SUPPORTING INFORMATION

Conversion of Pyrolysis Products into Volatile Fatty Acids with a Biochar-Packed Anaerobic Bioreactor

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1 Supporting Methodological Details

Pyrolysis Set-Up:

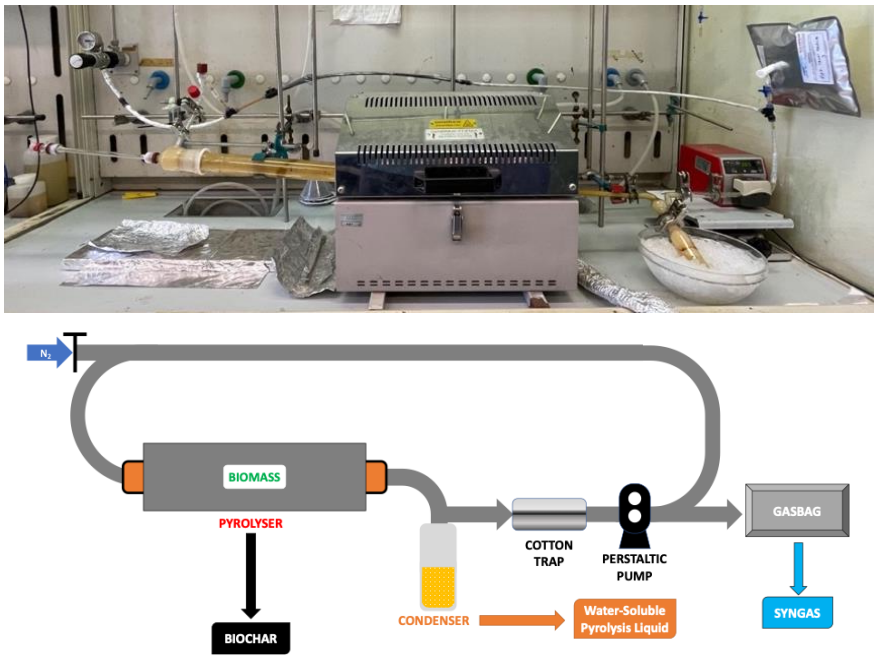

Figure S. 1: Pyrolysis set-up used in the study (above) and the flow-diagram (below).

Detailed Procedure For Pyrolysis:

A series of sequential pyrolyzes were done prior to the fermentation study and all obtained WS and syngas were stored under proper conditions throughout the biological study. About 5.0 grams of dry biomass were pyrolyzed at intermediate pyrolysis conditions for 30 minutes residence time at each batch run of the pyrolysis reactor (**Figure S.1)**. Constant temperature (550 °C) was maintained in the horizontal lab-scale tubular furnace. About 10 L of Nitrogen gas (N_2) was initially provided, at 1 L/min rate for 10 min, to purge the air from the pyrolysis system. Subsequently all available gas was continuously recirculated by a peristaltic pump (at 100 mL/min flow-rate) to avoid dilution of the produced syngas components during the pyrolysis of biomass. One impinger containing 50 mL of distilled water was connected and placed inside an ice-bucket to the outlet of the quartz reactor. In this way, all the water-soluble condensable part of pyrolysis products (i.e. aqueous pyrolysis liquid, APL) was collected inside the water trap and distinguished from acetone-soluble pyrolytic lignin portion which was named as water-insoluble fraction. A cotton trap was placed just after the water-trap to capture the fine aerosols. Gaseous pyrolysis products (syngas) were collected inside a laminated foil gasbag. The solid carbonaceous fraction (biochar) was collected at the end of each pyrolysis.

Medium Composition:

Table S.1: Cultivation medium.

