

Supplementary Material

Simulation of the Working Volume Reduction through the Bioconversion Model (BioModel) and Its Validation Using Biogas Plant Data for the Prediction of the Optimal Reactor Cleaning Period

Maria - Athina Tsitsimpikou ¹, Sotirios D. Kalamaras ¹, Antonios A. Lithourgidis ¹, Anastasios Mitsopoulos ², Lars Ellegaard ³, Irini Angelidaki ⁴, Thomas A. Kotsopoulos ^{1*}

¹ Department of Hydraulics, Soil Science and Agricultural Engineering, School of Agriculture, Aristotle University of Thessaloniki, GR 54124 Thessaloniki, Greece; tsitsimp@agro.auth.gr (M.A.T.); sklamaras@agro.auth.gr (S.D.K.); lithouraa@agro.auth.gr (A.A.L.);

² Biogas Lagada S.A., Biogas Plant, Organic Waste Treatment & Electricity Generation, Plot 677 Kolchikou, GR 57100 Kolchiko Lagada, Greece ; tmtsop@ergoplanning.gr (A.M.)

³ Burmeister & Wain Scandinavian Contractor, Gydevang 35, Allerød, 3450, Denmark ; lse@bwsc.dk (L.E.)

⁴ Department of Chemical and Biochemical Engineering, Technical University of Denmark, Kgs Lyngby, DK 2800, Denmark ; iria@kt.dtu.dk (I.A.)

* Correspondence: mkotsop@agro.auth.gr (T.A.K.)

Table S1. Biochemical reactions (B) and kinetic equations (K) used in the BioModel (Modified and Original) [1-3]*.

Hydrolysis	Carbohydrate hydrolysis	B	$(C_6H_{10}O_5)_{is} \rightarrow Y_c(C_6H_{10}O_5)_s + (1 - Y_c)(C_6H_{10}O_5)_{in}$
	Protein hydrolysis	B	$(Protein)_{is} \rightarrow Y_p(Amino\ acids) + (1 - Y_p)(Protein)_{in}$
	Lipid hydrolysis	B	$(Lipid)_{is} \rightarrow Y_L(GTO)_s + (1 - Y_L)(Lipid)_{in}$
		K	$R_s = k_0 \cdot [S] \cdot \frac{Ki}{Ki + [VFA]}$
Acidogenesis	Glucose fermenting acidogens (X_4)	B	$(C_6H_{10}O_5)_s + 0.1115\ NH_3 \rightarrow 0.1115\ C_5H_7NO_2 + 0.744\ CH_3COOH + 0.5\ CH_3CH_2COOH + 0.4409\ CH_3CH_2CH_2COOH + 0.6909\ CO_2 + 0.0254\ H_2O$
		K	$\mu_{X_4} = \mu_{max,X_4}(T) F_{pH,X_4} \left(\frac{[(C_6H_{10}O_5)_s]}{[(C_6H_{10}O_5)_s] + K_{s,X_4}} \right) \left(\frac{[NH_4]}{[NH_4] + K_{s,NH_4,X_4}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA,X_4}}} \right) - k_{d,X_4}$
	Amino acid degrading acidogens (X_5)	B	$CH_{2.03}O_{0.6}N_{0.3}S_{0.001} + 0.3006\ H_2O \rightarrow 0.017013\ C_5H_7NO_2 + 0.29742\ CH_3COOH + 0.02904\ CH_3CH_2COOH + 0.022826\ CH_3CH_2CH_2COOH + 0.013202\ CH_3CH_2CH_2CH_2COOH + 0.0752\ CO_2 + 0.28298\ NH_3 + 0.001\ H_2S$
		K	$\mu_{X_5} = \mu_{max,X_5}(T) F_{pH,X_5} \left(\frac{[(AA)_s]}{[(AA)_s] + K_{s,X_5}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA,X_5}}} \right) - k_{d,X_5}$
	GTO degrading acidogens (X_6)	B	$C_{57}H_{104}O_6 + 1.90695\ H_2O + 0.04071\ NH_3 + 0.0291\ CO_2 \rightarrow 0.04071\ C_5H_7NO_2 + 0.94184\ CH_3CH_2COOH + 3\ C_{18}H_{34}O_2$
		K	$\mu_{X_6} = \mu_{max,X_6}(T) F_{pH,X_6} \left(\frac{[(GTO)_s]}{[(GTO)_s] + K_{s,X_6}} \right) \left(\frac{[NH_4]}{[NH_4] + K_{s,NH_4,X_6}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA,X_6}}} \right) - k_{d,X_6}$

