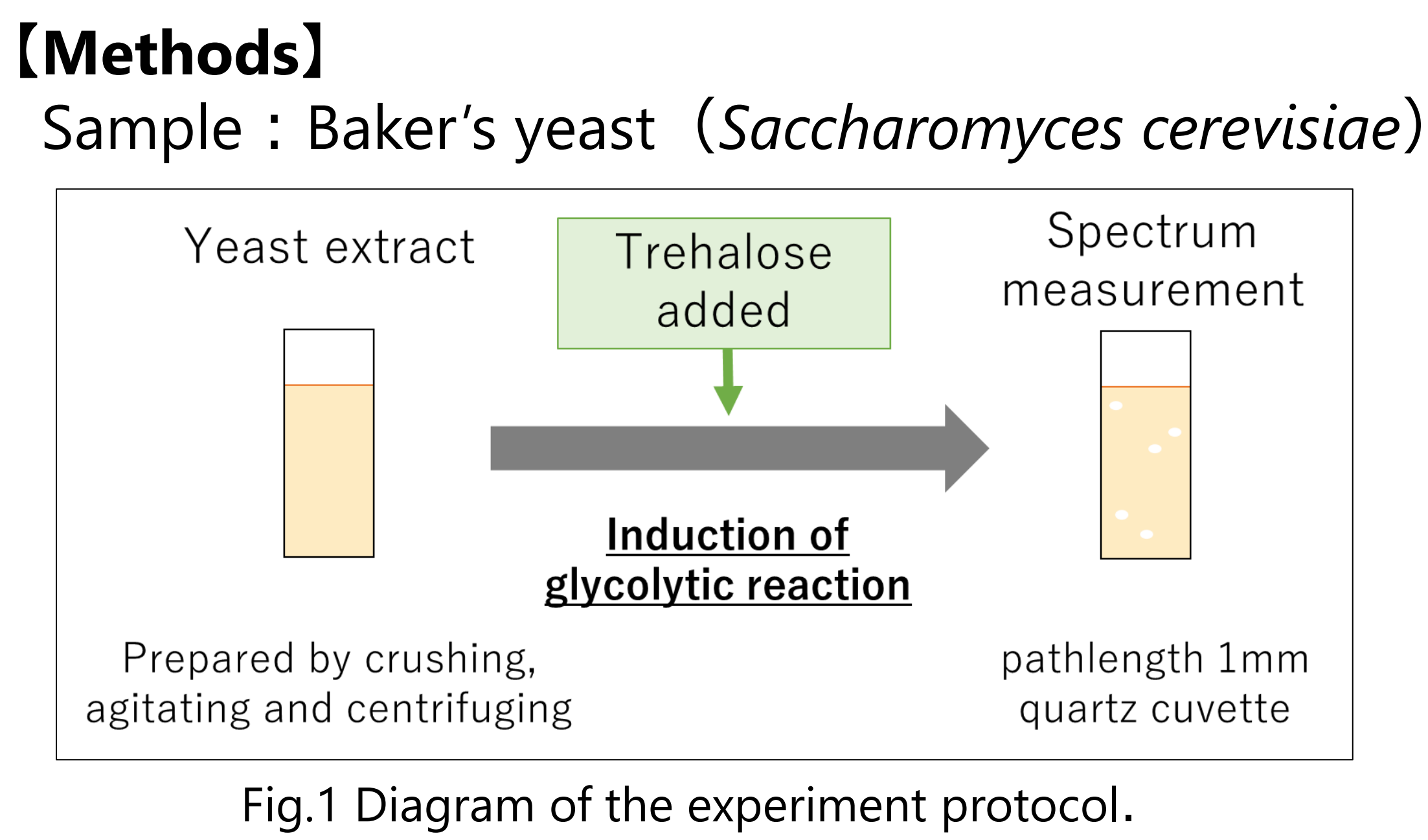


Real-time measurement and analysis of metabolic reactions using phase relationships between metabolites

【Object】
We measured the temporal oscillatory metabolic reactions of baker's yeast in real-time by using near-infrared spectroscopy. We reported the current results that we made a model based on the simultaneous measurement of near-infrared spectra and UV spectra and tried to classify the spectral patterns of each metabolite group.

