Amylolysis of eight species of starch with four different species of α-amylase

by

Robert Leonard Sheets

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Major: Biochemistry

Program of Study Committee:
Richard Honzatko, Major Professor
Mark Hargrove
Patricia Thiel

Iowa State University

Ames, Iowa

2016

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

ABSTRACT

Presented here are the action of four different α-amylases on eight different starches in their native granular form. Of the starches, potato and amylomaize-7 were slowest to degrade, but maize, wheat, rice, barley, and tapioca degraded at a similar (and faster) rates. Waxy maize degraded most rapidly compared to the other starches. Amylases from *Bacillus licheniformis* and human saliva were significantly more effective than amylases from *Bacillus amyloliquefaciens* and porcine pancreas. The broad range of outcomes indicates the correct choice of enzyme and starch is a critical factor in the design of efficient protocols for starch processing in the bio-renewable industry.

CHAPTER I: INTRODUCTION

Background

Starch is ubiquitous in the plant world, but human agricultural efforts historically have focused on starch-rich foods. Worldwide, more than half of all food calories comes from starch in the form of cereals and rhizomes (FAO and WHO, 1998). Starch is a glucose polymer of $\alpha(1\rightarrow4)$ glycosidic linkages with $\alpha(1\rightarrow6)$ glycosidic branch points. Amylose and amylopectin are the predominant constituents of starch. Amylose has very few branches, is insoluble in cold water. Amylopectin has significantly more branches, averaging between three to four percent, and is soluble in water. The rate of dissolution of amylopectin, however, is slow at low temperatures, a consequence in part to the semi-crystalline properties of this molecule (Gidley, 1987).

Although all starches are polymers of glucose, their secondary structures differ vastly due to different amylose/amylopectin ratios, crystallinity, branching percentages, granule sizes and granule shapes. Every starch-producing organism will produce a distribution of starch granules. But each distribution is characteristic of the starch type. For example, potato starch granules tend to be large and smooth, whereas rice starch granules are small and angular (Kimura, 1995; Jane, 1994). The granules of waxy maize are predominately amylopectin, whereas normal maize has an approximate ratio of 80:20 amylopectin to amylose (Lindenboom, 2004).

Starches are stored energy. That energy is tapped in plants through the degradation of starch into simple sugars by the action of starch phosphorylase or amylase (Rathore, 2009). Other organisms use amylases to hijack glucose equivalents from plant starches. Hence, a multitude of species-specific amylases exist all of which act upon $\alpha(1\rightarrow4)$ linkages. Some are endo-hydrolases (hydrolyzing linkages within a chain) and others are exo-hydrolases (hydrolyzing nonreducing ends of starch chains). Amylases also produce various products: glucose, maltose and

maltodextrins. Maltodextrins are also short-chained polymers of glucose ranging from three to twenty glucose units.

Most schemes for enzyme kinetics assume both enzyme and substrate are soluble. In the case of polymeric substrates, enzyme-substrate interactions exhibit one-dimensional Brownian motion and "sliding" (Callender, 2014; Breyer, 2001). Starch in nature, however, is rarely a solubilized substrate, being instead a semicrystalline or amorphous solid. Kinetics data gleaned from liquefied (solubilized) starch may allow the precise determination of catalytic parameters, but such parameters are not a faithful representation of enzyme action on insoluble starch granules.

As the secondary structure of native starch varies, as do amylases from each organism, one might expect a wide range in the efficiency of starch degradation. Many different amylases might rapidly degrade one starch-type, whereas another might be susceptible to the action of only one amylase. Knowing the best combination of starch-type and amylase could reduce costs associated with pre-liquefaction of starch.

Thesis Organization

This thesis begins (Chapter II) with a review of the literature related to the properties of glucose, the structure of starch granules, the structure of selected α-amylases and the ecological niche occupied by the selected amylase-secreting organisms. A presentation of research methods and summary of the results follows (Chapter III). The development of kinetic models appears in Chapter IV, with correlations between enzyme efficacy and starch-type in Chapter V. Finally, suggestions for future directions of research are in Chapter VI. Appendices and acknowledgements conclude the thesis.

CHAPTER II: REVIEW OF LITERATURE

Glucose

Sugars are carbohydrate, the simplest (monosaccharides) having the general formula $(CH_2O)_n$. Physically, monosaccharides consist of a carbon chain that is decorated with hydroxyl groups on each carbon except for one carbon which contains a relatively more reactive carbonyl group. The hydrophilic hydroxyl and carbonyl groups allow water molecules to solvate what would otherwise be an insoluble hydrophobic carbon chain, stabilize secondary structure via intra-molecular hydrogen bonding and be readily metabolized. The electrophilic carbon atom of the ketone or aldehyde group of monosaccharides reacts intra-molecularly and reversibly with a nucleophilic hydroxyl to form either a hemiketal or hemiacetal. The resulting cyclized the monosaccharide are of relatively low energy and the dominant state at equilibrium.

Glucose has six carbons, one of which is included in the terminal aldehyde group and the others having hydroxyl groups and specific chiralities. The chiral carbons are such that cyclization into the pyranose ring puts all but one oxygen atom in the same equatorial plane and all carbon-linked hydrogen atoms perpendicular that plane. The hydroxyl group associated with the hemiacetal can have conformations either α (axial) or β (equatorial). Having all of the relatively large hydroxyl groups in equatorial positions is preferred sterically, so β-pyranose is twofold more prevalent than α-pyranose at equilibrium. The six-atom cycle of pyranose is also sterically preferred to the five-atom cycle of furanose. Glucose then has hydrophilic equatorial plane and two hydrophobic surfaces above and beneath this plane. These hydrophobic surfaces favor the stacking of glucose with aromatic rings of amino-acid side chains and glucose polymers with helical and double helical conformations. Glucose helices can entrap relatively hydrophobic molecules, such as triiodide.

Although monosaccharides favor cyclized states, the cyclization reaction is reversible, and consequently a small percentage of uncyclized monosaccharide is present at equilibrium. In the case of glucose, this leaves the aldehyde group available for condensation reactions, process is called glycation. Frequently, amino groups condense with aldehyde groups of sugars in the Maillard Reaction (Mottram, 2002). After the initial formation of an imine group from the amino and aldehyde groups, the imine group tautomerizes with the adjacent alcohol to form amine and ketone groups (Amadori Rearrangement). Glycation of amino groups in proteins is a direct consequence of high serum glucose levels of hyperglycemia and advanced glycation end-products (AGEs) are associated with age related diseases such as Alzheimer's (Vistoli, 2013). What differentiates glucose from other sugars is its stability in the cyclized state, thus reducing glycation events.

The hemiacetal (or hemiketal) group generated by cyclization is also reactive, but the condensation of the hemiacetal (or hemiketal) group with the hydroxyl of another simple sugar results in a relatively inert glycosidic linkage. The formation of the glycosidic link, which generates a molecule of water as a product, is not energetically favored under aqueous conditions. Within organisms, this thermodynamically disfavored condensation is driven by nucleoside-diphospho sugars, and the hydrolysis of diphosphate. The hemiacetal (or hemiketal) is phosphorylated and then reacts with a nucleoside triphosphate to form a high-energy nucleoside-diphospho-sugars. The nucleoside-diphospho sugars are polymerized to form kinetically stable polymers (Ghosh, 1966). Amylose and amylopectin are $\alpha(1\rightarrow4)$ glycosidic polymers of glucose, and are both synthesized from adenosine-diphosphoglucose.

Starch Granule

Amylose and amylopectin are the two main components of starch. Amylose is a straight chain of $\alpha(1\rightarrow4)$ bound glucose molecules with very few $\alpha(1\rightarrow6)$ branch points. Amylose makes

up about one fifth of the total carbohydrate in starch with a few known exceptions. Amylopectin

contains an α(1→6) branch every 24 to 30 α(1→4) linked glucose units. Amylose is insoluble in

cold water, whereas amylopectin is soluble, the difference attributed to the frequency of α(1→6)

branches.

Both amylose and amylopectin form semi-crystalline single and double helices in solution.

Amylose can form left-handed rigid single helices, but also right-handed helices in the presence of

specific complexing agents (Gessler, 1999; Bulpin, 1982; Winter, 1974). Single-helix amylose is

called V-type. Double helices of amylose are left- and right-handed, are more rigid and stack

against each other (Sarko, 1978). A-type crystalline double-helical amylose is orthogonally

packed, whereas B-type is hexagonally packed. A-type amylose is less hydrated than B-type

(Blazek, 2011). When amylose crystallizes out of solution, the conditions associated with the

retrogradation determine the crystals form (Sarko, 1978).

Although amylose forms helices easily through retrogradation from solution, crystallinity is

actually hindered by the presence of amylose in starch granules. Amylopectin exhibits the

crystalline forms of amylose; however, because amylopectin is branched, crystallization is

intramolecular. Computational and NMR studies show that the branch points cause significant

conformational restrictions in starch that favor a left-handed helical model (Corzana, 2004).

Amylopectin within starch granules then may exist predominately as double helices with a left-

handed chirality. Amylose is presumably less able to form double helices, contributing to single

helical arrays that interrupt the crystallinity of amylopectin. This changes after retrogradation when

amylose becomes concentrated (Pérez, 2010), and becomes more crystalline than amylopectin

(Wang, 2015).

The suggestion that crystalline regions of starch degrade more slowly than amorphous

regions derives from the observation of "shelled" or "ringed" structures left behind after partial

degradation of starch granules by acid-based or amylase-based hydrolysis (Pérez, 2010). As noted above both amylose and amylopectin exist in crystalline and amorphous states, and these states may change due to treatment. For instance, after cooling, cooked starches increase in crystallinity with a concomitant loss of flavor. Nonetheless most agree that amylopectin, which is the major constituent of starch (around 80%), is also the major component of crystalline regions, proposed as double helices of amylopectin packed in either A-type or B-type arrangements. There are several models for covalent bonding of amylose and amylopectin, but no technology has yet to determine the composition of starch layers, and the characteristics of coupling between layers.

