Organic fraction of municipal solid waste recovery and valorisation by conversion into polyhydroxyalkanoates and biogas

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Abstract

The integrated-multistage process proposed herein is a practical example of a biorefinery platform, where the organic fraction of municipal solid waste (OFMSW) is used as value source for polyhydroxyalkanoates (PHA) and biogas production. Technical and economical feasibility of this approach have been demonstrated at pilot-scale providing a possible upgrade to traditional biowaste management practices presently based on anaerobic digestion (AD). A pH-controlled OFMSW fermentation stage produced a liquid VFA-rich stream with high VFA/COD_{SOL} ratio (0.90 COD/COD) that was easily used in following aerobic stages for biomass and PHA production. The solid fraction was valorized into biogas through AD, obtaining energy and avoiding secondary fluxes waste generation. The reliable aerobic biomass enrichment was demonstrated by stable feast-famine regime and supported by microbial community analysis. The selected consortium was able to accumulate PHA up to 55% wt. Compared to the traditional single stage AD process in an urban scenario of 900,000 AE, the integrated approach for OFMSW valorisation is preferable to biogas production only, being characterized by electrical energy production of 85.7 MWh/d and 1.976 t/d as PHA productivity. The proposed process has been also evaluated economically sustainable if PHA is marketed from 0.53 €/Kg, as minimum threshold, to higher market price.

Introduction

European food waste production approximately accounts for 90 million tons per year; this amount includes organic waste produced at household level (40%), bio-waste produced by the food service sector (15%) and at retail level (5%).[1] In the whole urban area, the total amount of organic waste includes also biodegradable garden and park waste. The disposal legislation for organic waste is progressively being less connected to landfill as best practises, and particularly in recent years it has become more expensive and restrictive.[2]

The possibility to recover added value products from biowaste could be a strategy for both decreasing cost of disposal and tackling the problems related to the increasing production of organic wastes, by using innovative technologies formally based on the circular economy concept.[3,4]

Even though biowastes have great variability in composition, they are characterized by high moisture content and biodegradability, both favoured by an efficient system of source separate collection. The high biological value of biowastes makes easier their valorisation with biological process; not only via composting or anaerobic digestion[5] but also with more recent biotechnologies that allow producing biopolymer and in particular the family of polyhydroxyalkanoates (PHA).[6,7] This group of biodegradable thermoplastic polyesters are biologically produced from specific bacteria strains, within their cell walls as carbon/energy source. The current industrial PHA production processes are based on pure cultures cultivation in sterile conditions.[8] Sterile condition causes an increase in production cost (up to 5.0 €/kg) that consequently renders these polymers not cost-competitive with conventional oil based polymers.[9] In order to decrease production costs, PHA can be produced from renewable organic resources using mixed microbial cultures (MMC) instead of pure cultures.[10,11] PHA-accumulating organisms can be selected from the waste activated sludge coming from the wastewater treatment, always available in the full-scale plants (WWTP), applying transient conditions such as aerobic dynamic feeding process (ADF). The selection of the MMC can be obtained through alternating feeding periods (feast and famine), with fermented feedstock rich in volatile fatty acids (VFA). The VFA are taken up very fast by PHA accumulating bacteria in the

feast phase and can be utilised to gain a competitive advantage during the subsequent famine phase, which are directly converted into PHA.[12] The typical process applied for PHA production from MMC is the so-called three-step process.[11] Many studies have been made in several years on PHA production by MMC using different types of waste, comprehensively listed in a recent review.[11] The interest for biopolymer production from urban waste valorisation is relatively recent, even if they have been recognized as key platform chemical raw material within biorefinery framework.[13,14] Few studies described different methods for PHA production, particularly from OFMSW sources, such as leachate,[15] percolate[16] and mixture of primary sludge and OFMSW.[17] These studies mainly concern laboratory scale tests. Pilot scale trials are unavoidably important to better understand process feasibility also for integration in existing WWTPs, as their further advancement.

The pilot scale study herein proposed is an example of an integrated approach to treat and simultaneously valorise the OFMSW (main constituent of urban organic waste) through the production of PHA (via open MMC) and biogas (via anaerobic digestion), without any generation of secondary waste fluxes. The piloting facilities are located in Treviso municipal Wastewater Treatment Plant (WWTP).

Experimental Section (Materials and Methods)

A process schematic of the units and concept at Treviso WWTP is presented in Figure 1. This integrated innovative scheme complies a first unit (Stage I) consisting in a Continuous Stirred Tank Reactor (CSTR) for OFMSW fermentation. The fermented OFMSW is then conveyed to the two following aerobic stages: a Sequencing Batch Reactor (SBR) for biomass selection/enrichment under feast-famine regime (Stage II); and a batch accumulation reactor for PHA production (Stage III). A final anaerobic digestion (CSTR) for residual and/or overflows valorisation is also included. The operation of each unit was automated via National Instrument™ cRIO device; centrifugation and feedstock filtration activities, VFA-rich stream feeding to Stage III were manual operations.

105 The renewable feedstock was the OFMSW coming from door-to-door collection of Treviso
106 municipality. A screw-press was used for feedstock pre-treatment and homogenization; pretreated
107 feedstock characteristics are given in following paragraph.
108 **Figure 1.**
109
110 *Anaerobic system for OFMSW fermentation and anaerobic digestion*
111 The pilot scale anaerobic process was characterised by a 200 L acidogenic fermentation CSTR with
112 a hydraulic retention time (HRT) of 3.3 d, and average organic load (OLR) of 20 KgVS/m^3 d (20.5
113 kgCOD/m^3 d). The downstream solids separation was conducted by means of a coaxial filter bag
114 (5.0 μm porosity) equipped centrifuge. The liquid fraction was intended to the use in the aerobic
115 PHA line.
116 The solid fraction of OFMSW fermentation was used as feed for 760 L CSTR anaerobic digester
117 (AD), operated at 3.9 KgVS/m^3d or 4.0 kgCOD/m^3d as OLR, and 12.7 d as HRT. The AD was
118 inoculated with digestate from Treviso full-scale plant. Fermentation and AD have been conducted
119 at thermophilic condition (55°C ± 0.1).
120 The optimal pH value at fermentation stage was controlled by the digestate recirculation from the
121 AD stage. Digestate recirculation, rich in buffer agent, was the strategy adopted. In detail, this
122 approach has been explained by Gottardo et al. (2017).[18]
123
124 *Aerobic stages for functionalized biomass and PHA production*
125 Stage II consisted in a 140 L working volume. The SBR was fed with different feeding solution: a)
126 synthetic acetic acid solution, days 1st-49th (start-up); b) pre-treated fermented OFMSW, days 50th-
127 129th. The synthetic solution consisting of acetic acid, diluted with tap water and anaerobic
128 digestate for nutrients supply.
129 The SBR was inoculated with thickened activated sewage sludge from Treviso WWTP. A single
130 run was conducted for approximately 4.5 month. HRT has been set at 1.0 d, equal to SRT (being

131 with no biomass settling phase), and cycle length at 6 h.[19] The reactor was aerated by means of
132 linear membrane blowers (Bibus EL-S-250), which operated also as stirring and heating system.
133 The temperature (T) and pH were continuously measured but not controlled. The temperature
134 changed seasonally between 14-18°C in March, and 26-29°C in July. The pH was maintained
135 between 8.0-8.5 in the whole SBR cycle. The applied OLR was initially equal to 3.0 $gCOD_{SOL}$/L d,
136 and then maintained around 2.5 $gCOD_{SOL}$/L d by using fermented feedstock.
137 The storage potential of the selected biomass was exploited through fed-batch accumulation tests,
138 performed with both synthetic (acetic acid), and pre-treated fermented OFMSW.
139 The operative conditions of both aerobic reactors have been chosen based on previous experimental
140 laboratory scale proofs of concept.[19,20] Dissolved Oxygen and pH were monitored by Hamilton®
141 industrial probes.
142
143 *Microbial community analysis in selection/enrichment SBR*
144 Fluorescence in Situ Hybridization
145 Aerobic sludge samples (10 mL) were taken over SBR operation at the end of feast phase and
146 immediately fixed in formaldehyde as previously described.[21] After fixation, the samples were kept
147 at -20°C to be further analyzed by Fluorescence In situ Hybridization (FISH). Oligonucleotide
148 probes targeting the Bacteria and Archaea domains and the main bacterial phyla were employed
149 following the hybridization conditions reported elsewhere.[22] The analysis was performed by
150 epifluorescence microscopy (Olympus, BX51). Images were captured with *Olympus F-View CCD*
151 camera and handled with *Cell˙F* software (Olympus, Germany).
152 DNA extraction
153 DNA extraction for NGS analysis was performed on samples collected throughout the SBR
154 operation. In detail, 10 ml sludge were collected and centrifuged at 15,000*g* for 15 min. Pellet was
155 processed for DNA extraction with Power Soil DNA extraction kit (*MoBio, Italy*) following the