【Background】
The calibration model (the regression coefficient) calculated by multivariate analysis includes information not only on the target component but also on other metabolites. In other word, when correctly understanding the calibration model, only spectral information on the target component is not sufficient; on the compounds involved in metabolic reactions is also necessary.
In this study, we tried to construct experimental systems and analysis methods for collecting spectral information on metabolites and coenzymes by using baker's yeast, whose metabolic pathways and fluxes are relatively clear.



NADH (Nicotinamide adenine dinucleotide)

- The coenzyme with an absorption peak at 340 nm.
- It is known to oscillate when the glycolytic reaction is working.
- The phase relationships with other metabolites and coenzymes working in the glycolytic reaction is clear (Fig.2 (b)).

- Near-infrared spectrum measurement
 - XDS near- infrared Rapid Liquid™ Analyser, (FOSS)
- UV spectrum measurement
 - Deuterium lamp (DH- 2000, Ocean Optics)
 - Spectrometer (HR4000CG-UV-NIR, Ocean Optics, wavelength range 200-1100 nm)
 - Measured every 90 seconds in transmittance
 - Cuvette temperature controlled at 30 °C by XDS system
- Data analysis
 - Partial least squares regression (PLS regression)
 - Objective variable Absorbance of NADH
 - Explanatory variable Near-infrared spectra
 - Pre-treatment : MSC (Multiplicative Scatter Correction)