The most widely accepted model for starch granule biosynthesis requires pre-existing amylopectin. The non-reducing ends of the amylopectin are lengthened with branching until steric crowding interferes with synthesizing enzymes. Debranching enzymes then remove excess branch points, allowing amylopectin to crystallize into double helices. Double helices pack together in either an A-type or B-type polymorph. Higher order structures may rise by combining packed double helices into a superhelix (Bertoft, 2004). Amylose appears when some chains of amylopectin become almost completely debranched.

The proposed model, however, fails to account for *de novo* synthesis in the absence of a primer or preexisting carbohydrate (Mukerjea, 2012). Such *de novo* synthesis requires starch synthase and adenosine-diphosphoglucose, and proceeds without starch-branching enzymes. The resulting product precipitates with amylose and n-butanol. The addition of branching enzyme leads to larger quantities of amylopectin. Models of elongation from the reducing ends of starch (widely accepted primer models) cannot account for the synthesis of starch as described above. The model suggested by John F. Robyt begins starch biosynthesis as amylose, which is in turn branched into amylopectin by branching enzyme. Starch is degraded to support metabolism by starch-

debranching enzymes (Mukerjea, 2013). Debranching occurs on oligosaccharides rather than the starch granule to avoid the formation of starch granules resistant to further degradation.

Regardless of how the starch granule is synthesized, there is a radial structure that is synthesized out of the hilum. The shell structure alternates between an easily degraded portion and a resistant potion. The resistant portion after hydrolysis is crystalline. Hydrolysis kinetics then, must account for fast and slow degrading portions (Buléon, 1998).

α-Amylase

α-Amylases are hydrolases that act upon α-linked glucose polymers. The standard definition restricts the action of α-amylase to α(1→4) glycosidic linkages; however, there are structural similarities and a conserved catalytic mechanisms with other amylases and transglycosylases (Kuriki, 1999; Svensson, 1994) . One common feature is a catalytic TIM barrel domain, which allows amylases and transglycosylases to be in an extended "α-amylase family". The reactions catalyzes are hydrolysis and trans-glycosylation, and the substrate types are α(1→4) glycosidic linkages and α(1→6) glycosidic linkages. Neopullanase is an example of an α-amylase that catalyzes all four reactions types. Cyclodextrinases, which cyclize starches into toroids by intramolecular transglycosylation, can also hydrolyze these same cyclodextrins and glycosylate a variety of hydroxyl groups presumably by a mechanism related to its activation of water.

Enzymes of the alpha-amylase family do not invert the chirality of the anomeric carbon after hydrolysis or transglycosylation, and possess a conserved pair (one Asp and one Glu) of essential catalytic side chains. Quantum mechanical simulations are consistent with one carboxylate poised to attack the anomeric carbon forming a β-linked glycosylated carboxyl group. The other carboxylic acid is in position to donate a proton to the leaving group. The β-linked glycosylated carboxyl group is hydrolyzed by water in a second reaction or by the hydroxyl group

of another sugar or acceptor molecule (transglycosylation). The double displacement reaction maintains the alpha configuration of the anomeric carbon (Pinto, 2015).

α-Amylases are a subset of the α-amylase family which selectively act upon α(1→4) glycosidic linkages. They hydrolyze starches to generate maltooligosaccharides. This is the first step in solubilizing starche. Once starches are degraded into soluble oligomers, enzymes degrade oligomers into disaccharides and monosaccharides.

Like other members of the α-amylase family, α-amylases contain a conserved TIM-barrel. They also contain a non-catalytic triad which is necessary for stability of the starches (Marx, 2008). They promiscuously use anions as activators for their catalytic mechanism (Aghajari, 2002). Anions, specifically chloride, properly orient the essential carboxylates at the active site and stabilize a helix, from which the substrate binding loop protrudes. Calcium also frequently stabilizes the enzyme (Ghollasi, 2013).

Aromatic residues play an important role in carbohydrate binding and are often present in active sites of carbohydrases (Matsui, 1994). Hydrophobic surfaces of glucose units stack with planar aromatic side chains. Computational models indicate a substantial free-energy decrease due to the displacement of water molecules from aromatic functional groups by methyl-glucose (Kumari, 2012). Amylases may have binding sites for carbohydrates that are not in the active site. Crystal structures of porcine pancreatic α-amylase show maltose on surfaces far away from the active site. Human salivary α-amylase contains possible sites for carbohydrate binding. Aromatic resides play an integral role in these binding interactions (Ramasubbu, 2003).

Ecological Niche

Bacillus amyloliquefaciens. Bacillus amyloliquefaciens (*BA*) is a soil-borne, spore-forming, rod-shaped microorganism that is in a symbiotic existence with the roots of plants. It was

named for its ability to liquefy starches in a Japanese publication (Fukumoto, 1943) with no

English translation. Liquefaction of starch by *BA* is not substantiated in this thesis.

BA in its symbiotic relationship with plants produces antifungals, antibacterials, and auxins

which promote plant growth. Moreover, *BA* produces a putative cellulase which facilitates the net

transfer of carbon from plant to microorganism (Kierul, 2013). In more direct carbon transfer

pathways, plants exude simple carbohydrates into the rhizosphere, as well as organic acids (Liu,

2014). It is, therefore, likely that cellulase products are not a major carbon source for *BA*.

Bacillus licheniformis. Bacillus licheniformis (*BL*) is also a soil-borne, rod-shaped,

microorganism. *BL* is pathogenic (Haydushka, 2012; Agerholm, 1997). Its spores travel by air, and

reside in bird plumage where it may play a role in molting (Burtt, 1999; Burtt, 2010). Enzymes

isolated from *BL* tend to be thermostable and effective on solid substrates. Subtilisin from *BL*

degrades feathers, one of the most stable and least soluble proteins in existence. Proper pre-

digestion with *BL* subtilisin transforms feathers into a food source. The same protein is used as a

high-pH protease for laundry detergents (Sellami-Kamoun, 2008). An exopolysaccharide isolated

from marine *BL* (Arena, 2006) may remove oral plaque by binding to degrading biofilms. *BL* also

secretes a thermostable amylase.

Human saliva. Saliva is the first digestive secretion that comes into contact with consumed

food. It must, therefore, act upon many solid substrates. The main constituents of saliva are

mucopollysaccharides, glycoproteins, buffering ions, and hydrolytic enzymes (Humphrey, 2001).

One of these hydrolytic enzymes is α-amylase encoded by the AMY1 gene. It is usually highly

glycosylated (Shou, 2012), binding tightly to teeth and bacteria. Aromatic residues responsible for

carbohydrate binding may be involved in binding bacteria (Ramasubbu, 2003). Because of this,

human salivary α-amylase (HSA) is a constituent of dental plaque (Scannapieco, 1995)

(Scannapieco, 1995). Since the pH optimum of HSA is 6.7-7.0, it is inactivated by stomach acid.

HSA must act then in the short time between chewing and swallowing. However, the presence of starch and maltooliogosaccharides can partially suppress the inhibition of a low pH (Rosenblum, 1988). Upon neutralization, HSA regains some activity. It can also have activity on starch within the small intestine.

The human diet, on average, receives most of its caloric intake from starches (FAO, 1998). This is due partially to humans breeding and cultivating starch-rich food sources in preference to other forms of food. Starches preserve easily (freeze drying potatoes in the mountains) and are versatile components of prepared food (bread products and flavor derived from saliva-based production of aromatic compounds). Humans, have therefore, devoted much effort and ingenuity into the manipulation of the starch granule.

Porcine pancreas. The porcine pancreas secretes many hydrolytic enzymes into the duodenum. By the time pancreatic enzymes come into contact with food, it is partially digested and liquefied into chyme. One of these pancreatic hydrolases is an α-amylase (PPA). PPA, in contrast to HSA, tends to generate smaller hydrolysis products.

CHAPTER III: METHODOLOGY & RESULTS

Data Collection

Four amylases and 8 species of starch were analyzed in the study. *Bacillus amyloliquefaciens* and *Bacillus licheniformis* were the bacterial sources of amylase. Human salivary and porcine pancreatic amylases served as mammalian sources. The eight starches were potato, waxy maize, maize, amylomaize-7, rice, wheat, tapioca, and barley.

The enzymes were assayed using a 10 mL starch solution prepared as follows: 110 mg of potato starch was autoclaved in 7 mL of water. The solution was buffered to a pH of 6.5 with imidazole hydrochloride. Calcium acetate was added as a source of calcium ions. The volume was raised to 10 mL, making a 10% (w/v) starch solution with 20 mM imidazole hydrochloride and 1 mM calcium acetate. Enzymes were diluted in 20 mM imidazole hydrochloride (pH, 6.5), 1 mM calcium acetate and 0.04% polyethylene glycol 1500 (PEG 1500). In a warm bath (37 °C), a vial containing 1.9 mL of the starch solution was heated and stirred. 100 μL of the diluted enzyme solution was added to the vial for a total volume of 2.0 mL. 100 μL aliquots were added to 900 μL of 0.1 M sodium hydroxide to quench reactions. The copper bicinchoninate method determined the number of reducing ends. A unit (IU) of activity is defined as 1 millimole hydrolysis reactions per minute. Enzymes were diluted to 2, 20, and 200 IU/mL for experiments described in the next paragraph. The dilutions also contained 20 mM imidazole hydrochloride, 1 mM calcium, and 0.04% PEG 1500.

