Improving of NIR in-line fermentation monitoring: one calibration set for different processes

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Introduction

Past experience have demonstrated that NIR, after an elaborate calibration phase, is a reliable tool for real-time monitoring and automated management of industrial bioprocesses[1]

In the biotecnological field it is very important to be able to measure in real time crucial process variables such as biomass, subrates and metabolic products, in order to maximise process yeld and product quality.

Using NIR in-line technique it is possible to monitor in real time and contemporaneously the concentration of the main biochemical parameters of a fermentation process.

The setting up of calibration models involves the acquisition of a lot of samples, and they have to be analyzed with a reference assay. The number of the samples depend on the growth and hydrodynamic conditions: a complicated matrix, such as a cultural broth, involves the acquisition of many samples.[2]

This means that NIR is not an immediately usable instrument but it needs a complicate and long step to fit the calibration models.[3-4]

In this work we employed NIR in-line instrument equipped with an immersion fibre optic probe.

Calibration curves were set up for monitoring *Staphylococcus xylosus* fermentation process, in which the depletion of glucose and the production of acetic and lactic acid and the concentration of biomass could be in real time and contemporaneously monitored.

We adapted the *Staphylococcus xylosus* calibration models in order to monitor the fermentation processes of other two different microganism: *Lactobacillus fermentum* and *Streptococcus thermophylus*.

In this way we spent little time in order to fit new calibration models for this two microganism, because we used the calibration data acquired during some fermentation processes of another microbe.

Experimental

Acquisition of NIR spectra

We used a Foss NIR Systems 6500. The steam-sterilisable fibre optic probe was inserted directly in the vessel of the fermenter, and, the spectra acquisition was in interactance mode (Figure 1). The slit was set to a 1 mm, thus proving an effective path optic of 2 mm. The culture broth flowed across the slit thanks to the mechanical stirring of the culture and was completely exposed to the prevailing hydrodynamic conditions.

Fermentation processes

The microrganisms growth conditions are showed in the table 1:

Near Infrared Spectroscopy: Proceedings of the 11th International Conference.

Table1. Fermentation conditions.

Medium composition		Chemical-fisical conditions	
Glucose	46,00 g/L	Temperature	37°C
KH_2PO_4	3,00 g/L	PH	7
$MgSO_4$	0,60 g/L	Stirring	500 rpm
$MnSO_4$	0,01 g/L	Gas flow	0-1.5 ml/min
NaCl	6,00 g/L		
$(NH_4)_2SO_4$	10,00 g/L		
$FeSO_4$	0,03 g/L		
Peptone	5 g/L		
Yeast Extract	10 g/L		
Vitamins Group B	50 mg/L		

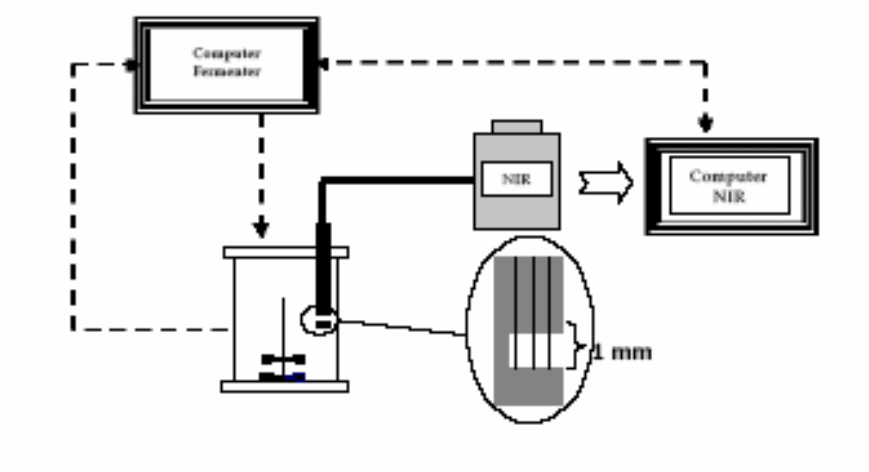

Figure 1. Instrument.

Reference assay.

The concentration of glucose, acetic and lactic acid was measured by HPLC (column: Aminex HPX-87H) analysis of a broth sample.

The biomass concentration was determined as the dry weight of the culture, measured by the membrane filtration method (cellulose acetate filters, pore size 0.45 μm).

Result and discussion

Staphylococcus xylosus calibration and validation.

Several fermentation processes of *S. xylosus* were made, in order to collect a lot of sample to insert into the calibration set.

The PCA analysis was applied to a second derivative spectra (122 samples), in order to find the outliers and the calibration model was fitted by PLS regression method.

