^{31}P Nuclear Magnetic Resonance Studies of Sugar Metabolism in *Zymomonas mobilis*

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Abstract

^{31}P-Nuclear magnetic resonance (NMR) spectroscopy is a valuable tool for continuous observation of the metabolic and energy status of the metabolically active cells. It enables to monitor the uptake rates of substrates as well as the formation rates of the various intracellular and extracellular phosphorylated metabolites. Glucose and xylose catabolism in *Zymomonas mobilis* strains were studied using ^{31}P-NMR spectroscopy in vivo. In vivo measurements revealed the noninvasive information about the kinetics of sugar utilization by *Z. mobilis* as well as the energetics of sugar metabolism of the cells grown on glucose and xylose.

What Can ^{31}P-NMR Tell Us?

^{31}P-NMR provides information on…

- the sugar uptake characteristics

  (*in Glucose, Fructose and Xylose metabolisms*)

- the intracellular phosphorylated pools

  (*and their variations with time*)

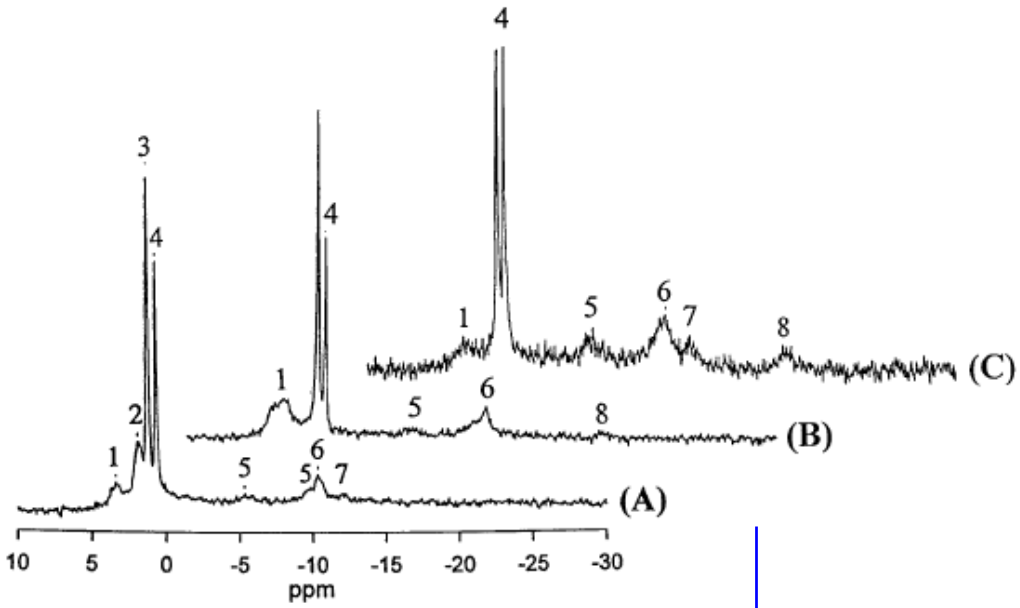
- the energy status of the cell

  (*by observing various nucleoside phosphates and other energy-rich compounds*)

- the intracellular pH

  (*from the chemical shifts of P_{in} and other phosphorylated metabolites with pK values near the physiological pH*)

^{31}P NMR Spectroscopy



Kim, I. S., K. D. Barrow and P. L. Rogers, *Appl. Environ. Microbiol.*, 66, 186-193, 2000.

1. sugar phosphates
2. intracellular phosphate
3. extracellular phosphate
4. TEP as the internal standard
5. NDP
6. NAD and NADP
7. UDP sugars
8. β-NTP

^{31}P NMR spectra of recombinant *Z. mobilis* ZM4(pZB5) cells at 30°C and pH 5.5:

(A) before the addition of sugars as the control;

(B) cells actively metabolizing xylose;

(C) cells actively metabolizing glucose.

Materials and Methods – *in vivo* ^{31}P NMR

Grow the cells to the late exponential phase

↓

Harvest the cells by centrifugation at 8,000 rpm and 4ºC for 15 min

↓

Wash the cells once with 100 mM MES buffer at 9,000 rpm and 4ºC for 15 min

100 mM MES, 5 mM KH_2PO_4, 4 mM $MgCl_2$, 2 mM Na_2EDTA, 50 mM glucose/xylose, pH 5.80