All the reactions took place at 37 °C. Flasks contained 19 mL of 1 g of one of eight starches in 20 mM imidazole hydrochloride (pH, 6.5) and 1 mM calcium acetate. Reactions were initiated by the addition of 1 mL of the diluted enzyme to the starch suspensions, giving final enzyme concentrations of 0.1, 1.0, and 10 IU/mL. 2 mL aliquots were removed from the reaction

digests at various time points and placed into an orange-capped tube containing 1 mL of 0.03 M trifluoroacetic acid (TFA) for a final pH about 2. Four enzymes, three concentrations, eight starch varieties, and 7 time points yielded a total of 96 reactions and 672 aliquots.

The aliquots were centrifuged at 10,000xg for 10 minutes. The supernatant was removed. Pellets were re-suspended in 2 mL of 0.01 M TFA and re-centrifuged. The supernatants were pooled. The volume of the pooled supernatants was brought up to 10 mL using 0.01 M TFA.

The supernatants were assayed using a modified sulfuric acid/phenol method to measure total carbohydrate content (TC) and a copper bicinchoninate method to measure the total reducing value (RV). For the total carbohydrate method, 25 µL of a properly diluted (10 – 200 µg/mL) sample was placed into a microwell plate in triplicate. 25 µL of a 5% (w/v) phenol solution was added to the wells over a bed of ice and agitated. 125 µL of concentrated sulfuric added was slowly added to the wells. The plate was then agitated. The plate was then floated upon an 80 °C hot bath for 30 minutes. The plate was then scanned at 492 nm.

For the reducing value, two stock solutions where prepared. The first was a bicinchoninate buffer solution which contained 5 mM disodium 2,2'-bicinchoninate, 64 g/L sodium carbonate monohydrate, and 24 g/L sodium bicarbonate. The second stock was a copper solution containing 5 mM copper sulfate and 12 mM L-serine. Equal parts of the stock solutions were mixed right before the assay was conducted. 100 µL of a properly diluted sample (1 – 20 mg/ml of maltose equivalents) was added to a microwell plate in triplicate. 100 µL of the binary stock solution was added to the microwell plate. The plate was agitated and then floated upon an 80 °C hot water bath for 35 minutes. The plate was then scanned at 560 nm. Both of these assays are in the literature (Fox, 1991).

Data Analysis

All data was recorded and exported to Microsoft Excel. Triplicate values for TC and RV were averaged and plotted as a function of maltose equivalents over time (Appendix A). TC evolution does not fit a single exponential decay model, and requires at least a double-exponential decay process. The RV plots are in Appendix B. RV plots were modeled using a single exponential decay function, although several time courses clearly undergo a double exponential decay process. The RV confirms the TC data by a second chemical assay, but provide no additional insight into the mechanism of starch degradation. Hence for the remainder of this thesis the focus is on the TC data.

Double exponential decay can come from two distinct mechanisms, which relate to the relative rates of the double-displacement reactions catalyzed by α-amylases. The first step results in a glycosylated enzyme active site and the release of the aglycone. As the aglycone in this case is a carbohydrate, it is measured by the TC and RV assays. If the first step is rate limiting (as is the case for soluble substrates), then different rates must be a consequence of different substrate types (for instance, amorphous and crystalline starch). The second step is the hydrolysis of the glycosylated enzyme intermediate, which in this study is a covalent complex between starch and enzyme, and technically no longer in solution. If the rate of hydrolysis is the slow step, then double exponential decay could reflect a rapid "burst" phase (step one) followed by a slow steady-state phase limited by the rate of hydrolysis of the enzyme-starch intermediate (step two). Hence, double exponential decay could be a consequence of starch structural heterogeneity or the α-amylase enzyme kinetics. In Chapter IV of this thesis we explore possible models that could be the basis for observed double exponential decay.

The fitted double exponential decay curves were used to determine a calculate value for TC after 24 hours. These values are summarize in Table 1.

	0.1 IU/ml			
	BLA	PPA	HSA	BAA
Potato	5.17	3.61	3.84	1.23
Waxy Maize	22.87	26.98	27.26	6.82
Maize	19.64	18.06	19.61	6.92
Amylomaize-7	7.37	5.42	6.09	2.22
Rice	22.26	22.89	31.66	13.63
Wheat	14.03	6.43	7.56	3.29
Barley	14.11	11.65	22.74	2.44
Tapioca	13.40	12.34	27.75	2.97

	1.0 IU/ml			
	BLA	PPA	HSA	BAA
Potato	8.13	7.07	7.66	2.39
Waxy Maize	55.24	55.12	64.84	17.40
Maize	36.04	34.51	36.36	16.16
Amylomaize-7	13.38	10.47	11.98	4.94
Rice	38.27	35.18	52.45	19.57
Wheat	29.95	29.35	36.10	10.59
Barley	32.11	25.83	42.66	8.40
Tapioca	40.22	26.62	58.00	9.38

	10.0 IU/ml			
	BLA	PPA	HSA	BAA
Potato	15.44	12.29	15.29	4.09
Waxy Maize	75.51	68.86	80.24	42.80
Maize	59.26	52.31	56.54	30.35
Amylomaize-7	29.86	21.30	22.27	8.57
Rice	60.81	58.53	81.11	30.15
Wheat	62.67	50.96	66.78	19.32
Barley	63.84	49.16	105.09	17.28
Tapioca	58.96	68.23	91.77	22.14

106
100
90
80
70
60
50
40
30
20
10
0

Table 1. Total carbohydrate (TC) after a 24 hours as determined by double exponential fits of observed data. Calculations and fits are in Appendix A. Values are in mg of TC as maltose, assuming 100 mg of starch produces 106 mg of maltose. The color scale emphasizes high TC values (purple) from low values (light green). Total enzyme concentrations are indicated at the top of data summaries as 0.1, 1.0, and 10.0 IU/mL..

CHAPTER IV: MODELS FOR STARCH DEGRADATION

Biphasic Kinetics Due to Slow Hydrolysis

If hydrolysis of the covalent complex between starch and enzyme is slow (the second step of the Ping Pong mechanism for α-amylase is rate-limiting), then a burst phase will appear in the data. The size of the burst is proportional to the amount of ES that forms, which in turn is proportional to the total enzyme used in the experiment. A burst greatly in excess of what the total enzyme can produce in a single turnover would disqualify a model based on rate-limiting hydrolysis. The theoretical limit (based on the highest enzyme concentration) for the experiments here is 20 mg of maltose-equivalent product. The burst concentrations of product for most reactions fall under 20 mg, and those that exceed 20 mg do so by no more than 50% (Appendix A). Hence, we cannot exclude a limiting hydrolysis mechanism as an explanation for biphasic kinetics. A direct determination of the rate of decay of the starch-enzyme complex is necessary in order to eliminate (or confirm) the possibility of rate-limiting hydrolysis. Confirmation of rate-limiting hydrolysis for insoluble carbohydrate-amylase complexes would impact the approach to the industrial processing of all carbohydrates, including cellulosic materials.

Biphasic Kinetics Due to Substrate Inhomogeneity

If the formation of the enzyme-intermediate complex is rate-limiting (first reaction of the Ping Pong mechanism), the Ping Pong mechanism becomes a Michaelis-Menten mechanism. Slow formation of the enzyme-starch covalent complex allows initial binding events between enzyme and starch to be at equilibrium. The reaction rate is determined by the k_{cat} multiplied by the concentration of the enzyme-substrate complex *ES*. As *ES* is in equilibrium with free enzyme *E* and free substrate *S*, $ES = E*S/K$, where *K* the dissociation constant governing the equilibrium. These assumptions carried forward result in the Michaelis-Menten equation: $-\frac{dS}{dt} = E_o k_{cat} S/(K + S)$. Integration of the Michaelis-Menten equation through separation of variables

results in the following: $(E_o k_{cat}/K)*t = \ln(S_o/S)+(S_o-S)/K$, where S_o is the concentration of substrate

at time zero. For a single substrate and single product P, $S_o-S=P$ for all times. Hence, the

integrated Michaelis-Menten equation giving the time dependence of product formation is as

follows: $(E_o k_{cat}/K)*t = -\ln[(S_o-P)/S_o]+P/K$. The latter equation reveals a complex relationship for

product formation with time; however, by exponentiation of the relationship, we find: $\exp[-$

$(E_o k_{cat}/K)*t]= [(S_o-P)/S_o]*\exp[-P/K]$. At early times $P\sim 0$, and $\exp[-P/K]$ approximates to unity.

The relationship reduces to a first-order exponential decay: $P = S_o-S_o*\exp[-(E_o k_{cat}/K)*t]$. Hence,

the use of exponential decay functions in data fitting is justified. The foregoing analysis assumes

the Michaelis-Menten reaction is irreversible. For starch hydrolysis reactions, the high

concentration of water drives the reaction to completion with almost no back reaction.

In the event of a rate-limiting first step in the Ping Pong mechanism, biphasic kinetics must

be due to starch heterogeneity. Heterogeneity can be represented by two substrate pools S^C

(crystalline) and S^A (amorphous). Assuming enzyme-starch binding to be at equilibrium, we can

define two dissociation constants K^C and K^A and two rate constants k^C and k^A for parallel

Michaelis-Menten mechanisms. The resulting equation is as follows: $\dfrac{dP}{dt} = \dfrac{E_o k^A S^A}{\left[\left(1+\frac{S^C}{K^C}\right)K^A+S^A\right]} +$

$\dfrac{E_o k^C S^C}{\left[\left(1+\frac{S^A}{K^A}\right)K^C+S^C\right]}$. This expression cannot be integrated by separation of variables, but we can explore

its complexity and behavior. For the first term, which governs the degradation of amorphous

starch, the constant (K^A) for the dissociation of ES^A (the complex of enzyme bound to amorphous

starch) is increased by a factor of $\left(1 + \frac{S^C}{K^C}\right)$. In other words, the presence of crystalline starch

enhances the dissociation of enzyme from amorphous starch. Inspection of the second term

indicates a reciprocal effect; the presence of amorphous starch enhances enzyme dissociation from

crystalline starch. These mutually antagonistic relationships are a consequence of separate

substrate pools competing for a single pool of enzyme. If we assume $k^A >> k^C$ (amorphous starch

degrades completely before significant loss of crystalline starch), the second term is small relative

to the first, and can be ignored. Also S^C is constant and equal to its initial concentration S^C_o. The

later allows the expression of S^A in terms of initial total substrate concentration S_o, product

formation P and the initial crystalline starch concentration S^C_o. In other words, $S^A = S_o - S^C_o - P$.

