Table S1. Relevant Deviations Matrix (RDM) listing the possible combinations of guidewords and process parameters to be used in BioHazop study.

RELEVANT DEVIATIONS MATRIX																				
Parameters																				
	Hydrolysis reactor								CSTR											
Guidewords	Flow (OFMSW)	Flow (sludge)	Composition (of feedstock mixture)	Temperature (thermal pre-treatment)	Duration (thermal pre-treatment)	Mixing		Flow (inlet mixture)	Temperature (mesophilic fermentation)	Pressure	HRT (Residence time of substrate)	TVS/TS (total solids) ratio	pH (of the substrate)	Mixing	Volume	COD_{VFA}/COD_{SOL} ratio	Composition of fermented mixture (liquid phase)	Composition of fermented mixture	
more	X	X						X												
no/less	X	X						X												
reverse	X	X						X												
lower				X					X	X	X	X	X		X	X				
higher				X					X	X	X	X	X		X	X				
other			X																	
shorter					X															
longer					X															
more CO_2																		X		
less CO_2																		X		
more CH_4																		X		
less CH_4																		X		
more H_2																		X		
less H_2																		X		
more H_2S																		X		

less H_2S																		X	
no						X								X					
more VFA																	X	X	
less VFA																	X	X	

Table S2. Worksheet obtained by BioHazOp analysis of node 1

Node 1: Hydrolysis reactor							
deviation	Engineering process		Biotechnology process		Existing counter measures	BioHazard	Proposed counter measures
	causes	consequences	causes	consequences			
Flow (OFMSW)							
MORE	-failure of IE4 -failure of E5	-alterations of the mixture composition to be transferred to CSTR -lowering of pH -excessive production of lactic acid -leakages from pipes -OFMSW spillage			-visual inspection - periodic maintenance of IE4 and E5 (check of centrifugal pump E5 sealing system) - IE4 and E5 setting check[1]		- install a flow (FT/FIC) transmitter (E5 outlet section) - transmitters redundancy
NO/LESS	-failure of IE4 -failure of E5	-alterations of the mixture composition to be transferred to CSTR -lower flow to hydrolysis reactor inlet			-visual inspection - periodic maintenance of IE4 and E5 -IE4 and E5 setting check		- install a flow (FT/FIC) transmitter (E5 outlet section) - transmitters redundancy
REVERSE	-failure of IE4 -failure of E5	-OFMSW spillage -lower flow to hydrolysis reactor inlet			- IE4 and E5 setting check		-install a non-return valve on the outlet section of E5 to ensure unidirectional flow

[1] See 5. Discussion

	-absence of non-return valve on the outlet section of E5	-damage to IE4 and to E5					
Flow (sludge)							
MORE	-failure of IE6 -failure of E6	-alterations of the mixture composition to be transferred to CSTR[2] -leakages from pipes			-visual inspection - periodic maintenance of IE6 and E6 -IE6 and E6 setting check		- install a flow (FT/FIC) transmitter (E6 outlet section) - transmitters redundancy
NO/LESS	-failure of IE6 -failure of E6	-alterations of the mixture composition to be transferred to CSTR			-visual inspection - periodic maintenance of IE6 and E6 -IE6 and E6 setting check		- install a flow (FT/FIC) transmitter (E6 outlet section) - transmitters redundancy
REVERSE	-failure of IE6 -failure of E6 - absence of non-return valve on the E6 outlet section	-sludge spillage					-install a non-return valve on the E6 outlet section to ensure unidirectional flow
Composition (of feedstock mixture)							
OTHER	-seasonal variation in the composition of the OFMSW[3]	-alteration of VFA yield maximization conditions			- a thickener ensures a constant		-monitoring of volatile solids in the feedstock (OFMSW),

[2] Mixture alterations do not lead to a lowering of the pH or undesirable production of lactic acid in the fermentation process

