Table 1: Monitoring targets to achieve process objectives

Monitoring target	Objective		
	High yield	**High productivity**	**Detect contamination**
pH	-	-	Reactor
Temperature	-	-	-
Cell biomass	-	Reactor	Reactor
Glucose	Reactor/inlet	-	Reactor/inlet
Xylose	Reactor/inlet	-	-
Ethanol	Reactor/off-gas	-	-
Acetic acid	Reactor/inlet	Reactor/inlet	-
Furfural	-	Reactor/inlet	
5-HMF	-	Reactor/inlet	
Lactic acid	-	-	Reactor
CO_2	Off-gas	Off-gas	-
O_2	Reactor/off-gas	-	-

Table 2: Overview of risks associated with cellulose to ethanol fermentation.

Risk	Consequence	Monitoring targets to diagnose and detect	Possible solution
Contamination by lactic acid bacteria (LAB)	The growth of LAB depletes some of the glucose and xylose decreasing the ethanol yield	pH, biomass or component dissolved in the liquid (fast detection desired)	Early stop of the batch in order to not use all the substrate
Inhibition by furfural and acetic acid	The ethanol production rate decreases significantly until detoxification	Components dissolved in the inlet (with constant inlet, slow detection sufficient) and dissolved in the reactor (fast detection required)	Control of the feed rate, to ensure the concentrations of inhibitors remain under a certain threshold
Presence of oxygen	The ethanol yield will decrease, as ethanol is produced in anaerobic conditions	The off-gas, or detection in the liquid (fast detection required)	The oxygen will need to be consumed

Table 3: The added value of (combinations of) different monitoring strategies of key process variables. -: does not monitor, +: monitors indirectly, ++: monitors indirectly through different models, +++: monitors directly. Each plus counts as one point, while the points of the standard setup (pH and temperature measurements) are subtracted from the total amount of gained points for each monitoring strategy.

	Monitor components in the off-gas	Monitor components in the reactor	Monitor components in the inlet	Monitor the biomass concentration	Detect contaminations	Total score
pH + Temperature	-	-	-	-	+	0
Off-gas	+++	+	-	+	+	5
Off-gas + inlet	+++	++	+++	++	+	10
Off-gas + reactor	+++	+++	-	++	++	9
Off-gas + biomass	+++	+	-	+++	+++	9
Inlet + reactor	-	+++	+++	++	++	9
Off-gas + inlet + reactor	+++	+++	+++	++	++	12
Off-gas + inlet + reactor + biomass	+++	+++	+++	+++	++	13

Table 4. Scoring matrix to evaluate the capabilities of the different methods to monitor the key compounds of the cellulose to ethanol fermentation. A method capable of monitoring all the relevant compounds would receive a score of 3, while a method unable to monitor any of the compounds would receive a score of 0.

	On-line NIR	In-line NIR	On-line MIR	In-line MIR	On-line Raman	In-line Raman	On-line UV-Vis	Biosensors*	At-line HPLC
Glucose	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Xylose	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Ethanol	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Acetic acid	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lactic acid	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Furfural	No	No	No	No	No	No	Yes	No	Yes
HMF	No	No	No	No	No	No	Yes	No	Yes
Total score	**1**	**1**	**2**	**2**	**2**	**2**	**1**	**2**	**3**

Table 5: Overview of all the techniques discussed to monitor components in the liquid phase. Scores from 0 to +++ are given for each criterion, 0 indicating a negative effect and +++ indicating a positive effect. The costs are evaluated with scores from --- to 0, --- indicating more costly and 0 less costly. A thorough description of the scoring system is provided in the supplementary material.

	Measured compounds	Sensitivity	Accuracy	Drift	Calibration & data analysis	Sample preparation	Response time	Industrial implementation	Costs	Total score
On-line NIR	+	+	++	+	+	++	++	++	-	**11**
In-line NIR	+	+	+	+	0	+++	+++	++	-	**11**
On-line MIR	++	++	++	+	+	++	++	++	- -	**12**
In-line MIR	++	++	+	+	0	+++	+++	++	-	**13**
On-line Raman	++	++	++	++	+	++	++	+	- -	**12**
In-line Raman	++	++	+	++	0	+++	+++	0	-	**12**
On-line UV-Vis	+	+	++	++	+	++	++	0	-	**10**
Biosensors	++	++	++	0	++	+	+	0	0	**10**
At-line HPLC	+++	+++	+++	++	++	0	0	+	- -	**12**

Table 6: Overview of all the techniques discussed to monitor the biomass concentration. Scores from 0 to +++ are given for each criterion, 0 indicating a negative effect and +++ indicating a positive effect. The costs are evaluated with scores from --- to 0, --- indicating more costly and 0 less costly. A thorough description of the scoring system is provided in the supplementary material.

