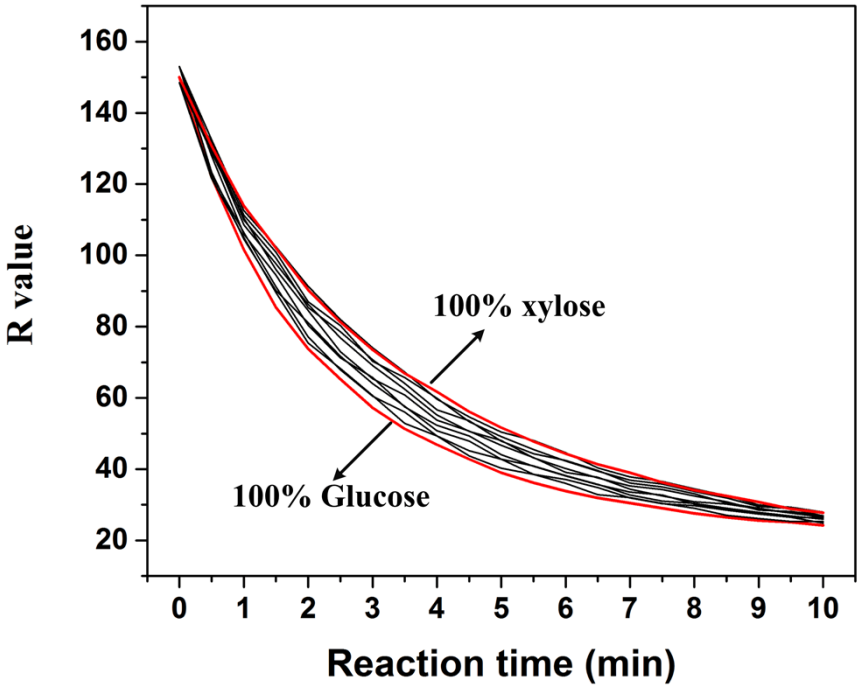
Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Appendix 6

The measurement of mixed sugar using digital color analysis method

In order to make sure whether the method is selective for a sugar or not, we designed the measurement experiments of glucose-xylose mixture. The chosen of glucose and xylose are because the two sugars were widely used in fermentation applications. The experimental program was designed as following:

The total amount-of-substance concentration of sugar mixture was 0.012 mol/L and the glucose-xylose ratios were set at 1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.3:0.7, 0.2:0.8, 0.1:0.9, 0:1. The mixtures were measured using our digital image process method, whose concrete methods were given in ***Experimental*** in our manuscript. The color development processes were shown as S6-1.



S6-1 the color development process of mixed sugar with different proportions of

glucose and xylose. The ratios between glucose and xylose are :1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.3:0.7, 0.2:0.8, 0.1:0.9, 0:1 respectively.

As S6-1 shown, the higher ratio of glucose in the mixture, the faster the color develops. It is mainly because that the reducibility of glucose is higher than that of xylose. At the same time, kinetics curves of the mixtures with different ratios overlapped during the process (0 - 10 min). Therefore, it is hard to determine the glucose and xylose concentration respectively only by color development curves. However, it can be found that the final R values of the mixtures (at 10 min) have little differences between each other. It means that the digital color analysis method can be used for total reducing sugar measurement.

To validation our hypothesis, glucose-xylose mixtures with different total concentration and ratios were measured. The measurement experiments were followed the method in ***Experimental*** in the manuscript and the ANNs model used was the one used for glucose measurement in our manuscript. The results were given in Table S6-1.

Table S6-1 the measurement of total sugar

The real Total concentration of sugar mixture (mol/L)	0.05	0.05	0.04	0.04	0.03	0.03	0.02	0.02
Ratio	9:1	8:2	7:3	5:5	7:3	4:6	3:7	2:8
The total concentration measured by DIAM (mol/L)*	0.048	0.048	0.038	0.037	0.028	0.032	0.018	0.019

The total concentration measured by OD (mol/L)	0.046	0.045	0.036	0.034	0.027	0.028	0.018	0.018

Note: DCAM was short for *digital color analysis method.* OD stands for the absorbance degree in the traditional method.

The detection error for total sugar was within 10%, which is higher than that of single sugar (6%), but much higher that the traditional OD method (15%). So, the digital color analysis method is more suitable for the measurement of single sugar (no other sugar exists). At the same time, it also can be used for the measurement of sugar mixture, which is more accurate than the traditional OD method.