



Effect of the temperature in a mixed culture pilot scale aerobic process for food waste and sewage sludge conversion into polyhydroxyalkanoates



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ABSTRACT

The utilisation of urban organic waste as feedstock for polyhydroxyalkanoates (PHA) production is growing since it allows to solve the main concerns about their disposal and simultaneously to recover added-value products. A pilot scale platform has been designed for this purpose. The VFA-rich fermentation liquid coming from the anaerobic treatment of both source-sorted organic fraction of municipal solid waste (OFMSW) and waste activated sludge (WAS) has been used as substrate for the aerobic process steps: a first sequencing batch reactor (SBR, 100 L) for the selection of a PHA-producing biomass, and a second fed-batch reactor (70 L) for PHA accumulation inside the cells. The SBR was operated at 2.0–4.4 kg COD/(m³ d) as OLR, under dynamic feeding regime (feast-famine) and short hydraulic retention time (HRT; 1 day). The selected biomass was able to accumulate up to 48% g PHA/g VSS. Both steps were performed without temperature (T) control, avoiding additional consumption of energy. In this regard, the applied OLR was tuned based on environmental T and, as a consequence, on biomass kinetic, in order to have a constant selective pressure. The latter was mainly quantified by the PHA storage yield ($Y_{P/S}^{feast}$ 0.34–0.45 COD_P/COD_S), which has been recognized as the main parameters affecting the global PHA productivity [1.02–1.82 g PHA/(L d)] of the process.

1. Introduction

Currently, municipal waste represents about 60% of the 140 tons/year of bio-waste produced in Europe (European Commission, 2010). This amount is constantly increasing and, up to now, only the upgrade of existing technologies, such as the anaerobic digestion (AD), has found the reliability in specific urban contexts (Bolzonella et al., 2006; Mattioli et al., 2017). Indeed, the valorisation of biological sludge and food waste through anaerobic co-digestion leads to the recovery of biogas and digestate as two final products. Moreover, this type of process is characterized by improved biogas production and digestate quality (Cabbai et al., 2016). In general, bio-wastes are characterized by high moisture content and biodegradability, low amount of inert material and high fraction of organic matter, when they are separate collected properly. In fact, with particular references to an urban scenario, increasing the biological fraction of bio-wastes through an efficient source separate collection makes easier their valorisation with different viable biological processes, not only anaerobic digestion or composting (Giroto et al., 2015). In this case, the potentiality of this bio-waste can be exploited also as valuable feedstock for biopolymers (e.g. polyhydroxyalkanoates, PHA) production (Pagliano et al., 2017).

The conversion of bio-wastes into added value products allows to both decrease the cost of their disposal and to limit their increasing production (Pfaltzgraff et al., 2013; Lieder and Rashid 2016). For most of the developing countries, this strategy could be particularly suitable, considering the concept of waste biorefinery as an innovative solution to the environmental and economic overburden caused by the current inefficient waste disposal practices. Moreover, the biorefinery approach may fulfil the increasing energy demand and at the same time support the development of new job markets, as well as the improvements in the public health and environment (Nizami et al., 2017). Several families of biopolymers, in particular polyhydroxyalkanoates (PHA), can be obtained by using innovative biotechnologies. In this case, a combined anaerobic-aerobic strategy can be applied to exploit fermentable and renewable organic feedstock (Valentino et al. (2015a)). In this view, an urban biorefinery can be implemented since a notable part of the bio-waste organic matter is directed to PHA production, instead of to traditional AD processes for biogas and bioenergy production (Valentino et al., 2018).

As largely known, PHA are a wide group of biodegradable thermoplastic polyesters synthesized as carbon source from specific microorganisms and stored as granules within the cells. A market scenario

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related to these biopolymers already exists and it is based on the industrial PHA production performed with pure cultures cultivation (Chen, 2010). However, the production based on pure cultures requires sterile conditions and refine feedstock, making these processes expensive and high energy demanding. These requirements cause a substantial increase in production cost (5.0-8.0 €/kg). Therefore, PHA are still not competitive with the widely marketed petroleum-based plastics (Gholami et al., 2016). An alternative and less expensive technology that can be applied for PHA production involves the use of mixed microbial culture (MMC). In fact, PHA-accumulating organisms can be enriched and selected from the typical activated sludge of a municipal wastewater treatment plant, by applying aerobic dynamic feeding (ADF) conditions. In this way, a good MMC selective pressure can be obtained through alternating feeding periods (feast and famine), exploiting a fermented organic waste feedstock rich in volatile fatty acids (VFA), typical election substrates for PHA-producing metabolism (Oliveira et al., 2017; Pardelha et al., 2012). In fact, VFA are rapidly consumed and converted into PHA in the feast phase; the stored polymer is in turn used as carbon source to gain a competitive advantage during the following famine phase, where no longer VFA are available in the culture medium (Salehizadeh and van Loosdrecht, 2004). In the last decade, many investigations have been focused on PHA production by MMC by using different types of organic waste, involving the typical three-step anaerobic-aerobic process: a) waste fermentation for VFA production; b) MMC selection/enrichment under ADF; c) PHA accumulation in batch reactor (Valentino et al., 2017). More recently, the PHA production from urban biowaste and in particular from the organic fraction of municipal solid waste (OFMSW) sources (Basset et al., 2016; Korkakaki et al., 2016; Colombo et al., 2017) has been described. A pilot plant platform has been also developed in order to assess the potentiality of PHA and bioenergy production in a specific territorial context (Valentino et al., 2018). However, the effect of temperature on process performance has never been discussed and, in this study, this process parameter has been related to its performances in terms of storage properties (rate and yield) and global polymer productivity, which is one of the most important aspect affecting the economic viability of the MMC-technology.

