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Research Paper

Influence of temperature on mixed microbial culture polyhydroxyalkanoate production while treating a starch industry wastewater

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ABSTRACT

The influence of temperature on the production of enrichment biomass and polyhydroxyalkanoates by activated sludge was evaluated within a practical case study. Two laboratory-scale sequencing batch reactors (SBRs) were operated in parallel over 131 days to treat a wastewater from a potato-starch modification facility, and produce surplus activated sludge biomass with PHA accumulation potential. The SBRs were operated similarly but at different temperatures (15 and 25 °C). Temperature did not influence wastewater treatment performance (average 97% COD removal).

Replicate PHA accumulation experiments were conducted on the SBR surplus biomass at 15, 20, 25 and 30 °C. Surplus biomass accumulated PHA with acetic acid to between 60 and 65%, gPHA/gVSS for all temperatures tested and with estimated Arrhenius temperature coefficients (θ) of 1.048 for the PHA production, and 1.062 for the COD consumption specific rates. The MMC PHA production process was adaptable within a common range anticipated for seasonal fluctuation of temperature for the influent wastewater and PHA production feedstocks without loss of performance. This outcome suggests a broad range in the practical feasibility and reliability with MMC method and process implementations. PHA production was predicted to be more efficient at lower temperatures. Strategies can be engineered in the bioprocess design and operations to accommodate for temperature shifts in practical applications of PHA production as a route to resource recovery from organic waste management services.

1. Introduction

Polyhydroxyalkanoates (PHAs) are a class of biodegradable polymers [1] with promise as ingredients for bioplastic manufacturing [2]. PHA production processes based on activated sludge (or mixed microbial cultures, MMCs) and using wastewater as carbon source have been widely investigated to produce PHAs more economically [3,4]. MMC PHA production processes can create synergistic benefits by integrating the regional needs of municipal and industrial wastewater treatment with a growing demand for biobased raw materials in regional, renewable-resource value chains [5,6].

Methods to obtain a mixed culture biomass with significant PHA-production potential entail creating reactor environments that select and enrich for the PHA-storing phenotype in the biomass. For this goal, a so-called feast and famine or aerobic dynamic feeding (ADF) approach has been commonly applied [7]. In an aerobic wastewater treatment process under ADF, activated sludge biomass is disposed repeatedly to feast environments, which are rich in readily biodegradable organic matter measured as chemical oxygen demand (RBCOD). Feast on RBCOD is followed repeatedly by famine environments with negligible availability of RBCOD. The excess activated sludge biomass from a biological wastewater treatment process can be harvested and be made to store PHA to more than its dry weight in a separate accumulation process under conditions of prolonged feast [8]. These ideas have been featured in the literature at bench scale [9], but also now increasingly at pilot scales [10–13].

Temperature is an important factor for the practical integration of MMC PHA production with wastewater treatment for both the biomass production and PHA accumulation processes. It is generally expensive to change the temperature of a wastewater for biological treatment at industrial scale. Some industrial wastewaters may be cooled down to suitable levels for biological treatment, but wastewaters are not

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typically heated nor their temperature controlled because of the high expenses that this would entail. Wastewater temperatures may drift due to seasonal changes, the operation of batch units, and shutdowns/start-ups within industrial processes. Gradual temperature variations are anticipated to naturally influence microbial community structure in biological wastewater treatment [14], and sudden temperature changes may also promote process instabilities and upsets [15]. In industrial practice for MMC PHA production, the temperature of biomass production may be different from that of PHA accumulation. A temperature difference is especially expected if different RBCOD sources are used for biomass production and for PHA production [5,11]. Since temperature may be imposed rather than offered as an adjustable process parameter for full-scale process implementations, we wished to more thoroughly understand the sensitivity of MMC PHA production to a given process temperature and to process temperature shifts. The present investigation has been an evaluation of the influence of temperature shift on MMC PHA production while treating an industrial process wastewater.

The effects of temperature in MMCs for PHA production have been reported to a limited extent in the research literature in the range of 15–35 °C, with aligned but also some mixed outcomes [16–19]. Johnson et al. [17] estimated Arrhenius temperature coefficients for PHA production ($\theta_p = 1.040$) and for substrate consumption ($\theta_s = 1.051$) for a pure acetate feedstock, which suggests the potential to engineer for process temperature in MMC PHA production. However, this and more recent work [19] have indicated challenges for MMC PHA production at lower temperature (< 20 °C).

Given the reported outcomes in the literature and that often municipal and some industrial wastewaters temperatures are below 20 °C for at least part of the year, our goal was also to evaluate and compare MMC PHA production above and below 20 °C. Wastewater treatment temperatures of 15 °C and 25 °C were selected within a practical industrial process integration case study, using a process water effluent from a potato-starch modification factory. The range from 15 to 25 °C is also typical for municipal wastewater temperatures in Northern climates [14]. We wished to corroborate the anticipated MMC PHA production process sensitivity to mesophilic temperature shifts for a real wastewater, especially given the reported challenges with respect to temperature in the literature. Two laboratory-scale sequencing batch reactors (SBRs) were operated with ADF, wherein one SBR was maintained at 15 °C and the other at 25 °C. The unfermented process water effluent from the potato starch modification factory was used as the carbon source for the MMC enrichment. The influences of temperature shifts on the respective community structures and the biomass PHA accumulation potentials were evaluated.