Acetogenesis	B	$C_{18}H_{34}O_2 + 7.7401 H_2O + 0.2537 NH_3 + 4.0834 CO_2 \rightarrow 0.2537 C_5H_7NO_2 + 8.6998 CH_3COOH + 3.4139 CH_4$
	K	$\mu_{X_7} = \mu_{max,X_7}(T) F_{pH,X_7} \left(\frac{[LCFA]}{[LCFA] + K_{s,LCFA,X_7} + \frac{[LCFA]^2}{K_{i,LCFA,X_7}}} \right) \left(\frac{[NH_4]}{[NH_4] + K_{s,NH_4,X_7}} \right) \left(\frac{1}{1 + \frac{[H_2]}{K_{i,H_2,X_7}}} \right) - k_{d,X_7}$
	B	$CH_3CH_2COOH + 0.314 H_2O + 0.06198 NH_3 \rightarrow 0.06198 C_5H_7NO_2 + 0.9345 CH_3COOH + 0.6604 CH_4 + 0.1607 CO_2$
	K	$\mu_{X_8} = \mu_{max,X_8}(T) F_{pH,X_8} \left(\frac{[HPr]}{[HPr] + K_{s,X_8}} \right) \left(\frac{[NH_4]}{[NH_4] + K_{s,NH_4,X_8}} \right) \left(\frac{1}{1 + \frac{[HAc]}{K_{i,HAc,X_8}}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA,X_8}}} \right) \left(\frac{1}{1 + \frac{[H_2]}{K_{i,H_2,X_8}}} \right) - k_{d,X_8}$
	B	$CH_3CH_2CH_2COOH + 0.8038 H_2O + 0.0653 NH_3 + 0.5543 CO_2 \rightarrow 0.0653 C_5H_7NO_2 + 1.8909 CH_3COOH + 0.4454 CH_4$
	K	$\mu_{X_9} = \mu_{max,X_9}(T) F_{pH,X_9} \left(\frac{[HBu]}{[HBu] + K_{s,X_9}} \right) \left(\frac{[NH_4]}{[NH_4] + K_{s,NH_4,X_9}} \right) \left(\frac{1}{1 + \frac{[HAc]}{K_{i,HAc,X_9}}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA,X_9}}} \right) \left(\frac{1}{1 + \frac{[H_2]}{K_{i,H_2,X_9}}} \right) - k_{d,X_9}$
	B	$CH_3CH_2CH_2CH_2COOH + 0.8045 H_2O + 0.0653 NH_3 + 0.5543 CO_2 \rightarrow 0.0653 C_5H_7NO_2 + 0.8912 CH_3COOH + 1.000 CH_3CH_2COOH + 0.4454 CH_4$
	K	$\mu_{X_{10}} = \mu_{max,X_{10}}(T) F_{pH,X_{10}} \left(\frac{[HVa]}{[HVa] + K_{s,X_{10}}} \right) \left(\frac{[NH_4]}{[NH_4] + K_{s,NH_4,X_{10}}} \right) \left(\frac{1}{1 + \frac{[HAc]}{K_{i,HAc,X_{10}}}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA,X_{10}}}} \right) \left(\frac{1}{1 + \frac{[H_2]}{K_{i,H_2,X_9}}} \right) - k_{d,X_{10}}$