The calibration and validation curves and statistic data are shown in table 2 and figure 2.

Table 2. Statistic calibration data Staphylococcus xylosus

CALIBRATION					
Costituent	**Range**	**R^2**	**SEC**	**F**	**SECV**
Glucose	0-58 g/L	0.9708	3.0192	508.6647	3.3493
Lactic Acid	0-23 g/L	0.9381	1.5061	190.6467	1.6665
Acetic Acid	0-19 g/L	0.9573	0.9588	307.5262	1.04745
Biomass	0-16 g/L	0.9506	0.9144	187.7451	1.1540
VALIDATION					
Costituent	**Range**	**R^2**	**SEP**		
Glucose	0-58 g/L	0.9710	2.6167		
Lactic Acid	0-23 g/L	0.9393	1.3669		
Acetic Acid	0-19 g/L	0.9010	0.6280		
Biomass	0-16 g/L	0.9535	0.8532		

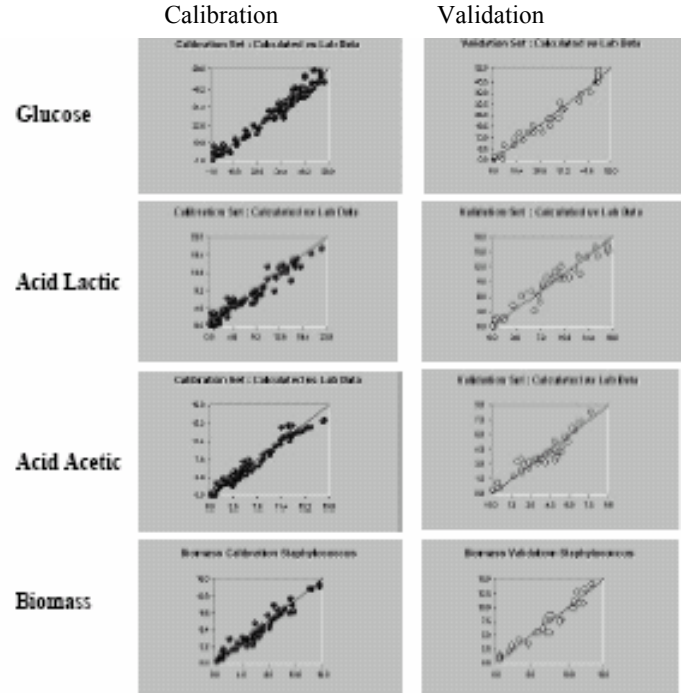

Figure 2. Calibration and validation curves of Staphylococcus xylosus.

Preliminary assay about Staphylococcus xylosus, Lactobacillus fermentum and Streptococcus thermophylus data set.

We used *Lactobacillus fermentum* and *Streptococcus thermophylus* because they grow in the same conditions of *S. xylosus*, but they present same differences: *L. fermentum* has a rod shape, and *S. thermophylus* is an homo-fermentative mocrorganism: it produces only lactic acid.

In order to show that the NIR would able or not to disinguish between different strains, the three set of spectra were analyzed by discriminant analysis (figure 3). We could note that the 3 cluster of spectra are perfectly superimposed. The main spectra for each data set were calculated and subtracted two by two, confirming the lack of any significant spectral difference specifically assignable to rod or spherical shape.

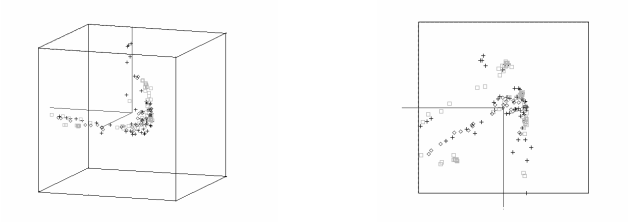

Figure 3. Discriminant analysis S. xylosus e di □ L. fermentum and O S. thermophylus.

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Validation of a S. xylosus calibration model with unknown samples of a Lactobacillus fermentum and Streptococcus thermophylus broth.

We predicted 43 unknown samples of *L. fermentum* using the calibration curves built for *S. xylosus* and we optained immediately good results (Table 3, figure 4).

Table 3. Prediction Statistic data of *L. fermentum*

	Biomass	Glucose	Lactic Acid	Acetic Acid
R^2	0.9227	0.9465	0.8774	0.8294
SEP	1.0272	2.6968	2.0142	0.5272
Range	0-20 g/L	0-51 g/L	0-21 g/L	0-6 g/L

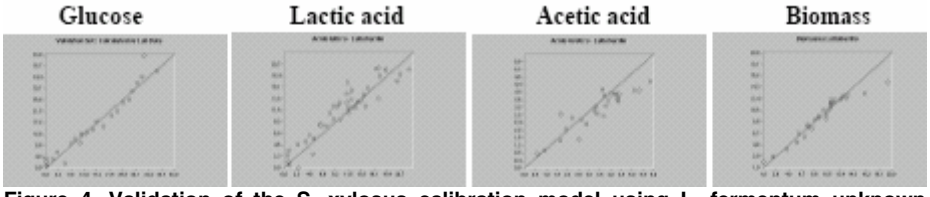

Figure 4. Validation of the S. xylosus calibration model using L. fermentum unknown samples.