Degradation of the amorphous component then is approximated by the following: $\frac{dP}{dt} =$

$\frac{E_o k^A (S_o - S^C_o - P)}{\left[\left(1 + \frac{S^C_o}{K^C}\right) K^A + (S_o - S^C_o - P)\right]}$. This expression can be integrated by separation of variables with a result

analogous to the integration of the single-component Michaelis-Menten expression. Hence, the

rapidly degrading component exhibits exponential decay early in the time course. When S^A is

exhausted (that is $S^A = 0$), the first term can be neglected relative to the second term. The

relationship becomes as follows: $\frac{dP}{dt} = \frac{E_o k^C (S^C_o - P)}{[K^C + (S^C_o - P)]}$, which again is analogous to a single component

Michaelis-Menten relationship, giving (after integration) an exponential decay function for

crystalline starch. The double-exponential decay function used in fitting seems justified not only

as an empirical functional form that captures the behavior of the data, but also by first principles of

kinetics.

The number 106 used in fitting derives from theoretical calculation that 100 mg of starch

will produce 106 mg of maltose equivalents, which we define here as the product of the reaction

measured by the TC assay. We note that for some starch-enzyme combinations, we are in reach of

106 mg of maltose from 100 mg of starch, whereas for others, we seem to fall short of the expected

yield. In certain instances the experimental determination of maximum yield may have greatly

improved double-exponential fits.

Additional Models for Starch Degradation

Appendices C, D and E explore other models and assumptions. In Appendix C, we assume

Michaelis-Menten kinetics, with total enzyme concentration equal to free enzyme concentration

($E_o = E$). The result is first-order exponential decay. (In early paragraphs or this chapter, we

explored the same model; however, we made no assumptions restricting the amount of free

enzyme relative total enzyme). This model did not account for data for biphasic starch-enzyme

reactions.

The model in Appendix D is a modified second-order model, in which the solution enzyme

must first associate with the granule. Not all of the associations between enzyme and starch result

in a productive *ES* complex. Only enzyme association with susceptible starch results in product

formation.

$$E_{soln} + S \leftrightarrow ES_{gran} + S_{susc(unbound)} \leftrightarrow ES_{bound} \rightarrow ES_{gran} + P$$

$$\phantom{E_{soln} + S \leftrightarrow ES_{gran}}K_1 \phantom{+ S_{susc(unbound)} \leftrightarrow}K_2$$
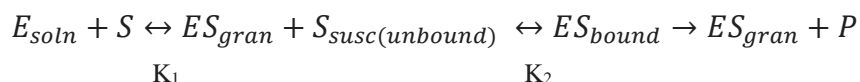
In essence, the model of Appendix D creates two pools of starch, both of which bind enzyme, but

only one of which allows product formation. The constant C transforms the total carbohydrate

mass into a concentration of hydrolysable starch, which is in equilibrium with enzyme. It is

assumed that the value of $K_2[ES]_{gran}$ is negligible in comparison to 1 as there is not a large

concentration of $[S]_{susc(unbound)}$. This is assumed because the solid polymer does not have a lot of

exposed surface area. If this assumption is false, there is no way to integrate the equation. The

concentration of $[E]_{tot}$ is assumed to be about the concentration of $[E]_{soln}$. Plots of the resulting

function and data are Appendix D.

The model in Appendix E allows the percentage of susceptible starch to change with time

(and hydrolysis). Portions of the starch granule are more susceptible to degradation, and as

degraded, would leave behind more resilient starch. The equation used to model this reduction is

an exponential decay function. The model is described as:

$$E_{soln} + S_{avail} \leftrightarrow ES \rightarrow E + P$$
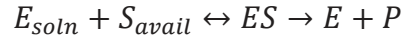
As before, one assumes $[E]_{soln} = [E]_{tot}$. The concentration of available substrate is a

function of the total starch multiplied by the mass concentration of the total starch minus the

starch-enzyme complex concentration. The function is an exponential decay function that stats at a

maximum value, C^0, which decays to a value of C_{min}. This decay happens over the course of the

digestion so that as P approaches 106 mg/ml, the C function approaches C_{min}.

The reaction rate is described as $k_{cat}[ES]$. Following the same steps as the derivation as in

Appendix C, results in an exponential integral function. The exponential integral function is a non-

elementary function that is described as $E_i(x) = -\int_{-x}^{\infty} \frac{e^{-t}}{t} dt$. According to this model, this

modified exponential integral function should linearize the TC values. A 12th power Taylor series

approximation is used on excel for this model. Using a one to twenty ratio as the decay function of

C, the data was better linearized than even the second order model, which was a more complicated

model to derive. The resulting graphs can be seen in Appendix E.

CHAPTER V: CORRELATIONS

The amylase from Bacillus amyloliquefaciens (BAA) was the slowest of all four enzymes. Porcine pancreatic amylase (PPA) had intermediate activity for some starch varieties. Both human salivary amylase (HSA) and Bacillus licheniformis amylase (BLA) were, in general, close in reactivity. BLA was slightly better than HSA at degrading potato and maize starches. HSA was slightly better than BLA at degrading wheat, but significantly better at degrading rice, barley, and tapioca.

Three trends are evidenced by the results: (1) Degradation proficiency depends modestly on the amylose/amylopectin ratio. (2) Degradation proficiency is strongly linked to the physiological or biological role of the enzyme. (3) Degradation proficiency is related to aromatic surface residues of the enzyme.

Amylose, being less soluble than amylopectin, should be more resistant to degradation, as is apparent for the three maize species. High-amylose starches reach the large intestine in larger quantities than low-amylase starches (Sandstedt, 1962). These resistant starches are important for the gut flora of both humans and pigs (Topping, 1997; Brown, 1997; Schulz, 1993). Intestinal microorganisms use these starches as a fuel, releasing short-chained carboxylic acids as waste. These acids, specifically butyric acid, play a large role in preventing colon cancer (Mandal, 2001; Bird, 2000; Van, 2010). The correlation between amylose content and degradability, however, is evident for only the maize subset of starches. For the entire set of starches, there is no clear correlation between the amylose/amylopectin ratio and degradation proficiency.

BA is a nonpathogenic soil organism, whereas *BL* is an opportunistic pathogen and saprophyte. *BA* lives symbiotically with starch producing plants and *BA* amylase is effective (high activity and specificity) in hydrolyzing dissolved (but not solid) starch. *BA* should avoid the

degradation of starch granules of its host, and prefer liquefied starch from decaying organisms external to the host. On the other hand, BLA is far more effective at degrading native (solid) starches. *BL* is spore forming (spread by air), and secretes enzymes that are effective at hydrolyzing other solid substrates, such as feathers of birds. *BL* may play a role in molting. *BL* is saprophytic; its ecological role is the degradation of dead organisms, and hence its secreted enzymes are be better suited for degrading solid materials.

The different proficiencies exhibited by PPA and HAS in degrading starch may be linked to the physiological role of each enzyme. HSA is secreted in the mouth, the first of many human digestive enzymes. Since food is eaten as a solid, HSA must handle a solid substrate. HSA has high affinity for dextrans (glucose-based polysaccharides common to dental plaque), and therefore adheres to dental plaque, facilitating HSA action by retaining the enzyme in the mouth. In contrast, PPA acts on partially digested substrates that emerge from the stomach.

The presence of aromatic residues on the surface of the enzyme correlate with the proficiency of solid starch degradation. Liquified starches bind to aromatic residues of the amylases (Matsui, 1994; Mishra, 2002; Asensio, 2012). Computational studies reveal favorable interactions between sugars and para-methyl phenol, toluene, and indole as analogues of tyrosine, phenylalanine, and tryptophan. Imidazole pi-stacks with hydrophobic surfaces of starch and hydrogen bonds with glucose (Chen, 2012). Molten imidazole is able to dissolve starch at even greater concentrations than water (Jordan, 2014). Computation studies of cellulose dissolved in imidazole-based ionic liquids indicate that the imidazole ring is responsible for stacking with the glucose monomers in cellulose (Mostofian, 2014; Swatloski, 2002). Is there a correlation in starch degradation proficiency and the number and type of aromatic surfaces residues of the enzyme?

HSA binds glucose-based carbohydrates effectively, and the study here shows that both HSA and BLA degrade solid starch effectively. To determine whether aromatic residues may be

playing a role, structures of amylases were inspected using UCFS Chimera. Phenylalanine and tryptophan represent hydrophobic aromatic residues, and tyrosine and histidine represent hydrophilic aromatic residues. Many surface hydrophobic residues of PPA are replaced by hydrophilic aromatic residues in HSA. The same pattern holds from BAA to BLA. Hydrophilic aromatic surface residues seemly favor solid starch as a substrate. When the starch is liquefied, hydrophobic aromatic residues are favored.

