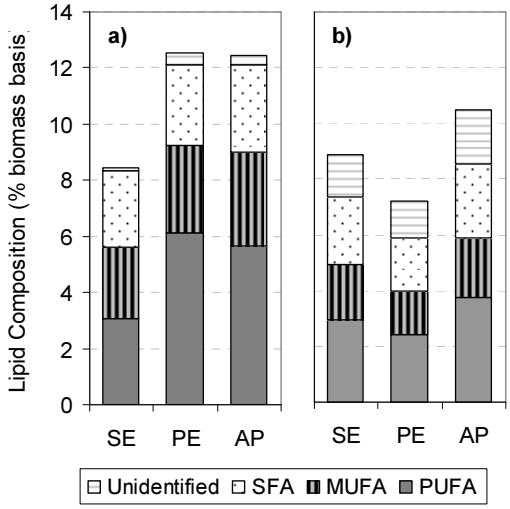

Figure 4: Microbial Biomass Lipids of a) Cold and b) Warm Studies.

Feed condition and climate did not appear to have an effect on either the

quantity or composition of the lipids. Due to the presence of comparable quantities of SFAs, MUFAs, and PUFAs for all mixtures, feedstock blending may not be required for optimal fuel performance, which could improve the economic considerations at large-scale production.

4. SUMMARY AND CONCLUSIONS

Primary wastewater was a better substrate for growth of microbial (microalgal-bacterial) biomass cultured in laboratory SBRs under warm and cold climate regimes compared to secondary wastewater on account of greater carbon concentration. Secondary wastewater was not a suitable substrate for microbial growth most likely because of limited CO_2 availability, bacterial inhibition, and photooxidative damage to microalgae.

Microbial biomass produced under cold climate was growth limited by light and/or temperature. Productivity was 42-193% greater under warm climate, confirming that microbial growth is highly sensitive to seasonal variations and that full-scale applications need to thoroughly prepare for such extremes.

In all cultures, microalgal photosynthesis supplied more than enough DO to meet oxygen demand of the feed wastewater and to sustain bacterial respiration without added aeration. This advantage supports the shift towards more sustainable wastewater engineering alternatives.

Settleability was governed by microbial aggregation into permanent, dense flocs and daily-induced bioflocculation, and it was greater and more consistent for primary wastewater cultures.

Lipids were similar among the cultures and did not appear to be impacted by climate regime. The inclusion of substantial portions of SFAs, MUFAs, and PUFAs in the biomass indicates that extracted oils may be useable in their neat (unblended) form, which could improve large-scale economics. The lipid, nutrient recycling, and oxygenation potential of microalgal-bacterial cultures over conventional activated sludge processes could have far-reaching benefits.

A mixed culture of activated sludge and microalgae provided the best overall performance for biomass productivity, settleability, and lipid content. These advantages outweighed any disadvantage of reduced light penetration to microalgae from shading by bacteria and other suspended solids.

Future research will focus on the effects of operational parameters (e.g., recycle rate, HRT, sludge age) on the performance of primary wastewater fed microbial biomass cultures.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of Lucy Pooch and Franz Resl at CWTP. Additional assistance by Peter McGuigan, David MacPherson, Paul Broady, and David Conder at the University of Canterbury was greatly appreciated. Financial support was provided by the Department of Civil and Natural Resources Engineering and a Doctoral Scholarship from the University of Canterbury.

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