Methanogenesis	Acetolactic methanogens (X ₁₁)	B	$\text{CH}_3\text{COOH} + 0.022 \text{ NH}_3 \rightarrow 0.022 \text{ C}_5\text{H}_7\text{NO}_2 + 0.945 \text{ CH}_4 + 0.066 \text{ H}_2\text{O} + 0.945 \text{ CO}_2$
		K	$\mu_{X_{11}} = \mu_{max,X_{11}}(T) F_{pH,X_{11}} \left(\frac{[HAc]}{[HAc] + K_{s,X_{11}}} \right) \left(\frac{[NH_4]}{[NH_4] + K_{s,NH_4,X_{11}}} \right) \left(\frac{1}{1 + \frac{[NH_3]}{K_{i,NH_3,X_{11}}}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA,X_{11}}}} \right) - k_{d,X_{11}}$
	Hydrogenotrophic methanogens (X ₁₂)	B	$\text{H}_2 + 0.0058 \text{ NH}_3 + 0.2644 \text{ CO}_2 \rightarrow 0.0058 \text{ C}_5\text{H}_7\text{NO}_2 + 0.2355 \text{ CH}_4 + 0.517 \text{ H}_2\text{O}$
		K	$\mu_{X_{12}} = \mu_{max,X_{12}}(T) F_{pH,X_{12}} \left(\frac{[H_2]}{[H_2] + K_{s,X_{12}}} \right) \left(\frac{[NH_4]}{[NH_4] + K_{s,NH_4,X_{12}}} \right) \left(\frac{1}{1 + \frac{[NH_3]}{K_{i,NH_3,X_{12}}}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA,X_{12}}}} \right) - k_{d,X_{12}}$
pH calculation	$F_{pH,Xj} = \frac{(1+2)10^{0.5(pK_{low,Xj} - pH)}}{(1+10)^{pH-pK_{high,Xj}} + 10^{pK_{low,Xj}-pH}} \quad j \in [4,12], \quad pH = -\log[H^+]$		

*Where: **is**, **s**, **in** is the abbreviation of insoluble, soluble and inert, respectively, **Y_{C,P,L}** is the degradability of carbohydrates, proteins and lipids, respectively, **R_s** is the substrate utilization rate, **k₀** is the uninhibited reaction rate constant, **S** is the substrate concentration, **K_i** is the specific inhibition constant to the given process step, **[VFA]** is the sum of the concentration of acetic, propionic, butyric and valeric acid, **μ** is the microorganism growth rate, **μ_{max,Xj}(T)** is the maximum specific growth rate at temperature T, **F_{(pH),Xj}** is the pH term of each microbial group, **K_s** is the half-saturation constant, **K_d** indicates cell death, **[NH₄]** is the concentration of total ammonia, **[LCFA]** is the concentration of long chain fatty acids, **[AA]** is the concentration of amino acids, **[GTO]** is the concentration of Glycerol trioleate, **[H₂]** is the concentration of hydrogen, **[HVa]** is the concentration of valeric and iso-valeric acid, **[HBu]** is the concentration of butyric and iso-butyric acid, **[HPr]** is the concentration of propionic acid, **[HAc]** is the concentration of acetic acid, **pK_{low}** is the lower pH value and **pK_{high}** is the higher pH value.

Table S2. Kinetic constants used in both simulation conditions of the BioModel (Modified and Original) [1-3]**.