156 manufacturer's instructions. Purified DNA from each sample was eluted in 100 µL sterile *Milli-Q*
157 water and 10 ng of extracted DNA was used for the following NGS analysis.
158 Next Generation Sequencing (NGS)
159 16S rRNA Amplicon Library Preparation (V1-3) was performed as detailed in Matturro et al.
160 (2016).[22] 10 ng of extracted DNA was used as template in the PCR reaction (25 µL) containing
161 dNTPs (400 nM of each), MgSO4 (1.5 mM), Platinum® Taq DNA polymerase HF (2 mU), 1X
162 Platinum® High Fidelity buffer (Thermo Fisher Scientific, USA) and barcoded library adaptors
163 (400 nM) containing V1-3 primers (27F:5'-AGAGTTTGATCCTGGCTCAG-3'; 534R:5'-
164 ATTACCGCGGCTGCTGG-3'). All PCR reactions were run in duplicate and pooled afterward.
165 The amplicon libraries were purified using the Agencourt® AMpure XP bead protocol (Beckmann
166 Coulter, USA). Library concentration was measured with Quant-iTTM HS DNA Assay (Thermo
167 Fisher Scientific, USA) and quality validated with a Tapestation 2200, using D1K ScreenTapes
168 (Agilent, USA).
169 The purified sequencing libraries were pooled in equimolar concentrations and diluted to 4 nM. The
170 samples were paired end sequenced (2 × 301 bp) on a MiSeq (Illumina) using a MiSeq Reagent kit
171 v3, 600 cycles (Illumina) following the standard guidelines for preparing and loading samples on
172 the MiSeq. 10% Phix control library was spiked in to overcome low complexity issue often
173 observed with amplicon samples. Data analysis was performed as detailed in Matturro et al.
174 (2016).[22]
175
176 *Analytical methods and calculation*
177 Suspended solids (total and volatile, TSS and VSS), ammonia and PHA analysis have been
178 performed as described in Valentino et al. (2014);[19] COD, VFA and phosphate have been quantified
179 as illustrated by Micolucci et al. (2014).[23]
180 Gas production in anaerobic reactors was monitored by two flow meters (Ritter CompanyTM,
181 drum-type wet-test volumetric gas meters). The percentages carbon dioxide was determined by an

infrared gas analyser portable GA2000TM (Geotechnical InstrumentsTM). Hydrogen and methane percentage were determined by a gas chromatograph GC Agilent Technology 6890NTM equipped with HP-PLOT MOLESIEVE^{TM} column and thermal conductivity (TCD) detector.

SBR and accumulation stages calculations were made as previously indicated.[19] Detailed explanations are also given in Supporting Information.

For energy balance, analytical data of the present pproposed study process (for proposed system) aand for of single stage anaerobic digestion process have been used to compare energy yields of the proposed platform with a classical single stage anaerobic digestion process. The parameters used to perform the final balance are those recently illustrated by Micolucci et al. (2018) and adapted to a scaled up version of both processes.[24] More technical details about parameters and boundary conditions are given in Supplementary Information.

Results and Discussion

Characteristics of the OFMSW and the effluent obtained after controlled fermentation

The OFMSW was given on a weekly basis by Treviso Municipality (Italy) from the separate collection. Before biological treatments, the OFMSW was pre-treated with the aim to remove inert materials, as plastic and metal. As for the general chemic-physical characteristics, pre-treated OFMSW showed an average dry matter content of 28%, of which 90% volatile solids. The COD values were typically greater than 900 gCOD/kgTS. The content of nitrogen and phosphorus in pre-treated OFMSW was of 27 gN/kgTS and 4 gP/kgTS on average respectively. As for the nutrients, this substrate showed a COD:N:P ratio of 100:2.9:0.7 (on average), not particularly rich in nutrients but potentially usable for the following aerobic stages. In addition, the alkalinity of fermented feedstock (around 900 mg $CaCO_3$/L, pH 5.0) had also excluded the necessity of NaOH addition in the medium, eventually necessary to avoid fast pH decrease (inhibiting for the biomass) during SBR feeding time.

By controlled fermentation process it was possible to obtain a liquid stream corresponding to 3.85 kgCOD/d of which 1.05 kgCOD/d in the soluble fraction and 2.81 kg COD/d in the particulate fraction. Therefore, it can be deduced that through a stable pH control of the fermentation there was a high VFAs production by microorganisms. The 90% of COD_{SOL} product was represented by VFA equal to 16.0 ± 0.4 gCOD/L (on average), and mainly consisting of butyric (38.0% COD basis), acetic (21.5%), propionic (12.7%), valeric (11.6%) and caproic (10.0%) acids (Figure 2). Lower levels of isobutyric (3.8%), isovaleric (1.6%) and isocaproic (0.7%) acids were also detected.

Figure 2.

Anaerobic digestion stage.

The fermented product was subjected to a solid/liquid separation process by centrifuge. The filtered mass flow was sent to a PHA production unit and the solid mass flow fraction ("cake") was sent to the anaerobic digestion stage.

As for the general chemical-physical characteristics, the cake showed an average dry matter content of 20% and a VS/TS ratio of 88%, with a COD:N ratio of 37. Therefore, regarding to nutrient content, this kind of substrate did not show any limitations. This assumption was demonstrated by ammonia concentration value of digestate, which was less than 900 mg NH_4^+-N/L, abundantly lower than inhibition value for the methanogenic activity.[23] The stability of the process was also proved from the VFA/partial alkalinity ratio, which was less than 0.3 for the overall period of experimentation (around 120 days).[24]

The average specific biogas production (SGP) was 0.71 Nm^3/KgVS fed with a composition of 65% v/v and 35% v/v of methane and carbon dioxide respectively. Considering the overall process (fermentation and AD stages), the composition of biogas was 53% methane 44% carbon dioxide and 3% H_2, as volume based percentage.

Biomass selection/enrichment in SBR (Stage II)

233 The start-up of the aerobic stages of the platform has been made in more easily controlled
234 conditions, by using acetate synthetic feeding solution. Thus, the applied OLR was stable and
235 initially set to 3.0 gCOD/L d. Since the system was not equipped with temperature (T) control, at
236 the start-up, the SBR temperature was slightly higher than 10°C (end winter) achieving almost 30°C
237 in the last part of experimentation (midsummer). This difference strongly affected the process of
238 culture adaptation to the newly imposed feast-famine conditions. More than 40 STRs were needed
239 to achieve a feast/cycle length ratio below 0.2 h/h, necessary condition for a stable feast-famine
240 regime with a satisfying biomass selective pressure.[11,12] Most of the studies approaching to the
241 MMC selection with the same process configuration were performed under T-control at 25°C; these
242 examples reported 10-15 SRTs maximum,[19,25,26] as window time to achieve a stable storage
243 response, also in agreement with a change in microbial community and stabilization of one major
244 PHA-storing phylotype.[19]
245 The length of the feast phase stabilized after about 50 SRTs (or days of operation) exhibiting values
246 consistently lower than 0.2 h/h (with respect to the overall aerobic cycle length), as temperature
247 started to increase from around 20°C above (Figure 3A). The temperature values, reported as daily
248 average values, progressively increased until the end of operation, generating an increasing
249 selective pressure over the biomass as demonstrated by the decreasing feast/cycle length ratio, often
250 below 0.1 h/h in the last 50 days of operation.
251 Before a reasonable feast-famine pressure was reached, strong fluctuations were observed for both
252 feast/cycle length ratio and PHA concentration (Figure 3B), with values abundantly higher than
253 average trends, which also positively correlated each other. Indeed, feast phase increased (i.e.
254 COD_{SOL} uptake rate decreased) as PHA concentration in the medium increased. When feast phase
255 started to be consistently short, PHA concentration profiles displayed net and constant differences
256 between the end of feast and the end of the cycle, as an indication of a stable storage response. This
257 evidence was already identified in previous lab-scale SBR experiments, and usually discussed based
258 on PHA concentration[26,27] or storage yield trends:[28] in the acclimation process, the biomass fits

259 faster to storing PHA than to consume it, bringing a remarkable increase of PHA cell content. The
260 biomass storage capacity is consequently saturated, but recovered when PHA concentration
261 decreases, and stabilized if process conditions remains unchanged. Similar biomass behaviour was
262 observed in this pilot scale approach, but with longer adaptation period, probably affected by the
263 relatively low temperature in the start-up.

264 **Figure 3.**

265 The feed shift from acetic acid to fermented OFMSW caused another fluctuation in the biomass
266 storage response (after day 49th). In this case the feast/cycle length ratio increased up to 0.4 h/h;
267 however, the functional feast-famine regime was rapidly re-established since the biomass was
268 already largely acclimated to the process condition and the applied OLR was slightly decreased to
269 2.5 $gCOD_{SOL}$/L d with respect to the use of synthetic feed. Indeed, the process conditions remained
270 substantially unchanged, except for medium temperature, whose progressive increase (up to
271 29.5°C) positively affected the biomass selective pressure.

272 Table 1 summarizes the main parameters that have been monitored and quantified in SBR, in both
273 periods where acetic and fermented feedstock were used as substrate. In the second period (day
274 50st-129th), the length of the feast phase was much shorter with respect to the first period and
275 abundantly lower than the largely recognized threshold value of 0.20 h/h,[11] indicating the
276 establishment of the 'feast-famine' conditions, required to enrich the culture in PHA-storing
277 microorganisms. The quantification of biomass storage properties, in terms of yields ($Y_{P/S}^{feast}$,
278 $Y_{P/VFA}^{feast}$) and rate (qP^{feast}), confirmed the efficiency of selective pressure over the biomass when
279 SBR was fed with fermented OFMSW. These parameters were comparable with previous
280 investigations applying the same three-step based technology.[29,30] Conversely, very lower values
281 were obtained by using acetate feeding, being selection/enrichment performance primary affected
282 by temperature.

283 **Table 1.**

284

285 *Fed-batch PHA accumulation reactor (Stage III)*

286 Preliminary accumulations have been conducted in order to exploit the storage potential of selected
287 consortium by using acetate synthetic feeding without nutrient addition. Then, more tests have been
288 carried out with fermented OFMSW assessing the biomass PHA production capacity and process
289 productivity. All the fed-batch tests were performed after the 50th days of SBR operation, when the
290 imposed selective pressure was stable and high enough to ensure a satisfying PHA accumulation
291 performance.

