

AN INDUSTRY PERSPECTIVE

**USP Enzyme Workshop
July 8, 2009
Presented on Behalf of
Enzyme Technical Association
by Gary L. Yingling**



Agenda

- ETA Background
- ETA View of FCC
- Enzymes
- A Concern
 - Harmonized Method
 - Standards for Testing Enzymes
 - Single enzyme monograph



Statement of Purpose

The Enzyme Technical Association (ETA) represents manufacturers and distributors of enzyme preparations for food, feed and industrial uses. The ETA's membership represents a majority of the North American enzyme industry with members located in the United States, Canada, and Mexico. ETA has been in existence since 1970 and has taken an active role in providing information to support the development of regulations and policies that affect the enzyme industry both in North America and internationally.



ETA Membership (19)

Members

AB Enzymes
Amano Enzyme USA
Bio-Cat, Inc.
Chr. Hansen
Deerland Enzymes
DSM Food Specialties USA, Inc.
Enzyme Development Corp.
Enmex SA de CV
Genencor, a Danisco Division
logen Corp.
Kerry Ingredients & Flavours
National Enzyme Company
Neova Technologies Inc.
Novozymes North America, Inc.
Specialty Enzymes & Biochemicals Co.
Syngenta Biotechnology
Verenium Corp.


Associate Members

Ajinomoto Corporate Services LLC
Emerald Foam Control



ETA Committees

- Executive
- Food and Feed
 - Canada Subcommittee
 - Feed Subcommittee
- BioTech
- Codex
- Dietary Supplements
- Safety/Communication
- Specifications/USP
- Website



Food Chemicals Codex US Pharmacopoeia

- Supports the availability of good quality and safe food ingredients
- A compendium of standards for food chemicals
- First edition published by National Academy of Sciences in 1966



ETA has supported FCC over the years

- ETA member company representatives serve on FCC committees
- Develop new methods for testing
- Participate in FCC collaborative studies



Enzyme Technical Association

Thrilled that USP has taken over FCC

In the past:

- Methods were not updated
- Publications were not completed on time
- Management of FCC activities was hit and miss



What are Enzymes?

● Proteins

- Made up of building blocks of 20 amino acids
- Linked by peptide bonds
- Defined shape, size, molecular weight

● Biological catalysts

- Highly specialized catalytic functions
- Specific reactions
 - Basis for classification
 - IUBMB system (International Union of Biochemistry and Molecular Biology)
<http://www.chem.qmul.ac.uk/iubmb/enzyme/>

● Indispensable part of every living cell



Enzymes in nature with a given designated activity

- Can be from a diverse set of organisms
- Have divergent amino acid sequences but often have conserved active (catalytic) site sequences
- Are naturally adapted to the environment of the host organism and therefore may have wide variation in pH optima and temperature optima



Enzymatic Activity

- Enzymes of a given activity catalyze the same reaction. For example:
 - Alpha-amylase
 - Endohydrolysis of 1,4- α -D-glucosidic linkages in polysaccharides containing three or more 1,4- α -linked D-glucose units
 - IUBMB Number 3.2.1.1

Alpha-amylases in nature have divergent amino acid sequences

% amino acid sequence identity	<i>B. amyloliquefaciens</i>	<i>B. licheniformis</i>	<i>G. stearothermophilus</i>	<i>A. niger</i>	<i>A. oryzae</i>	<i>Z. mays</i>	<i>O. sativa</i>	<i>H. vulgare</i>	<i>P. vulgaris</i>	<i>H. sapiens</i>
<i>Bacillus amyloliquefaciens</i>	100									
<i>Bacillus licheniformis</i>	80	100								
<i>Geobacillus stearothermophilus</i>	65	65	100							
<i>Aspergillus niger</i>	21	21	22	100						
<i>Aspergillus oryzae</i>	23	24	24	66	100					
<i>Zea mays</i> (corn)	24	26	25	28	27	100				
<i>Oryza sativa</i> (rice)	25	27	25	27	26	89	100			
<i>Hordeum vulgare</i> (barley)	25	23	24	25	28	70	69	100		
<i>Phaseolus vulgaris</i> (bean)	26	27	25	24	27	67	65	64	100	
<i>Homo sapiens</i> (human)	25	33	29	22	28	23	22	23	24	100

but have the same catalytic activity and IUBMB number



A Concern:

**The proposal to create
a
harmonized method.**



A Concern – Harmonized Method

What is a harmonized method?

