

PROLUNG

DEGRADATION

ACTIVITY TESTING

LAB BOOK 4

Biobrick sialidase assay test

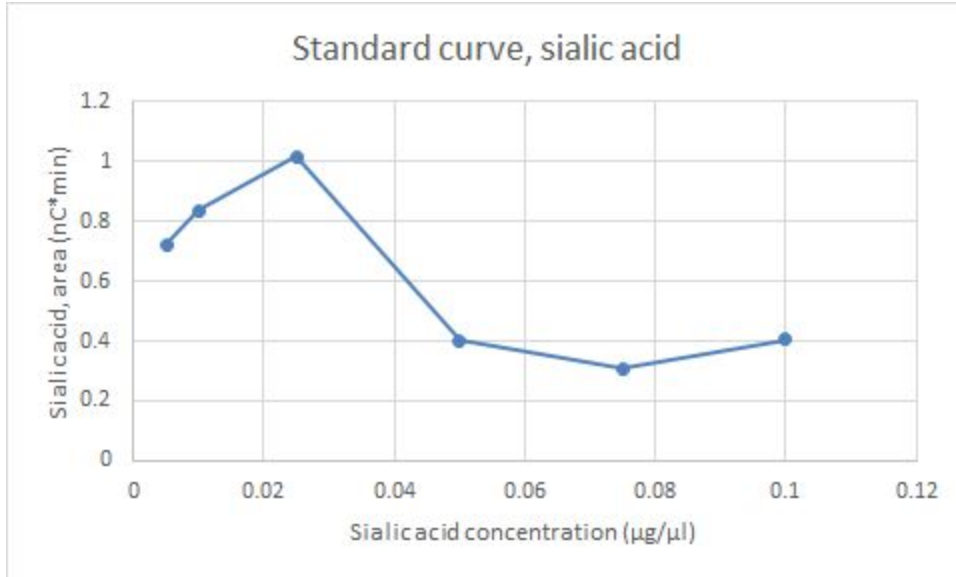
Objective:

- To test the purified biobrick sialidase that was produced the previous week.
- Background information
 - The protocol for the assay has been created and optimized by iGEM Stockholm 2017.
 - The sialidase activity was tested on bovine submaxillary mucin (BSM). Sialic acid degraded from the mucin is first filtered and later quantified using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

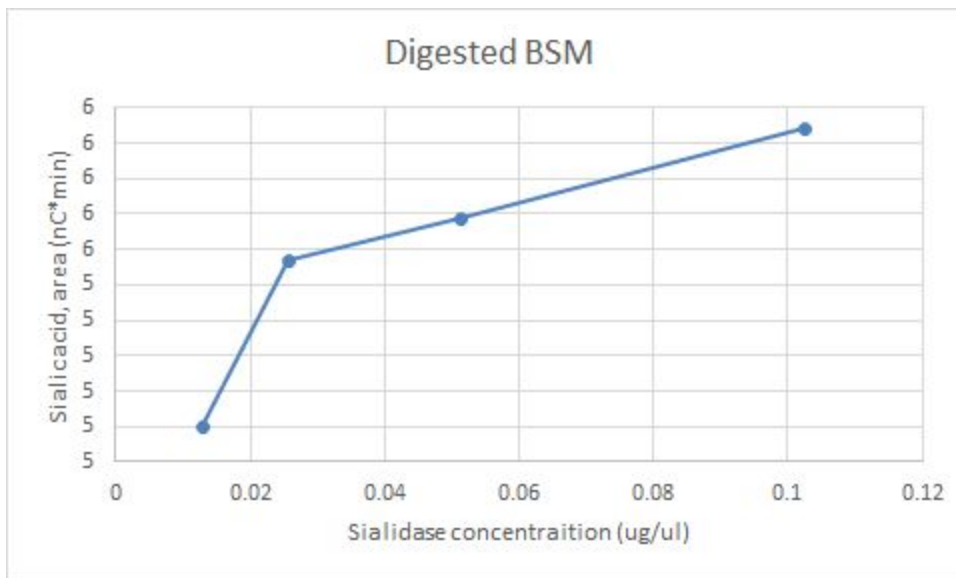
Procedure:

- Sialidase assay protocol by iGEM 2017 was followed.
- 4 digested samples were tested with different concentrations of sialidase enzyme.
- No negative control was performed. Only a positive control using sulfuric acid, as mentioned in the protocol.
 - The positive control had half the mucin solution volume in comparison to the rest of the samples.

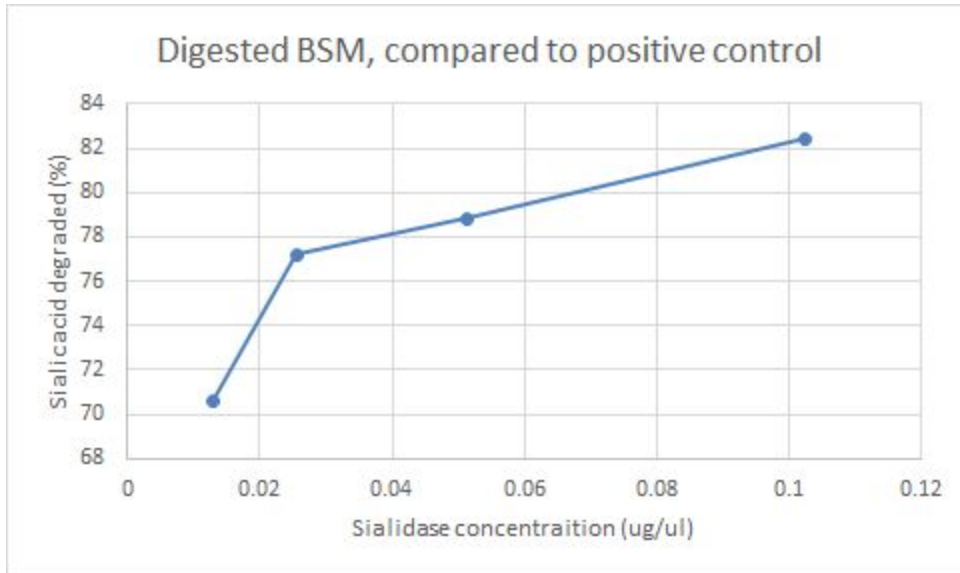
Results:



The standard curve of sialic acid concentration compared to the signal strength from the HPAEC.



The enzymatically digested BSM's signal from the HPAEC.



Using the positive control as a reference (100% digestion) the signal strength from the degraded BSM is each compared to the other digested samples.

Discussions:

The standard curve in this experiment did not show linearity. Therefore, it could not be used for quantification of degraded sialic acid. This may be caused by poor dilution and pipetting when working with small volumes.

Instead an additional graph was added using the positive control as a reference.

Calculations:

100% digest from positive control

*=> 3.5431 nC*min*

The signal needs to be doubled as a result of the halved volume

*=> 3.5431 * 2 = 7.0862 nC*min*

As an example, comparison of highest sialidase concentration digested sample

*=> (5.8411/7.0862)*100% = 82.42923%*

Conclusions:

- The sialidase enzyme produced by the biobrick in *E.coli* BL21 is active.
- The next step would be repeating the experiment with a better standard curve and a negative control.