[3] In case of a centralised purification plant, the sludge comes from a thickener, that guarantees a mixture with a constant composition (2-3% solids)

					composition of sludge mixture		using multi-parameter probes[4]
Temperature (thermal pre-treatment)							
LOWER temperature (< 70°C)	-lower flow rate of water circulating in the thermostatic jacket (failure of E10 and IE3) -lower temperature of the water circulating in the jacket (boiler failure); -failure of the temperature detector installed in the reactor -poor stirring of the mixture	- incomplete solubilisation of the organic matrix		-incomplete inactivation of faecal bacteria	-visual inspection - periodic maintenance of IE3 and E10 - periodic maintenance of reactor stirrer		- install a transmitter, which monitors the temperature of the water circulating in the thermostatic jacket - transmitters redundancy - electric stirrer motor provided with preferential power supply (generator or uninterruptible power supply)
HIGHER temperature (> 70°C)	-higher flow rate of water circulating in the thermostatic jacket (failure of E10 and IE3); -higher temperature of the water circulating in the jacket (boiler failure); -failure of the temperature	- hydrolysis is not inhibited by T > 70°C - low economic sustainability of the process			-visual inspection - periodic maintenance of IE3 and E10 - periodic maintenance of reactor stirrer		- install a transmitter, which monitors the temperature of water circulating in the thermostatic jacket - transmitters redundancy - electric stirrer motor provided with preferential power supply (generator or

[4] Regulating the concentration of volatile solids could ensure a constant organic load and allow the subsequent fermentation process to be controlled

	detector installed in the reactor						uninterruptible power supply)
Duration (thermal pre-treatment)							
SHORTER duration (< 48 h)	-failure of feeding pumps timers[5] -failure of E5, E10 and E6 -failure of IE6, IE3 and IE4	-lowering of hydraulic residence time (HRT) - incomplete hydrolysis -alterations of the mixture composition to be transferred to CSTR			-check of the volumetric flow rate of E5, E10 and E6 -check of the volumetric flow rate of E7 -visual inspection -periodic maintenance of E5, E6, E7, E10, IE1, IE4, IE6, IE3 -periodic check and maintenance of timers for switching pumps on and off		- install a Distributed Control System (DCS) for switching automatically pumps on and off - install flow transmitters (E5, E6 and E10 outlet section) - transmitters redundancy
LONGER duration (> 48 h)	-failure of feeding pump timers[6] -failure of E5, E10 and E6 -failure of IE6, IE3 and IE4	- HRT increase			-check of the volumetric flow rate of E5, E10 and E6 -check of the volumetric flow rate of E7		- install flow transmitters (E5, E6 and E10 outlet section) - transmitters redundancy

[5] The reactor feeding process is currently semi-continuous: E5 and E6 are activated once a day. E5 and E6 fed the reactor for a longer time than planned

[6] E5 and E6 fed the reactor for a shorter time than planned

					-visual inspection -periodic maintenance of E5, E6, E7, E10, IE1, IE3, IE4 and IE6		
Mixing							
NO	-failure of the electric motor powering the stirrer -mechanical stirrer failure	-lowering of the heat transfer coefficient -change in hydrolysis temperature					-install a temperature transmitter to adjust the water mass flow to the thermostatic jacket - transmitters redundancy - electric stirrer motor provided with preferential power supply (generator or uninterruptible power supply)
Node 1: CSTR (continuous stirred tank reactor)							
Flow (inlet mixture)							
MORE	-failure of IE1 -failure of E7	-lowering of HRT			-visual inspection - periodic maintenance of IE1 and E7 (check of centrifugal pump E7 sealing system)		-install a level transmitter (LT/LIC) - transmitters redundancy

					- IE1 and E7 setting check		
NO/LESS	-failure of IE1 -failure of E7	- HRT increase			-visual inspection - periodic maintenance of IE1 and E7 -IE1 and E7 setting check		- install a level transmitter (LT/LIC) - transmitters redundancy
REVERSE	-failure of IE1 -failure of E7 -absence of non-return valve on the E7 outlet section	-fermented mixture spillage -lower flow to fermenter inlet -damage to IE1 and to E7					-install a non-return valve on the E7 outlet section to ensure unidirectional flow
Temperature (mesophilic fermentation)							
LOWER (<37°C)	-lower flow rate of water circulating in the thermostatic jacket (failure of E9 and IE5) -lower temperature of the water circulating in the jacket (boiler failure)[7]; -failure of the temperature detector installed in the reactor	-lowering of VFA concentrations		-different microbial community -lowering of microbial community concentration	-visual inspection - periodic maintenance of IE5 and E9 - periodic maintenance of reactor stirrer		-install a temperature transmitter (TT/TIC) - transmitters redundancy -electric stirrer motor provided with preferential power supply (generator or uninterruptible power supply)