Comparison of human pancreatic α-amylase (HPA) to HAS limits the effect of species variation. Although the residue differences are not as substantial as HSA versus PPA, there are more hydrophobic aromatic residues on the surface of HPA relative to HSA, a change of an HPA tyrosine to a HAS histidine, and an additional tyrosine on HSA. The differences are consistent with the correlation of hydrophilic aromatic surfaces residues and proficiency in starch degradation. *Human Salivary Amylase (HAS) exhibits multiple anomalies.* HSA degrades barley, tapioca, and rice with unprecedented efficiency in comparison to the other starches and enzymes. HSA action on starch from barley approached and surpassed 106 mg/mL (theoretical maximum yield from 100 mg/mL starch). HSA was slightly better than BLA at digesting wheat and waxy maize, but worse than BLA at digesting starches from potato and the maize varieties. Barley and primitive wheat are among the oldest of cultivated grains. Starch granules from both barley and wheat have been found fossilized in Iranian Neanderthal calculus. Granules are both undamaged (evidence of raw consumption) and damaged (evidence of cooking) (Henry, 2011), indicating the consumption of raw and cooked grain in the humanoid diet well before the advent of agriculture. Evidently, barley, wheat, and related grains were selected for their food (caloric) value by prehistoric humanoids. Moreover, HSA may have evolved to bind selectively and degrade the starches from these grains. The data here suggest one or more feature of starches from wheat and barley causes proficient action by HSA relative to BLA. This feature may have been bred into waxy maize while being

absent in maize and amylomaize. The proficient action of HSA on rice is also apparent in the data

of this thesis; however, direct evidence for rice in the diet of Asiatic hominids has yet to surface.

Nonetheless, as cultivated rice originated in the orient, it is likely that rice has been in the diet of

ancient human and pre-human species. The data suggest HSA evolved to degrade minimally

processed forms of barley, wheat and perhaps rice starch granules, whereas human evolution has

not adjusted to starch from potatoes, amylomaize, and maize, crops subject to relatively recent

cultivation. However, there was still a significant amount of time for maize to be bred for the

human palette. A question is, "Why have maize and potato not been selected for HSA binding

degradation the same way barley, wheat, and rice have?" It may be due to the processing of maize.

An alternative explanation for the lack of proficiency in HSA degradation of starch from

potato and maize may stem by the processing of these grains early on. In the Americas, maize has

historically gone through a process called "nixtamalization." (boiling maize starch in lime water).

This process avoided malnutrition caused by eating non-nixtamalized maize. Pellegra and

Kwashiorkor affected Europeans who consumed maize without processing. Although these forms

of malnutrition are associated with the inability to degrade the hull of the maize, it is known that

heat and basic water transforms the starch granule into a more liquid form. Potatoes, historically

were freeze-dried. Freeze drying (in contrast to simply drying) greatly affects the starch granule

structure and its susceptibility to hydrolysis (Zhang, 2014). In the Andes, where potatoes

originated, potatoes where left to dry at high altitudes and used as a preserved foodstuff.

The proficient action of HSA on starch from tapioca, however, stands without a definite

explanation; however, as a rhizome-based starch, tapioca does not undergo harsh treatment in food

processing (mashing, washing, and dried as a thinly spread paste or slightly fermented). Hence,

starch granules retain their original structure and properties, requiring a proficient enzyme for its

degradation.

The selectivity of BLA for corn starch over HSA was most apparent in amylomaize-7, indicating that structures generated by amylopectin provide recognition sites for HSA. This may be why waxy maize was similarly digested by both BLA and HSA.

Unexpected lack of correlations. The lack of correlation between measurable properties of starch and proficiency of enzyme action is surprising. Water content, amylose/amylopectin ratios, crystallinity, and granule size do not contribute to the hydrolytic susceptibility of the starches. Among the maize varieties, there was a dependence on amylose/amylopectin content, but this was not a trend for starches investigated here. The poor performances of the amylases on potato starch, which has a similar amylose/amylopectin ratio as maize, showed that other factors play bigger roles. The lack of correlation between crystallinity and hydrolytic proficiency is surprising. Resistant starches left after hydrolysis are enriched in crystallinity relative to their original state. Conceivably, "trimming" effects due to hydrolysis may promote crystallinity, in which case a model based on static pools of amorphous and crystalline components will fail. Another is one of experimental reproducibility. In general, BAA was worse at hydrolyzing solid starch relative to PPA, which is in conflict with earlier results from the Robyt lab (Yook, 2002). No enzyme stabilizing agent (PEG 1500) was added in earlier experiments. As PPA is loses activity over time, it is likely that PPA instability gave the appearance of reduced activity on the solid substrate. BAA, being a secreted stable enzyme, would retain its activity for the time course of the experiment (Yoon, 2005).

CHAPTER VI: FUTURE STEPS AND IMPORTANCE

That the amylose/amylopectin ratio influences starch synthesis or degradation is unclear. Future work should probe multiple species of starch with almost identical amylose/amylopectin ratios. The best approach is to treat starch from the same species by breaking the granule into smaller pieces, adding lipids that bind to the granule, and sifting the granules into various sizes before hydrolysis. Other experiments would include starch from the same species, but exhibiting different amylose/amylopectin ratios, and then compares to another species exhibiting different amylose/amylopectin ratios.

The hypothesis that enzymes have evolved to act on specific starches could be addressed by examining α-amylase varieties and determining whether the biological/physiological role of each enzyme would have it act on solid or liquid starch. Once completed, the analysis of surface residues could provide evidence in support of hydrophobic aromatic residues favoring liquid substrates and hydrophilic aromatic residues favoring solid substrates. Confirmation of the latter findings would point the way to engineering efficient degradative enzymes for solid substrates. This is not limited to starch, but could extend to hemicellulose, cellulase, and lignases

The efficient degradation of barley, tapioca, rice, and wheat starches by HSA begs the question: Is there a special feature common to all of these starches or is there a special attribute of HSA not shared by other amylases? Although it appears amylopectin structures are what is allowing HSA to have a selective advantage, more testing should be done to verify if that is the case.

Conclusions. These results indicate that although enzymes can be standardized to have the same activity toward a solubilized substrate, their activities are altered in a way that is not easy to predict when acting upon solid granules. BLA and HSA were able to work on the solid substrate

with the greatest efficacy. *Bacillus licheniformis* and the human saliva amylases may have evolved

to act on solid substrates. If so, surface aromatic resides may be playing a role in recognition of

solid as opposed to liquid substrates.

REFERENCES

Agerholm, J. S., et al. "A preliminary study on the pathogenicity of Bacillus licheniformis bacteria in immunodepressed mice." Apmis 105.1-6 (1997): 48-54.

Aghajari, Nushin, et al. "Structural basis of α-amylase activation by chloride." Protein Science 11.6 (2002): 1435-1441.

Arena, Adriana, et al. "Antiviral and immunoregulatory effect of a novel exopolysaccharide from a marine thermotolerant Bacillus licheniformis." International immunopharmacology 6.1 (2006): 8-13.

Asensio, Juan Luis, et al. "Carbohydrate–aromatic interactions." Accounts of chemical research 46.4 (2012): 946-954.

Bertoft, Eric. "On the nature of categories of chains in amylopectin and their connection to the super helix model." Carbohydrate Polymers 57.2 (2004): 211-224.

Bird, Anthony R., Ian L. Brown, and David L. Topping. "Starches, resistant starches, the gut microflora and human health." Current issues in intestinal microbiology 1.1 (2000): 25-37.

Blazek, Jaroslav, and Elliot Paul Gilbert. "Application of small-angle X-ray and neutron scattering techniques to the characterisation of starch structure: A review." Carbohydrate Polymers 85.2 (2011): 281-293.

Breyer, Wendy A., and Brian W. Matthews. "A structural basis for processivity." Protein Science 10.9 (2001): 1699-1711.

Brown, Ian, et al. "Fecal numbers of bifidobacteria are higher in pigs fed Bifidobacterium longum with a high amylose cornstarch than with a low amylose cornstarch." The Journal of nutrition 127.9 (1997): 1822-1827.

Buléon, A., et al. "Starch granules: structure and biosynthesis." International journal of biological macromolecules 23.2 (1998): 85-112.

Bulpin, P. V., E. J. Welsh, and E. R. Morris. "Physical Characterization of Amylose-Fatty Acid Complexes in Starch Granules and in Solution." Starch-Stärke 34.10 (1982): 335-339.

Burtt Jr, Edward H., and Jann M. Ichida. "Occurrence of feather-degrading bacilli in the plumage of birds." The Auk (1999): 364-372.

Burtt, Edward H., et al. "Colourful parrot feathers resist bacterial degradation." Biology letters (2010): rsbl20100716.

Callender, Robert, and R. Brian Dyer. "The dynamical nature of enzymatic catalysis." Accounts of chemical research 48.2 (2014): 407-413.

Chen, Mo, et al. "Molecular dynamics simulations of the interaction of glucose with imidazole in aqueous solution." Carbohydrate research 349 (2012): 73-77.

Corzana, Francisco, et al. "Hydration of the amylopectin branch point. Evidence of restricted conformational diversity of the α-(1→ 6) linkage." Journal of the American Chemical Society 126.40 (2004): 13144-13155.

FAO, and WHO Expert Consultation. "Carbohydrates in human nutrition (FAO Food and Nutrition Paper 66)." *Rome, Italy: FAO* (1998).

Fox, Jeffrey D., and John F. Robyt. "Miniaturization of three carbohydrate analyses using a microsample plate reader." Analytical biochemistry 195.1 (1991): 93-96.

Fukumoto, J. "Studies on the production of bacterial amylase. I. Isolation of bacteria secreting potent amylases and their distribution." J. Agr. Chem. Soc. Japan 19 (1943): 487-503.

Gessler, Katrin, et al. "V-Amylose at atomic resolution: X-ray structure of a cycloamylose with 26 glucose residues (cyclomaltohexaicosaose)." Proceedings of the National Academy of Sciences 96.8 (1999): 4246-4251.

Ghollasi, Marzieh, Maryam Ghanbari-Safari, and Khosro Khajeh. "Improvement of thermal stability of a mutagenised α-amylase by manipulation of the calcium-binding site." Enzyme and microbial technology 53.6 (2013): 406-413.

