Immobilization of fungal α-amylase on Graphene Oxide-Fe_3O_4 magnetite nanoparticles for yielding more production of high maltose containing syrup

Dave Dolly[1], Desai Rucha[2*] and Kikani Bhavtosh[3*]

[1]*Department of Biological Sciences, P.D. Patel Institute of Applied Sciences, Charotar University of Science and Technology, Changa – 388 421, Gujarat, India (davedolly709@gmail.com)*

[2] *Department of Physics, P.D. Patel Institute of Applied Sciences, Charotar University of Science and Technology, Changa – 388 421, Gujarat, India (ruchadesai.neno@charusat.ac.in)*

[3] *Department of Biological Sciences, P.D. Patel Institute of Applied Sciences, Charotar University of Science and Technology, Changa – 388 421, Gujarat, India (bhavtoshkikani.bio@charusat.ac.in)*

Abstract

Among hydrolytic enzymes, α-amylase (EC 3.2.1.1) shares almost 38% of global market. Being an endoamylase, it catalyses starch into maltose (major end product) and glucose (trace amount). Amylases share range of industrial applications, yet, industries demand for more robust and thermostable amylases for better applications. Therefore, a range of approaches is employed to meet the industrial demands. In the current study, we tried immobilize fungal α-amylase on Graphene Oxide-Fe_3O_4 magnetite nanoparticles with significant practical yield. Due to covalent binding, detachment of the amylase was greatly reduced. Therefore, the immobilized amylase was re-used for 9 subsequent cycles very efficiently. The temperature and pH range for amylase catalysis and stability was also be broadened upon immobilization. Alongside, the temperature and pH optima for catalysis shifted from 50˚C to 80˚C and pH 7 to pH 5, respectively. Calculation of various thermodynamic parameters, such as enzyme half-life, deactivation rate constant, changes in enthalpy, entropy, activation energy and Gibb's free energy also suggest stable enzyme-substrate reactions at higher temperatures. The immobilized amylase was also employed to produce high maltose containing syrup, consisting of three stages: gelatinization (90˚C for 2 h), liquefaction (75˚C for 6 h) and saccharification (50˚C for 24 h). At each step, the amylase hydrolyzed starch to produce 1.4, 4.06 and 7.06 g/ml reducing sugars, respectively. Eventually, the structural stability of immobilized amylase was deduced and established using FTIR and TGA analysis. Overall, the attributes of immobilized amylase: better immobilization yield, higher thermostability, broad pH stability, better operational stability (re-useability) and efficient starch hydrolysis highlights its future commercial applications in the production of high maltose containing syrup.

Materials and Methods

Activation of Graphene Oxide (GO):

1mg/mL concentration of GO powder was ultrasonicated for 30min at 30mV.

Synthesis of Fe_3O_4nanoparticles by co-precipitation:

In 4mL of distilled water, 1mL of activated GO. Then 10mL of FeCl2.4H2O and 10mL of FeCl3.6H2O added into the mixture under constant stirring condition followed by addition of 5mL of ammonia solution and mixture was stirred for 5min. Generated particles were washed by 10Mm phosphate buffer(pH-7.0) and with distilled water. Stored at 10°C with final volume of 100mL of 10Mm phosphate buffer (pH-7.0)

Immobilization of α-amylase on synthesized Fe_3O_4 –GO nanoparticles:

For immobilizing 5mL of mixture; 1µL to 1000µL of Fe_3O_4 –GO was used with constant enzyme volume of 4mL (1mg/mL) and rest of volume was adjusted with 10mM phosphate buffer (pH-7.0) and was kept under shaking condition at 20°C-25°C. GO and Fe_3O_4

Optimization of pH

To examine the effect of pH, Fe_3O_4 –GO immobilized with α-amylase was incubated with 2% starch as a substrate. The reaction mixture was prepared in following buffer of 10mM concentration; sodium acetate buffer pH-5.0, phosphate buffer pH-6.0 and pH-7.0, TrisHCl buffer pH-8.0, glycine NaOH buffer pH-9.0 and pH-10.0. After incubation at 37°C for 20min, enzyme activity assay was performed by DNSa method and graph of pH vs. relative activity was plotted. Experiments were performed in triplicates.

Optimization of temperature

To check effect of temperature Fe_3O_4 –GO immobilized with α-amylase was incubated with 2% starch as a substrate at temperature from 10°C to 100°C for 20min and enzyme activity assay was performed by DNSa method. Graph of temperature vs. relative activity was plotted Experiments were performed in triplicates.

Determination of substrate concentration in the assay

To confirm the substrate concentration required for the system, assay was performed of 0.2%,0.4%,0.6%,0.8%,1.0%,1.2%,1.4%,1.6%,1.8 %,2.0%,2.2%,2.4%,2.6%, 2.8% and 3.0% of starch concentration and enzyme activity assay was done by DNSa method. Graph of 1/[S] vs. 1/V was plotted to calculate Km and Vmax. Experiments were performed in triplicates.