Chemical Compounds	Molecular Formula	Concentration (g/L)
Ammonium chloride	NH4Cl	13.425
Potassium dihydrogen phosphate	KH2PO4	7.815
Sodium chloride	NaCl	2.919
Sodium sulfate decahydrate	Na2SO4*10H2O	0.573
Magnesium chloride hexahydrate	MgCl2*6H2O	1.201
Ferrous sulfate heptahydrate	FeSO4*7H2O	0.031
Calcium chloride	CaCl2	0.006
Tri-tert-butyl borate	H3BO4	0.001
Sodium molybdate dihydrate	Na2MoO4*2H2O	0.001
Zinc sulfate heptahydrate	ZnSO4*7H2O	0.032
Cobalt (II) chloride monohydrate	CoCl2*H2O	0.009
Copper (II) chloride dihydrate	CuCl2*2H2O	0.022
Manganese (II) chloride tetrahydrate	MnCl2*4H2O	0.025
Nickel (II) chloride hexahydrate	NiCl2*6H2O	0.005
EDTA	C10H16N2O8	0.500

2 Formulas and Calculations

In this section all the calculation methods used in this study will be explained in detail with corresponding formulas. Each equation will be followed by its unit-based formulation to provide a clear presentation. First formula is related to medium concentration level calculation:

$$20.0\frac{\text{gCOD}}{\text{Liters}}\ \text{x}\frac{15\ \text{gCOD Microbial Mass}}{100\ \text{gCOD}}\ \text{x}\ \frac{5\ \text{g Nitrogen}}{100\ \text{g Microbial Mass}} = \frac{0.15\ \text{g Nitrogen}}{L}$$

$$\frac{0.15\ \text{g Nitrogen}}{L}\ \text{x}\ 53.5\ \frac{\text{g}\ NH_4Cl}{\text{mole}\ NH_4Cl}\ \text{x}\left(14.0\ \frac{\text{g Nitrogen}}{\text{mole}\ NH_4Cl}\right)^{-1} = \frac{0.57\ \text{g}\ NH_4Cl}{L}$$

$$\frac{0.57\ \text{g}\ NH_4Cl}{L}\ \text{x}\left(13.425\ \frac{\text{g}\ NH_4Cl}{\text{Liters medium}}\right)^{-1} \cong \frac{42.5\ \text{mL Medium}}{L}$$

(1)

Hydraulic retention time (HRT) of the continuous reactor operation was calculated as follow, where [V_{Liq}] corresponding the total wet-volume (i.e. active volume) of the bioreactor set-up, and [Q_{Liquid}] as the daily liquid feeding/discharging rate:

$$\mathbf{HRT} = \frac{\boldsymbol{V_{Liq}}}{\boldsymbol{Q_{Liquid}}}$$

$$days = \frac{mL}{\frac{mL}{day}}$$

(2)

Organic loading rate (OLR) were calculated as follow, where [COD_X] as the measured COD concentration of each substrate material and [$\%_X$] as the substrate ratio depending on the feeding regime.

$$\mathbf{OLR} = \left[\left(\mathbf{COD_{Aq-Oil}} \times \%_{\mathbf{Aq-Oil}}\right) + \left(\mathbf{COD_{GLU}} \times \%_{\mathbf{GLU}}\right) + \left(\mathbf{COD_{CH_4}} \times \%_{\mathbf{CH_4}}\right)\right] \times (\boldsymbol{HRT})^{-1}$$

$$\frac{gCOD}{L \cdot day} = \left[\left(\frac{gCOD}{L} \times \%\right) + \left(\frac{gCOD}{L} \times \%\right) + \left(\frac{gCOD}{L} \times \%\right)\right] \times (days)^{-1}$$

(3)

COD concentration of the gas input (syngas) and gas output (biogas) were calculated as follows, where [COD_{GAS}] as the overall COD concentration of the gaseous mixture, [C_X] as the percent concentrations of each gas component measured by GC-TCD, and [COD_X] as the COD constant of each gas component in g-COD/L unit (e.g. H_2: 0.71 , CO: 0.71, CH_4: 2.85).

$$\mathbf{COD_{GAS}} = \left(\mathbf{C_{H_2}} \times \mathbf{COD_{H_2}}\right) + \left(\mathbf{C_{CO}} \times \mathbf{COD_{CO}}\right) + \left(\mathbf{C_{CH_4}} \times \mathbf{COD_{CH_4}}\right) \quad \textbf{(4)}$$

$$\frac{gCOD}{L} = \left(\% \times \frac{gCOD}{L}\right) + \left(\% \times \frac{gCOD}{L}\right) + \left(\% \times \frac{gCOD}{L}\right)$$