	Differentiate cells and particles	**Assess cell viability**	**Detect contaminations**	**Sample preparation**	**Calibration & data analysis**	**Industrial availability**	**Costs**	**Total score**
OD probes	0	0	0	++	+++	++	0	**7**
IR spectroscopy	0	0	0	+++	+	+	-	**4**
2D fluorescence	+++	0	0	+++	+	+	-	**7**
Bio-calorimetry	+++	0	0	+++	+++	0	-	**8**
Flow cytometry	+++	+++	+++	0	++	0	- - -	**8**
Dielectric spectroscopy	+++	+++	0	+++	+	++	0	**12**
Microscopy and imaging analysis	+++	+++	+++	++	0	+	-	**11**

Table 7: Overview of the process models researched in this study.

Reference	Model type	State variables	Substrate	Product	Inhibitors
Krishnan *et al.* [16]	Monod form expression for substrate inhibition. Two constant model for product inhibition	Glucose Xylose Ethanol Cell biomass	Glucose Xylose	Ethanol	Glucose Xylose Ethanol
Navarro [90]	Polynomial inhibition	Furfural	Glucose	Ethanol	Furfural
Palmqvist *et al.* [92]	Mechanistic model taking stoichiometric balances and metabolic pathways into account	Glucose Ethanol Cell biomass Glycerol Furfural	Glucose Furfural	Ethanol Glycerol Furfuryl alcohol	Furfural Glucose
Zhang *et al.* [94]	Monod type kinietics with competitive substrate inhibition for glucose and xylose, and an additional term for ethanol inhibition	Glucose Xylose Ethanol Cell biomass	Glucose Xylose	Ethanol	Glucose Xylose Ethanol
Luong [95]	Monod type kinetics with Levenspiel product inhibition	Ethanol (main component) Glucose	Glucose	Ethanol	Ethanol
Starzak *et al.* [96]	Unstructured models for kinetics of cellular metabolism. Monod type kinetics for biomass growth and exponential ethanol inhibition.	Sucrose Ethanol Cell biomass	Sucrose	Ethanol	Ethanol
Hanly and Henson [55]	Monod type kinetics including several inhibition functions	Glucose Xylose Ethanol Oxygen Furfural HMF Furfuryl alcohol 2,5-bis-hydroxymethylfuran Acetate Cell biomass	Glucose Xylose	Ethanol Furfuryl alcohol 2,5-bis-hydroxymethylfuran Acetate	Ethanol Furfural HMF Furfuryl alcohol 2,5-bis-hydroxymethylfuran Acetate
Phisalaphong *et al.* [97]	Monod type kinetics including	Sugar (cane molasses) Ethanol	Sugar	Ethanol	Sugar Ethanol

	several inhibition functions	Cell biomass			
Pinelli *et al.* [98]	Monod type kinetics with product inhibtion	Glucose Lactic acid Cell biomass	Glucose	Lactic acid	Lactic acid
Athmanathan *et al.* [99]	Monod type kinetics with the Levenspiel product inhibition function	Glucose Xylose Ethanol	Glucose Xylose	Ethanol	Ethanol
Leksawasdi *et al.* [20]	Monod type kinetics with substrate limitation and inhibition, and product inhibition	Glucose Xylose Ethanol	Glucose Xylose	Ethanol	Glucose Xylose Ethanol
Mauricio-Iglesias *et al.* [89]	Monod type kinetics including several inhibition functions	Reactor holdup Glucose Xylose Furfural Acetate HMF Ethanol Furfuryl alcohol Base conjugated cations Total inorganic carbon Cell biomass	Glucose Xylose	Ethanol	Glucose Xylose Ethanol Furfural Acetic acid HMF
Wang *et al.* [100]	Segregated model with 2 populations (active and inactive) controlled by acetic acid. Active type is a Monod type kinetics with glucose and xylose as substrate. Inactive type only consumes glucose.	Glucose Xylose Ethanol Acetic acid Cell biomass	Glucose Xylose	Ethanol	Glucose Acetic acid