Reversibility of enzymatic reactions might limit biotransformation of organic micropollutants

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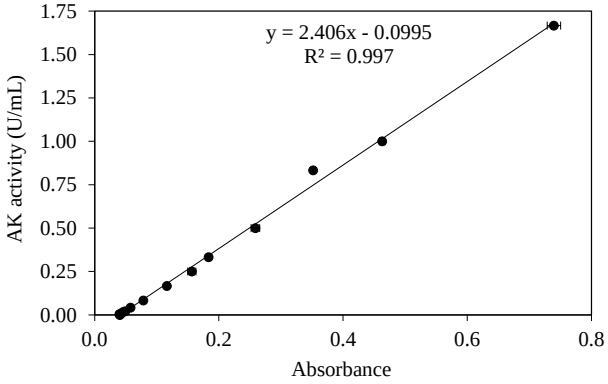

Figure S1. Standard curve to correlate absorbance and AK activity from *E. coli* (n = 4).

Section II. Measurement of HK activity

HK activity was measured by the hexokinase colorimetric assay kit. As summarized in Figure S2, glucose is converted to glucose-6-phosphate by the enzyme in the sample; the glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to form NADH, which reduces a colourless probe to a coloured product (450 nm) proportional to the HK activity. This procedure is conducted in 96-well plates. Briefly, 5–10 µL of sample from the assays with OMPs were diluted with the provided buffer to obtain 50 µL of sample (in duplicate), then 50 µL of reaction mix (prepared with the provided buffer, enzyme mix, developer, coenzyme and substrate) was added. The plate was incubated at room temperature for 30 min and the absorbance (450 nm) was measured in kinetic mode each 2 min in a BioTek Synergy 2 plate reader (BioTek Instruments Inc., USA). The slop (absorbance/min) obtained from the linear range of the curve was substituted in the standard curve equation (Figure S3) and divided by the volume of sample (5–10 µL) to obtain the activity. Blanks (without substrate) and positive controls (with purified HK provided in the kit) were performed in each measurement. The absorbance values of the blanks were subtracted from all the readings to correct the sample background. Positive controls were used as a benchmark sample to verify the kit is working properly.

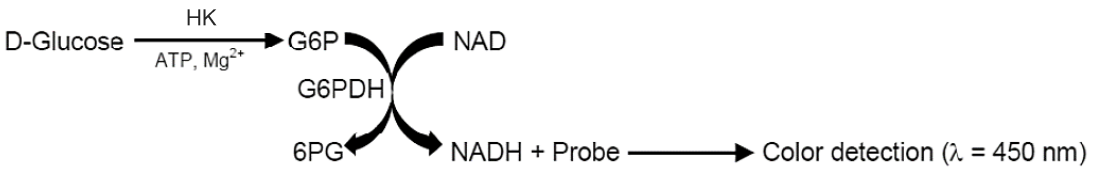

Figure S2. Schematic representation of the coupled enzyme assay to measure HK activity (abcam protocol).

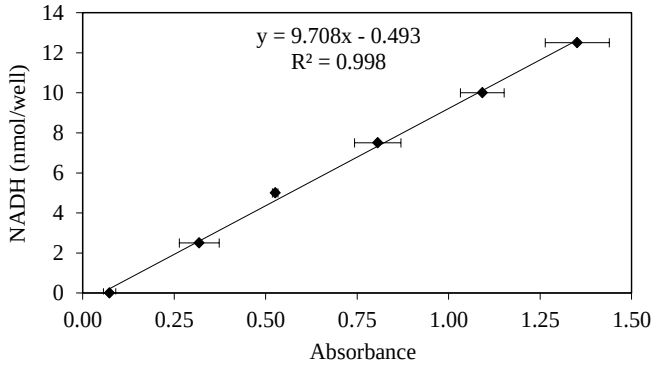

Figure S3. Standard curve to correlate absorbance and the production of NADH in the HK activity assays (n = 4).