292 Acetate accumulations led to 0.37-0.42 gPHA/gVSS as PHA content, consistent with results
293 previously reported by using synthetic acetate (0.12-0.76 gPHA/gVSS)[31,32] or VFA mixture
294 solution (0.14-0.51 gPHA/gVSS).[19] The accumulation capacity of the biomass was better expressed
295 with fermented OFMSW, being PHA content in the range 0.39-0.52 gPHA/gVSS. However, PHA
296 saturation levels in the biomass was not achieved, even though storage response was prevailing
297 mechanism of substrate removal alongside biomass growth.

298 These performances were in line with many examples demonstrating the three-step process
299 feasibility by using renewable fermented feedstock.[11] In particular, recent investigations
300 approaching the use of fermented OFMSW or similar sources reported a wide range of different
301 MMC-PHA accumulation capacities. Amulya and co-workers achieved a maximum level of 0.24
302 gPHA/gVSS with fermented oil free filtered food waste.[33] The use of percolate was more
303 comparable with the present investigation; the PHA biomass content was in the range 0.40-0.48
304 gPHA/gVSS.[16] Korkakaki and co-workers (2016)[15] achieved even better performances by using
305 pre-treated leachate (close to 0.80 gPHA/gVSS), even though the biomass selection step was
306 performed with a solution largely made up of synthetic VFA (75%-90% volume based).

307 Although with some variability due to fermentation performance and/or maximum VFA content
308 achieved, fed-batch accumulations indicated that fermented OFMSW triggered higher accumulation
309 rates and productivities than those with acetate with no nitrogen and phosphorus addition (Table 2).

310 **Table 2.**

311 Such differences in accumulation response to different levels of nutrients have been widely
312 investigated and even the presence of nutrients at certain levels were associated with increased
313 polymer productivities due to concurrent PHA storage and active biomass growth.[11,34] Indeed, PHA
314 storage was contingent upon nutrient level as well as growth response. Higher biomass growth
315 response and yields ($Y_{X/S}^{batch}$ = 0.19-0.28 COD_{Xa}/COD_{SOL}) were obtained with fermented OFMSW
316 with respect to strongly growth-limiting nutrient levels of acetate solution ($Y_{X/S}^{batch}$ = 0.12-0.15
317 COD_{Xa}/COD_{SOL}). PHA storage response was greater in acetate accumulations and higher in terms
318 of yield ($Y_{P/S}^{batch}$ = 0.61-0.64 COD_{PHA}/COD_{SOL}); however, the lack of nutrient availability strongly
319 limited the PHA production to large extent, since PHA storage was poorly supported by new storing
320 active biomass growth. For this reason, the PHA productivities were doubled or even tripled when
321 using fermented OFMSW (0.28-0.49 vs 0.16-0.18 gPHA/L h with OFMSW and acetate
322 respectively) as a result of a not negligible growth response and faster kinetics for both substrate
323 uptake and storage specific rates (Table 2). In these cases, it is reasonable to suppose that a
324 sustained PHA content (apparently not at saturation level as shown in Supporting Information;
325 Figure S1) alongside growth of PHA-storing biomass increased PHA productivities.
326
327 *PHA-accumulating microbial community in SBR*
328 The microbial composition and structure of the communities selected in SBR were estimated by
329 using *in situ* detection methods for cell-based quantification and high-throughput sequencing.
330 Overall, bacteria were the main microbial component accounting for 50-80% of the total biomass
331 (Supporting Information; Figure S2). FISH analysis revealed a marginal presence of archaeal cells,
332 approximately 4% with acetate feeding (days 28, 50) and 10% of total population with fermented
333 OFMSW (days 62, 82, 91 and 108).
334 The analysis with bacterial phylum specific probes revealed the dominance of *betaproteobacteria*
335 during acetate feeding: 70% of total bacteria (day 28; Supporting Information, Figure S3) and was
336 almost constituted by members of *Azoarcus*/*Thauera* group (Supporting Information; Figure S4).

337 From the beginning of SBR operation, a six-fold increase of the *Azoarcus*/*Thauera* relative
338 abundance was observed indicating members of this group as the main PHA-accumulating
339 microorganisms in the SBR with acetate as the sole carbon source. The latter finding is in line with
340 previous experiences that showed the dominance of *Thauera* and *Azoarcus* species in the PHA
341 accumulation under ADF conditions with acetate as feedstock.[28,35,36,37,38]
342 Temporal fluctuations of the microbial population were observed during the operation with
343 fermented OFMSW (Supporting Information; Figure S5). Members of *Proteobacteria* and
344 *Cythophaga*/*Flexibacte*r/*Bacteroidetes* represented the main components at day 91 whereas a
345 marked shift towards the dominance of *Betaproteobacteria* was found at day 108 along with the
346 increase of temperature most likely driving the observed changes of the PHA accumulating SBR
347 biomass. Diversely from start-up phase with synthetic feeding, *Thauera*/*Azoarcus* group
348 represented only a portion of total *Betaproteobacteria* (Supporting Information; Figure S3) and
349 gradually decreased until reaching the lowest value (~12% of total *Betaprotebacteria*) at day 108.
350 High-throughput sequencing showed the occurrence of known PHA-accumulating microorganisms
351 including *Acidovorax* spp. and *Hydrogenophaga* spp., the latter representing 52-79% of the OTUs
352 affiliated to *Betaproteobacteria* (Supporting Information, Figure S5-A). Additionally, genera
353 *Amaricoccus* spp., *Meganema* spp. *Rhizobium* spp. and *Rhodobacter* spp. were found (Supporting
354 Information, Figure S5-B) as well as a variety of other *Alphaproteobacteria* occurring at very low
355 relative abundance.
356 Some of the taxa found to dominate with fermented OFMSW, such as *Acidovorax* and
357 *Hydrogenophaga*, were previously found prevailing in MMC fed with synthetic soluble fraction of
358 municipal wastewater[39] under ADF conditions or with fermented waste activated sludge under
359 aerobic/anaerobic operating conditions.[40] Both genera, commonly found in activated sludge, are
360 aerobic even though some species are capable of heterotrophic denitrification of nitrate. In addition
361 to chemoorganotrophic metabolism, some strains of *Hydrogenophaga* are chemolithoautotrophic,
362 using the oxidation of H_2 as an energy source and CO_2 as a carbon source.

363 To date, only very little is known regarding the key-microbes catalyzing the PHA storage from
364 OFMSW and most of the indications were obtained by adopting low-resolution monitoring tools
365 such as DGGE without providing any quantitative data as, instead, has been performed in this
366 study. In particular, *Brachymonas denitrificans*, *Corynebacterium*, *Xanthobacter* and *Azorhizobium*
367 were found with raw or pre-treated leachate obtained from OFMSW with VFA mainly composed
368 by acetate, propionate and butyrate.[15] The influence of aerobic and anoxic conditions on PHA
369 production was evaluated in SBRs treating un-fermented and fermented food waste.[41] DGGE
370 analysis was performed only on the biomass selected in SBR treating un-fermented food waste
371 under anoxic conditions and revealed the occurrence of genera belonging to *gammaproteobacteria*
372 (e.g. *Pseudomonas*, *Aeromonas* and *Acinetobacter*) followed by members of *betaproteobacteria*,
373 *epsilonproteobacteria*, *Bacteroidia* and *Firmicutes*.
374
375 *Mass and energy balance assessment of the integrated platform*
376 Data analysis of each separated pilot units have been transferred to a single industrial scheme,
377 ideally identified in an urban scenario of 900,000 PE with a specific OFMSW production of 0.3
378 kg/PE d.[24] As consequence, considering a recovery of 75% TS from pre-treatment screw-press stage
379 and a dry matter content of 28%, the amount of OFMSW to be treated is 60,143 kgTS/d which
380 corresponding to 53,865 kgTVS/d. The mass balance discussed in this paragraph is illustrated in
381 detail in Figure 4.
382 **Figure 4.**
383 The pre-treated OFMSW stream conveyed to the industrial plant for PHA and biogas production
384 needs to be abundantly diluted with tap water in order to reduce TS level to 7% w/w and to
385 maintain the applied OLR in the fermenter around 20 kgVS/m^3 d. The gaseous effluent flow rate
386 out of acidogenic fermenter is 8,080 Nm^3/d, as product of SGP value (0.15 Nm^3/kgTVS). As
387 expected, it is mainly composed by CO_2 (75% v/v or 6,060 Nm^3 CO_2/d), and in minor part by H_2