[7] The plant includes two boilers serving the hydrolysis reactor and the CSTR

	-poor stirring of the mixture/stirrer failure						
HIGHER (>37°C)	-higher flow rate of water circulating in the thermostatic jacket (failure of E9 and IE5); -higher temperature of the water circulating in the jacket (boiler failure); -failure of the temperature detector installed in the reactor	-uncontrollable alterations in VFA concentration values (hyperthermophilic conditions)[8] - low economic sustainability of the process -decrease of the lower flammability limit (T≈ 55°C) - hydrogen concentration increase		-different microbial community	-visual inspection - periodic maintenance of IE5 and E9 - periodic maintenance of reactor stirrer		-install a temperature transmitter (TT/TIC) - transmitters redundancy -monitoring of flammable gases concentrations
Pressure							
LOWER (<20 mbar)	-variation of the composition of the inlet mixture -variation of process temperature	-formation of an explosive atmosphere (the overpressure absence allows O_2 to enter the reactor)			- gaseous mixture control (sampling with gas bag and laboratory GC analysis)		-install a pressure transmitter with alarm thresholds -transmitters redundancy
HIGHER (>20 mbar)	- Occlusion of the gas withdrawal line				-hydraulic seal [9] - gaseous mixture control (sampling with gas bag and		-install a pressure transmitter with alarm thresholds -transmitters redundancy

[8] The pH control could compensate the lower VFA production

[9] In case of overpressure, the gaseous mixture is conveyed into the hydraulic guard (normally water with anti-freeze glycol)

					laboratory GC analysis)		- install safety valves -safety valves redundancy
HRT (Residence time of substrate)							
LOWER (<5.5 d)	-failure of E7 -failure of IE1 -failure of feeding pump (E7) timer	- inhibition of the fermentation process - alterations of the mixture composition -lowering of VFA concentrations			-periodic check and maintenance of timers for switching pump on and off		- install a Distributed Control System (DCS) for switching automatically pump on and off and adjusting the degree of valve opening (level sensor)
HIGHER (> 5.5 d)	-failure of E7 -failure of IE1 -failure of feeding pump (E7) timer	- alterations of the mixture composition					- install a Distributed Control System (DSC) for switching automatically pump on and off and adjusting the degree of valve opening (level sensor)
TVS/TS (total solids) ratio							
LOWER	- changes in the composition of the mixture, feeding the hydrolysis reactor and fermenter (see OFMSW and sludge flows)[10]	- reduction of VFA production and lower PHA yields		-slowing down of fermentation kinetics			- install a pH-meter to monitor pH - pH-meter redundancy

[10] The mixture composition in the fermenter corresponds to the per cent composition of OFMSW and sludge at the outlet of the hydrolysis reactor

HIGHER	- changes in the composition of the mixture feeding the hydrolysis reactor and fermenter (see OFMSW and sludge flows)	- uncontrolled changes in the composition of the mixture due to a pH decrease and an excess of TVS concentration with a possible increase in VFA production		- inhibition of the fermentation process of the substrate by fermentative microorganisms			- install a pH-meter to monitor pH - pH-meter redundancy - install a storage tank and a dosage pump to carry out pH adjustments[11]
pH (of the substrate[12])							
LOWER	- possible and unlikely pre-fermentation activity	- inhibition of the fermentation process		- possible triggering of fermentation processes by hyperthermophilic microorganisms			- install a pH-meter to monitor mixture pH inside the hydrolysis reactor[13] - pH-meter redundancy - install a storage tank and a dosage pump to carry out pH adjustments
HIGHER	- almost totally unlikely						
Mixing							
NO	-failure of the electric motor powering the stirrer	- inhomogeneity and instability of the fermentation mixture		- accumulation of microorganisms in particular sections of the fermenter,			- electric stirrer motor provided with preferential power supply (generator or

[11] pH adjustment consists of an alkalinity control by addition of reagents or anaerobic digestate

[12] Mixture leaving the hydrolysis reactor and entering the CSTR

[13] Controlling the pH in the fermentation reactor may also be appropriate for monitoring the composition of the VFA mixture. Indeed, pH can modify metabolic pathways: an alkaline pH (>7) produces more propionic or valeric acid, while a more acidic pH (5-6) produces more acetic and butyric acid

	-mechanical failure of the stirrer	- variable and slower fermentation activity - formation of sedimentable solids[14] - formation of stagnant zones with higher HRT -increase of CH_4 production -decrease of VFA production		that can go into methanogenesis - formation of different microbial consortia, including methanogenic ones			uninterruptible power supply)
Volume[15]							
LOWER	- design choice[16]	- variation of HRT from the optimization value of the fermentation process - biomass washout [17] -decrease of VFA production - uncontrolled change in gaseous phase composition					- monitoring of flammable gases concentrations with alarm thresholds
HIGHER	- design choice	- variation of HRT from the					