Total COD concentration of VFAs in the WS and fermentation effluents were calculated by below equation, where [COD_{VFA}] as the overall COD concentration of the liquid sample, [C_X] as the concentration of each VFA component measured by GC-MS, and [COD_X] as the COD constant of each VFA component in g-COD/g unit (e.g. Acetic: 1.1 , Propionic: 1.5 , Caproic: 2.2).

$$\mathbf{COD_{VFA}} = \left(\mathbf{C_{Acetic}} \times \mathbf{COD_{Acetic}}\right) + \left(\mathbf{C_{Propionic}} \times \mathbf{COD_{Propionic}}\right) + \cdots \quad \textbf{(5)}$$

$$\frac{gCOD}{L} = \left(\frac{g}{L} \times \frac{gCOD}{g}\right) + \left(\frac{g}{L} \times \frac{gCOD}{g}\right) + \cdots + \left(\frac{g}{L} \times \frac{gCOD}{g}\right)$$

Total input [M_{In}] values were calculated by following equation, where [$t_{Experiment}$] represents the total duration of the experiment, and [Σt_{Batch}] corresponds the duration of the batch mode operation when no feeding was provided to the bioreactor system:

$$\mathbf{M_{IN}} = \left[\mathbf{OLR} \times \left(\boldsymbol{t_{Experiment}} - \sum \boldsymbol{t_{Batch}}\right)\right] \quad \textbf{(6)}$$

$$gCOD = \left[\left(\frac{gCOD}{L - day}\right) \times \left(days - \sum days\right)\right]$$

Total output [M_{Out}] value which is corresponding the sum of the removal of both gas and liquid materials in line with the principle of continuous operation were calculated by the following equation **(7)**. [$C_{Liq\text{-}Out}$] is the measured COD concentration of the effluent liquid and [$V_{Liq\text{-}Out}$] is the amount of discharged liquid at its corresponding day, while [$C_{Biogas\text{-}Out}$] as the measured concentration of the bioreactor system's off-gas and [$V_{Biogas\text{-}Out}$] is the total volume of the discharged gas on that day.

$$\mathbf{M_{OUT}} = \sum \left[\left(\boldsymbol{C_{Liq-Out}} \times \boldsymbol{V_{Liq-Out}}\right) + \left(\boldsymbol{C_{Biogas-Out}} \times \boldsymbol{V_{Biogas-Out}}\right)\right] \quad \textbf{(7)}$$

$$gCOD = \sum \left[\left(\frac{gCOD}{L} \times L\right) + \left(\frac{gCOD}{L} \times L\right)\right]$$

COD recovery as an indicator parameter is included to the calculations for showing the COD balance efficiency of the experiment which takes into account of 'Total Input' and 'Total Output' parameters. Given the fact that the COD trapped inside the packed-bed was not monitored, this definition does not fully correspond the total COD balance, yet it still provides beneficial information about the recovered overall materials in terms of COD.

$$\mathbf{COD\ Recovery} = \frac{\mathbf{M_{OUT}}}{\mathbf{M_{IN}}} \mathbf{x\ 100} \quad \textbf{(8)}$$

$$\% = \frac{gCOD}{gCOD} \times 100$$

Daily net VFA [L_{VFA}] production has found a critical monitoring parameter by the authors, since it provides a direct tool to observe the target products' productivity and estimated by the following equation. In the formula, [$Cvfa_T$] represents the current (last) measured COD-eq VFA concentration, [$Cvfa_{T\text{-}1}$] is the one previous VFA measurement and [$V_{Effluent}$] is the discharged amount of liquid from the bioreactor which is basically based on the HRT.

$$\boldsymbol{L_{VFA_{DAY-T}}} = \left[\left(\boldsymbol{V_{Liq}} \times \left(\boldsymbol{C_{VFA_T}} - \boldsymbol{C_{VFA_{T-1}}}\right)\right) + \left(\boldsymbol{C_{VFA_{T-1}}} \times \boldsymbol{V_{Effluent}}\right)\right] \quad \textbf{(9)}$$

$$\frac{gCOD}{day} = \left[\left(L\left(\frac{gCOD}{L} - \frac{gCOD}{L}\right)\right) + \left(\frac{gCOD}{L} \times L\right)\right]$$