In the case of *S. thermophylus* we didn't have immeditely sactisfactory results, because of the *S. xylosus* calibration models needed a little bias and slop adjustement (Table 4, Figure 5), so the adaptation was made in two steps.

In the first step we acquired 36 unknown samples in order to adjust the bias and slope of the original calibration curves.

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Table 4. Prediction Statistic data of *S. thermophylus*

	Biomass	Glucose	Lactic acid
R^2	0.8577	0.9384	0.8996
SEP	0.6692	5.0937	1.6613
Range	0-8 g/L	0-35 g/L	0-21 g/L

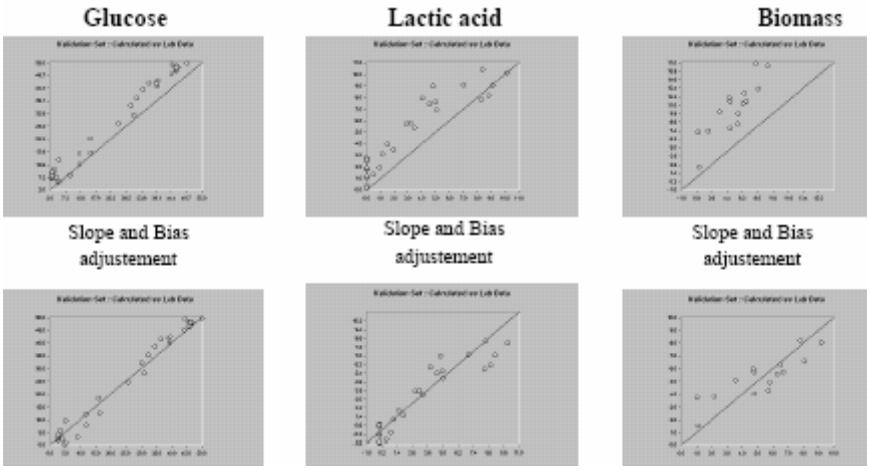

Figure 5. Validation of the S. xylosus calibration model usingS. thermophylus unknown samples –1° Step.

The second step was to predict other 18 unknown samples of *S. thermophylus*, using the *S. xylosus* adjusted calibration models. In this case the results were more satisfactory (Tabella 5, figure 6).

Table 5. Prediction Statistic data of *S. thermophylus*, after bias slope adjustment

	Biomass	Glucose	Lactic acid
R^2	0.9093	0.9178	0.9450
SEP	0.6935	3.6328	1.5198
Range	0-8 g/L	0-50 g/L	0-24 g/L

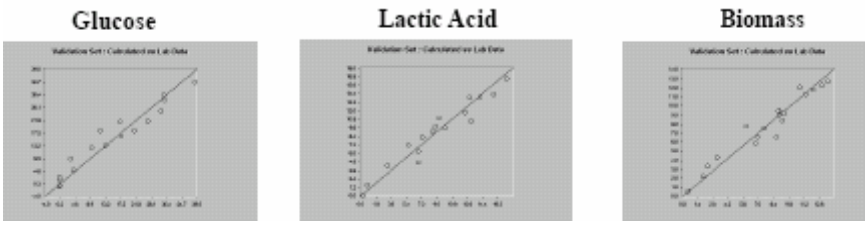

Figure 6. Validation of the S. xylosus calibration model usingS. thermophylus unknown samples – 2° Step.

Conclusions

The NIR spectroscopy is a reliable tool for monitoring, in real time and contemporaneously, the concentration of main biochemical parameters, such as glucose, lactic and acetic acid and biomass, during a bioprocess.

The long phase of the calibration model built was overcome by the adaptations of the *S. xylosus* calibration curves for monitoring fermentation processes of other two different microrganisms: *L. fermentum* and *S. thermophylus*.

For building the *S. xylosus* curves, we acquired 122 samples and another 30 for validating the models. In order to calibrate the NIR instrument for *L. fermentum* and *S. thermophylus* we collected respectively only 43 and 54 spectra.

In this way we were able to demonstrate that it is possible to reduce the great work spent to calibrate the instrumentation, adapting the calibration model of ine microorganisms to the other ones .

References

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