388 (15%, 1,212 Nm^3 H_2/d) and CH_4 (10%, 808 Nm^3 CH_4/d), corresponding to 10,479 kgTVS/d in the
389 effluent gas phase, almost 20% of TVS influent amount (53,865 kgTVS/d).
390 The solid/liquid separation unit allows recovering more than 70% of the volumetric OFMSW liquid
391 flow rate: 574,839 Kg/d of fermented stream with a TS content around or even below 0.5% w/w
392 and VFA/COD_{SOL} ratio of 0.90 can be used for both aerobic PHA production steps. On the other
393 hand, the more concentrated TS stream (cake) can be further valorised into biogas, once diluted
394 before feeding in anaerobic digestion unit.
395 Regarding PHA production line, the liquid fermented OFMSW needs to be split in two secondary
396 fluxes, properly quantified based on OLR applied on both SBR and accumulation reactors. The
397 SBR step accounts for 49% of influent carbon source (281,671 kg/d) and 100% of dilution water
398 consumption, as largely proven in this PHA process configuration.[11] Dilution water is included
399 because SBR has been modelled at 3.0 $kgCOD_{SOL}/m^3$ d as OLR (similar to this study), lower than
400 40 $kgCOD_{SOL}/m^3$ d, which is the current OLR applied in PHA accumulation reactor. Accordingly,
401 the volume of selection and accumulation reactors is 1,690 m^3 and 136 m^3 respectively. The storage
402 yield for both aerobic stages is 0.35 COD_{PHA}/COD_{SOL}, based on consumed $kgCOD_{SOL}$/d for
403 biomass production (in SBR) and PHA synthesis (accumulation). The modelled multi-stage process
404 has a production potential of 1,976 kgPHA/d; this means an overall polymer productivity of 1.08
405 $kgPHA/m^3$ d.
406 For overflows valorisation, the diluted cake previously discharged out of PHA line represents the
407 feed for anaerobic digestion. Based on 0.69 Nm^3/kgTVS as digester SGP, the anaerobic digestion
408 process produces around 28,410 Nm^3/d of biogas, mainly composed by CH_4 (65% v/v or 18,467
409 Nm^3CH_4/d) and then CO_2 (35% v/v, 9,944 Nm^3CO_2/d).
410 The energy balance comparison between the proposed process and the classical single stage
411 anaerobic digestion process (CSSP) was carried out using the same full-scale scenario of mass
412 balance (900,000 PE basin). All the thermal and electrical energy items are summarized in Table 3
413 (reference parameters and boundary conditions are given in Supporting Information; Table S1). The

aim of this comparison is to evaluate the different energetic yields between the two scenarios and estimate the minimum economic value of PHA produced to cover the economic income from the amount of biogas that would has been produced in a single stage anaerobic digestion platform. The specific SGP of CSSP, 0.75 Nm^3/kgTVS, was determined from Micolucci et al. (2018).[24]

Table 3.

Considering the thermal yield of the Combined Heat & Power unit (CHP) of 0.5,[24] the thermal energy produced is approximately 464,828 MJ/d and 419,854 MJ/d from the CSSP and the proposed process respectively. In both scenarios, the thermal balance is closed positively because the estimate thermal energy request is roughly 40% (185,774 MJ/d) and 82% (342,314 MJ/d) of the thermal energy produced for the CSSP single stage and the proposed multi-stage process respectively.

Regarding the economic income from the electrical energy produced, a production of approximately 103.4 MWh/d for the CSSP and 93.4 MWh/d for the proposed process has been estimated. By considering the overestimated electrical energy consumption for the oxygenation in the two aerobic steps (7.7 MWh/d), the net production is 85.7 MWh/d (Table 3). The electrical CHP yield of 0.4 has been considered for the thermal energy balance as well.[24] Assuming 130 €/MWh (no incentives) and 100 €/t for the disposal cost of the digestate (25% TS),[24] a gap of approximately 378,193 €/y exists between the CSSP and the proposed process. However, this gap can be easily covered because 1.976 t/d of produced PHA (as indicated in mass balance) has to be marketed at the low economic value of 0.53 €/Kg, as minimum threshold. A higher but still reasonable market price making it easier to overcome the economic income from that part of the biogas not produced in the platform with respect to CSSP, giving a practical evidence of the economical sustainability (in addition to the demonstrated technical one) of proposed multi-stage process.

Acknowledgments

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Figure Captions

Figure 1. Schematic overview of the pilot scale multi-steps process for PHA and biogas production from the OFMSW

Figure 2. VFA evolution in the fermenter CSTR (Stage I)

Figure 3. Feast phase/cycle length ratio and temperature monitored in SBR (A); PHA concentration at the end of feast and at the end of cycle (B) (Stage II)

Figure 4. Mass balance of the proposed multi-stage process currently developed at pilot-scale

Table Captions

Table 1. Main parameters monitored in SBR, in the two representative periods with different feed solutions

Table 2. Summary of PHA accumulation fed-batch tests performed with synthetic acetate solution and fermented OFMSW

Table 3. Assessment of energy balance of proposed integrated platform in comparison with the traditional anaerobic digestion process

681
682 **Figure 1.**
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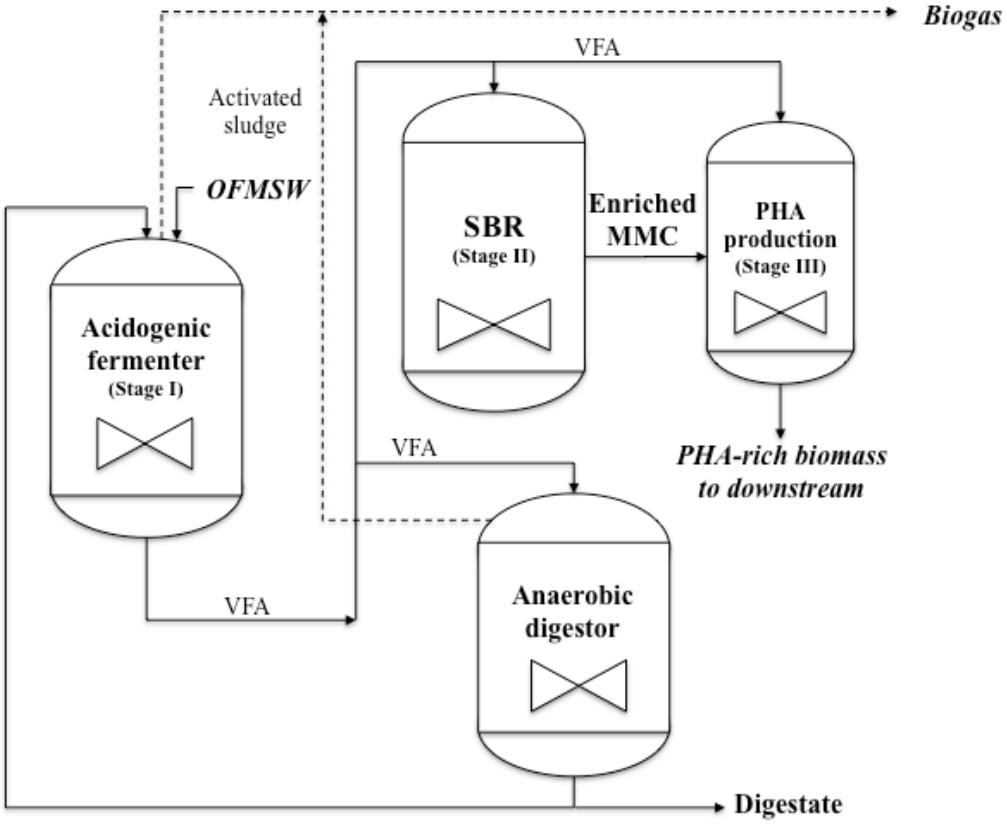

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687 **Figure 2.**
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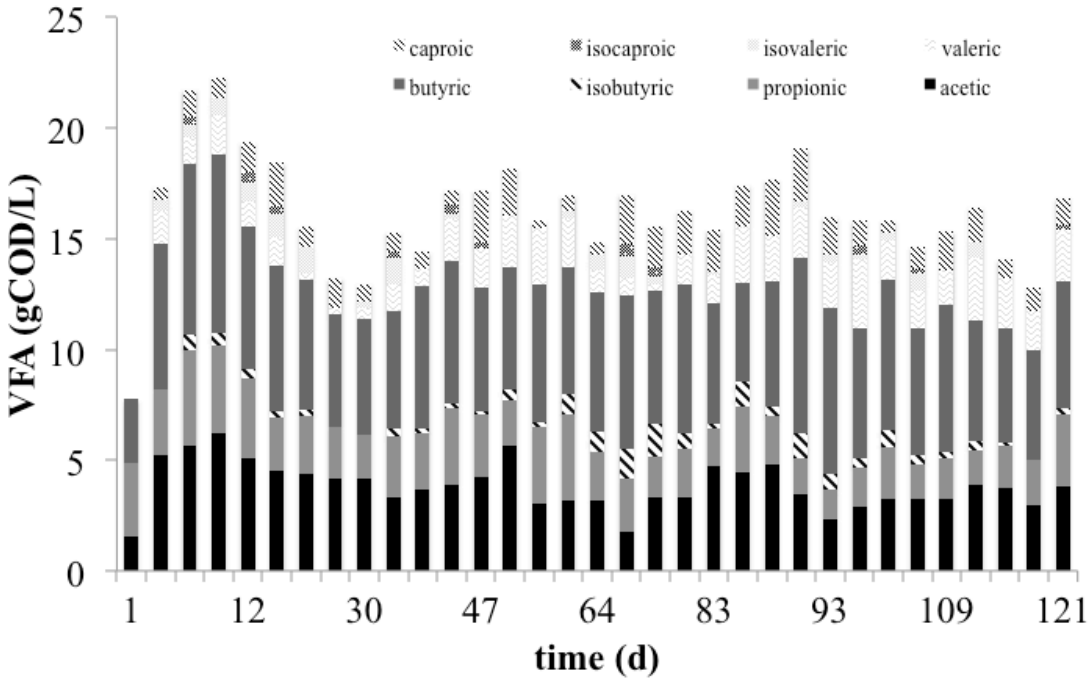