↓

Concentrate the cells by 1 M MES buffer for ^{31}P NMR analysis

1.0 M MES, 50 mM KH_2PO_4, 40 mM $MgCl_2$, 20 mM Na_2EDTA, pH 5.80

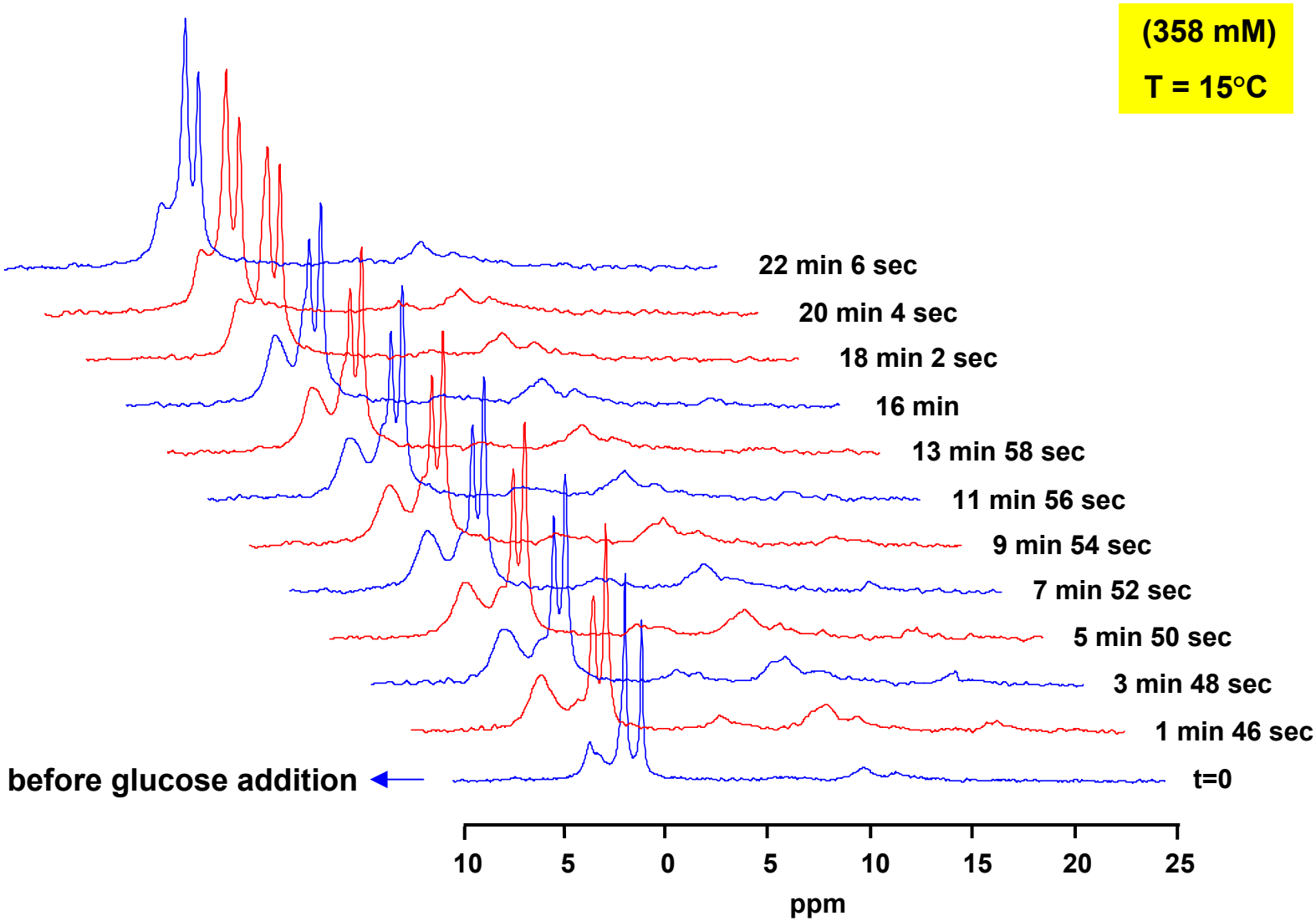
↓

Mix 2.1 ml cell suspension, 450 μl D_2O, 60 μl 1 M triethylphosphate (TEP) and 400 μl 50% (w/v) glucose/xylose solution at zero time for in vivo measurements

→ Strohhacker, J., A. A. de Graaf, S. M. Schoberth, R. M. Wittig, H. Sahm, *Arch. Microbiol.*, 15, 484-490, 1993.