2. Materials and methods

2.1. Fermented feedstock as substrate for biomass cultivation

The renewable organic feedstock was the OFMSW coming from door-to-door collection of Treviso municipality (northeast Italy) and the thickened waste activated sludge (WAS) produced in Treviso municipal wastewater treatment plant (WWTP). A screw-press was used for OFMSW pre-treatment and homogenization and the squeezed liquid fraction has been used in this work as discussed in Moretto et al. (2020). A pilot scale Continuous Stirred Tank Reactor (CSTR, $V = 380$ L) was used for the fermentation of the squeezed OFMSW alone, and the OFMSW mixed with thickened WAS. The fermented stream was characterized by a high VFA content on the soluble COD (COD_{SOL}), in the range 0.73 - 0.85 COD_{VFA}/COD_{SOL} , a crucial characteristic for a renewable feedstock to be valorized in the MMC-PHA production technology (Valentino et al., 2017). A detailed description of both fermented streams is available in Table 1.

Before to be used in the aerobic stages for MMC cultivation and PHA accumulation, both streams were subjected to a two-stages filtration system made by a coaxial centrifuge equipped with a nylon filter bag of 5.0 μ m porosity, and a tubular ceramic membrane of 0.2 μ m porosity. This system was necessary for a complete solids and anaerobic microorganisms removal.

2.2. Aerobic stages for MMC selection/enrichment and PHA production

The MMC cultivation was performed in a 100 L working volume

Table 1

Physical-chemical parameters of the fermented streams used for MMC cultivation

Parameter	OFMSW [†]	OFMSW-WAS mixture [‡]
Total Solids (TS, g/kg)	75 ± 4	56 ± 1
Volatile Solids (VS, g/kg)	68 ± 2	46 ± 1
COD_{SOL} (g/L)	20 ± 3	27 ± 4
COD_{VFA} (g/L)	17 ± 2	20 ± 1
COD_{VFA}/COD_{SOL}	0.85 ± 0.09	0.73 ± 0.08
Ammonium ($N-NH_4^+$; mg/L)	615 ± 37	673 ± 72
Phosphate ($P-PO_4^{3-}$; mg/L)	140 ± 13	119 ± 11
$COD_{VFA}/N/P$ (g)	100/3.6/0.8	100/3.4/0.6

[†] squeezed OFMSW (after mechanical pre-treatment).

[‡] squeezed OFMSW and thickened WAS (30%-70% v/v respectively).

SBR, which was inoculated with thickened activated sewage sludge from Treviso WWTP. Two SBR runs were conducted at similar hydraulic retention time (HRT) or sludge retention time (SRT) of 1.0 d, and operating cycle length (6 h). The reactor was equipped with linear membrane blowers (Bibus EL-S-250), which operated as aeration and stirring system. The pH and T were continuously measured but not controlled. The pH evolved steadily (8.0-9.0) in the whole SBR operation; T in the aerated medium changed seasonally. In the first SBR run (Run I), T decreased from a maximum of 28 °C to a minimum of 15.5 °C. In the second SBR run (Run II), T increased from 28 °C to 34 °C. The applied organic loading rate (OLR) was regulated based on the feast phase length, which was identified by the dissolved oxygen (DO) concentration profile, daily recorded for each SBR cycle (Valentino et al., 2014). Depending on the substrate consumption kinetic, the OLR was set in order to maintain a short and stable feast phase, ensuring an appropriate selective pressure over the biomass. The OLR was changed in the range 2.0 - 4.4 g $COD_{SOL}/(L d)$.

The SBR performance was monitored by measurement of biomass concentration, as volatile suspended solid (VSS, samples taken at the end of the feast phase), and PHA (samples taken at the end of the feast phase and at the end of the famine phase).

The storage potential of the biomass was explored in fed-batch accumulation tests ($V = 60-80$ L), which were performed at the same T and pH of the SBR. The fermented streams were dosed with a multi-pulses addition as described in Campanari et al. (2014). The mixed liquor was sampled at the beginning of the test and at regular intervals until the end for analytical determinations of VFA and PHA. The VSS were quantified at the beginning and at the end of each test.

The operation of reactors was automated with a programmable logic controller (myRio Labview by National Instrument) except for the feeding of the VFA-rich streams into the batch reactor, which was manually conducted.

2.3. Analytical methods

The SBR was monitored measuring total and volatile suspended solids (TSS and VSS), VFA and PHA as characterizing parameters. The suspended solids were determined following the Standard Methods (APHA 1998).

The quantification of VFA was conducted using a gas chromatography (GC) method (Carlo Erba instruments) with hydrogen as gas carrier. The GC was equipped with a Fused Silica Capillary Column (Supelco Nukol TM, 15 m - 0.53 mm - 0.5 mm film thickness) and with a flame ionization detector (200 °C). The analysis was conducted under increasing temperature (T) from 80 °C to 200 °C with a ramp rate of 10 °C/min.