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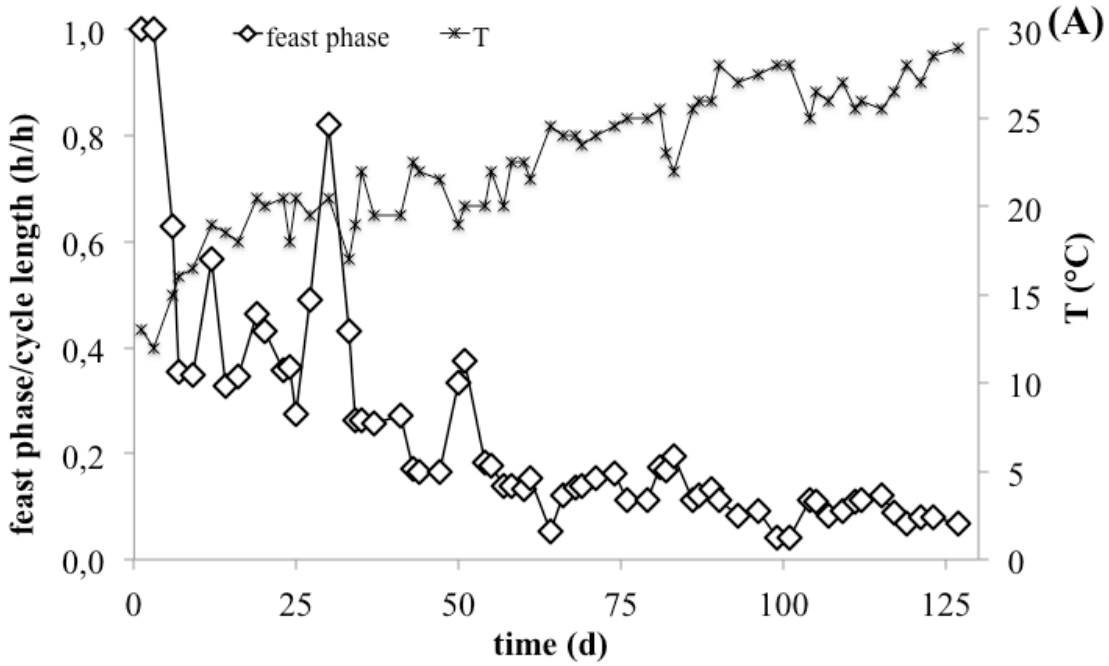

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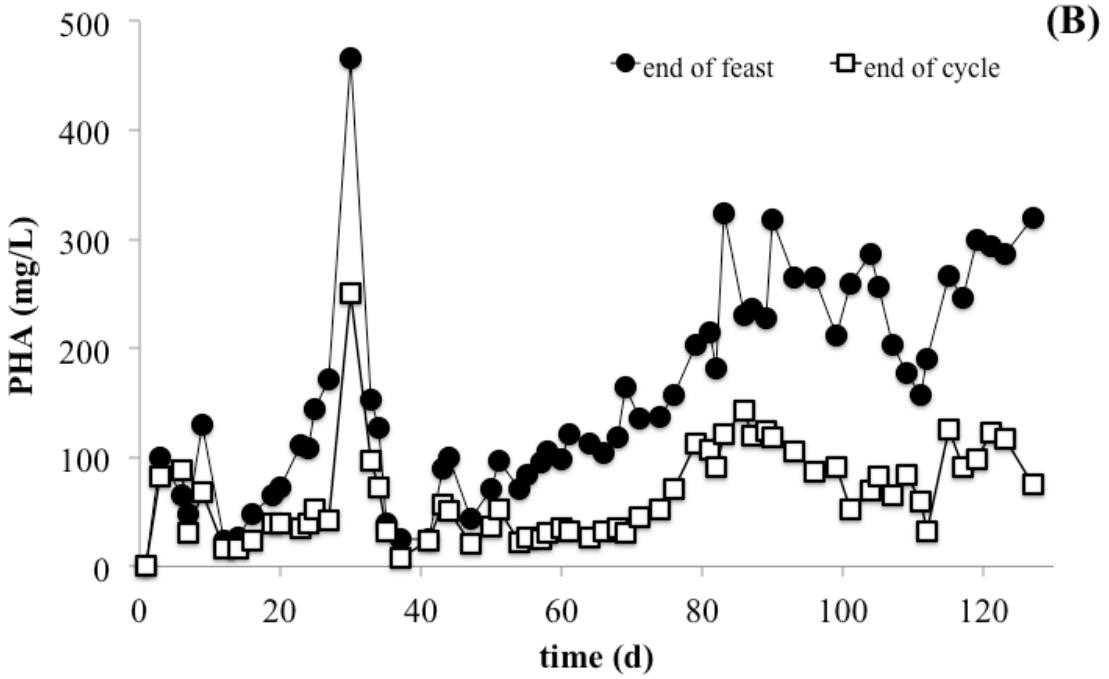

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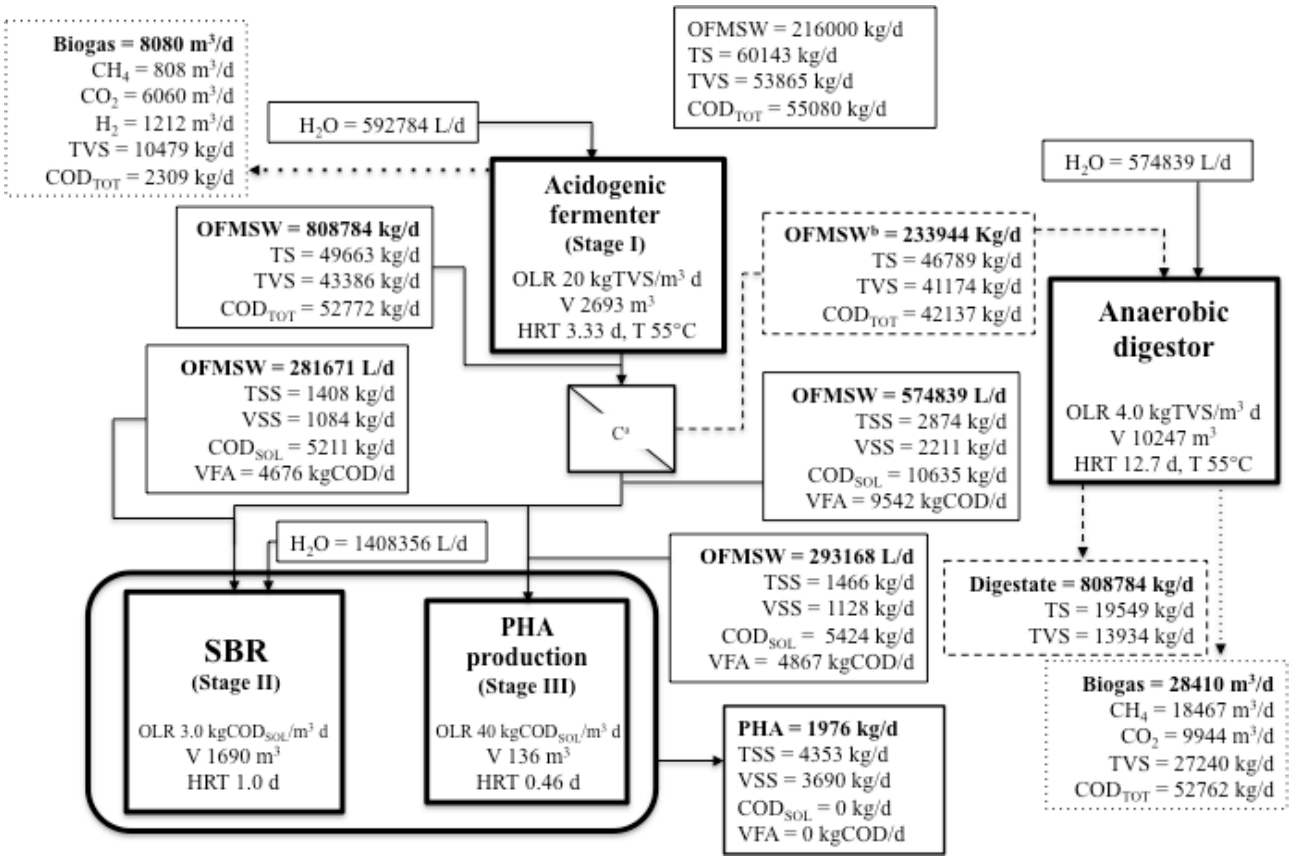
Figure 4.



[a]Coaxial filter-bag equipped centrifuged (solid/liquid separation unit)
[b]Cake

Table 1.

Parameter	Unit	Synthetic acetate solution (days 1st-49th)	Pre-treated fermented OFMSW (days 50st-129th)
VSS (end of feast)	mg/L	1227 ± 144	1405 ± 132
PHA (end of feast)	mg/L	119 ± 55	200 ± 19
PHA (end of cycle)	mg/L	55 ± 23	69 ± 8
HB:HV content (end of feast)	Mass (g) %	100:0	88 ± 1:12 ± 1
Feast phase/cycle length ratio	h/h	0.42 ± 0.07	0.13 ± 0.02
Substrate uptake rate ($-qS^{feast}$)	$mgCOD_{SOL}/gCOD_{Xa}/h$	320 ± 32	578 ± 77
Storage rate (qP^{feast})	$mgCOD_{PHA}/gCOD_{Xa}/h$	46 ± 22	225 ± 33
Storage yield ($Y_{P/S}^{feast}$)	COD_{PHA}/COD_{SOL}	0.16 ± 0.08	0.36 ± 0.04
($Y_{P/VFA}^{feast}$)	COD_{PHA}/COD_{VFA}		0.41 ± 0.05
Observed yield (Y_{OBS}^{SBR})	COD_{VSS}/COD_{SOL}	0.40 ± 0.03	0.59 ± 0.05
Temperature range	°C	10.5 – 22.5	20 – 29.5
pH		7.2-8.2	7.8-8.4
Applied OLR	$gCOD_{SOL}/L\ d$	3.0	2.7 ± 0.2
	$gCOD_{VFA}/L\ d$		2.3 ± 0.2