Total produced net VFA [M_{VFA}] amount is also estimated for each set or phase of experiment to calculate further critical parameters such as VFA productivity and VFA yield. This defined parameter is calculated by following the next equation.

$$\mathbf{M_{VFA}} = \sum[(\boldsymbol{L_{VFA_{DAY1}}}) + (\boldsymbol{L_{VFA_{DAY2}}}) + \cdots + (\boldsymbol{L_{VFA_{DAYn}}})]$$

(10)

$$gCOD = \sum\left[\left(\frac{gCOD}{day}\right) + \left(\frac{gCOD}{day}\right) + \cdots + \left(\frac{gCOD}{day}\right)\right]$$

Volumetric productivity [Q_P] is defined directly based on the net VFA production and estimated by the following equation.

$$\mathbf{Q_P} = \frac{\mathbf{M_{VFA}}}{\boldsymbol{t_{Experiment}} \times \boldsymbol{V_{Liq}}}$$

(11)

$$\frac{gCOD}{L - day} = \frac{gCOD}{day \times L}$$

One another critical parameter for the performance evaluation of the target products is the net VFA yield [ε_{VFA}] which is calculated by this following equation.

$$\boldsymbol{\varepsilon_{VFA}} = \frac{\mathbf{M_{VFA}}}{\mathbf{M_{OUT}}} \times \mathbf{100}$$

(12)

$$\% = \frac{gCOD}{gCOD} \times 100$$

Formulas related to overall COD mass balance estimations will be presented in the following equations. Total adsorbed organic material [A] in terms of COD was based on a measurement. It is simply calculated by the total COD mass detected by subsequential dual washing of packing-bed with excess amount of distilled water **(13)**. While microbial growth [*l*] is a hypothetical estimation value, defined by the difference between the total input and the sum of total output and adsorbed material **(14)**. Lastly, another hypothetic parameter was defined to estimate unreacted portion (ω) of substrates **(15)**.

$$\boldsymbol{A} = (\mathbf{C_{Wash1}} \times \mathbf{V_{Wash1}}) + (\mathbf{C_{Wash2}} \times \mathbf{V_{Wash2}})$$

(13)

$$gCOD = \left[\left(\frac{gCOD}{L} \times L\right) + \left(\frac{gCOD}{L} \times L\right)\right]$$

$$\boldsymbol{l}\ (\mathbf{gCOD}) = \boldsymbol{M_{IN}} - (\boldsymbol{M_{OUT}} + \boldsymbol{A})$$

(14)

$$gCOD = [gCOD - (gCOD + gCOD)]$$

$$\boldsymbol{\omega}\ (\mathbf{gCOD}) = \sum(\boldsymbol{C_{Liq-Out}} \times \boldsymbol{V_{Liq-Out}}) - \boldsymbol{M_{VFA}}$$

(15)

$$gCOD = \sum\left[\left(\frac{gCOD}{L} \times L\right) + \left(\frac{gCOD}{L} \times L\right)\right] - gCOD$$

3 WS Mono-Substrate Fermentation (Preliminary Tests)

As mentioned in the introduction and confirmed by several preliminary fermentation tests, the most challenging part aspect of PyP fermentation is the strong inhibition arising from WS's phenols and furans (Facchin 2021; Torri and Fabbri 2014). For this reason, in this set of preliminary tests, WS was used as a solo substrate material to investigate its acidogenic bioconversion capability in presence of biochar (Active Reactor) and without biochar (Control Reactor).

To investigate the effect of lower OLR on the inhibition effect (**Table S.2**), the influent concentration level [$C_{SUBSTRATE}$] was kept constant at 5 g-COD/L during the whole test, while decreasing OLR from 0.50 to 0.25 g-COD/L-day in the 2nd half of the test (Phase II). Each phase of the tests has ended up with a batch period which is shown on the profile graphs. Consequential to the

complete inhibition of MMC, detected as accumulation of GC-MS detectable WS constituents, the fermenters were switched to batch mode phases (**Figure S.5**) until complete levoglucosan biodegradation was back detected.