PHA concentration was determined from the analysis of 5.0 mL of mixed liquor treated with 1.0 mL of NaClO solution (5% active Cl_2) and then stored at -4 °C. In accordance to Braunegg et al. (1978), the polymer was subjected to an acid-catalyzed methanolysis in order to

obtain the 3-hydroxyacyl methyl esters directly solubilized in Chloroform and then quantified by GC method. The abundance of hydroxybutyric (HB) and hydroxyvaleric (HV) monomers were quantified using P(3HB-co-3 HV) Sigma-Aldrich standard polymer at 5 wt% HV content.

2.4. Calculations

All the parameters characterising reactor performances were calculated after pseudo-steady state was achieved. Such pseudo-steady state was recognized when the feast phase length remained approximately stable (within a 10% deviation from average) for at least 5 SRTs. In each SBR cycle, the end of the feast phase (complete depletion of VFAs) was identified by a rapid increase of the DO concentration, continuously monitored. The non-polymer biomass (or active biomass, X_A) and the biomass PHA content (g PHA/g VSS) were calculated as reported elsewhere (Valentino et al., 2014). Then, the mass values of X_A , PHA and VFA were converted into COD units by using the relative conversion factor from oxidation stoichiometry in order to calculate rates and yields (Valentino et al., 2013). The specific VFA (or substrate) uptake rate was calculated as ratio between the removed VFA and the length of feast phase (t), per unit of X_A : $(-qS^{feast}) = \Delta S / (tX_A)$. The specific PHA production rate was calculated as the ratio of stored PHA and the length of feast phase (t), per unit of X_A : $qP^{feast} = \Delta PHA / (tX_A)$. The storage yield in the feast phase was determined as the ratio between the amount of stored PHA and the amount of the removed VFA ($Y_{P/S}^{feast} = \Delta PHA / \Delta S$).

In fed-batch accumulations, the specific PHA storage rate (qp^{batch}) was calculated by linear regression of data versus time (by considering the initial period, approximately the first 2 h at constant rate). Storage yield was quantified at the end of accumulation (6 h) by using the same mathematical formula described above. The PHA content in the biomass at the end of the test (g PHA/g VSS) was calculated by dividing the measured PHA by the biomass concentrations.

The global PHA productivity was calculated as the amount of produced PHA per unit of overall volume of both reactors and per unit of time [g PHA/(L d)].

Statistics data analysis was performed using the open source software R version 3.5.0 (2018-04-23).

3. Results and discussion

Under the well-known feast-famine condition, the literature body suggests that biomass selection/enrichment depends on many process parameters such as the SRT (Dionisi et al., 2001), OLR (Villano et al., 2010), feeding frequency (Moralejo-Gárate et al. (2013)) and temperature (Jiang et al., 2011). Currently, a multi-parametric model to predict the performance of the biomass enrichment reactor has not yet been developed. However, it is also largely recognized that a low feast-to-aerobic reaction time ratio plays a pivotal role for an effective MMC selection with significant PHA storage capacity (Morgan-Sagastume et al., 2015). In the pilot scale investigation herein proposed, the feast phase length has been maintained between 12–15% of the overall aerobic cycle length. The values in this range were lower than the largely recognized threshold of 20%, generally reported as limit value, below which the selective pressure over the biomass is enhanced (Valentino et al., 2017).

3.1. Aerobic SBR runs for biomass selection/enrichment by using OFMSW as substrate

According to environmental T, the SBR had an initial operating T of 28 °C. Over the course of operation, T of the system decreased, even though not at constant rate. Therefore, three different periods were recognized since they were characterized by a narrow range of T variation (maximum 3.0 °C). The first period is indicated in Fig. 1A as Run

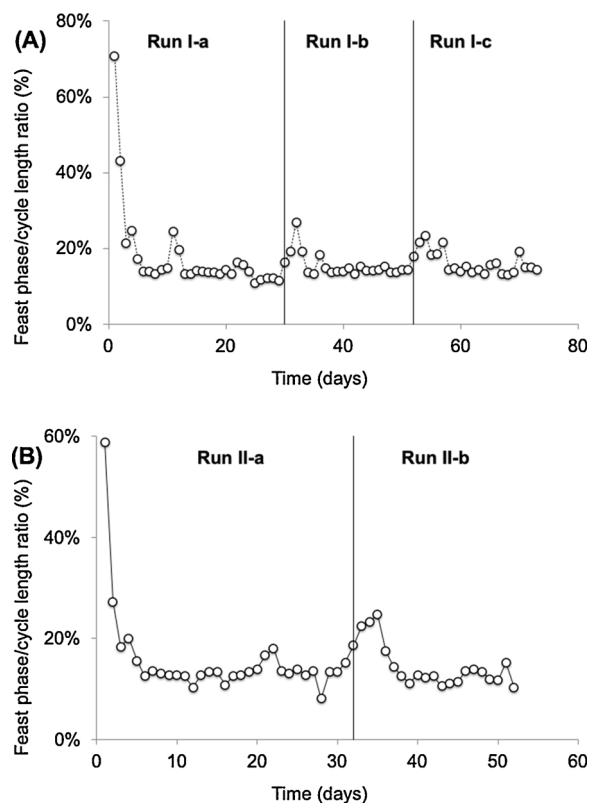


Fig. 1. Trend of the feast phase length in the three stability periods of Run I (A) and in the two stability periods of Run II (B).