741 **Table 2.**

Substrate		Unit	Accumulation						
Number			Acetate		OFMSW				
Parameter			1	2	1	2	3	4	5
COD:N:P ratio		Mass basis	100:0:0	100:0:0	100:2.6:0.6	100:3.0:0.7	100:2.6:0.6	100:2.8:0.5	100:2.5:0.5
Temperature		°C	22.5	22-24	24-26	23.5-26	24-26.5	25-27	25-27
pH			7.8-8.5	8.0-8.8	8.0-8.8	7.4-8.7	7.5-8.9	7.4-9.0	7.5-9.0
Feast phase length (SBR cycle)*		h/h	0.13	0.16	0.08	0.11	0.13	0.09	0.11
Accumulation time		h	6	6	4.3	5	6	5.3	6
PHA content		g/gVSS	0.42	0.37	0.52	0.45	0.47	0.39	0.44
Storage yield	$Y_{P/S}^{batch}$	COD_{PHA}/COD_{SOL}	0.64	0.61	0.45	0.39	0.46	0.40	0.47
	$Y_{P/VFA}^{batch}$	COD_{PHA}/COD_{VFA}			0.57	0.49	0.55	0.43	0.50
Growth yield	$Y_{X/S}^{batch}$	COD_{Xa}/COD_{SOL}	0.07	0.09	0.18	0.21	0.21	0.24	0.17
	$Y_{X/VFA}^{batch}$	COD_{Xa}/COD_{VFA}			0.20	0.24	0.25	0.28	0.19
Storage rate (qP^{batch})		$COD_{PHA}/COD_{Xa}/h$	264	232	436	388	255	294	367
Substrate uptake rate ($-qS^{batch}$)		$COD_{SOL}/COD_{Xa}/h$	404	389	746	702	520	501	662
PHA productivity		gPHA/L h	0.18	0.16	0.49	0.40	0.28	0.31	0.36
HB:HV content		Mass%	100:0	100:0	92:8	88:12	87:13	93:7	92:8

742 * corresponding to the SBR cycle in which the biomass was sample

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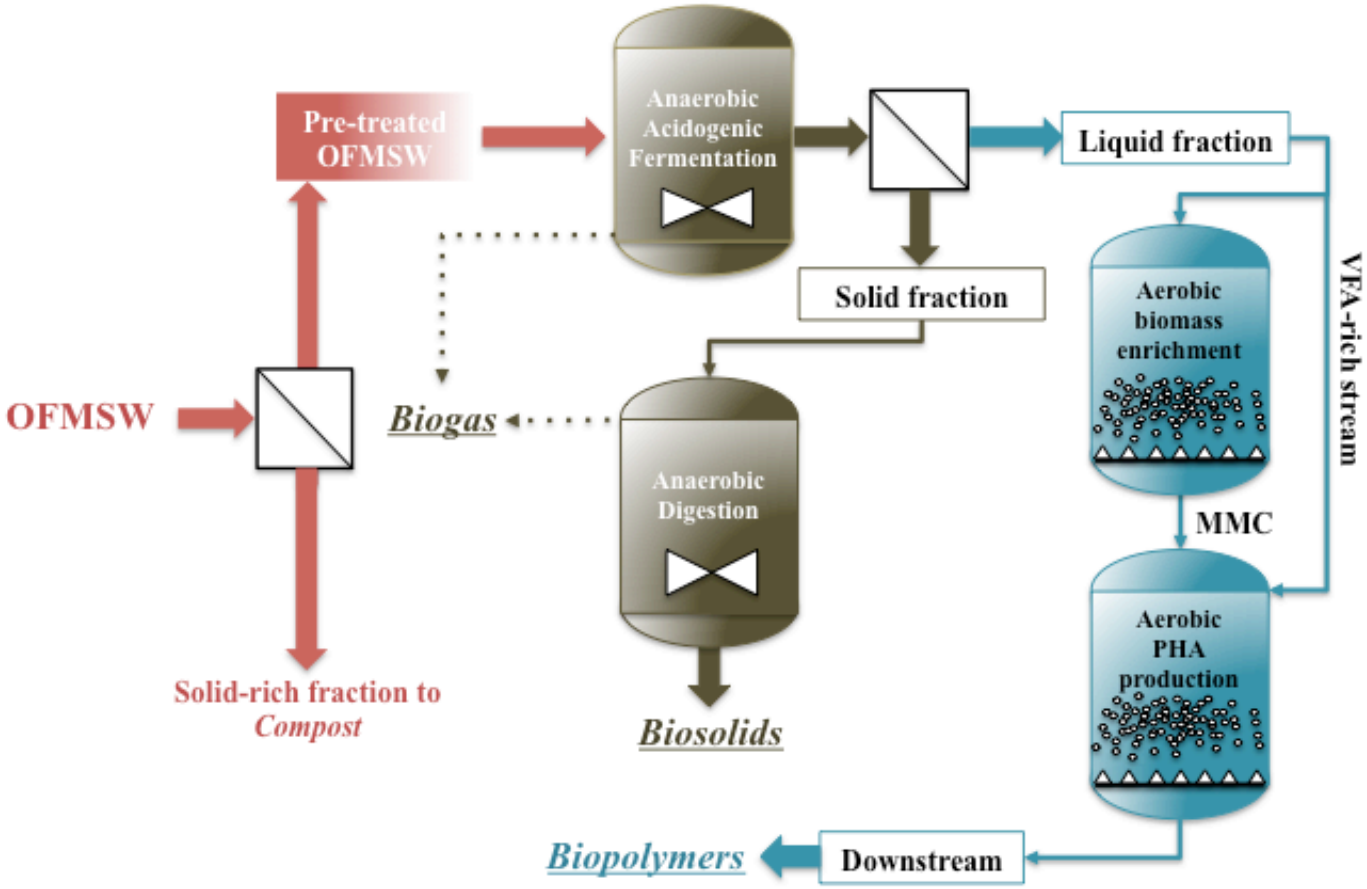
Table 3.

Process	Energy and process units			Unit
CSST	Thermal Energy Required	Digester	Heating Water	173,989 MJ/d
			Thermal Dissipation	10,491 MJ/d
			Total	184,481 MJ/d
	Energy produced from CHP		Thermal	464,828 MJ/d
			Electrical	103.4 MWh/d
Integrated multi-stage process	Thermal Energy Required	Fermentation Reactor	Heating Water	135,358 MJ/d
			Thermal Dispersion	3,282 MJ/d
		Aerobic Reactors (PHA line)	Heating Water	58,926 MJ/d
		Digester	Heating Water	135,358 MJ/d
			Thermal Dispersion	8,000 MJ/d
		Whole platform	Total	340,924 MJ/d
	Energy Produced from CHP		Thermal	438,804 MJ/d
			Electrical	97.6 MWh/d
	Energy consumed for aerobic reactors (PHA line)		Electrical	7.7 MWh/d

Abstract Art



Synopsis

Recovering bio-based products from the conversion of bio-waste organic fraction allows solving open issues about bio-waste treatment and disposal.

Supporting Information

Organic fraction of municipal solid waste recovery and valorisation by conversion into polyhydroxyalkanoates and biogas

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Methods

Analytical method for PHA monomeric composition

For PHA determination, the mixed liquor sample was treated immediately with a NaClO solution with 7% active Cl_2 (1 mL NaClO per 5 mL mixed liquor) and stored at 48°C for the following analysis. PHAs were extracted, hydrolyzed and esterified to 3-hydroxyacyl methyl esters and determined by gas chromatography following the method illustrated by Braunegg et al. (1978).[1] The relative abundance of HB and HV monomers were quantified using a commercial polymer P(3HB-co-3HV) of known HV content as the standard (Poly(3-hydroxybutyric acid-co-3- hydroxyvaleric acid), with a PHV content of 12 wt%, Sigma-Aldrich).

Calculation

For SBR calculation, the observed and storage yield as well as specific rates of storage and substrate were quantified in terms of COD units. Active biomass (or non-PHA biomass) was consistently expressed as X_A=(VSS-PHA)•1.42, by using COD conversion factor of 1.42 gCOD/gX_A. PHA was also converted into COD according to the following oxidation stoichiometry: 1.67gCOD/gHB and 1.92gCOD/gHV monomer. The specific COD_{SOL} removal rate was calculated as ratio between consumed COD_{SOL} (ΔS) and feast phase length (t), per unit of active biomass (X_A): $(-qS^{feast})=\Delta S/(t \bullet X_A)$. Stored polymer ($\Delta$PHA) was calculated as difference between PHA concentration at the end of feast and at the end of cycle; consequently, the specific storage rate was expressed as follows: $(qP^{feast})=\Delta PHA/(t \bullet X_A)$. The storage yield in the feast phase was quantified based on removed COD_{SOL} ($Y_{P/S}^{feast}$) and on removed VFA ($Y_{P/VFA}^{feast}$). Observed yield was calculated between the ratio of VSS concentration and the applied OLR: Y_{OBS}^{SBR}=VSS/(OLR•HRT).

In batch tests, the specific storage rates (qP^{batch}) and substrate consumption ($-qS^{batch}$) were calculated by linear regression of the data versus time by considering the initial period at constant rate (2.5 h approximately). Biomass growth yield was calculated from ammonia consumption by

considering the mean nitrogen content in the biomass (10% as gN/gX_A). Growth yields were calculated as the ratio between the new produced X_A (ΔX_A) and the consumed COD_{SOL} ($Y_{X/S}^{batch}$) or VFA ($Y_{X/VFA}^{batch}$) as given in the following equations: $Y_{X/S}^{batch}=\Delta X_A/\Delta S$; $Y_{X/VFA}^{batch}=\Delta X_A/\Delta VFA$.