Ghosh, Hara Prasad, and Jack Preiss. "Adenosine diphosphate glucose pyrophosphorylase a regulatory enzyme in the biosynthesis of starch in spinach leaf chloroplasts." Journal of Biological Chemistry 241.19 (1966): 4491-4504.

Gidley, Michael J., and Paul V. Bulpin. "Crystallisation of malto-oligosaccharides as models of the crystalline forms of starch: minimum chain-length requirement for the formation of double helices." Carbohydrate Research 161.2 (1987): 291-300.

Haydushka, Irina A., et al. "Recurrent sepsis due to Bacillus licheniformis." Journal of global infectious diseases 4.1 (2012): 82.

Henry, Amanda G., Alison S. Brooks, and Dolores R. Piperno. "Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium)." Proceedings of the National Academy of Sciences 108.2 (2011): 486-491.

Humphrey, Sue P., and Russell T. Williamson. "A review of saliva: normal composition, flow, and function." The Journal of prosthetic dentistry 85.2 (2001): 162-169.

Jane, Jay-Lin, et al. "Anthology of starch granule morphology by scanning electron microscopy." Starch-Stärke 46.4 (1994): 121-129.

Jordan, Torsten, et al. "Molten imidazole–a starch solvent." Green Chemistry 16.4 (2014): 1967-1973.

Kierul, Kinga. Comprehensive proteomic study of Bacillus amyloliquefaciens strain FZB42 and its response to plant root exudates. Diss. Humboldt-Universität zu Berlin, Mathematisch-Naturwissenschaftliche Fakultät I, 2013.

Kimura, Atsuo, and John F. Robyt. "Reaction of enzymes with starch granules: kinetics and products of the reaction with glucoamylase." Carbohydrate Research 277.1 (1995): 87-107.

Krause, Johannes, et al. "Neanderthals in central Asia and Siberia." Nature 449.7164 (2007): 902-904.

Kumari, Manju, Raghavan B. Sunoj, and Petety V. Balaji. "Exploration of CH··· π mediated stacking interactions in saccharide: aromatic residue complexes through conformational sampling." Carbohydrate research 361 (2012): 133-140.

Kuriki, Takashi, and Tadayuki Imanaka. "The concept of the α-amylase family: structural similarity and common catalytic mechanism." Journal of Bioscience and Bioengineering 87.5 (1999): 557-565.

Lindeboom, Nienke, Peter R. Chang, and Robert T. Tyler. "Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review." Starch-Stärke 56.3-4 (2004): 89-99.

Liu, Yunpeng, et al. "Enhanced rhizosphere colonization of beneficial Bacillus amyloliquefaciens SQR9 by pathogen infection." FEMS microbiology letters 353.1 (2014): 49-56.

Mandal, Mahitosh, et al. "Butyric acid induces apoptosis by up-regulating Bax expression via stimulation of the c-Jun N-terminal kinase/activation protein-1 pathway in human colon cancer cells." Gastroenterology 120.1 (2001): 71-78.

Marx, Jean-Claude, et al. "The noncatalytic triad of α-amylases: A novel structural motif involved in conformational stability." Proteins: Structure, Function, and Bioinformatics 70.2 (2008): 320-328.

Matsui, Ikuo, et al. "Roles of the aromatic residues conserved in the active center of Saccharomycopsis. alpha.-amylase for transglycosylation and hydrolysis activity." Biochemistry 33.2 (1994): 451-458.

Mishra, Prasunkumar J., Chandran Ragunath, and Narayanan Ramasubbu. "The mechanism of salivary amylase hydrolysis: role of residues at subsite S2′." Biochemical and biophysical research communications 292.2 (2002): 468-473.

Mostofian, Barmak, Jeremy C. Smith, and Xiaolin Cheng. "Simulation of a cellulose fiber in ionic liquid suggests a synergistic approach to dissolution." Cellulose 21.2 (2014): 983-997.

Mottram, Donald S., Bronislaw L. Wedzicha, and Andrew T. Dodson. "Food chemistry: acrylamide is formed in the Maillard reaction." Nature 419.6906 (2002): 448-449.

Mukerjea, Rupendra, and John F. Robyt. "De novo biosynthesis of starch chains without a primer and the mechanism for its biosynthesis by potato starch-synthase." Carbohydrate research 352 (2012): 137-142.

Mukerjea, Rupendra, and John F. Robyt. "Tests for the mechanism of starch biosynthesis: de novo synthesis or an amylogenin primer synthesis." Carbohydrate research 372 (2013): 55-59.

Pérez, Serge, and Eric Bertoft. "The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review." Starch-Stärke 62.8 (2010): 389-420.

Pinto, Gaspar P., et al. "Establishing the Catalytic Mechanism of Human Pancreatic α-Amylase with QM/MM Methods." Journal of chemical theory and computation 11.6 (2015): 2508-2516.

Ramasubbu, Narayanan, Chandran Ragunath, and Prasunkumar J. Mishra. "Probing the role of a mobile loop in substrate binding and enzyme activity of human salivary amylase." Journal of molecular biology 325.5 (2003): 1061-1076.

Rathore, R. S., et al. "Starch phosphorylase: role in starch metabolism and biotechnological applications." Critical reviews in biotechnology 29.3 (2009): 214-224.

Rosenblum, JERRY L., CARL L. Irwin, and DAVID H. Alpers. "Starch and glucose oligosaccharides protect salivary-type amylase activity at acid pH." American Journal of Physiology-Gastrointestinal and Liver Physiology 254.5 (1988): G775-G780.

Sandstedt, Rudolph M., et al. "The digestibility of high-amylose corn starches compared to that of other starches. The apparent effect of the ae gene on susceptibility to amylase action." Cereal Chem 39 (1962): 123-131.

Sarko, A., and H-CH Wu. "The Crystal Structures of A-, B-and C-Polymorphs of Amylose and Starch." Starch-Stärke 30.3 (1978): 73-78.

Scannapieco, Frank A., G. I. Torres, and M. J. Levine. "Salivary amylase promotes adhesion of oral streptococci to hydroxyapatite." Journal of dental research 74.7 (1995): 1360-1366.

Schulz, AGl, J. M. Van Amelsvoort, and A. C. Beynen. "Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats." The Journal of nutrition 123.10 (1993): 1724-1731.

Sellami-Kamoun, Alya, et al. "Stability of thermostable alkaline protease from Bacillus licheniformis RP1 in commercial solid laundry detergent formulations." Microbiological Research 163.3 (2008): 299-306.

Shou, Takashima, and Amano Junko. "Glycosylation and secretion of human α-amylases." Advances in Biological Chemistry 2012 (2012).

Svensson, Birte. "Protein engineering in the α-amylase family: catalytic mechanism, substrate specificity, and stability." Plant molecular biology 25.2 (1994): 141-157.

Swatloski, Richard P., et al. "Dissolution of cellulose with ionic liquids." Journal of the American Chemical Society 124.18 (2002): 4974-4975.

Topping, David L., et al. "A high amylose (amylomaize) starch raises proximal large bowel starch and increases colon length in pigs." The Journal of nutrition 127.4 (1997): 615-622.

Van Immerseel, Filip, et al. "Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease." Journal of medical microbiology 59.2 (2010): 141-143.

Vistoli, G., et al. "Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation." Free radical research 47.sup1 (2013): 3-27.

Wall, Jeffrey D., et al. "Higher levels of Neanderthal ancestry in East Asians than in Europeans." Genetics 194.1 (2013): 199-209.

Wang, Shujun, et al. "Starch Retrogradation: A Comprehensive Review." Comprehensive Reviews in Food Science and Food Safety 14.5 (2015): 568-585.

Winter, William T., and A. Sarko. "Crystal and molecular structure of V-anhydrous amylose." Biopolymers 13.7 (1974): 1447-1460.

Yook, Cheol, and John F. Robyt. "Reactions of alpha amylases with starch granules in aqueous suspension giving products in solution and in a minimum amount of water giving products inside the granule." Carbohydrate research 337.12 (2002): 1113-1117.

Yoon, Seung-Heon, and John F. Robyt. "Activation and stabilization of 10 starch-degrading enzymes by Triton X-100, polyethylene glycols, and polyvinyl alcohols." Enzyme and Microbial Technology 37.5 (2005): 556-562.

Zhang, Bin, et al. "Freeze-drying changes the structure and digestibility of B-polymorphic starches." Journal of agricultural and food chemistry 62.7 (2014): 1482-1491.