I-a, where the average T was 26.1 ± 0.8 °C. The pseudo steady state was achieved in 5 days (or SRTs) after inoculum. Apart from the fluctuations at day 11, Run I-a exhibited stable feast phase values (13.4 ± 1.0 % h/h) approximately for 25 days of operation. Hence, the mixed culture was selected with a robust and stable selective pressure, under an average OLR of 3.3 ± 0.2 g $COD_{SOL}/(L d)$. The solids concentration quantified in this run was equal to 1.40 ± 0.02 g VSS/L. The progressive decreased of the temperature led to a decrease of the process kinetics; in fact, temporary extension of the feast phase was observed (up to 27% h/h, at day 32). In order to avoid the prolonged feast, with consequent loss of the selective pressure, the applied OLR was progressively decreased, until a new stationary phase. This was identified as Run I-b. A stable feast phase (14.2 ± 0.6 % h/h) was maintained for more than 15 days after a transition period of 5 days approximately. In Run I-b, the applied OLR was equal to 2.8 ± 0.1 g $COD_{SOL}/(L d)$; the selected biomass had an average VSS concentration of 1.24 ± 0.03 g VSS/L, consistent with the lower applied OLR if compared to Run I-a. In this period, the maximum and minimum recorded T were 23.0 °C and 20.2 °C respectively; on average, the operating T was equal to 21.3 ± 0.9 °C. With the progress of decreasing T, the applied OLR was set at 2.0 ± 0.1 g $COD_{SOL}/(L d)$. As previously observed, the decrease of T negatively affected the substrate consumption rate; hence, a lower OLR was necessary to maintain a selective pressure as stable as possible. This last stability period (Run I-c) lasted for almost two weeks and it was characterized by an average operating T of 16.9 ± 0.7 °C, and a feast phase length of 14.5 ± 0.9 % h/h. Lower OLR corresponded to lower VSS (1.02 ± 0.04 g VSS/L).

As indicated in Fig. 1A, the feast phase length was maintained approximately constant and similar in the three runs. Therefore, a good selective pressure for PHA-producing microorganisms was maintained also at operating T lower than 20 °C. This finding is not often reported in the literature body, where the process T is generally controlled around 25 °C (Kourmentza et al., 2017).

3.2. Aerobic SBR runs for biomass selection/enrichment by using OFMSW-SS mixture as substrate

The methodology applied in Run I was also replicated in Run II. In this case, the range of explored T was different (28–34 °C) and two stability periods were identified. The selection of the biomass was started from lower T. Fig. 1B shows the two periods where operating T was different as well as the applied OLR. Run II-a was characterized by an average T equal to 29.2 ± 0.8 °C and OLR of 3.7 ± 0.2 g COD_{SOL}/(L d). In Run II-b, operating T was higher (33.0 ± 0.8 °C) as well as the average OLR [4.4 ± 0.2 g COD_{SOL}/(L d)]. The feast phase length was maintained stable in both periods. After the start-up and the achievement of the pseudo steady state at day 6, the average value in Run II-a was $13 \pm 1\%$ h/h; following a transition period of one week approximately (from day 31 to day 37), a new stable feast-famine regime was achieved with an average feast phase length of $12 \pm 1\%$ h/h. The two periods were characterized by an average VSS level of 1.64 ± 0.03 (Run II-a) and 1.80 ± 0.05 g VSS/L (Run II-b).

3.3. Effect of the temperature on the performances of the selected MMC

To the best knowledge of the authors, there are no experiences in the literature that discuss how the microbial selection performs at temperature higher than 30 °C. Basically, in all T-controlled system, a maximum value of 30 °C has been investigated (De Grazia et al., 2017; Johnson et al., 2009; Jiang et al., 2011). In the most recent investigation (De Grazia et al., 2017) the influence of T was more related to the PHA accumulation capacity rather than selection efficiency: the produced biomass accumulated PHA up to 0.60–0.65 g PHA/g VSS in all tested T (from 15 °C to 30 °C). The interesting and practical outcomes of the work of De Grazia et al. (2017), in agreement to this study, was that the MMC PHA production process has been demonstrated to be adaptable within a predicted range of T fluctuations due to the seasonality.

As previously explained, the applied OLR was regulated based on seasonal T fluctuations. However, the system was not equipped with a specific OLR set up and its changes were manually made. Despite some practical difficulties, the following Fig. 2 shows the good correlations between operating T (average daily T, not controlled) and the OLR (manually set). The data reported in this figure are related to the periods of stability only (in both Run I and Run II), excluding the start-up periods and the transitions periods of the OLR changes.

Since the feast phase length was maintained at similar values in the five SBR runs, it is reasonable to suppose that the selective pressure on the biomass was similar in all the investigated cases, independently from the applied OLR or operating T.

Surprisingly, a deep analysis of the selection performances revealed that T affected the specific substrate uptake rate ($-qS^{\text{feast}}$) and the specific storage rate (qP^{feast}) in different manner (Table 2). The ($-qS^{\text{feast}}$) showed an increasing monotonous trend, from 388 ± 53 to