The maximum polymer content in the biomass was given by the ratio between PHA and VSS concentrations at the end of each accumulation test: %PHA = PHA/VSS = PHA/(XA+PHA).

For the final energy balance, the parameters and boundary conditions (e.g. temperature of water and air, etc.) that have been used are reported in Table S1.

Table S1

Results and Discussions

In the PHA accumulations with fermented OFMSW, a maximum plateau of PHA content was not always reached since growth and storage response simultaneously occurred. However, PHA contents were similar or even higher than those obtained in acetate accumulations, where both PHA concentration and PHA biomass content did not increase linearly, especially after 3 h of accumulation.

The following figure S1 illustrates the trends of PHA, X_A, VFA concentration and PHA biomass content in two representative batches carried out with synthetic acetate solution (A) and fermented OFMSW (B).

Figure S1

The following figures S2-S5 illustrate microbial population (bacteria and archea, figure S2) selected in the SBR with particular emphasis to the composition of total bacteria (figure S3) and β-proteobacteria (figure S4, S5).

Figure S2

Figure S3

Figure S4

Figure S5

Figure and Table captions

Figure S1. Concentration trends of PHA, VFA and active biomass together with PHA biomass content in two explicative examples of accumulation test performed with synthetic acetate solution (A) and fermented OFMSW (B) (Stage III).

Figure S2. Relative abundance of total bacteria and archaea estimated in selected samples taken in SBR during acetate (sampling days: 1 and 28) and fermented OFMSW feeding (sampling days: 58, 62, 91 and 108).

Figure S3. Composition of total bacteria estimated by FISH analysis with group specific oligonucleotide probes in selected samples throughout the reactor operation.

Figure S4. Relative abundance of *Betaproteobacteria* and *Thauer*a/*Azoarcus* estimated by FISH analysis at different sampling times (days) over the reactor operation.

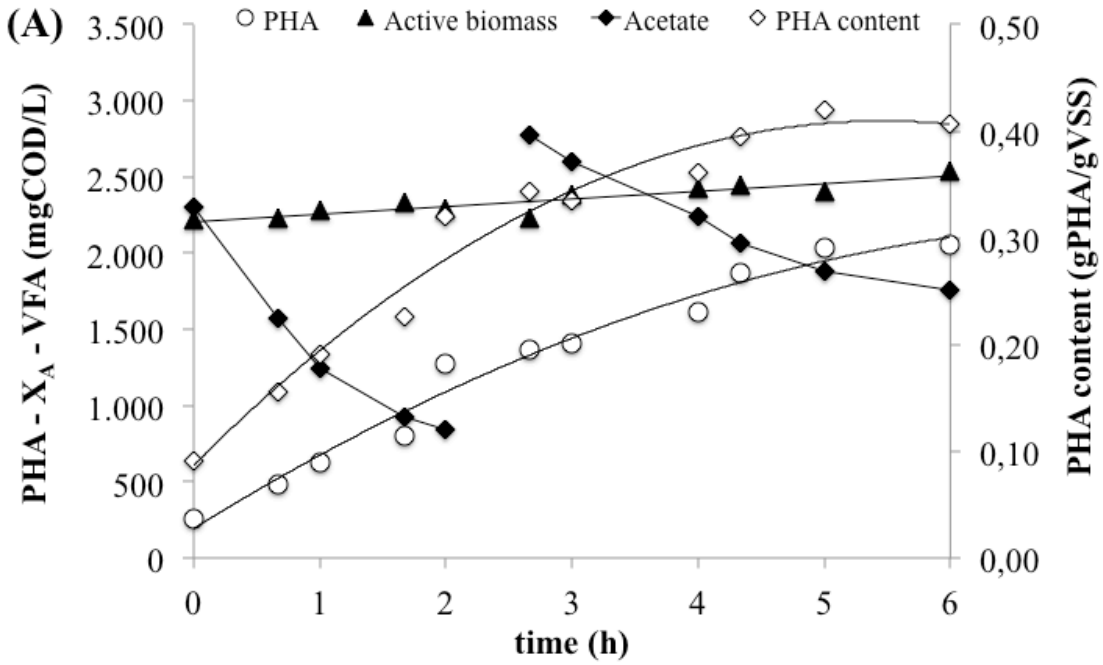
Figure S5. Affiliation of the main OTUs belonging to A) *betaproteobacteria* and B) *alphaproteobacteria* estimated by Illumina sequencing in samples taken in SBR during fermented OFMSW feeding (sampling days: 91, 108 and 119).

Table S1. Reference parameters and boundary conditions for energy balance

Table S1

Parameter	Unit	Value
	BIOGAS[2]	
Low Heat Value Biogas	kJ/Nm3	23,012
	COMBINED HEAT AND POWER (CHP)[2]	
Termical Energy yield	-	0.5
Electrical Energy yield	-	0.4
Total Energy yield		0.9
	BOUNDARY CONDITIONS[2]	
Operative Temperature Anaerobic Processes	°C	55
Operative Temperature Aerobic Processes	°C	25
Water Temperature	°C	15
Air Temperature	°C	20
Ground Temperature	°C	25
	HEAT TRANSFER COEFFICIENT[3]	
Outer Concrete Reactor Wall	W/(m^2°C)	0.7
Inner Concrete Reactor Wall	W/(m^2°C)	1.2
Floor	W/(m^2°C)	2.85
	AERATION SYSTEM[3]	
Electric Motor Adsorbed Power	kWh/kgCOD rimoso	0.753

150 **Figure S1**



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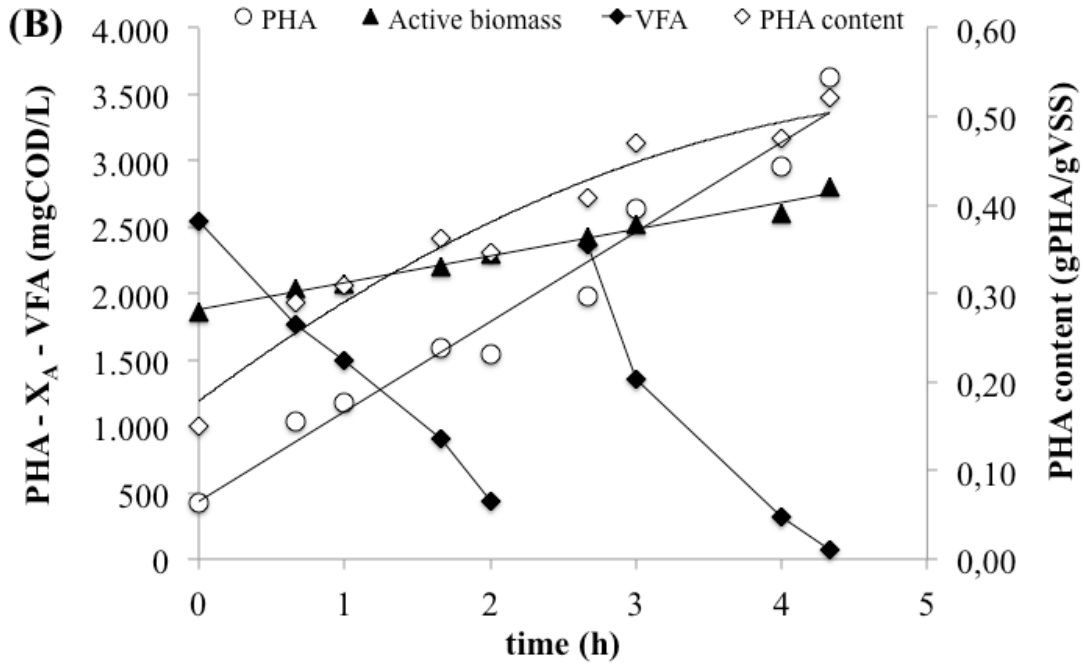

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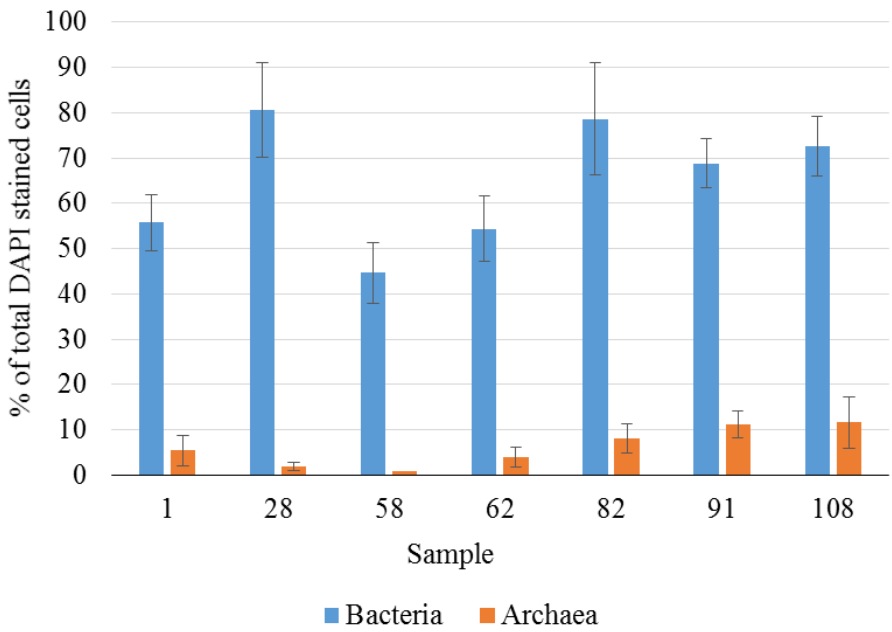
164 **Figure S2**