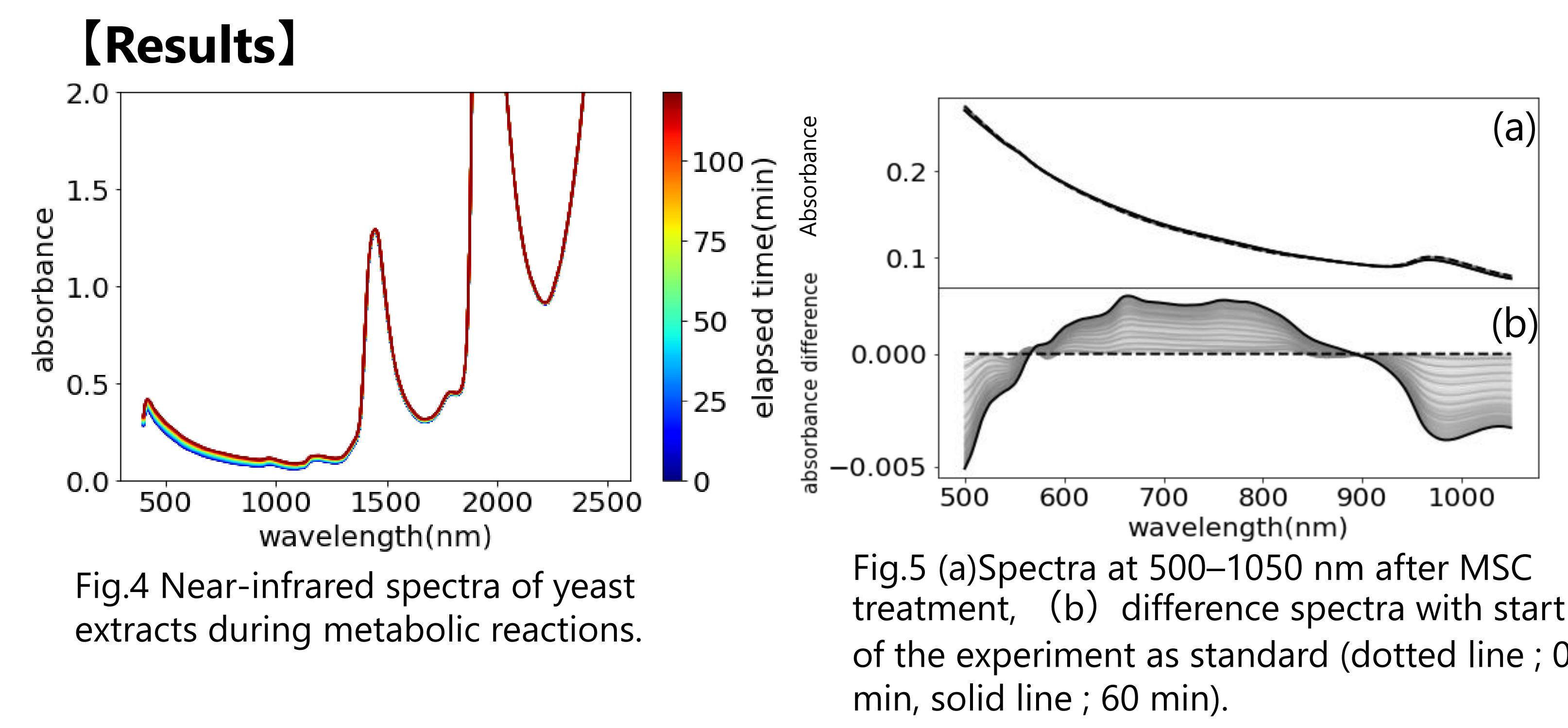
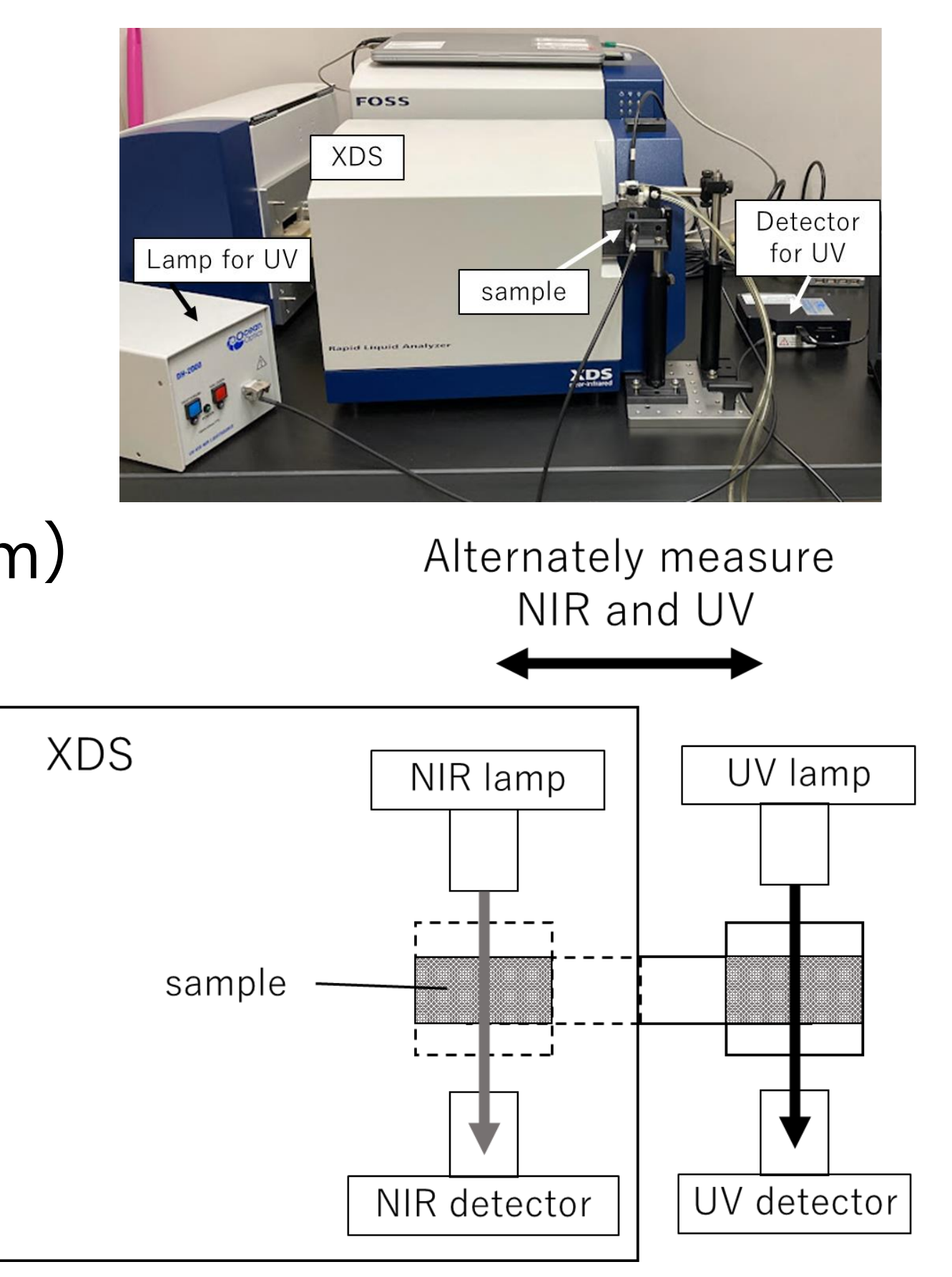