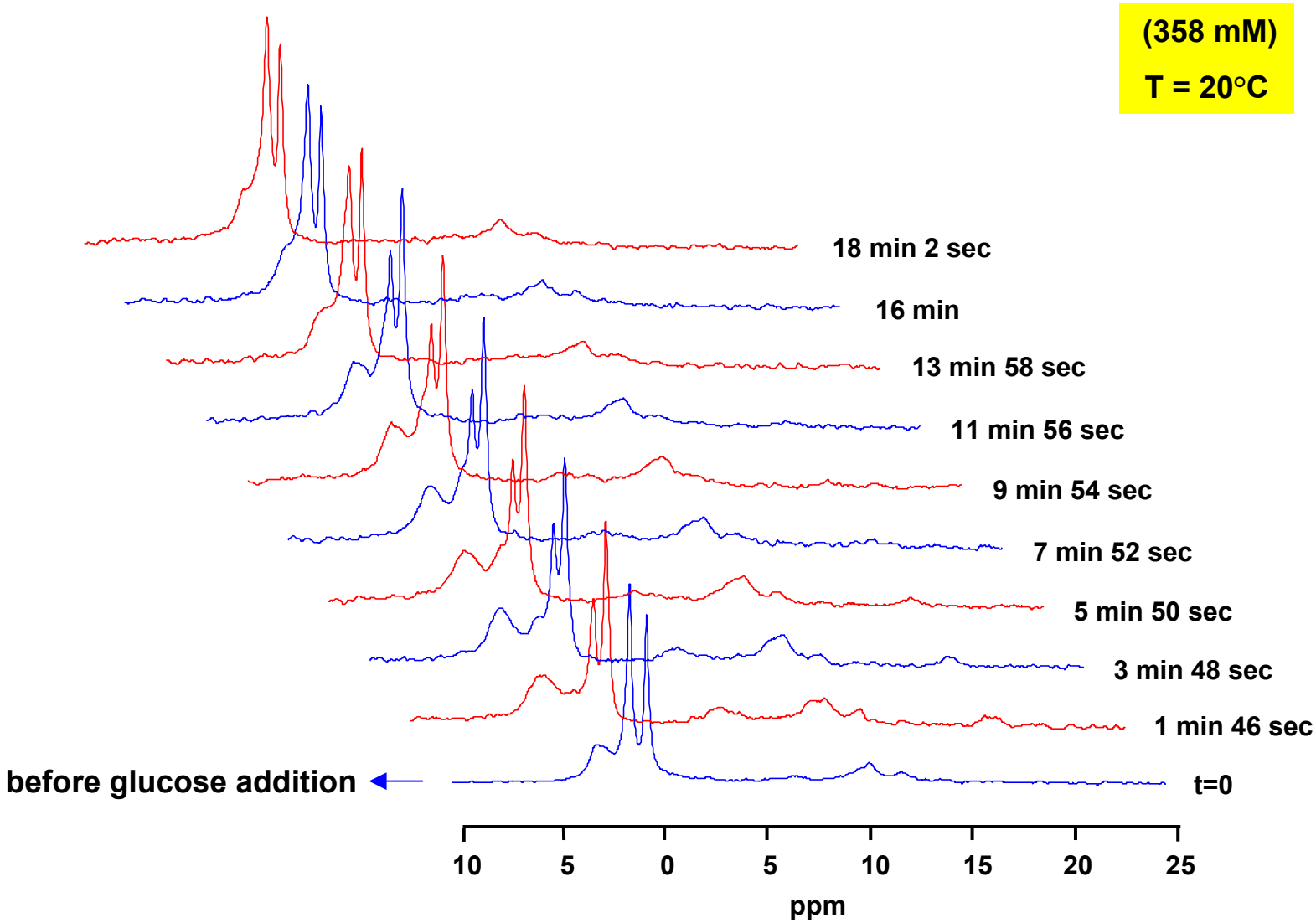
^{31}P NMR Spectroscopy – Glucose 1

when the cells were grown on glucose & the cell suspension was spiked with glucose



^{31}P NMR Spectroscopy – Glucose 2

when the cells were grown on glucose & the cell suspension was spiked with glucose