725 ± 70 mg COD/(g COD_{Xa} h) as T increased from 16.9 to 33 °C. A different trend was observed for the storage rate (qP^{feast}), which variation was at lower extent and not linearly correlated with T variation. A remarkable increase of qP^{feast} was quantified from 143 ± 49 to 275 ± 55 mg COD_{PHA}/g COD_{Xa}/h in the range of intermediate explored T of 21.3–29.2 °C. Higher T seemed not to further stimulate the storage response, being qP^{feast} equal to 289 ± 47 mg COD_{PHA}/g COD_{Xa}/h. At lowest T investigated, qP^{feast} was equal to 167 ± 35 mg COD_{PHA}/(g COD_{Xa} h), 15% higher than value obtained at 21.3 °C. In light of these numbers, lower temperature (below 20 °C) appeared to be in favour of the storage response, since the highest storage yield was obtained at lowest T ($Y_{P/S}^{\text{feast}} 0.45 \pm 0.02$ COD_{PHA}/COD_{SOL}). This was in agreement with a previous funding (De Grazia et al., 2017), where it was demonstrated how the COD consumption rate was more strongly affected by an increase in temperature than PHA storage, and the storage yields were predicted to decrease with increasing T. Therefore, this study showed how the MMC-PHA production could be more efficient at lower T from a substrate utilization perspective. The higher kinetics for substrate consumption ($-qS^{\text{feast}}$) than that for PHA production (qP^{feast}) suggested that, on average, the PHA yield on consumed substrate should be observed to decrease with increasing T since higher T can lead to a greater quantity of organic carbon channelled to cell maintenance. However, the estimated PHA yield did not provide for a well-defined or monotonic trend as a function of T and, even though the highest PHA yields were obtained at lowest T (16.9 °C), temperature alone did not seem to solely govern the biomass response and other factors related to the biomass physiological state may also play a role.

The storage ability of the sludge selected in the SBR was evaluated in the fed-batch accumulation tests, which were performed at the same temperatures of those monitored in SBR. The main quantified parameters are reported in Table 2. The data reflected what was previously observed in SBR; the storage performances were not linearly correlated with T. Even though an average T of 26.1 °C and 29.2 °C led to a better result with respect to intermediate T (21.3 °C) and high T (33.0 °C), a lower T was even better performing since the final storage yield ($Y_{P/S}^{\text{batch}}$) and PHA biomass content were the highest achieved: 0.47 ± 0.02 COD_{PHA}/COD_{SOL} and 48 ± 2 g PHA/g VSS respectively (Table 2). It is noteworthy that, independently from the substrate that has been used, the biomass behaviour over the course of the accumulations could be differently described according to two different T ranges. More in detail, the following Fig. 3 describes the trends of the specific cumulative produced polymer (per unit of active biomass), which represents the true capacity of the biomass to produce PHA for a large extent. Under low T interval (Run I-b and Run I-c), the experimental data are linearly correlated with time (Fig. 3A) for the duration of the tests (7–8 h). This is quite relevant since the biomass seemed not to be affected by any PHA saturation phenomena, even at relatively high PHA content (48% g PHA/g VSS; Run I-c). Hence, in the hypothesis that the accumulation test lasts longer, higher level of intracellular PHA may be achieved. Differently, for higher T interval (26–33 °C), the data were better described by logarithmic trends: for the three different biomasses, the specific amount of produced polymer was higher in the first two hours of each test, achieving values between 0.26–0.32 g PHA/g X_A (Fig. 3B); almost three times higher than the corresponding values obtained at lower T (0.10–0.12 g PHA/g X_A; Fig. 3A). For longer time (up to 6 hours), the specific storage activity showed a remarkable decrease, which was most likely due to an increase of the growth response more than an effect of polymer saturation. In fact, in the three cases the final PHA content achieved was between 39–44% g PHA/g VSS, below the highest level measured in Run I-c.

Even with different trends, in all cases the selected biomass expressed a reproducible extant PHA accumulation potential. Basically, this was guaranteed by the combination of two factors: the efficient selective pressure established (short feast phase, below 15% h/h, compared to the overall cycle length; Albuquerque et al., 2013) and the presence of nutrients (at similar level) in the fermented carbon sources,

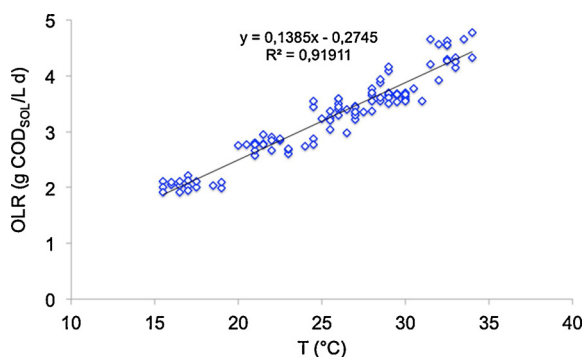


Fig. 2. Correlation between the average daily T and the applied OLR in the SBR in both Run I and Run II.

Table 2
Main parameters and performances observed in the two aerobic steps of MMC-PHA production process.