[14] The fermentation does not take place in precipitation accumulations

[15] Nominal CSTR volume

[16] It depends on mass flows and HRT values. These parameters have to be set according to the desired process efficiency (PHA yield)

[17] Biomass doesn't undergo fermentation

		optimization value of the fermentation process -decrease of VFA production - uncontrolled change in gaseous phase composition					
COD_{VFA}/COD_{SOL} ratio							
LOWER (<100%)	-chemical composition of the matrix - high protein content in the OFMSW/sludge mixture[18]	- incomplete fermentation - decrease in VFA production (and PHA yield) -excessive production of lactic acid, alcohols (ethanol, butanol) and other organic substrates		- fermentation mechanisms by micro-organisms, which include metabolic pathways and speeds other than the optimal ones[19]			-periodic measurement of soluble COD -periodic measurement of VFA[20]
HIGHER (>100%)	- impossible condition						
Composition of fermented mixture (liquid phase)							
MORE VFA	see discussion on HRT, TVS/TS ratio, pH, mixing, Volume						- pH control from the hydrolysis step

[18] Secondary sludge finds it harder to undergo fermentation process than OFMSW

[19] There may be an accumulation of substances, which don't come from the breaking down of high molecular weight substrates and don't reach the metabolic pathway to be converted into VFA

[20] The measurement could be carried out once a day, but counter measures, which increase the ratio (temporary change of temperature and feed rate), take longer to be effective

LESS VFA	see discussion on HRT, TVS/TS ratio, pH, mixing, Volume						
Composition of fermented mixture (gaseous phase)[21]							
MORE CO_2	- operating temperature - % composition of OFMSW and sludge	- increase of the lower flammability limit (safer condition)					- install sensors for gases concentration measurements -sensors redundancy - setting of low and high concentration alarm thresholds for checking flammability limits
LESS CO_2	- operating temperature - % composition of OFMSW and sludge	- decrease of the lower flammability limit (less safe condition)					same as above
MORE CH_4	- operating temperature - % composition of OFMSW and sludge	- increase of the lower flammability limit (safer condition)					same as above
LESS CH_4	- operating temperature - % composition of OFMSW and sludge	-decrease of the lower flammability limit (less safe condition)					same as above
MORE H_2	- operating temperature	-decrease of the lower flammability					same as above

[21] The gases concentrations reciprocally affect each other

	- % composition of OFMSW and sludge	limit (less safe condition)					
LESS H_2	- operating temperature - % composition of OFMSW and sludge	-increase of the lower flammability limit (safer condition)					same as above
MORE H_2S[22]	- operating temperature - % composition of OFMSW and sludge	-less safe conditions due to the gas toxicity					same as above
LESS H_2S	- operating temperature - % composition of OFMSW and sludge	-safer conditions due to the gas toxicity					same as above

[22] The gas is present in traces (< 0.4% v/v). The toxic effects (health hazards) may be considered to outweigh the flammability effects (safety hazards)

Figures S1 and S2 show the P&IDs related to first node. Figure S1 represents the existing pilot plant, whereas figure S2 shows the design intention of the node. In particular, this last figure is derived from BioHazop and minutely reports the instrumentation, which is able to measure quantities, such as reagents flow, temperature, pressure, gases concentrations, etc., and alarm levels linked to main operating parameters. Indeed, in order to control the process and avoid parameters deviations from design intention, the instrumentation and alarm levels play a fundamental role. The diagram, reported in figure S2, represents the milestone in order to ensure the best performances of first node in terms of operability, efficiency and safety. More in detail, in order to ensure a safe fermenter operation and achieve high performance of VFA production, it is recommended to set two alarm levels:

1) low level alarm (AL);
2) high level alarm (AH).