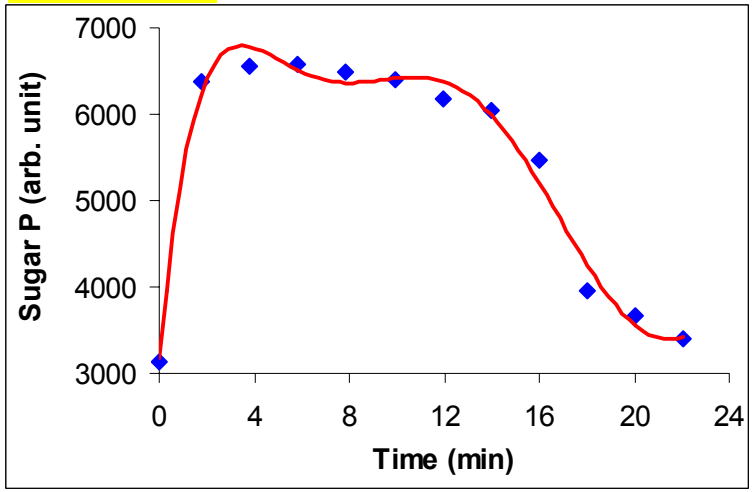


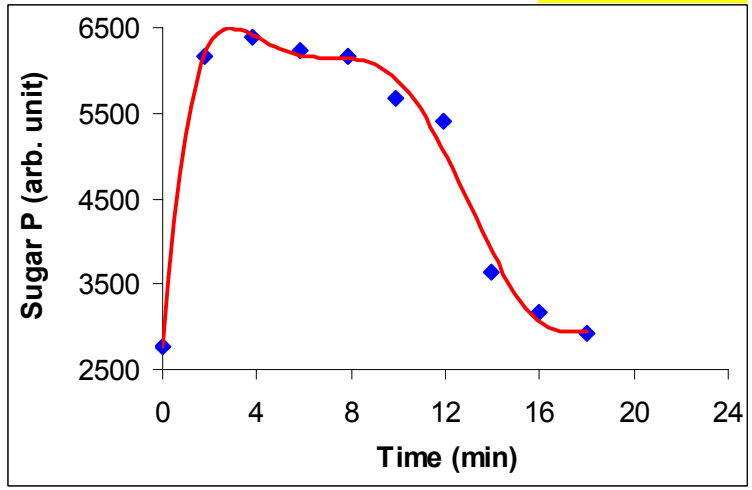
Glucose Metabolism

(358 mM)
T = 15°C

Sugar Phosphates

(358 mM)
T = 20°C

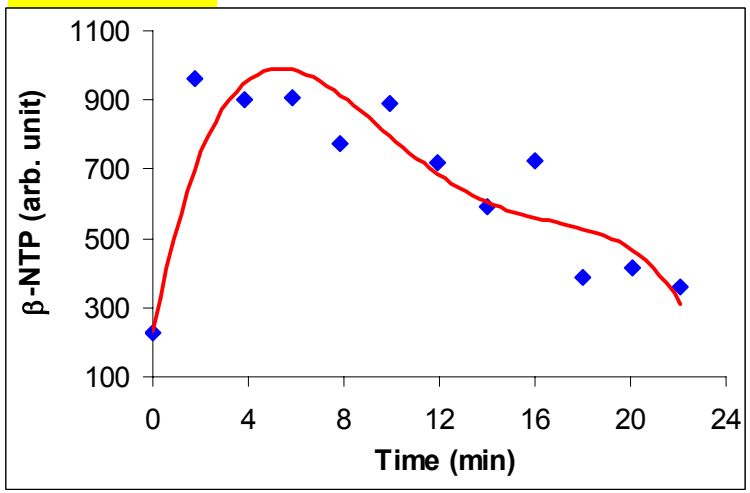


