Unveiling the influence of carbon nanotube diameter and surface modification on the anchorage of L-asparaginase

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ASNase activity determination:

One unit of free ASNase activity is defined as the enzyme amount that releases 1 µmol of ammonia per minute at 37 ℃ (Eq. S1):

$$ASNase\ activity\ \left(\frac{U}{mL}\right) = \frac{\left[NH_3\right]\left(\frac{\mu mol}{mL}\right) \times V_{Nessler}(mL) \times f_d}{t_r(\min) \times V_{Enzyme}(mL)} \tag{S1}$$

where $V_{Nessler}$ is the volume of the Nessler solution, f_d is the sample dilution factor, t_r is the reaction time, and V_{Enzyme} is the volume of enzyme solution.

One unit of immobilized ASNase activity corresponds to the enzyme amount that releases 1 µmol of ammonia *per* minute and support mass at 37 ℃ (Eq. S2).

$$ASNase\ activity\ \left(\frac{U}{mg}\right) = \frac{\left[NH_3\right]\left(\frac{\mu mol}{mL}\right) \times V_{Nessler}(mL) \times f_d}{t_r(\min) \times m_s(mg)} \tag{S2}$$

where m_s is the mass of the support.

Immobilization Yield (IY) determination:

The immobilization yield, *IY* (%), corresponds to the difference between free ASNase activity before immobilization and free ASNase activity that remains in the supernatant after immobilization divided by free ASNase activity before immobilization (Eq. S3).

$$IY\ (\%) = \frac{Free\ ASNase\ Activity\left(\frac{U}{mL}\right) - Supernatant\ ASNase\ Activity\left(\frac{U}{mL}\right)}{Free\ ASNase\ Activity\left(\frac{U}{mL}\right)} \times 100 \tag{S3}$$

Relative recovered activity (RRA) determination:

The relative recovered activity, *RRA* (%), of the immobilized ASNase (Eq. S4) was determined as the ratio between the effectively immobilized ASNase activity and the maximum theoretical activity that would exist if the free ASNase was completely immobilized (Eq. S5).

$$RRA \ (\%) = \frac{Immobilized \ ASNase \ activity\left(\frac{U}{mg}\right)}{Maximum \ ASNase \ activity\left(\frac{U}{mg}\right)} \times 100 \qquad\qquad (S4)$$

where:

$$Maximum \ ASNase \ activity \ \left(\frac{U}{mg}\right) = \frac{[NH_3]_{free \ ASNase}\left(\frac{\mu mol}{mL}\right)\times V_{Nessler}(mL)\times f_d}{t_r(\min)\times m_s(mg)} \qquad (S5)$$
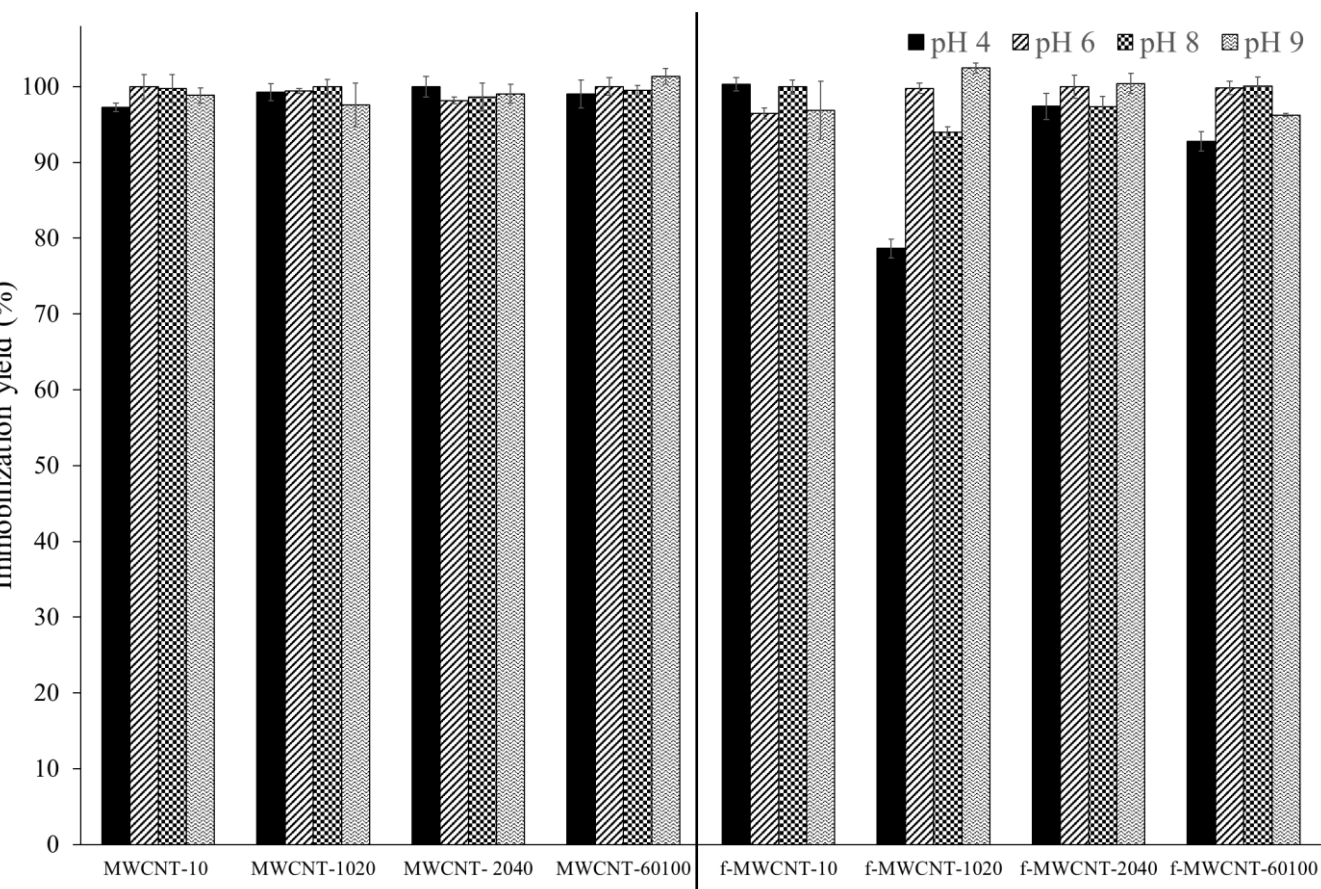
Figure S1 - Effect of pH and diameter of multi-walled carbon nanotubes (MWCNTs) on the immobilization yield (*IY*). Immobilization of 0.086 mg/mL of L-asparaginase (ASNase) onto 2 mg of pristine (MWCNTs) and functionalized MWCNTs (f-MWCNTs) for 60 min of contact time. Error bars correspond to the standard deviation between replicates.
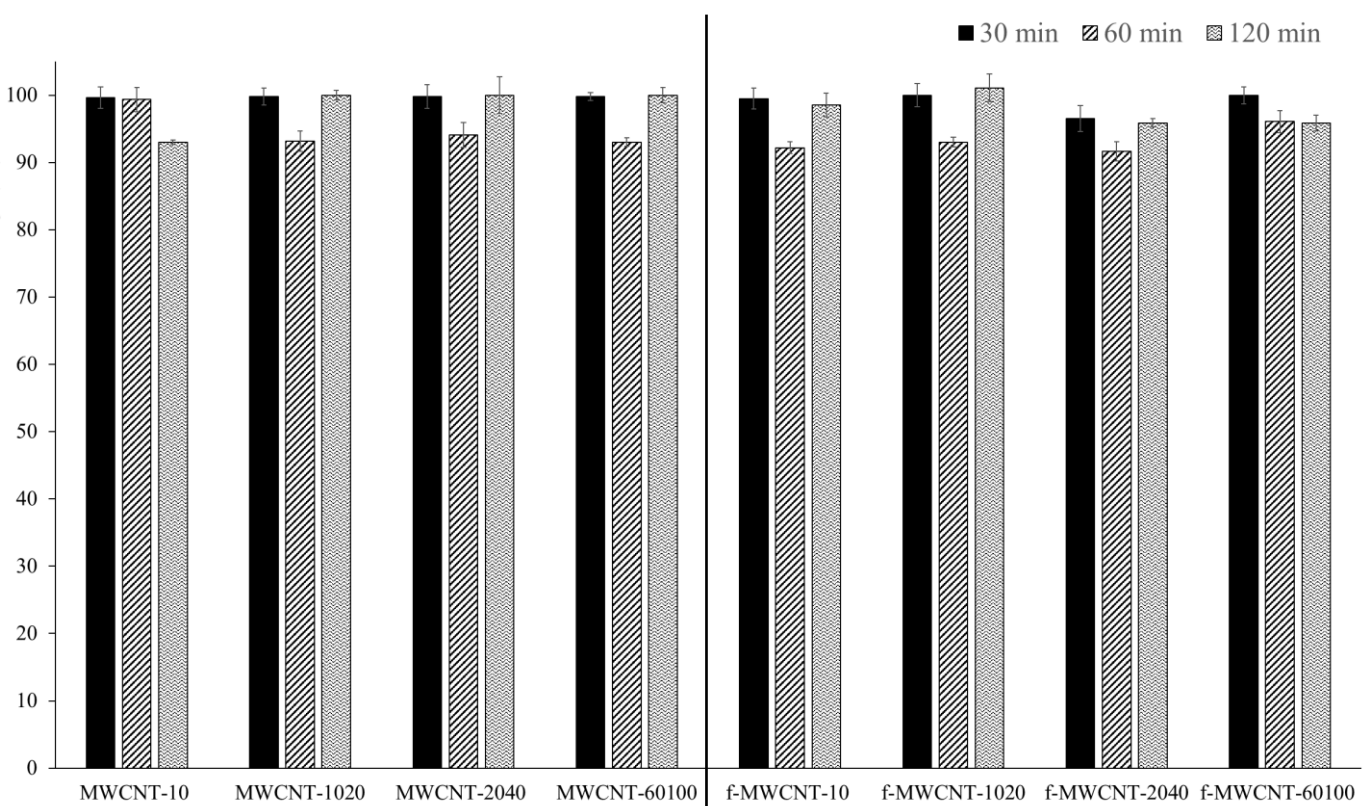
Figure S2 - Effect of immobilization time and diameter of multi-walled carbon nanotubes (MWCNTs) on the immobilization yield (*IY*). Immobilization of 0.086 mg/mL of L-asparaginase (ASNase) onto 2 mg of pristine (MWCNTs) and functionalized MWCNTs (f-MWCNTs) at pH 8. Error bars correspond to the standard deviation between replicates.