2. Materials and methods

2.1. Experimental approach

Two sequencing batch reactors (SBRs) were operated for 131 days in parallel at 15 °C (SBR15) and 25 °C (SBR25). Eighty liters of process effluent from the KMC factory (Brande, Denmark) were delivered fresh by courier once per month to Veolia Water Technologies (Lund, Sweden). The received factory process water effluent was stored at 4 °C pending its use as the SBR feedstock. Acetate fed-batch PHA accumulation tests were performed to evaluate and compare the MMC PHA accumulation potential (PAP) response as a function of time and accumulation temperature for the harvested surplus activated sludge biomass from the two SBRs. In these PAP tests, PHA storage capacity, kinetic parameters, specific PHA storage and specific active biomass growth yields were evaluated. These evaluations were made for SBR15 and SBR25 surplus biomass at their respective acclimation feast-famine temperatures (15 and 25 °C), and at accumulation temperatures corresponding to a sudden 10 °C up-shift and down-shift (25 and 15 °C, respectively). Additional PAP tests were conducted for the SBR15 biomass at 20 and 30 °C to further evaluate temperature sensitivity. The PAP response of the 15 °C biomass with acetic acid at 30 °C was also compared with that under the same accumulation conditions but with the factory process effluent as feedstock.

2.2. Wastewater characterization

The industrial process water effluent from KMC was pretreated prior to use as a feedstock by centrifugation (9 min at 3500 × *g*) to remove residual suspended solids (starch particles). The centrate SBR feedstock presented relatively constant soluble COD (sCOD) levels of 13.1 ± 1.7 g-COD/L (n = 6). About 56 ± 8% of the sCOD was due to acetic acid, and this represented the source of volatile fatty acid (VFA) with 7.1 ± 0.9 gHAc-COD/L. No significant levels of other VFAs were detected in the process water. The remaining sCOD was not identified specifically but it was nevertheless found to be readily biodegradable during feast in SBR15 and SBR25. The process water was further characterized by relatively low levels of soluble nitrogen (14 ± 8 mgN/L; with less than 0.02 mg-NH_4^+-N/L; n = 6) and phosphorus (26.8 ± 1.6 mgP/L with 18 ± 5 gPO_4^{3-}-P/L; n = 6).

2.3. SBR biomass production

The SBRs were with a working volume of 3.5L. Water jackets around the SBRs connected to temperature-regulated water baths (Julabo, F12-ED refrigerated/heating circulator) were used to control the SBR temperatures at 15 °C (SBR15) and 25 °C (SBR25). SBR operation, including timing of mechanical mixing (magnetic stirrer), air supply, and influent/effluent pumping, was automated by programmable logic control (GE Fanuc VersaMax). The SBRs were inoculated with activated sludge from Klagshamn wastewater treatment plant (Malmö, Sweden). The reactor dissolved oxygen (DO) levels were monitored and logged at sampling rates between 0.033 to 0.100 Hz (HQ40d, Hach) and were with DO greater than 1 mg-O_2/L during feast.

The SBR influent was KMC process water effluent centrate diluted with tap water down to 2 gCOD/L. Additional nitrogen and phosphorus were added as NH_4Cl and KH_2PO_4 in order to establish an SBR feedstock carbon to nutrient mass ratio of COD:N:P of 200:7:1. A feedstock ratio of COD:N of 200:7 was determined during start-up and over the first 50 days of operation to be sufficient for reliable COD removal without supplying excess ammonia that would support nitrification activity (based on effluent nitrate and nitrite concentrations). No nitrification inhibitors were added to the feed. The evaluations of the influence of temperature on biomass enrichment and PAP were conducted after the initial tuning of nutrient balance, during 81 days of steady state operations after day 50. Trace nutrients were provided to the SBR feedstock as 3 mL/L from a trace elements stock solution ($FeCl_3$·6H2O, 1500 mg/L; H_3BO_3, 150 mg/L; $CoCl_2$·$6H_2O$, 150 mg/L; $MnCl_2$·$4H_2O$, 122 mg/L; $ZnCl_2$·$7H_2O$; $NaMoO_4$·$2H_2O$, 60 mg/L; $CuSO_4$·$5H_2O$, 30 mg/L; KI, 180 mg/L; EDTA, 10000 mg/L).

The SBR cycles were the same (8 h) for both SBR15 and SBR25. With three cycles per day, waste activated sludge (WAS) was withdrawn for solids retention time (SRT) control in one cycle per day. In cycles without WAS withdrawal, each cycle began at the end of the ADF famine phase and comprised: (1) a process sequence for evaluation of endogenous respiration and oxygen transfer lasting 14 min, (2) biomass settling during 30 min without mixing and aeration, (3) effluent (supernatant) discharge, (4) 1L influent pumping by rapid (dump) feeding over 2 min without mixing and aeration, (5) mixing and aeration for the remainder of the eight hours. The above-mentioned process sequence (1) consisted of 7 min mixing with no aeration, followed by 7 min with mixing and aeration. From the period with no aeration, the endogenous respiration of the biomass was estimated from the rate of decrease of dissolved oxygen (DO) concentration over time. The trend in DO increase for the subsequent 7-min period with mixing and aeration was used to estimate the oxygen transfer coefficient. Volume exchanges