APPENDIX A: TOTAL CARBOHYDRATE GRAPHS

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Porcine Pancreas treatment on Potato Starch

Porcine Pancreas treatment on Waxy Maize Starch

Porcine Pancreas treatment on Maize Starch

Porcine Pancreas reatment on Amylomaize-7

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.
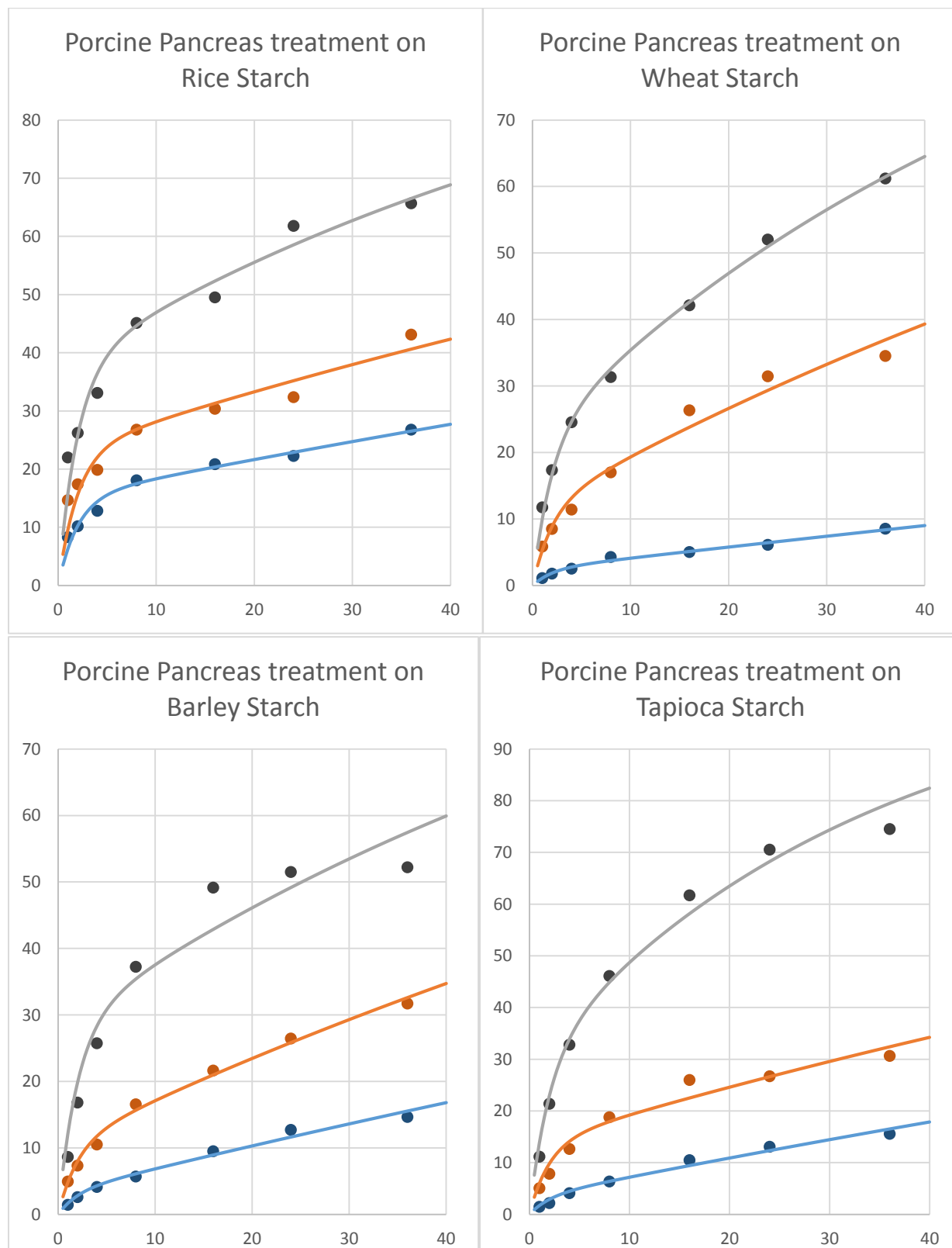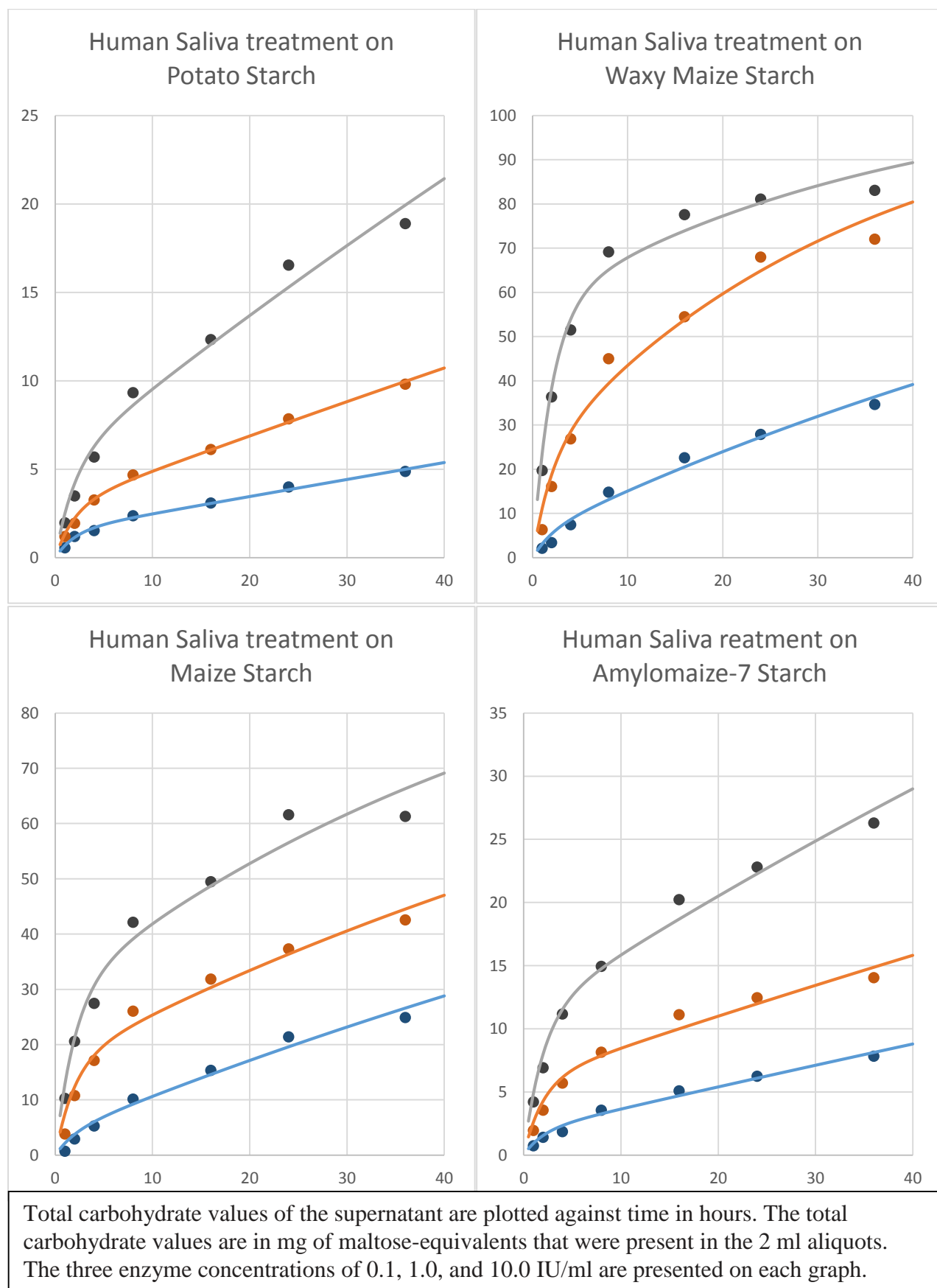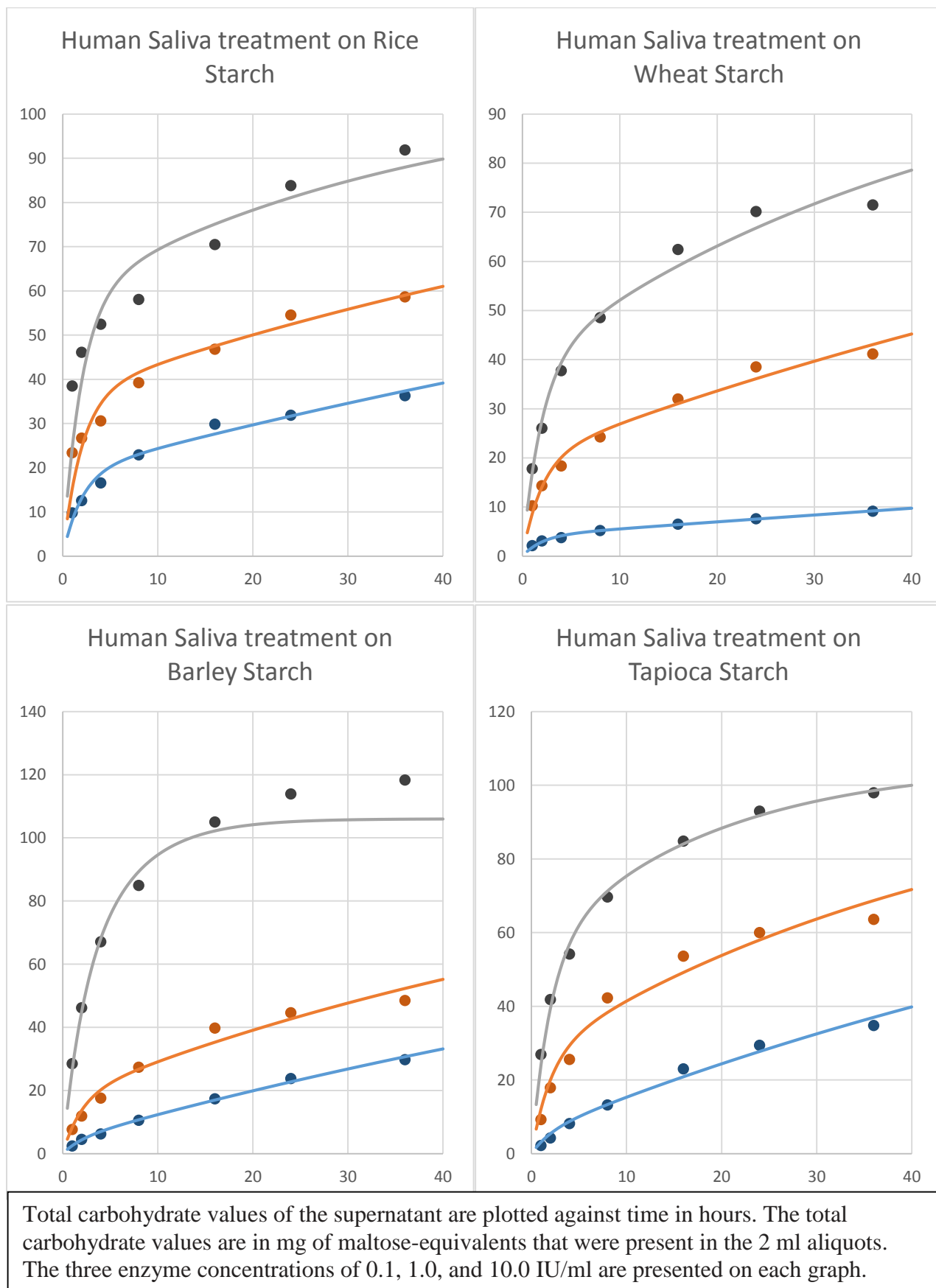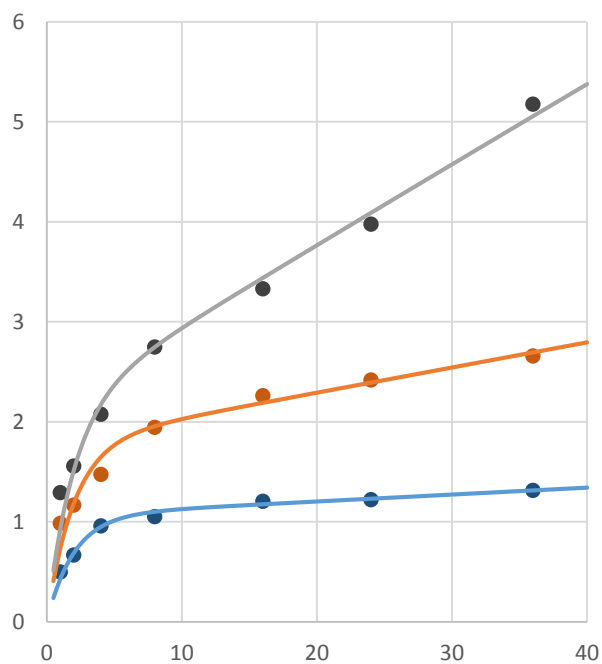
Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Curve fits for the total carbohydrate values are described by $106\ mg * (R * e^{Arg*t} + (1 - R) * e^{0.5t})$ with R, and Arg being parameters. Rationale for having two exponential decays for the curve fitting is due to single decay curves not fitting the data well. The presence of a quickly-degrading component is the rational for the $e^{0.5t}$ term. R denotes the ratio between the quickly degrading starches and the slowly degrading starches. A table with these arguments is presented below.