The low level alarm can act as a warning of a potential problem requiring investigation. On the contrary, the high level alarm can trigger an emergency response, such as evacuation or shutdown. With reference to alarm levels, a particular attention has been addressed to fermenter, because the acidogenic fermentation produces a gaseous flammable mixture. On this subject, the low level alarm should be set as low as practicable, preferably no higher than 10-15% of the lower flammability limit (LFL) of gaseous mixture, whereas the high level alarm should be no more than 50% LFL of the mixture. These alarm levels are strictly dependent on oxygen presence (in the fermenter), which is extremely improbable (the acidogenic fermentation is an anaerobic process). However, the oxygen presence inside the fermenter cannot be categorically excluded. Indeed, in case of seals failure and overpressure absence, air could enter the fermentation reactor and a potentially explosive atmosphere could be generated. The fixed gas detectors have to be included in the plant maintenance schedule, because the performances of most detectors deteriorate with time and the operating conditions, as it could occur in case of dusty, corrosive or damp environment. These factors strongly influence the frequency of inspection and maintenance. In order to ensure a continuous monitoring of H_2, CH_4, H_2S, CO_2 and O_2 concentrations, the sensors number has to be considered. On this subject, the duplication (or triplication) of sensors and control apparatus is recommended to ensure a continuous monitoring of first node operation. In the pilot plant, located at Treviso, the fermenter is equipped with an hydraulic seal for preventing dangerous overpressures. In the full-scale plant, it is recommended to install safety valves (figure S2) in order to protect the fermentation reactor from the overpressures. Indeed, compared with the hydraulic seal, the safety valve allows to decrease the pressure inside the fermenter in a shorter time. The safety valves duplication is required to ensure the safety devices operating in case of valve failure. The safety valve operation pressure must be lower than fermenter design pressure in order to preserve its structural resistance. Finally, non-return valves are reported in figure S2. A non-return valve is designed to allow fluid to flow in

one direction only, therefore preventing the liquid or gas from flowing back valve upstream. Their use ensures the right flow of sludge and OFMSW to hydrolysis reactor and fermenter. Furthermore, the non-return valves also ensure the right water flow to the thermostatic jackets. The right flows of reagents and water are not secondary topics, because they influence the process efficiency (VFA production) and safety (LFL of flammable gaseous mixture produced by acidogenic fermentation).

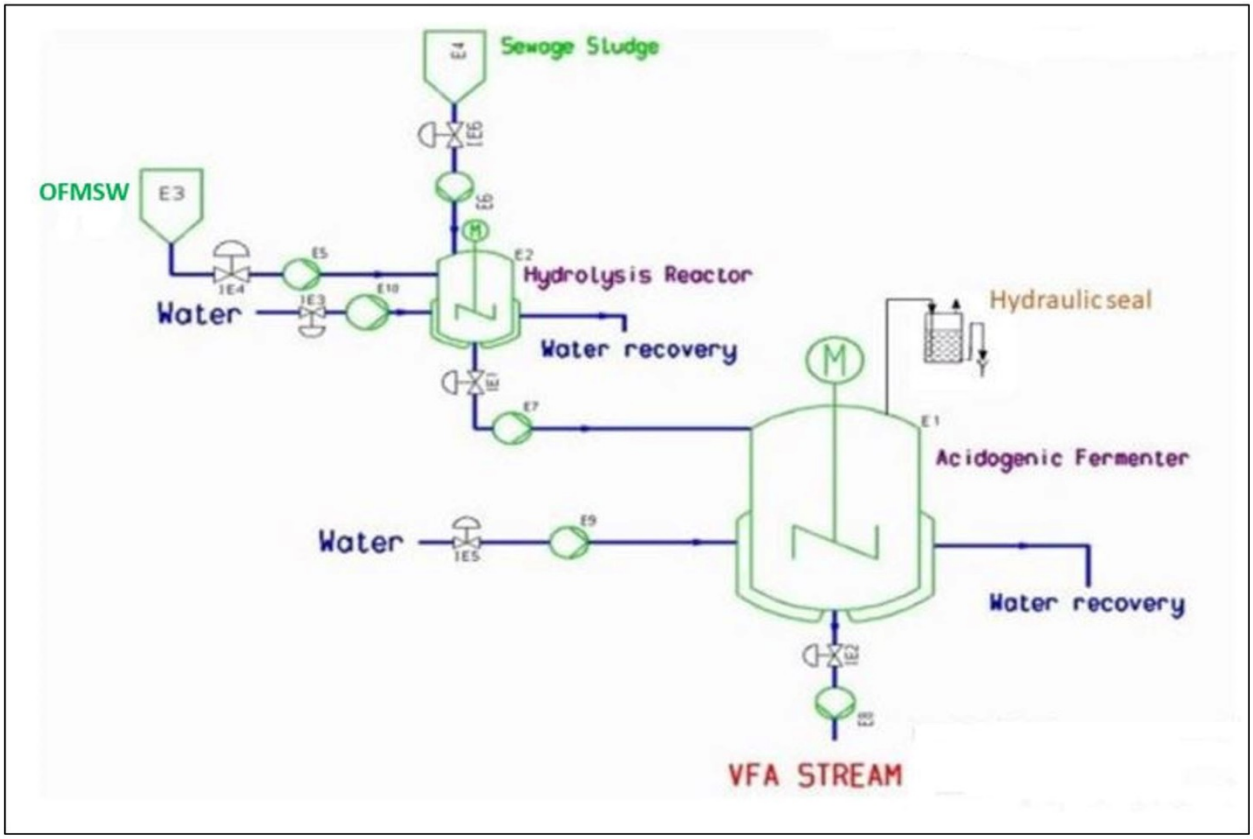


Figure S1. P&ID of node 1 (hydrolysis reactor and CSTR).