Parameter	SBR Run					References		
	Run I-a	Run I-b	Run I-c	Run II-a	Run II-b	Valentino et al. (2014)	Campanari et al. (2014)	Albuquerque et al. (2010)
OLR g COD/(L d)	3.3 ± 0.2	2.8 ± 0.1	2.0 ± 0.1	3.7 ± 0.2	4.4 ± 0.2	8.5	4.7	2.2-4.4
Temperature °C	26.1 ± 0.8	21.3 ± 0.9	16.9 ± 0.7	29.2 ± 0.8	33.0 ± 0.8	25	25	-
Feast phase/cycle length (%) h/h	13.4 ± 1.0	14.2 ± 0.6	14.5 ± 0.9	13 ± 1	12 ± 1	19-20	5.6 ± 0.3	17-50
VSS concentration (end of feast) g/L	1.40 ± 0.02	1.24 ± 0.02	1.02 ± 0.01	1.64 ± 0.03	1.80 ± 0.03	2.3-2.5	-	3.5-4.4
PHA concentration (end of feast) g/L	0.22 ± 0.04	0.15 ± 0.01	0.15 ± 0.02	0.23 ± 0.03	0.25 ± 0.02	0.09-0.18	-	-
Specific storage rate (q_P^{feast}) [mg COD _P /(g COD _X h)]	223 ± 31	143 ± 49	167 ± 35	275 ± 55	289 ± 47	164-190	339 ± 48	-
Specific substrate consumption rate ($-q_S^{\text{feast}}$) [mg COD _S /(g COD _X h)]	560 ± 35	450 ± 66	388 ± 53	659 ± 40	725 ± 70	628-648	-	-
Storage Yield ($Y_{P/S}^{\text{feast}}$) COD _P /COD _S	0.41 ± 0.01	0.34 ± 0.02	0.45 ± 0.02	0.42 ± 0.02	0.39 ± 0.03	0.26-0.32	0.56 ± 0.05	0.43-0.70

Parameter	Accumulation Performance					References		
	Run I-a	Run I-b	Run I-c	Run II-a	Run II-b	Valentino et al. (2014)	Campanari et al. (2014)	Albuquerque et al. (2010)
Final PHA content (%) g PHA/g VSS	42 ± 2	36 ± 3	48 ± 2	44 ± 4	39 ± 2	0.45-0.46	0.32 ± 0.05	0.38-0.52
Storage Yield ($Y_{P/S}^{\text{batch}}$) COD _P /COD _S	0.40 ± 0.03	0.33 ± 0.02	0.47 ± 0.02	0.42 ± 0.05	0.38 ± 0.04	0.45-0.53	-	0.26-0.48
Global PHA Productivity g PHA/(L d)	1.37 ± 0.16	1.02 ± 0.12	1.40 ± 0.22	1.77 ± 0.24	1.82 ± 0.17	1.25-1.70	1.50 ± 0.01	0.85

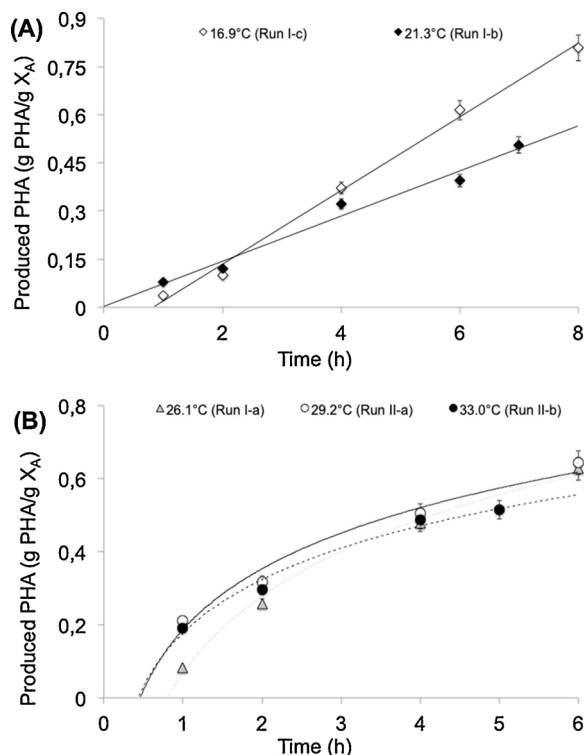


Fig. 3. Mass of produced PHA normalized per gram of active biomass over the course of the accumulations performed in the five SBR runs, with biomass acclimated at different operating T and different feedstock in Run I (A) and Run II (B).

which stimulated a concurrent PHA-storing biomass growth. A deep analysis of the effect of nutrients level (N/COD and P/COD) on the biomass storage response was performed in a past experience (Valentino et al. (2015b)), with synthetic medium. A restricted interval of N/COD (2-15 mg/g) and P/COD (0.5-3 mg/g) values were identified as main feedstock characteristic for the simultaneous maximization of the PHA biomass content and volumetric polymer productivity. From the practical point of view, the regulation of nutrients content in a real feedstock implies additional operation cost that may impact on the market price of the final product or on the economic feasibility of the whole process (Fernández-Dacosta et al. (2015)).

Both feedstock used in this study contained nutrients at higher levels than those reported in Valentino et al. (2015b). Hence, the simultaneous additions of carbon and nutrient source were unavoidable. However, the application of an appropriate OLR and its regulation with environmental T allowed maintaining an efficient biomass selection. In turn, over the course of accumulations, the biomass maintained its storage ability and, the increase of the process productivity (the mass of produced PHA increased in time) while still maintaining significant PHA biomass content was demonstrated to be technically feasible. Comparable results have been obtained in previous and relevant laboratory scale studies, carried out in more controlled process conditions (Table 2).

3.4. Fundamentals of modelled approach for PHA productivity maximization

By considering both SBR and accumulation reactors in sequence, the process polymer productivity was calculated as the amount of produced PHA per unit of overall volume of both aerobic reactors and per unit of time. Even though the storage response was better express at lower T, the higher PHA productivity was obtained at 29.2 °C and 33.0 °C

[1.77 ± 0.24 and 1.82 ± 0.17 g PHA/(L d) respectively; Run II-a and Run II-b]. This result is related to the OLR applied in SBR, which affects the biomass productivity of the selection stage. Generally, biomass productivity in the SBR almost linearly increased at increasing OLR (Villano et al., 2010; Lorini et al. 2020). In the runs I-a, I-b and I-c the OLR has been decreased from 3.3 to 2.0 g COD_{SOL}/(L d), and, as a consequence, the biomass level decreased from 1.41 to 1.02 g VSS/L. However, the higher PHA productivity among the three runs was quantified at the lower applied OLR (Run I-c; T 16.9 °C; 1.40 ± 0.22 g PHA/L d). In practice, the decrease of biomass production was counterbalanced by the higher efficiency of substrate utilization (Run I-c) compared to the lower efficiency exhibited at higher T (e.g. storage yield).