Table S.2: An overall summary data of the preliminary mono-substrate fermentation tests

Parameters		Phase I	Phase II	Phase I	Phase II
Reactor Type		Control Bioreactor		Active Bioreactor	
Packing Material		Only Glassbeads		Biochar + Glassbead	
$C_{SUBSTRATE}$	*(g-COD/L)*	5,0		5,0	
OLR	*(g-COD/L-day)*	0,50	0,25	0,50	0,25
HRT	*(days)*	10,0	20,0	10,0	20,0
Operational Time	*(days)*	52	31	38	45
Total Input [M_{IN}]	*(g-COD)*	3,0	1,1	3,0	1,8
Total Output [M_{OUT}]	*(g-COD)*	2,8	1,1	2,8	2,0
COD Recovery	*(%)*	95%		101%	
Produced VFA [M_{VFA}]	*(g-COD)*	1,0		2,0	
VFA Productivity [Q_P]	*(g-COD/L-day)*	0,06		0,12	
VFA Yield [ε_{VFA}]	*(%)*	27%		41%	

First weeks of operation at the active reactor, total VFA were found quite stable over 4 g-COD.L^{-1}. However, an inhibition was occurred and VFA values approximately halved. Whenever the continuous feeding has stopped and the first batch mode started after the 3rd week of continuous operation, VFA values were started to increase back and reached to the 3 g-COD.L^{-1} (**Figure S. 2b**). In the meantime, all the available levoglucosan content was completely degraded in a very short time (**Figure S.4**). This was showing that toxification of MMC due to the WS's components was not irreversible. Later on, HRT was doubled from 10 days to 20 days with the same feeding concentration, meaning that OLR was halved. Interestingly, VFA values have started to decrease again immediately after, even though the levoglucosan and mannosan levels were still quite low which was indicating a continuous upgrade of the WS molecules. This phenomenon might be explained by another reason rather than inhibition by WS components, which could be the insufficient nutrition amount due to the extremely low OLR. A clear outcome is, lowering the OLR can be a solution of inhibitory effects of WS, yet is not productive. In case of the experiments without biochar (control), a similar situation was observed in terms of general trend of overall VFA amounts. However, considerably lower VFA values were monitored throughout the experiments as compared to active reactor (**Figure S. 2a**). In addition, levoglucosan levels were higher during Phase-I with a higher OLR. In contrast to the active reactor, detoxification of MMC appearing in the control reactor has taken longer times during the first batch period (**Figure S. 5**). In **Table S.2**, COD based estimations were presented to reveal an overall performance of the mono-substrate tests. COD recovery parameter **(8)** was found critical for this closed loop anaerobic system where all input and output materials should be identical in terms of total COD since there is no oxidative agent that can consume COD. In this matter, both tests were shown an extraordinary performance and resulted in COD recoveries over 95%. In case of VFA production performances of the tests, active reactor with biochar has ended up with a double Q_P and considerably higher ε_{VFA} values.