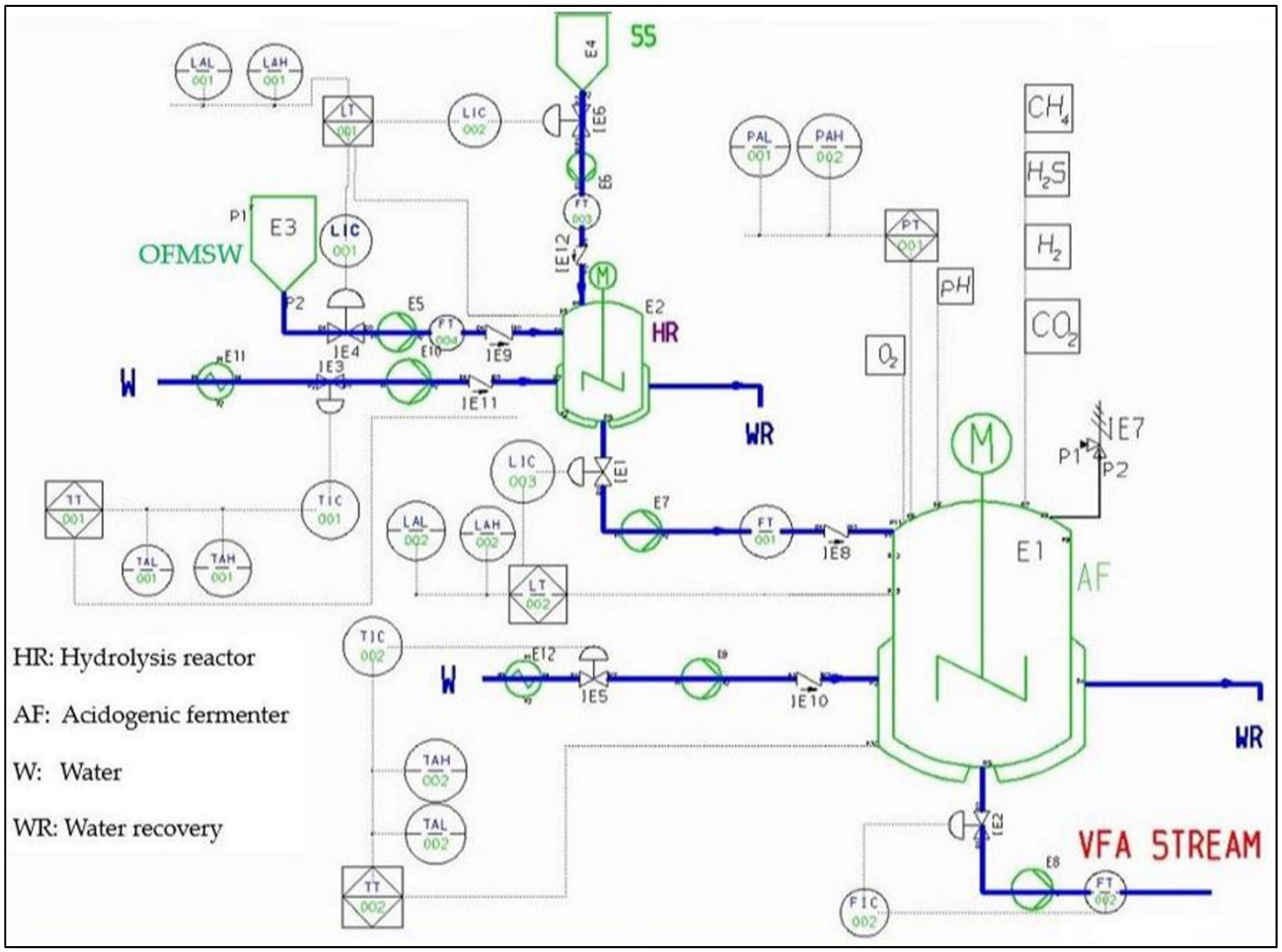


Figure S2. Final P&ID of node 1.