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166 **Figure S3**

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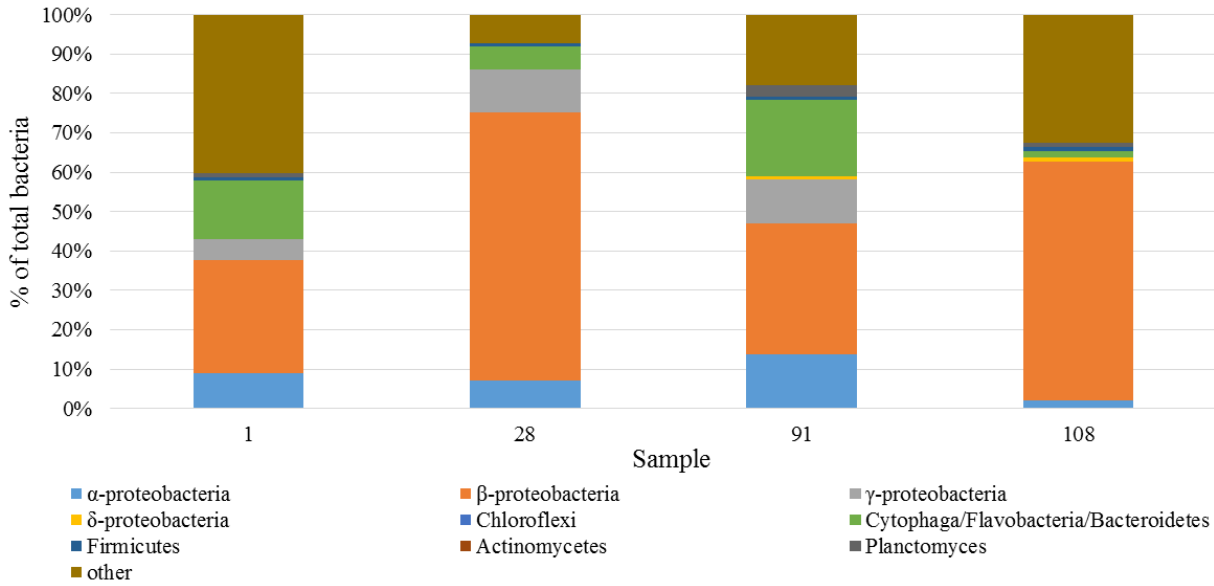

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181 **Figure S4**
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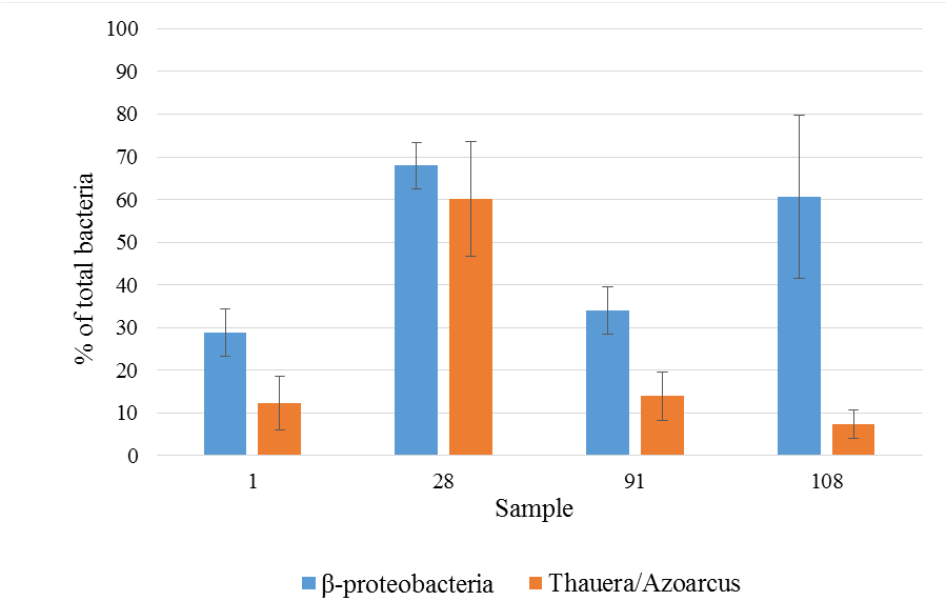

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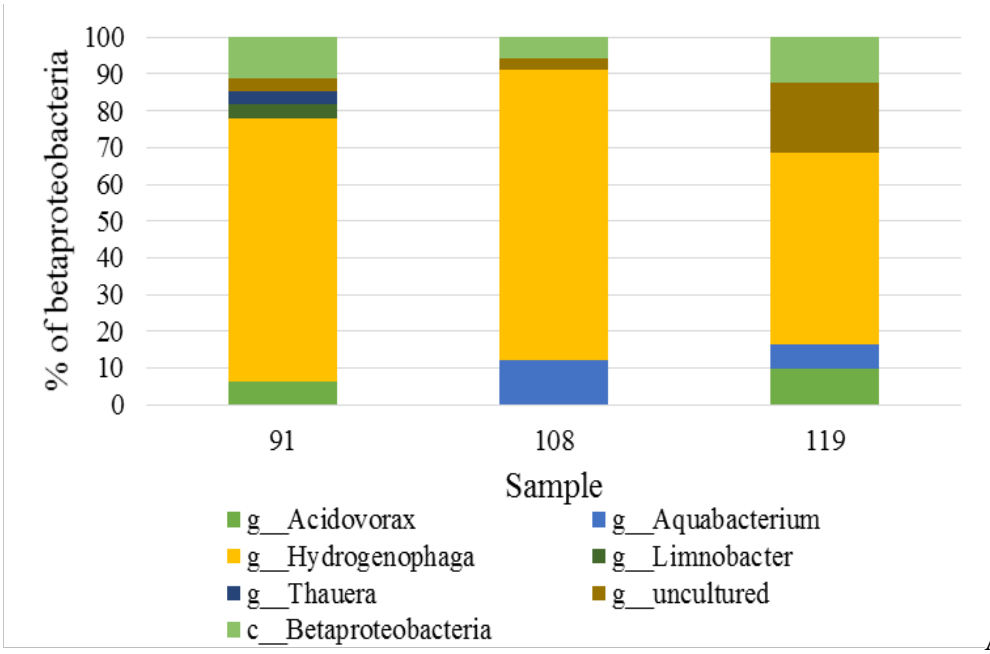
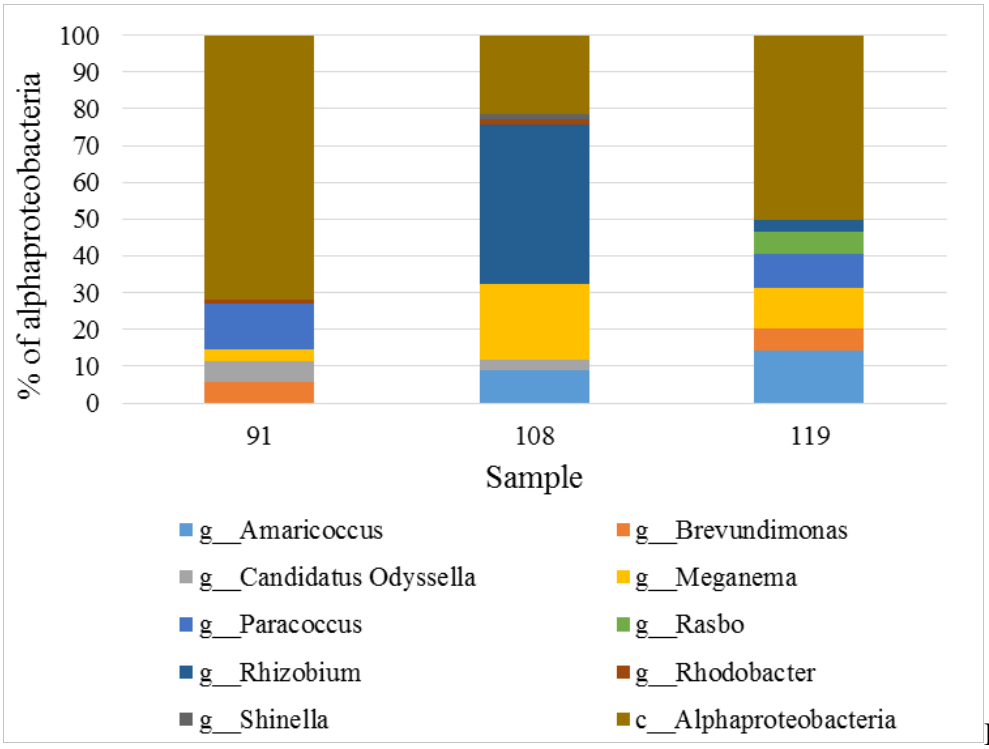
Figure S5


% of betaproteobacteria
100
90
80
70
60
50
40
30
20
10
0
91
108
119
Sample
g__Acidovorax
g__Aquabacterium
g__Hydrogenophaga
g__Limnobacter
g__Thauera
g__uncultured
c__Betaproteobacteria

A)


% of alphaproteobacteria
100
90
80
70
60
50
40
30
20
10
0
91
108
119
Sample
g__Amaricoccus
g__Brevundimonas
g__Candidatus Odyssella
g__Meganema
g__Paracoccus
g__Rasbo
g__Rhizobium
g__Rhodobacter
g__Shinella
c__Alphaproteobacteria

B)

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