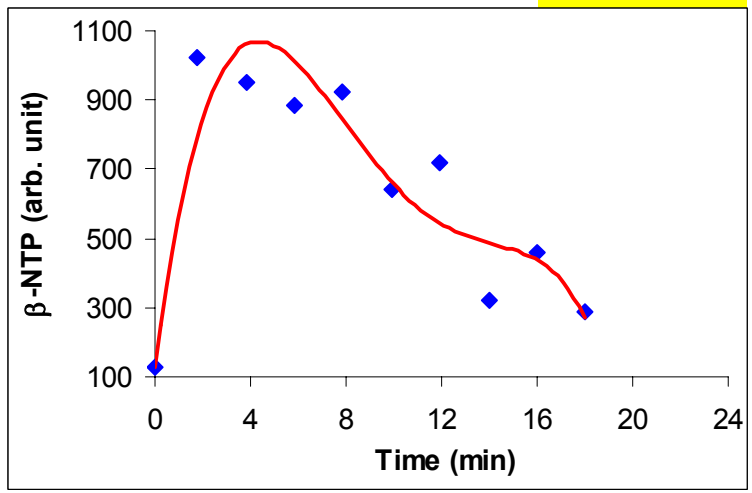


(358 mM)
T = 15°C

Energy Levels

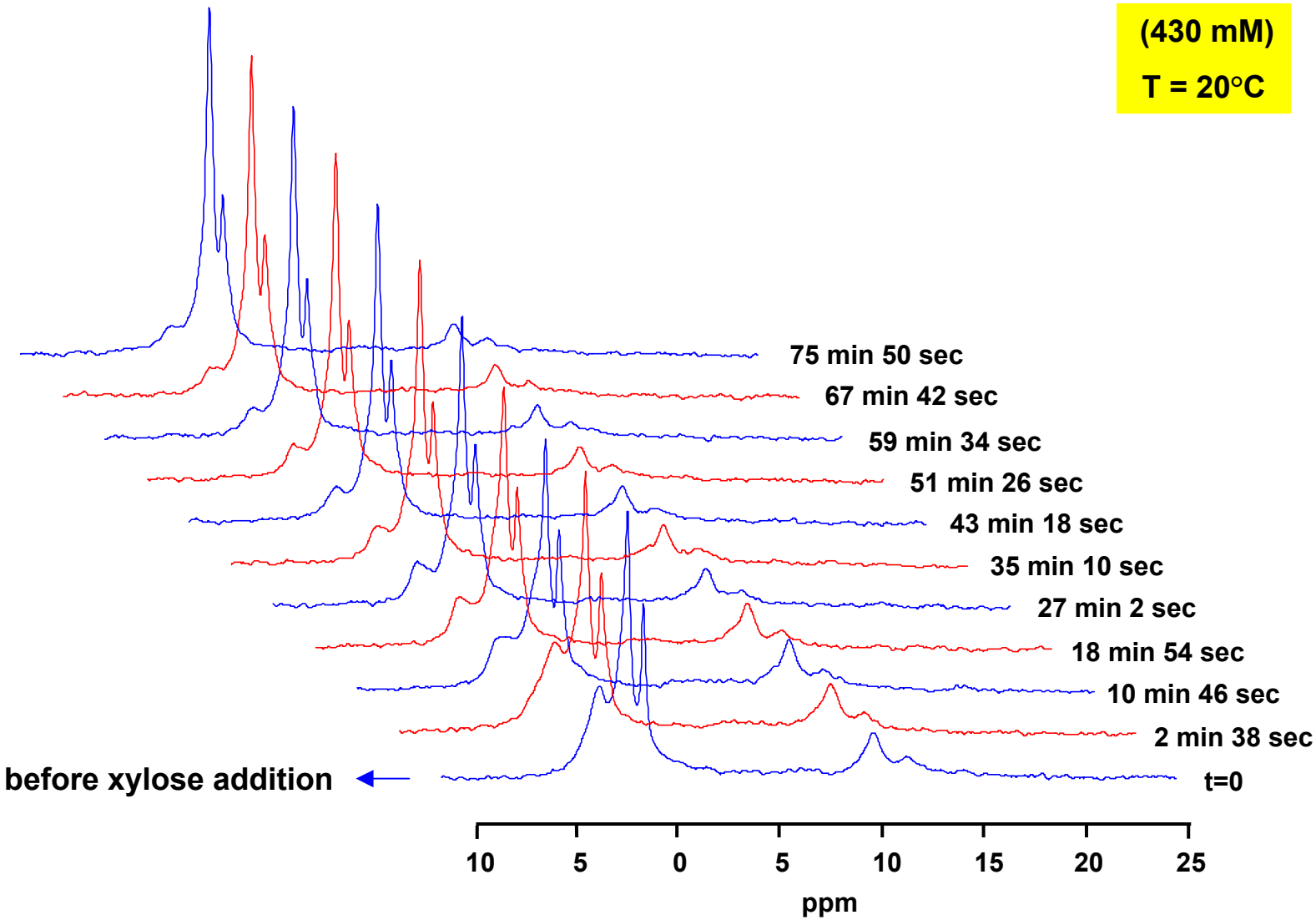
(358 mM)
T = 20°C





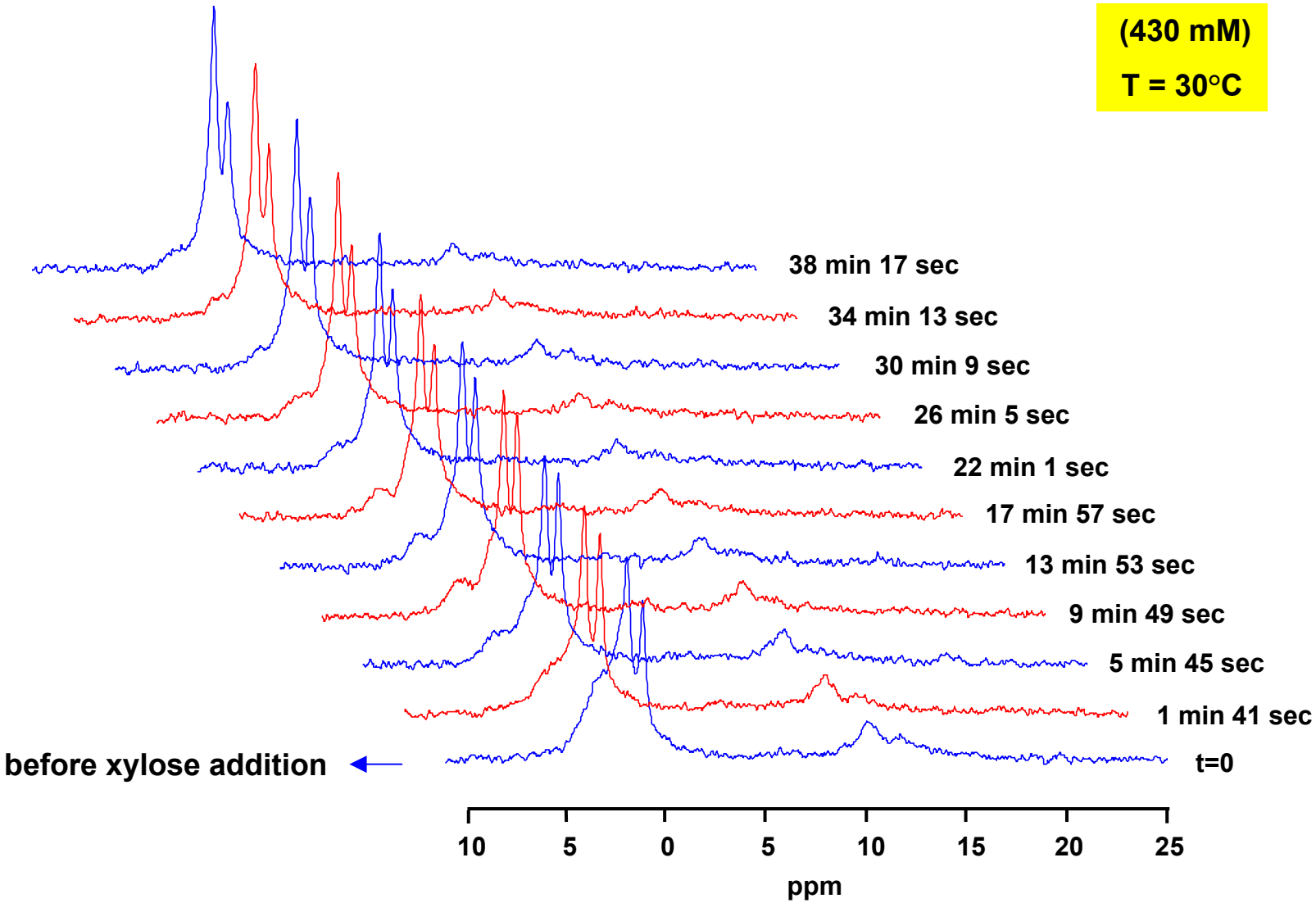
^{31}P NMR Spectroscopy – Xylose 1