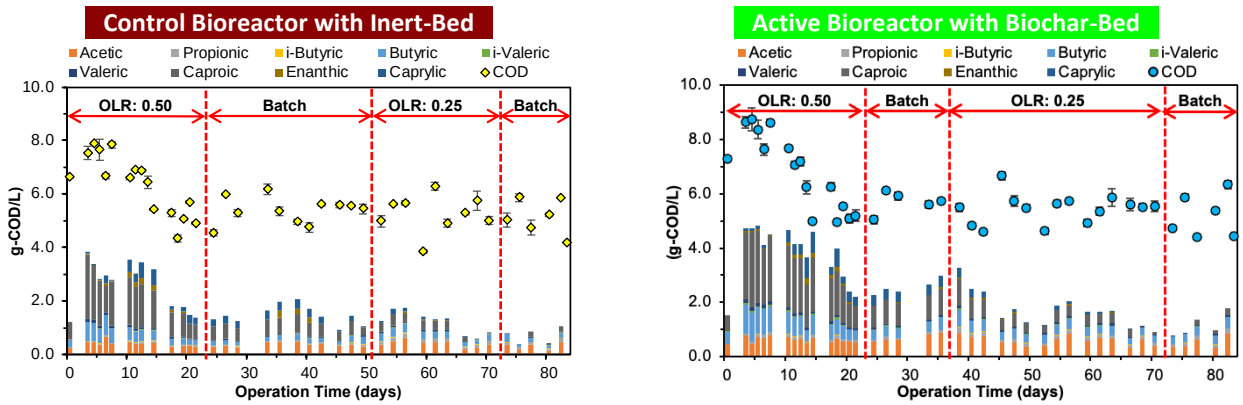

Figure S. 2: Composition of available VFA types and COD profile

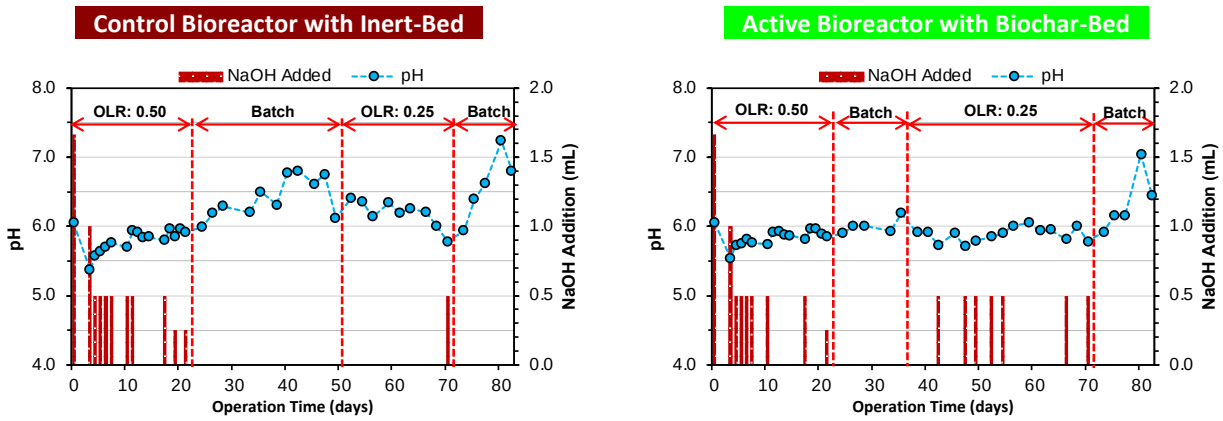

Figure S. 3: Alkaline additions and pH profile

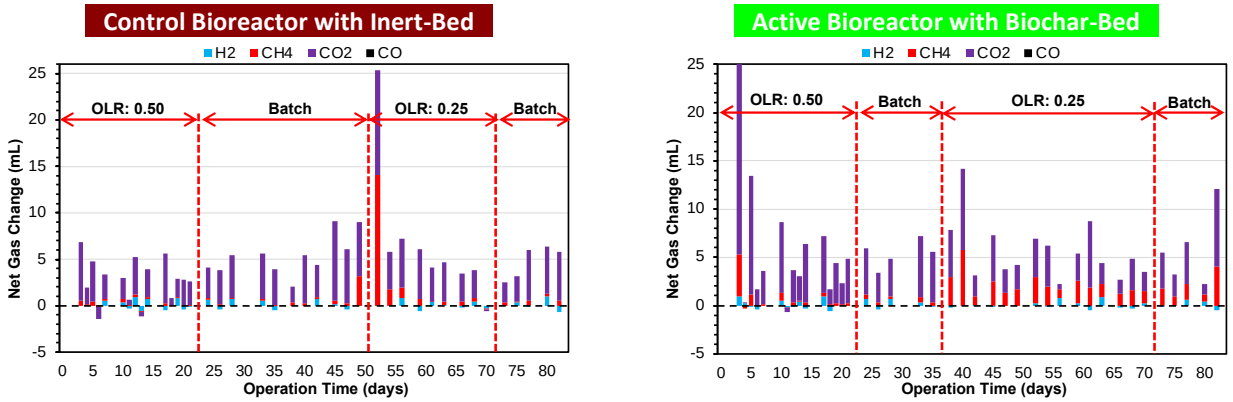

Figure S. 4: Produced and consumed net gas amounts

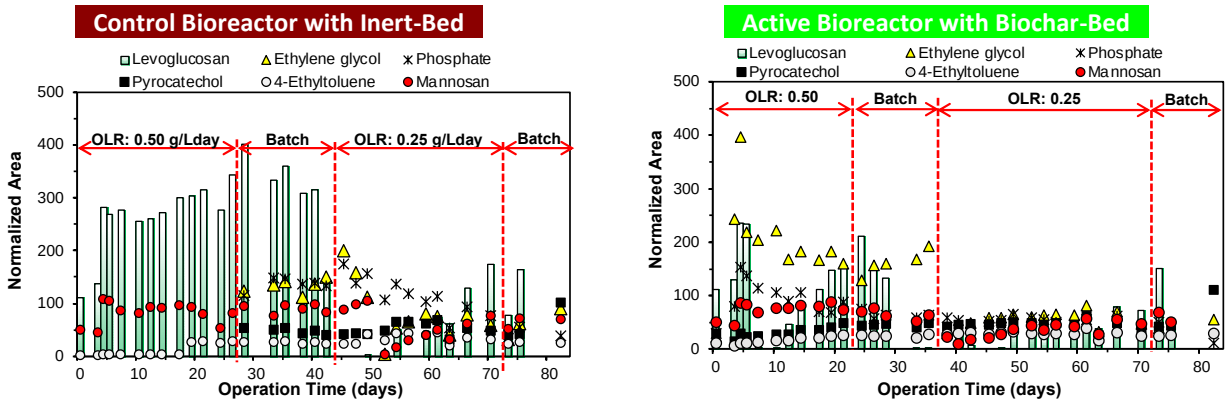

Figure S. 5: Profile of the selected PyP molecules via silylation analysis

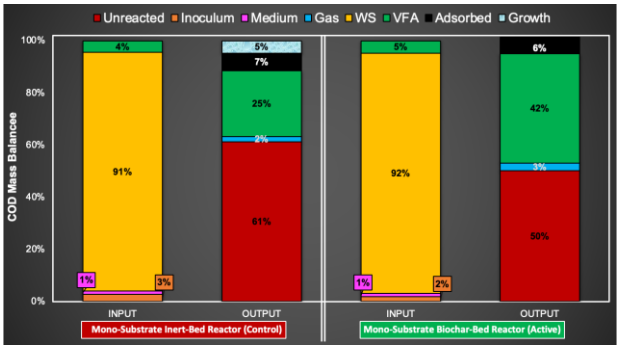

Figure S. 6: Overall COD balance by percentage for mono-substrate tests

4 Additional Information on the Co-fermentation Experiment

Table S.3: Overall balances of different phases of acclimatization experiment

Parameters		Phase I	Phase II	Phase III	Phase IV	Phase V	Phase VI	Overall
Total Input [M_{IN}]	*(g-COD)*	5,0	3,9	3,5	2,2	2,6	1,1	24,1
Total Output [M_{OUT}]	*(g-COD)*	3,9	3,7	3,8	2,3	2,4	1,1	19,8
COD Recovery	*(%)*	77%	93%	108%	104%	90%	96%	82%
Produced VFA [M_{VFA}]	*(g-COD)*	2,2	1,6	1,8	1,5	0,7	0,5	8,9
VFA Productivity [Q_P]	*(g-COD/L-day)*	0,35	0,39	0,49	0,56	0,28	0,34	0,39
VFA Yield [ε_{VFA}]	*(%)*	58%	43%	46%	63%	31%	44%	45%