All the collected data from the five different runs have been taken into account; specifically, the global PHA productivity (GP) trend has been evaluated as a function of the operating T. The results showed a 2nd degree polynomial correlation and the best fitting polynomial regression was expressed as follows:

$$GP = 0.008496 T^2 - 0.36216 T + 5.032464$$

Moreover, this function was statistically significant according to the applied F test ($p < 0.05$). The data correlation is shown in Fig. 4; in the whole T range (15–30 °C), the GP function has a minimum around 21–22 °C. Data obtained at the highest T (33.0 ± 0.8 °C) have been excluded from this mathematical treatise since recognized as not significative for the GP function interpretation.

An analogous relation has been found for the SBR storage yield ($Y_{P/S}^{\text{feast}}$) and operating T (Fig. 5A). As observed in Fig. 4, the $Y_{P/S}^{\text{feast}}$ shows a minimum in the same range (21–22 °C), equal to 0.27 COD_P/COD_S (predicted value). On the other hand, the storage yield obtained for the batch step only ($Y_{P/S}^{\text{batch}}$) has no correlation with temperature as shown by the high data dispersion in Fig. 5B.

The global productivity is the sum of two contributions representing the selection/enrichment step (necessary for the PHA-accumulating biomass production) and the batch accumulation step (necessary for the PHA production). Based on the previously observed correlations, however, it is reasonable to suppose that the relation between GP and T, expressed through equation (1), can be mainly derived from the similar correlation that has been observed between $Y_{P/S}^{\text{feast}}$ and T. Indeed, the storage yield of the selection stage strongly affects the GP. Given the necessity to carry out the accumulation step for the PHA synthesis, a multivariate linear model has been developed to better correlate the three cited parameters (i.e. $Y_{P/S}^{\text{feast}}$, $Y_{P/S}^{\text{batch}}$ and GP) (Fig. 6):

$$GP = 3.86Y_{P/S}^{\text{feast}} + 2.52Y_{P/S}^{\text{batch}} - 1.1$$

The previous model has been developed to confirm what has been observed from the previous correlations: the GP is more dependent from the SBR storage performance (or the storage response of the selected biomass) as demonstrated by the regression coefficients associated to the independent variables. These coefficients are equal to 3.86 and 2.52 for the $Y_{P/S}$ of the selection and accumulation stages respectively; they have been derived and then tested through ANOVA

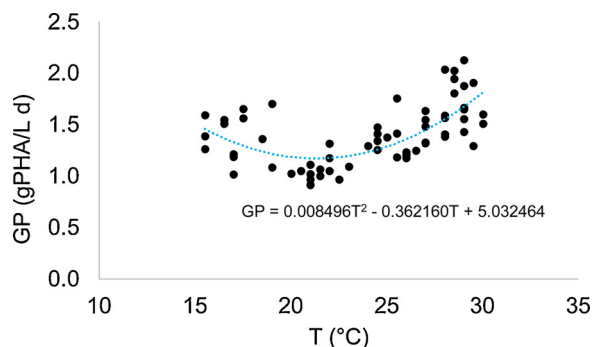


Fig. 4. Correlation between the global PHA productivity (GP) and the temperature (T) of the process.

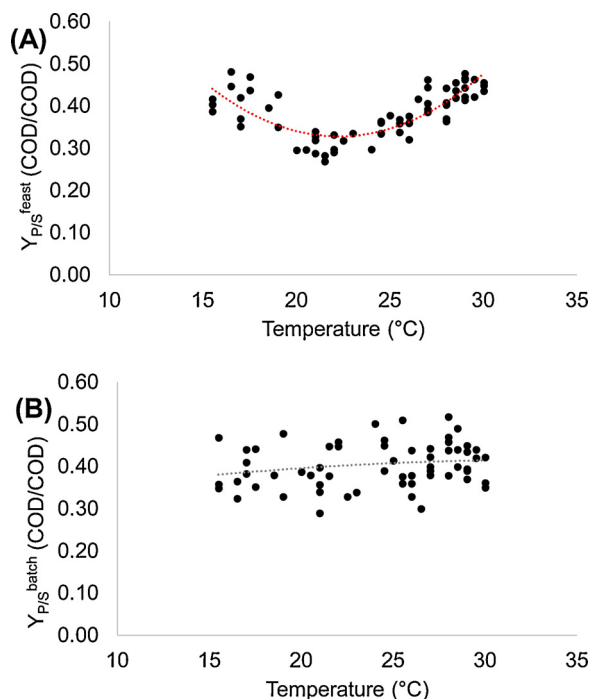


Fig. 5. Correlation between the storage yield in the SBR ($Y_{P/S}^{\text{feast}}$) and the operating T (A); correlation between the storage yield in the accumulations ($Y_{P/S}^{\text{batch}}$) and the operating T (B).