when the cells were grown on xylose & the cell suspension was spiked with xylose



^{31}P NMR Spectroscopy – Xylose 2

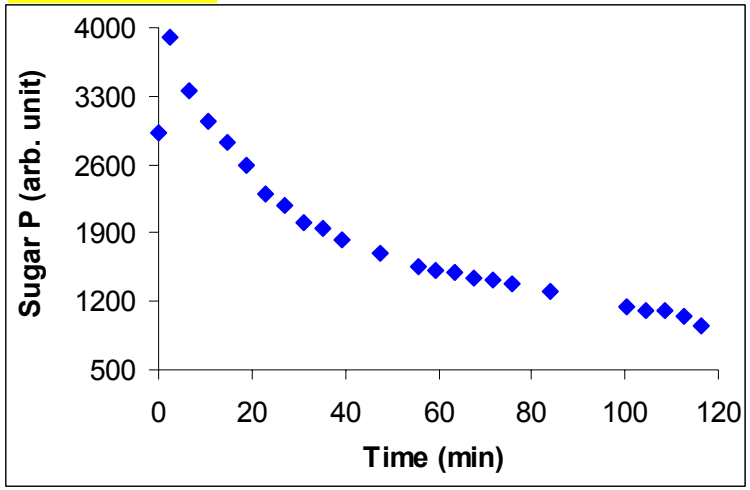
when the cells were grown on xylose & the cell suspension was spiked with xylose