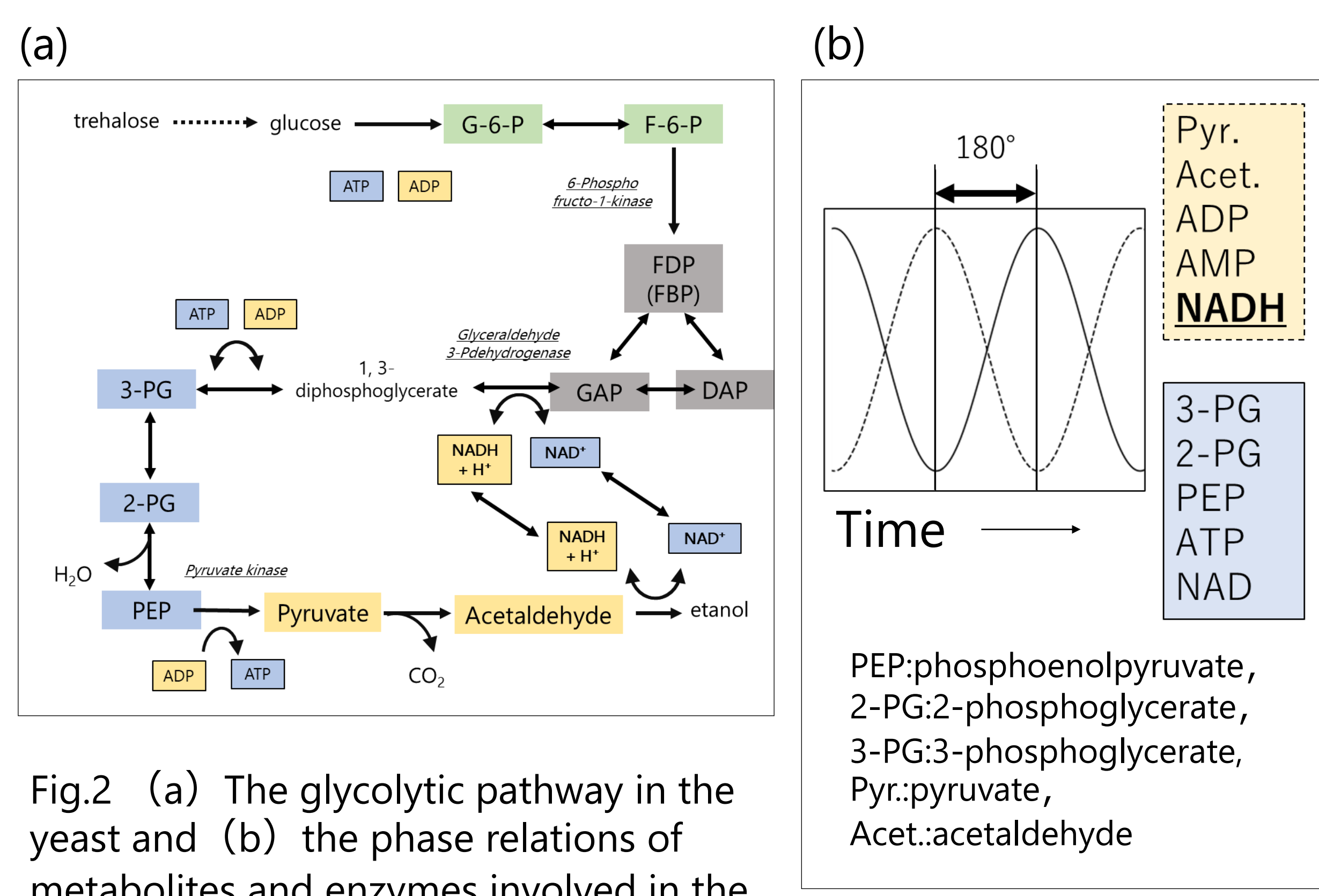