	$\mu_{\max}(T_{ref})$ (h ⁻¹)	alpha (h ⁻¹ °C ⁻¹)	T _{opt} (°C)	T _{max} (°C)	K _{s,x} (g L ⁻¹)	K _{s,NH3,X} (g L ⁻¹)	K _{i,0,X} (g L ⁻¹)	K _{i,LCFA,X} (g L ⁻¹)	K _{i,H2,X} (g L ⁻¹)	pK _{low}	pK _{high}	cell death (% h ⁻¹)
Carbohydrate enzymes	4.17 · 10 ⁻²	7.65 · 10 ⁻⁴	55	65	0.000	0.000	0.333	0.000	0.000	0.000	0.000	0.000
Protein enzymes	4.17 · 10 ⁻²	7.65 · 10 ⁻⁴	55	65	0.000	0.000	0.333	0.000	0.000	0.000	0.000	0.000
Lipid enzymes	4.17 · 10 ⁻²	7.65 · 10 ⁻⁴	55	65	0.000	0.000	0.333	0.000	0.000	0.000	0.000	0.000
Glucose degraders	2.12 · 10 ⁻¹	3.88 · 10 ⁻³	55	65	0.500	0.050	0.000	5.000	0.000	0.000	14.000	5.000
Amino acid degraders	2.66 · 10 ⁻¹	4.87 · 10 ⁻³	55	65	2.328	0.000	0.000	5.000	0.000	0.000	14.000	9.900
Lipid (GTO) degraders	2.21 · 10 ⁻²	4.05 · 10 ⁻⁴	55	65	0.010	0.050	0.000	5.000	0.000	6.000	8.500	5.000
LCFA degraders	2.3 · 10 ⁻²	4.22 · 10 ⁻⁴	55	65	0.020	0.050	0.000	5.000	1.73 · 10 ⁻⁶	6.000	8.500	5.000
Propionate degraders	2.04 · 10 ⁻²	3.74 · 10 ⁻⁴	53	65	0.125	0.050	0.960	5.000	2.31 · 10 ⁻⁶	6.000	8.500	13.20
Butyrate degraders	2.81 · 10 ⁻²	5.15 · 10 ⁻⁴	60	70	0.176	0.050	0.720	5.000	5.50 · 10 ⁻⁶	6.000	8.500	6.500
Valerate degraders	2.89 · 10 ⁻²	5.30 · 10 ⁻⁴	60	70	0.106	0.050	0.400	5.000	4.90 · 10 ⁻⁶	6.000	8.500	8.400
Acetate degraders	2.55 · 10 ⁻²	4.68 · 10 ⁻⁴	55	65	0.537	0.050	0.275	5.000	0.000	6.000	8.8300	2.700
Hydrogen degraders	3.33 · 10 ⁻¹	6.11 · 10 ⁻³	55	70	0.000	0.050	0.500	5.000	0.000	6.000	8.500	5.000
Syntrophic acetate oxidizers	1.57 · 10 ⁻²	2.88 · 10 ⁻⁴	55	65	0.537	0.050	0.56	5.000	7.29 · 10 ⁻⁶	5.500	8.500	5.000

**Where $\mu_{\max}(T_{ref})$ is the maximum specific growth rates (at reference temperature), alpha is the correlation with temperature, T_{opt} is the temperature for optimal growth rate, T_{max} is the temperature for maximum growth rate, K_{s,x} is the half-saturation constant, K_{s,NH3,X} is the ammonia inhibition constant, K_{i,0,X} is the inhibition constant, K_{i,LCFA,X} is the LCFA inhibition constant, K_{i,H2,X} is the hydrogen inhibition constant, pK_{low} is the lower pH value and pK_{high} is the higher pH value.

Table S3. Yield coefficients (g gbiomass⁻¹) used in both simulation conditions of the BioModel (Modified and Original) [2, 3].

Metabolism	Glucose insoluble	Glucose inert	Glucose soluble	Protein insoluble	Protein inert	Protein soluble	Lipid insoluble	Lipid inert	Lipid soluble	LCFA	Hpr	HBut	HVal	HAc	NH ₃	CH ₄	CO ₂	H ₂ S	H ₂
Cell decay	0.36	0.00	0.00	0.82	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.001	0.00
Carbohydrate enzymes	-1.00	0.30	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Protein enzymes	0.00	0.00	0.00	-1.00	0.24	0.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lipid enzymes	0.00	0.00	0.00	0.00	0.00	0.00	-1.00	0.200	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glucose degraders	0.00	0.00	-12.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.94	3.08	0.00	3.54	-0.12	0.00	2.41	0.00	0.00
Amino acid degraders	0.00	0.00	0.00	0.00	0.00	-14.49	0.00	0.00	0.00	0.00	1.12	1.04	0.00	9.28	2.06	0.00	1.72	0.02	0.00
Lipid (GTO) degraders	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-192.16	183.9	15.15	0.00	0.00	0.00	-0.12	0.00	-0.28	0.00	0.00
LCFA degraders	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-14.67	0.00	0.00	0.00	27.16	-0.12	0.00	-0.57	0.00	1.51
Propionate degraders	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-14.3	0.00	0.00	0.00	10.83	-0.12	0.00	7.67	0.00	1.08
Butyrate degraders	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-14.31	0.00	0.00	18.46	-0.12	0.00	-0.39	0.00	0.61
Valerate degraders	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.03	0.00	-13.82	7.25	-0.12	0.97	-3.30	0.00	0.00
Acetate degraders	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-24.13	-0.12	6.08	16.73	0.00	0.00
Hydrogen degraders	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.12	5.79	-17.83	0.00	-3.09	
Syntrophic acetate oxidizers	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-4.25	-0.12	0.00	4.30	0.00	0.39