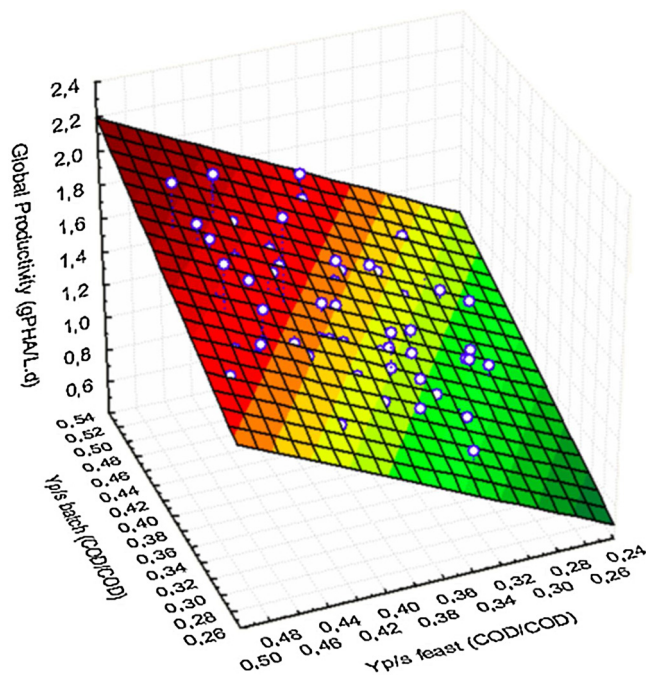


Fig. 6. Multivariate model for the correlation between the global PHA productivity (GP) and the storage yields of both aerobic stages ($Y_{P/S}^{\text{feast}}$ and $Y_{P/S}^{\text{batch}}$).

analysis. As a main result, the coefficients were significantly nonzero (p value < 0.05), confirming the effectiveness of the multivariate model. Moreover, the F test has been applied and also the overall regression coefficient resulted nonzero (p value < 0.05).

3.5. An effective strategy for the management of PHA production process with no T-control

From the practical point of view, the management of the process without T control can be advantageous in terms of energy saving, especially in this kind of process which is totally aerobic and needs continuous air supplying (Morgan-Sagastume et al., 2016). On the other hand, the selective pressure on PHA-accumulating biomass needs to be maintained and the OLR regulation as a function of operating T can be considered as the optimal strategy. The importance of an efficient selective pressure is not only related to the PHA accumulation performance (in batch mode), where usually the final PHA biomass content is taken into account (Rodriguez-Perez et al., 2018). As confirmed by the multivariate linear model, it has been demonstrated how the stimulated storage capacity in the biomass (specifically the yield $Y_{p/s}^{feast}$ occurring during the feast phase) also affects the volumetric polymer productivity of the whole process, or at least of the two aerobic stages [gPHA/(L d)]. In this way, both the final PHA biomass content and the global PHA productivities, usually considered as the main targets to look at from the technical and economic viability of MMC-PHA technology (Valentino et al., 2017; Lorini et al., 2020), are taken into account. It is necessary to consider that the MMC approach needs to be included in a scenario where PHA is not the only product obtainable from waste/wastewater treatment. The example described in Moretto et al. (2020) shows the biorefinery approach within an urban environment, where also biogas production is evaluated. Hence, even though MMC-PHA productivities are generally lower than pure culture productivities, the realistic possibility to recover other bioproducts (e.g. biogas) makes the MMC approach economically attractive. These considerations need to be supported by an ad-hoc business model (developed for a specific waste stream) and an estimation of the capital cost for the realization of new reactors and the trend of cumulative income coming from the sale of each bioproducts.

This study furnishes a useful tool for process management since it has been developed at pilot-scale with real VFA-rich carbon source. In principle, all explored T were found suitable for process operation, provided that the OLR needed to be modulated according to operating T itself. Under this assumption, the development of the MMC-PHA production in such conditions cannot be characterized by similar performance over the course of entire year, especially for the global PHA productivity more than the achievable PHA content.

4. Conclusions

The outcomes of this pilot scale study furnish a method for the practical reliability of MMC-PHA production technology in parallel to the valorisation of organic waste, in particular the organic material produced in an urban scenario. The temperature variation over the course of the year should not be seen as an input to control in order to maintain the mixed culture active and stable in its storage response. Changing the OLR according to the temperature fluctuations is a strategy to carry out the facilities without the control of this parameters and to save energy, whose overconsumption negatively affect the operating cost of the process (OPEX). In addition, it has been demonstrated that lower temperature did not necessarily correspond to lower storage performance (e.g. storage yield and maximum PHA content in the biomass below 20 °C); this further strengthens the idea to adapt the process (e.g. OLR) according to the temperature variation.

As demonstrated in this study, different operating temperature (16–33 °C) also corresponds to different PHA productivities [1.02–1.82 g PHA/(L d)] with narrow variation of final PHA content achievable (36–48% g PHA/g VSS). Hence, it should be taken into account that the process profitability may not always be the same over a wide period of time operability. An in deep analysis of process implementation and scale up as well as business model adapted to a particular scenario of bio-waste production, may help for MMC-PHA bioprocess design as a

route for resource recovery from the services of the urban organic waste management.

CRediT authorship contribution statement

Francesco Valentino: Conceptualization, Writing - original draft, Writing - review & editing. **Laura Lorini:** Data curation. **Marco Gottardo:** Formal analysis. **Paolo Pavan:** Supervision. **Mauro Majone:** Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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