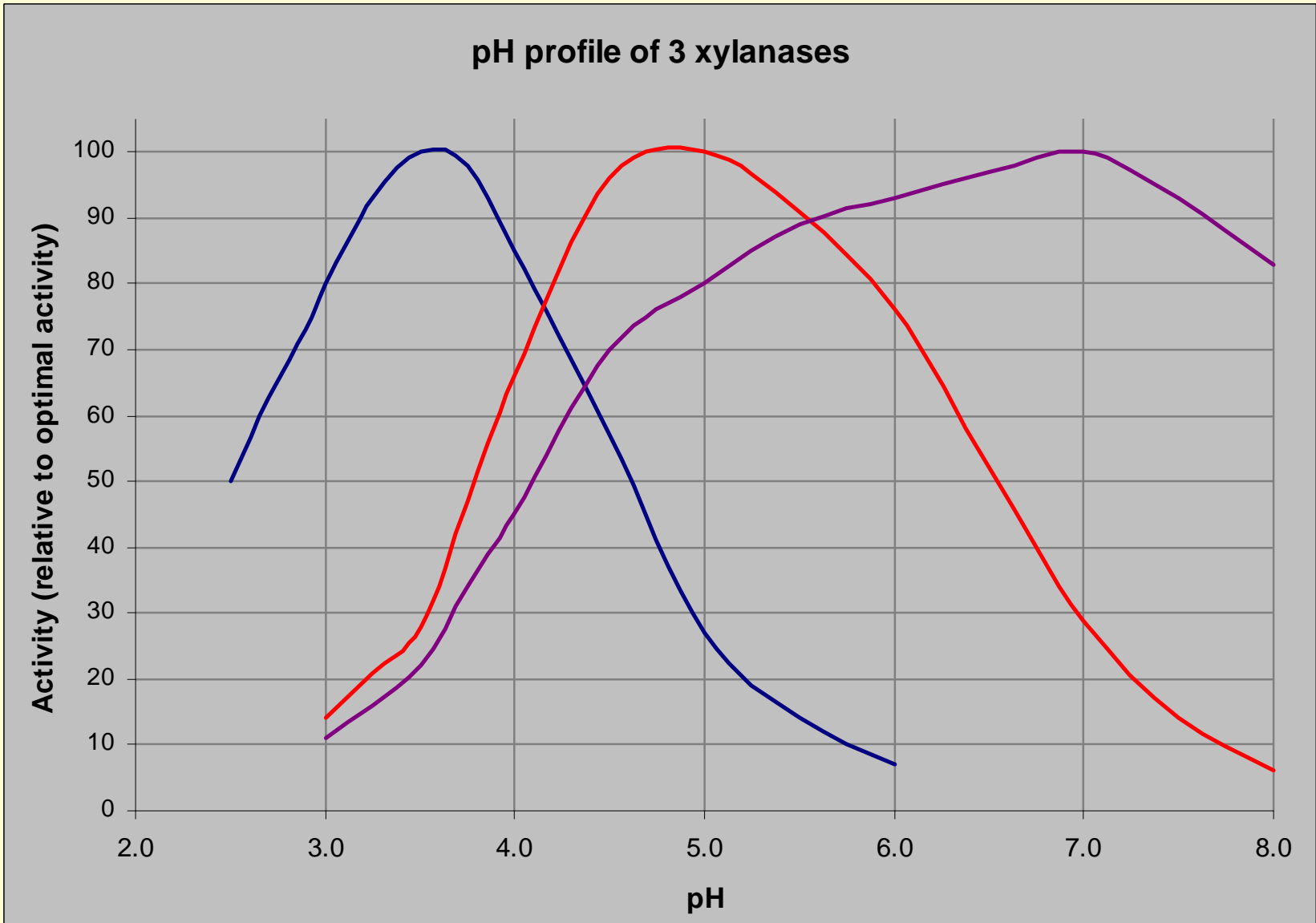
- A single method with fixed parameters
 - pH
 - Temperature
 - Reaction time
 - Substrate
 - Buffer
 - Standards
 - Other



A Concern – Harmonized Method

Too much variation

- pH vs. Activity
- pH vs. Stability
- Temperature vs. Activity
- Temperature vs. Stability
- Cofactors: e.g. metal ions
- Substrate specificity






A Concern – Harmonized Method

- Different enzymes of same type (class) have different pH profiles
- Different enzymes of same type (class) have different temperature profiles
- Even modest shifts from the pH and temperature optima will significantly affect the activity response



A Concern – Harmonized Method

- Different enzymes in a class will have a different activity response to given substrates
- Different enzymes in a class will give varying results depending upon the detection method (e.g. viscometric, colorimetric)



A Concern – Substrate Standards for Testing Enzymes

- USP has been suggesting substrate standards.
- They are not necessary or supported by the industry.
- Many enzymes are substrate specific



A Concern – Enzyme Standards for Testing

- There is no universal standard for a class of enzymes (proteases, amylases, lipases, pectinases, xylanases, cellulases....)
- Because of protein's nature, standards are difficult to produce and maintain
- There can be no standard
 - Enzymes are not distinct chemicals
 - Enzymes are complex, UVCB biochemicals




A Concern – The Proposal of USP to Create a Monograph for Every Enzyme Product

● *Industrial enzymes are classified by functional activity*

- Amylase
- Glucoamylase
- Protease
- Lipase
- Cellulase

but enzymes in a class are highly diverse

● *Current single monograph works and is referenced by regulators*



A Concern – Heavy Metals

USP Heavy Metal List

- Creation of a list suggests the need for a standard
- Appearance in standard-setting publication suggests a testing requirement
- Safety concerns for heavy metals is a function of ingestion, not a function of the quantity in a product
 - Amount ingested dependent upon amount of product consumed
 - For most enzymes, amount consumed is negligible



Conclusions

- Appreciate USP
- Harmonized assays are not needed or workable
- Substrate standards are not needed
- Beyond existing enzyme standards, no new ones are needed
- Current, general enzyme monograph works
- Continue updating Appendix V and assay library