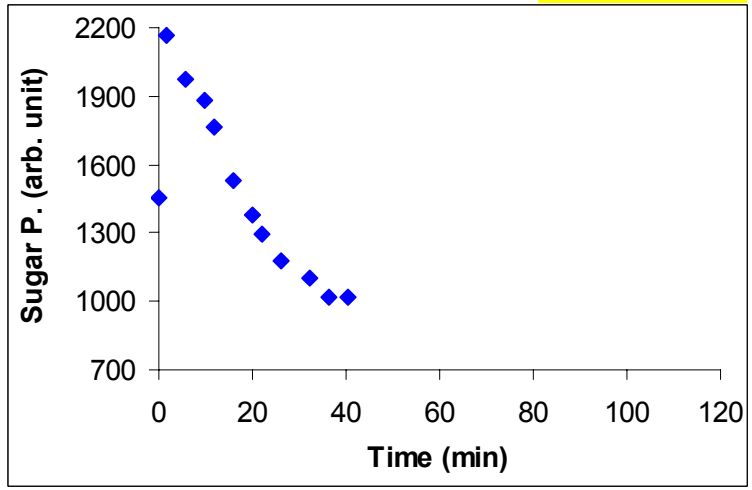
Xylose Metabolism

Sugar Phosphates

(430 mM)
T = 20°C

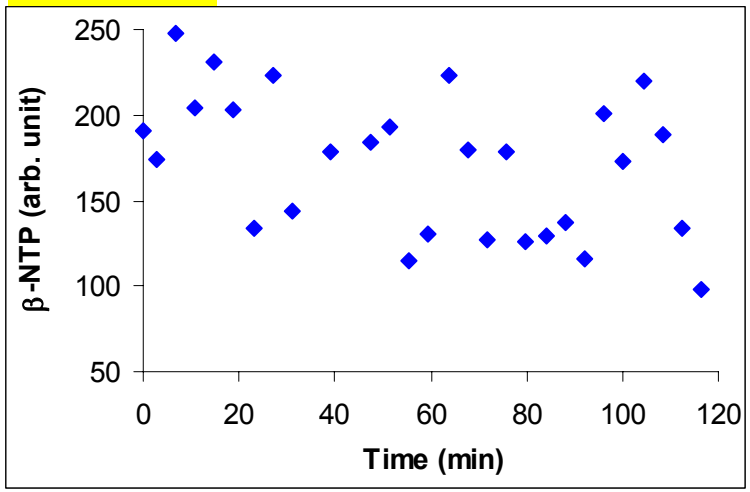


(430 mM)
T = 30°C

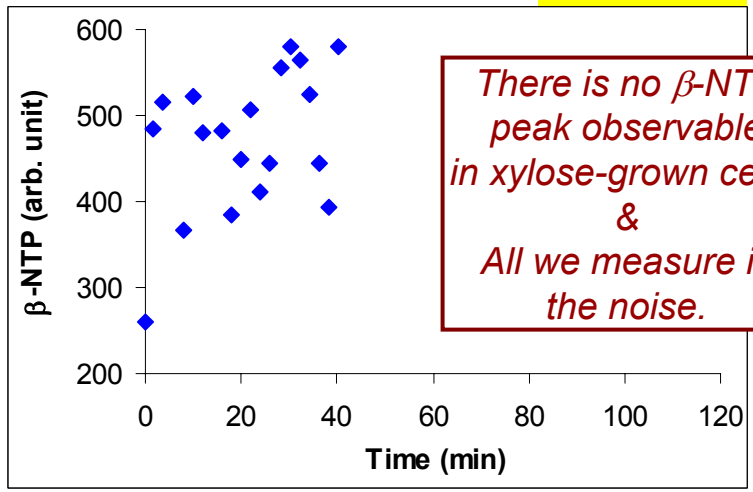


Energy Levels

(430 mM)
T = 20°C



(430 mM)
T = 30°C



There is no β-NTP peak observable in xylose-grown cells.
&
All we measure is the noise.