Thermal and pH stability analysis of Fe_3O_4 – GO immobilized with α-amylase

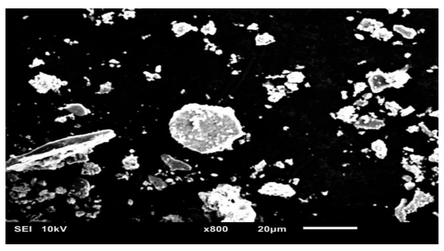
To determine the thermal and pH stability,Fe_3O_4 – GO immobilized with α-amylase was incubated at 37°C, 50°C, and 70°C at pH-5.0, pH-7.0,pH-9.0. at regular interval of 0min,1hr,3hr,6hr,24hr,27hr samples were withdraw and enzyme activity was performed by DNSa method. Graph of incubation time vs. residual activity was plotted. Experiments were performed in triplicates.

Bradford method to estimate protein concentration.

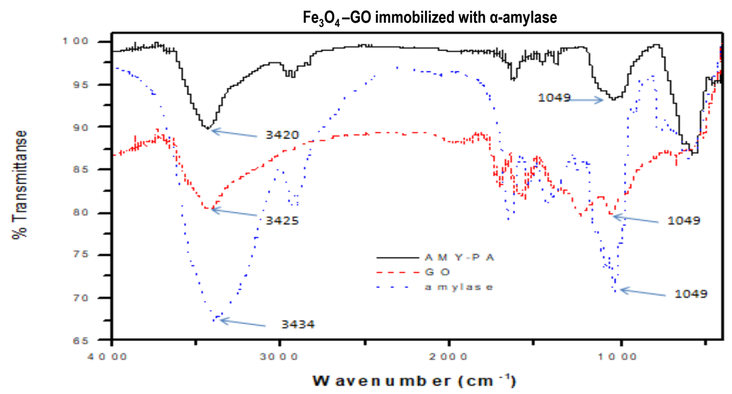
Results and Conclusions

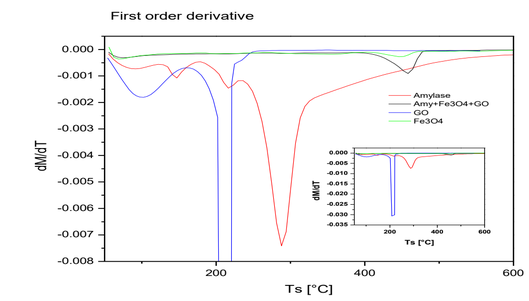
Fig.3.1.2 Fe_3O_4 –GO magnetite nanoparticles suspended in 10mM phosphate buffer (pH-7.0) without magnetic field.

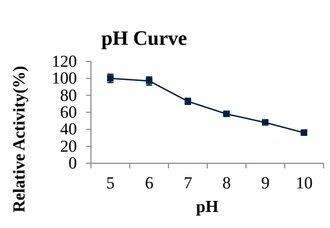
Fig 3.1.2 Fe_3O_4–GO magnetite nanoparticles suspended in 10mM phosphate buffer (pH-7.0) with magnetic field.

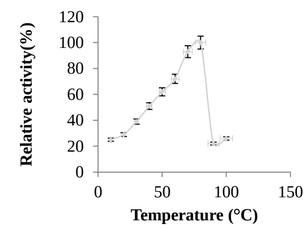


Fe_3O_4–GO immobilized with α-amylase

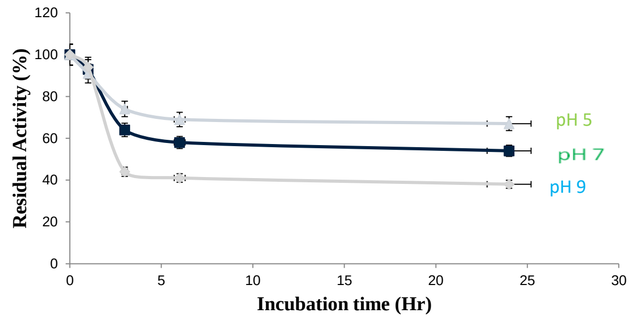


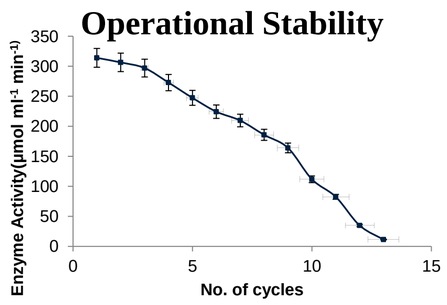






70°C





Acknowledgment:

Authors would like to thank P.D.Patel institute of applied sciences for providing infrastructural support to conduct research.