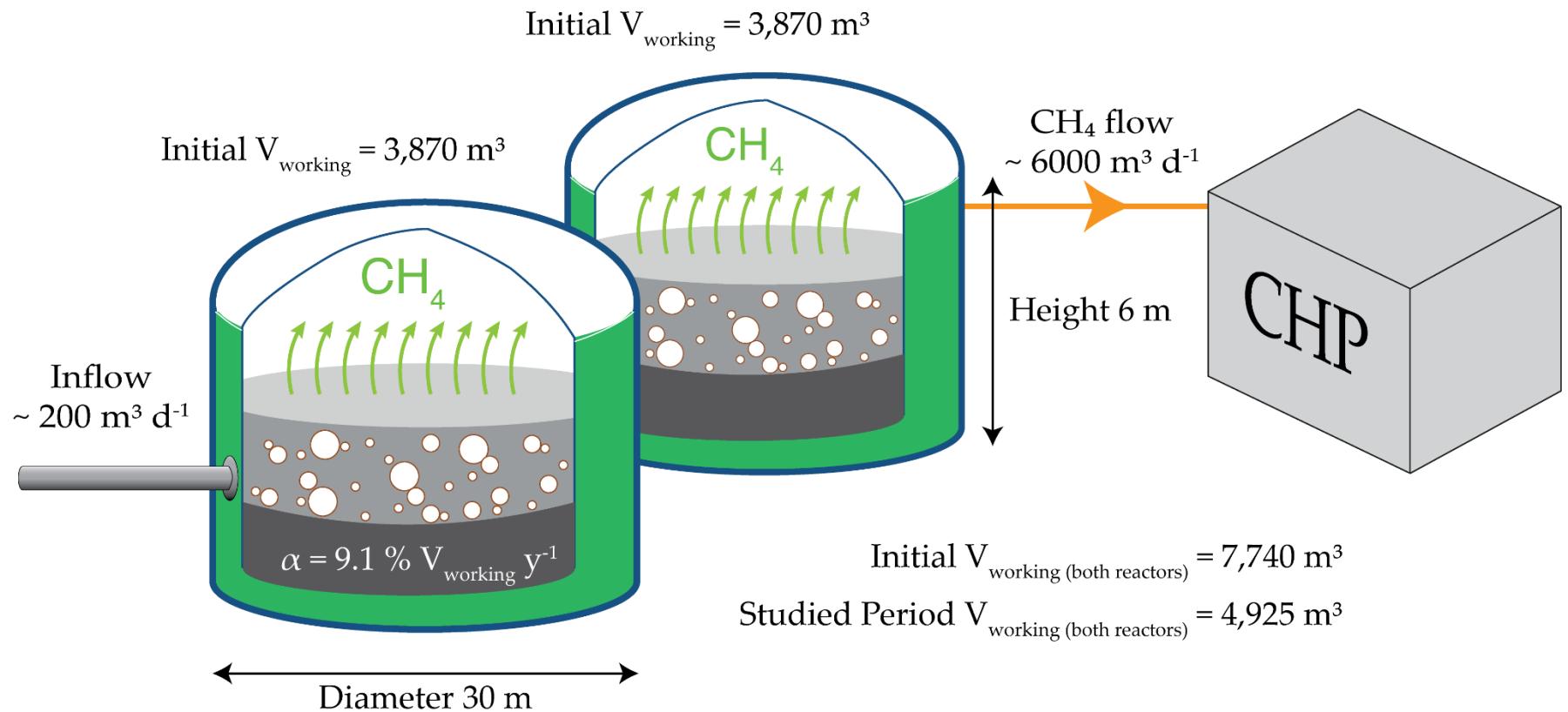


Fig. S1. An illustration of the two reactors, indicating the main operational parameters: inflow rate, dimensions, CH₄ flow, working volume and accumulated rate.