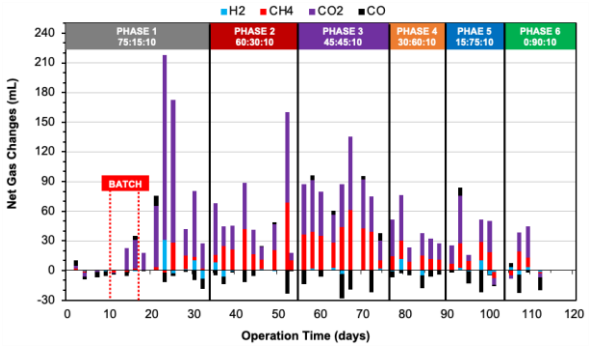

Figure S. 7: Produced and consumed net gas amounts

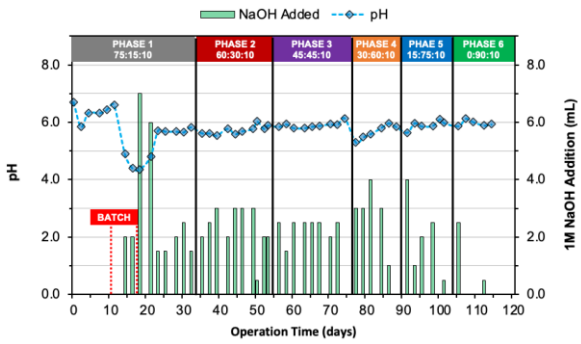

Figure S. 8: pH profile and NaOH additions

5 SEM of Biochar Packing Material

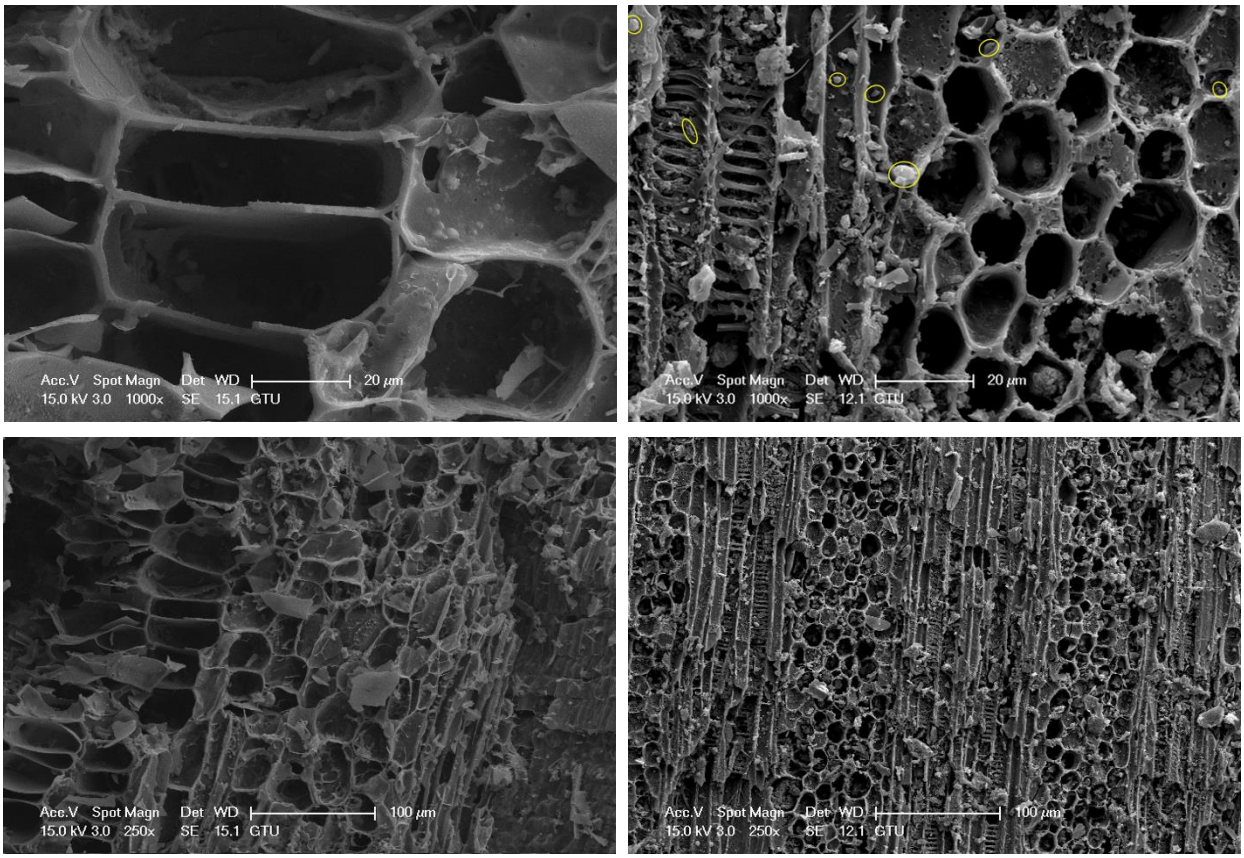

Figure S. 9: SEM images of the biochar grains: images before the application (clean) on the left-side, and images after the application (microbially-dirtied) as a packing material on the right-side.