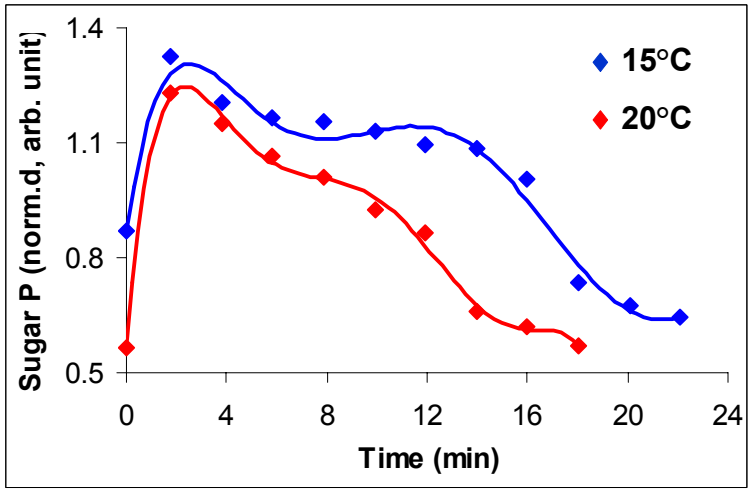
Glucose vs Xylose Metabolism

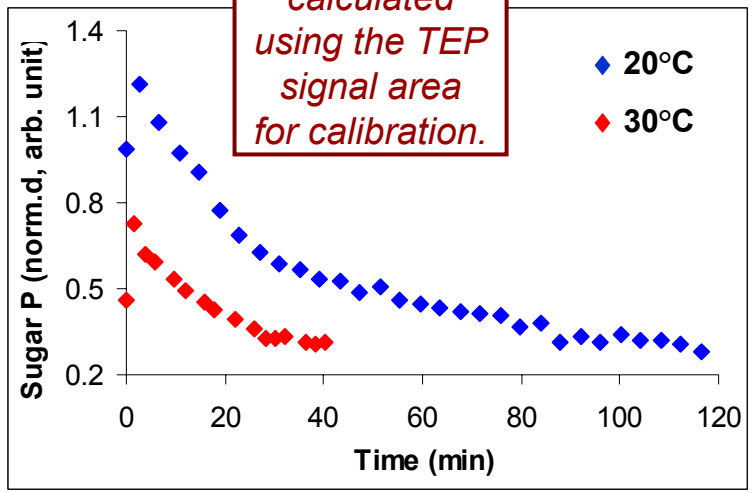
Glucose

Sugar Phosphates

Levels were calculated using the TEP signal area for calibration.

Xylose

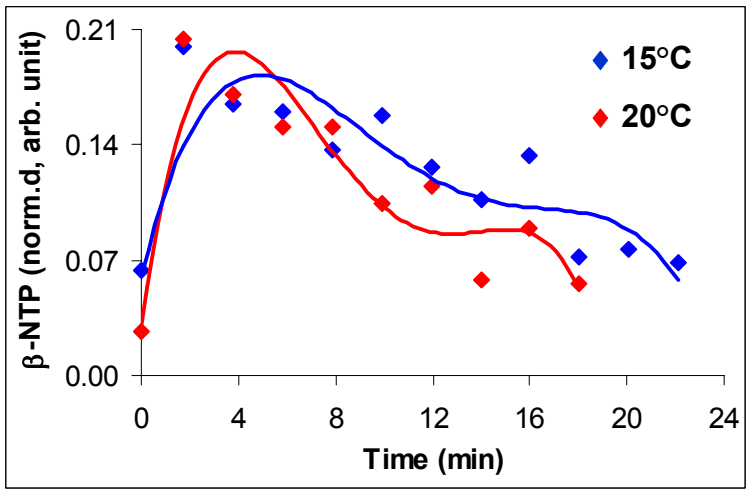


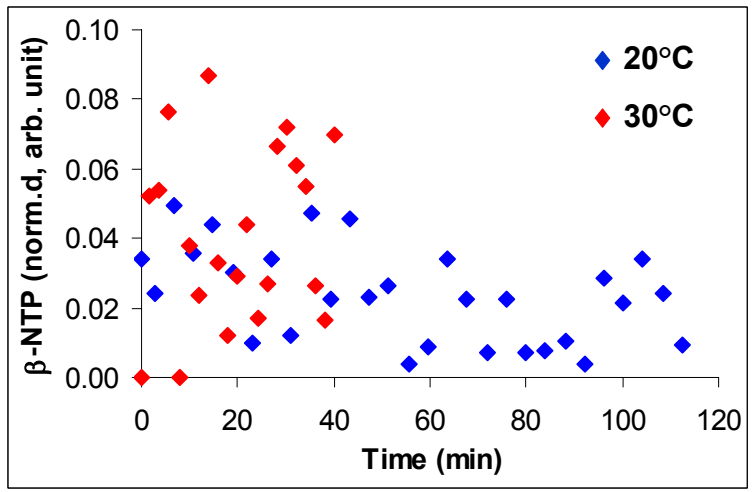


Glucose

Energy Levels

Xylose





Conclusions

- Recombinant cells utilize glucose faster than xylose at 20°C. Xylose metabolism is slower than the glucose metabolism.
- There is no 'β-NTP' peak observable in xylose-grown cells. The 'β-NTP' peaks appear after the cells metabolize glucose and the their levels are similar at 15 and 20°C.
- Xylose-fermenting recombinant cells were significantly less energized than those fermenting glucose.

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