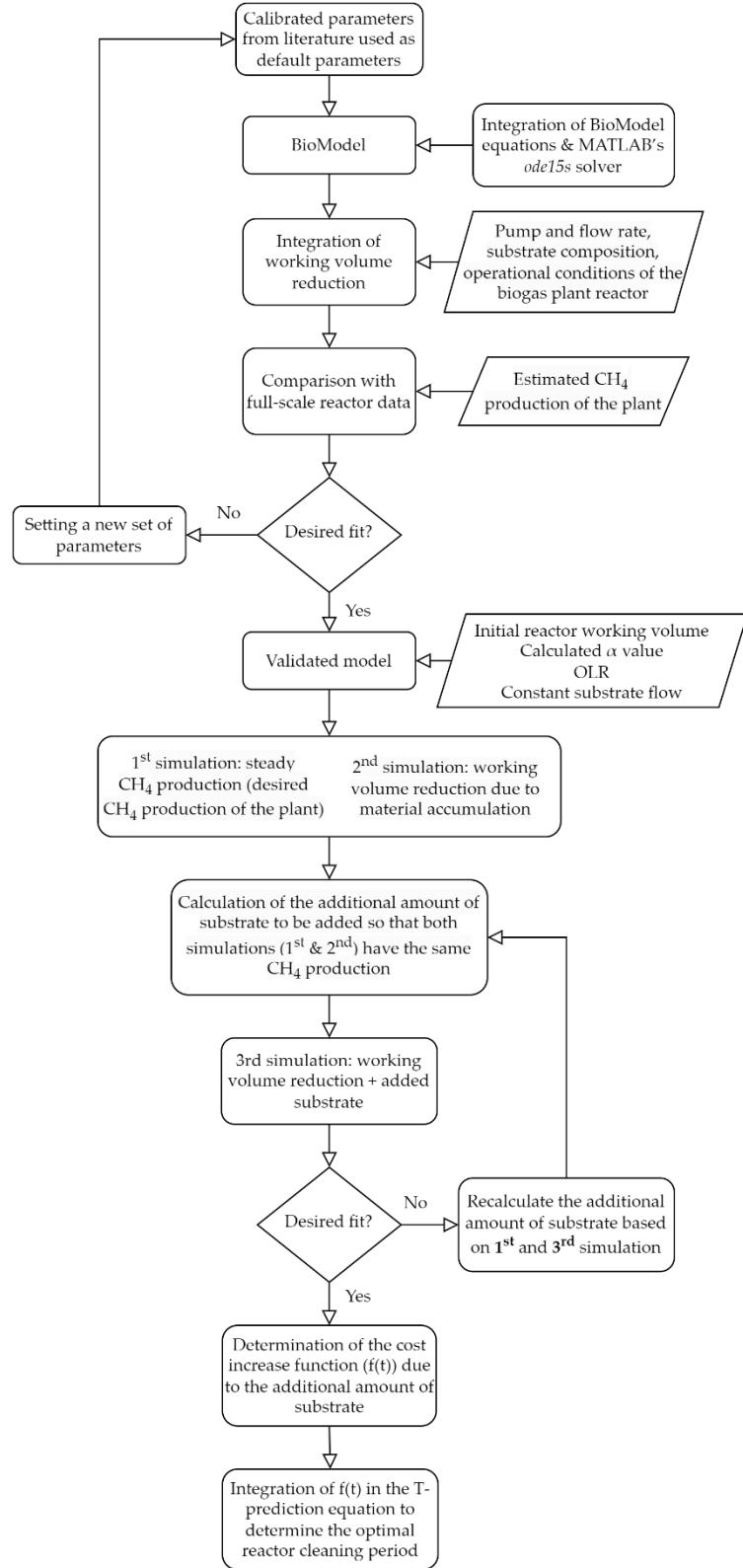


Fig. S2. A flowchart of the proposed methodology and simulation steps.

References

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