Table1 Predictive accuracy results for each latent variables at 500-1050 nm.

	Number of latent variables				
	1	2	3	4	5
R ²	0.065	0.172	0.459	0.702	0.773
RMSE	7.61×10 ⁻³	7.16×10 ⁻³	5.79×10 ⁻³	4.30×10 ⁻³	3.75×10 ⁻³

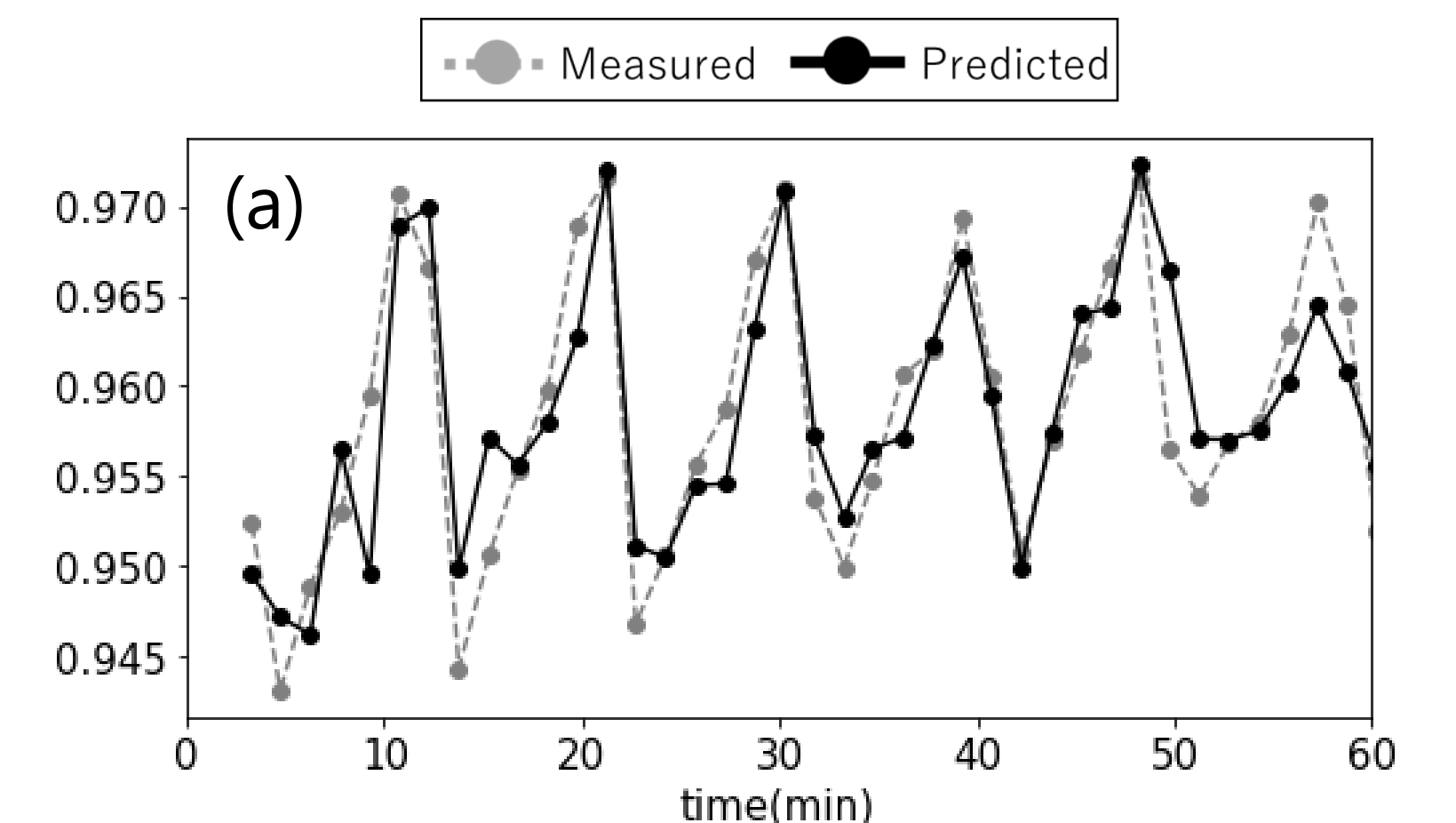
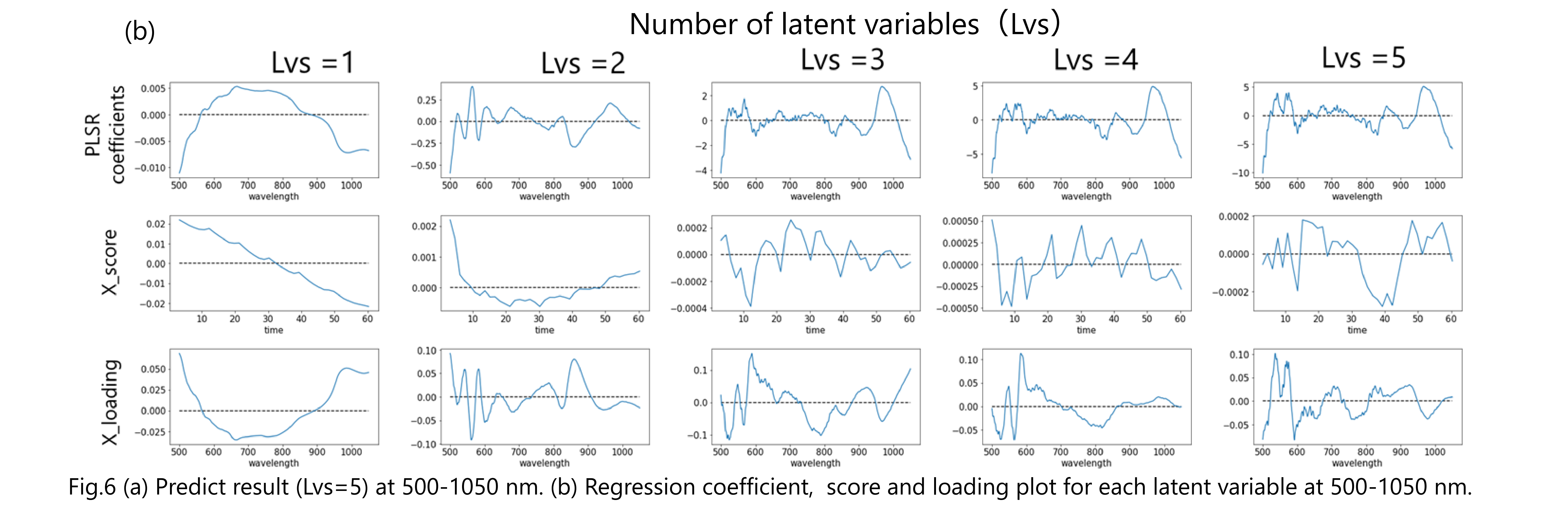


Fig.6 (a) Predict result (Lvs=5) at 500-1050 nm.



- Absorbance of NADH oscillated with a period of about 9.5 min.
 - the glycolytic reaction worked normally during the experiment.
- PLS regression results showed high performance in the 500-1050 nm, 1200-1800 nm and 2100-2300 nm.
 - Lvs=1 : the contribution of explanatory variable is more 90%.
 - the shape of the loading was similar with the difference spectra based on starting of the measurement.
 - Lvs=3, 4 : the contribution of explanatory variable was significantly lower.
 - the score showed oscillations in the same phase or the opposite phase with NADH.
 - The regression coefficient and the loading may contain the spectral information of the metabolite group synchronizing with NADH (Fig.2(b)) .

【Conclusion and future】
We measured the glycolytic reaction of baker's yeast in real time by using near-infrared spectroscopy and created the PLS regression model for the absorbance of NADH. As a result, the oscillation of glycolytic reaction was estimated from near-infrared spectra, and the score oscillated the same period with NADH.
Next, we plan to compare with pure spectra of related metabolites and measure by ¹H-NMR.