BLA

R values

	Potato	Waxymaize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.979	0.937	0.943	0.977	0.851	0.958	0.980	0.960
1.0 IU/ml	0.967	0.812	0.855	0.942	0.777	0.912	0.934	0.849
10.0 IU/ml	0.959	0.731	0.698	0.897	0.700	0.804	0.836	0.795

Arg values (in hours^{-1})

	Potato	Waxymaize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.0012	0.0074	0.0061	0.0021	0.0031	0.0041	0.0051	0.0040
1.0 IU/ml	0.0019	0.0220	0.0108	0.0031	0.0082	0.0100	0.0122	0.0131
10.0 IU/ml	0.0048	0.0389	0.0192	0.0093	0.0207	0.0282	0.0310	0.0243

PPA

R values

	Potato	Waxymaize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.983	0.940	0.943	0.987	0.858	0.977	0.969	0.968
1.0 IU/ml	0.969	0.743	0.867	0.963	0.784	0.891	0.902	0.871
10.0 IU/ml	0.968	0.697	0.754	0.909	0.647	0.793	0.734	0.724

Arg values (in hours^{-1})

	Potato	Waxymaize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.0007	0.0097	0.0054	0.0017	0.0037	0.0016	0.0035	0.0038
1.0 IU/ml	0.0016	0.0182	0.0105	0.0028	0.0066	0.0087	0.0073	0.0063
10.0 IU/ml	0.0038	0.0287	0.0166	0.0054	0.0153	0.0176	0.0131	0.0295

Scale	R	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.0
	Arg	0.50	0.40	0.30	0.20	0.10	0.05	0.04	0.03	0.02	0.01	0.00

A quickly degrading portion and a slowly degrading portion are considered in the curve fitting. The curve fitting is in the form $106\ mg * (R * e^{Arg*t} + (1 - R) * e^{0.5t})$. The variable t is the time is hours, R is a ratio of the slow to fast degrading component, and Arg is the decay rate of the slowly degrading portion of the starch.

HSA
R values

	Potato	Waxymaize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.986	0.950	0.965	0.982	0.822	0.961	0.960	0.950
1.0 IU/ml	0.973	0.792	0.843	0.944	0.657	0.813	0.832	0.750
10.0 IU/ml	0.951	0.468	0.725	0.896	0.449	0.633	0.633	0.490

Arg values (in hours^{-1})

	Potato	Waxymaize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.0009	0.0102	0.0070	0.0017	0.0066	0.0014	0.0084	0.0105
1.0 IU/ml	0.0020	0.0297	0.0104	0.0026	0.0109	0.0087	0.0138	0.0210
10.0 IU/ml	0.0044	0.0273	0.0184	0.0052	0.0270	0.0224	0.1792	0.0540

BAA
R values

	Potato	Waxymaize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.990	0.965	0.975	0.988	0.906	0.998	0.999	0.993
1.0 IU/ml	0.983	0.946	0.944	0.982	0.862	0.997	0.995	0.978
10.0 IU/ml	0.980	0.821	0.893	0.971	0.817	0.930	0.954	0.962

Arg values (in hours^{-1})

	Potato	Waxymaize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.0001	0.0013	0.0018	0.0004	0.0016	0.0012	0.0009	0.0009
1.0 IU/ml	0.0002	0.0051	0.0045	0.0012	0.0023	0.0043	0.0032	0.0030
10.0 IU/ml	0.0008	0.0133	0.0093	0.0023	0.0055	0.0054	0.0054	0.0082

Scale	R	1.00	0.90	0.80	0.70	0.60	0.50	0.40	0.30	0.20	0.10	0.00
	Arg	0.50	0.40	0.30	0.20	0.10	0.05	0.04	0.03	0.02	0.01	0.00

A quickly degrading portion and a slowly degrading portion are considered in the curve fitting. The curve fitting is in the form $106\ mg * (R * e^{Arg*t} + (1 - R) * e^{0.5t})$. The variable t is the time is hours, R is a ratio of the slow to fast degrading component, and Arg is the decay rate of the slowly degrading portion of the starch.

Bacillus licheniformis

	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.18	0.51	0.68	0.42	1.11	0.68	0.77	1.16
1.0 IU/ml	0.40	2.29	1.44	0.48	2.27	1.30	2.10	2.54
10.0 IU/ml	0.58	1.70	2.16	1.59	3.10	3.76	4.66	4.06

Porcine pancreas

	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.24	1.52	2.66	0.29	1.14	0.29	0.66	0.75
1.0 IU/ml	0.24	2.57	3.38	0.70	2.84	1.87	0.85	1.94
10.0 IU/ml	0.87	1.51	1.27	0.56	3.41	1.00	4.14	3.01

Human Saliva

	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.13	1.67	1.35	0.32	1.51	0.24	0.67	1.56
1.0 IU/ml	0.19	4.12	2.25	0.75	4.07	1.70	2.86	3.91
10.0 IU/ml	0.85	3.51	3.61	0.96	7.88	2.87	6.66	2.09

Bacillus amyloliquefaciens

	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.04	0.32	0.25	0.11	1.35	0.15	0.08	0.13
1.0 IU/ml	0.13	0.53	0.48	0.09	1.57	0.64	0.39	0.51
10.0 IU/ml	0.18	1.66	1.37	0.23	2.37	1.39	1.22	1.21

The above table includes the square root errors in the calculations of the curve fits. The tabulated data is in units of mg.

43

Bacillus licheniformis

Potato Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.00	2.32	2.70
2	1.65	2.55	4.08
4	2.29	3.32	4.99
8	3.13	5.01	8.80
16	4.57	6.53	11.18
24	5.00	8.48	15.28
36	6.57	10.19	20.85

Bacillus licheniformis
Waxy Maize Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	3.46	9.73	14.41
2	5.10	14.25	23.31
4	8.15	23.11	33.57
8	12.78	34.55	50.50
16	18.24	49.84	66.41
24	23.28	53.49	75.49
36	29.40	65.64	84.70

Bacillus licheniformis

Maize Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	3.03	7.31	16.11
2	4.85	12.00	21.42
4	7.86	16.92	30.59
8	10.78	23.02	42.72
16	14.26	27.43	54.11
24	20.84	38.53	60.43
36	25.35	43.78	66.45

Bacillus licheniformis
Amylomaize-7 Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.19	2.71	5.37
2	1.89	4.04	7.79
4	2.69	6.15	11.25
8	4.14	9.13	17.74
16	5.41	10.82	27.17
24	8.20	14.02	29.54
36	9.35	16.40	36.65

Bacillus licheniformis
Rice Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	7.93	14.50	18.95
2	11.36	17.55	25.06
4	13.31	20.83	33.87
8	16.98	27.27	39.75
16	20.82	33.07	50.12
24	23.26	39.70	59.21
36	24.57	44.24	74.30

Bacillus licheniformis
Wheat Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.65	4.13	8.90
2	3.11	7.30	15.46
4	4.90	11.02	23.09
8	7.99	16.29	42.26
16	11.83	25.31	56.13
24	14.60	31.56	62.92
36	17.68	36.70	70.69

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Bacillus licheniformis Barley Total Carbohydrate				*Bacillus licheniformis* Tapioca Total Carbohydrate		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.46	2.80	4.34	1	1.78	5.56	9.09
2	2.30	4.65	11.64	2	2.77	10.05	14.98
4	3.52	9.84	26.02	4	4.54	16.44	25.49
8	6.35	17.20	41.32	8	6.95	27.52	35.98
16	9.81	26.28	52.63	16	11.90	34.94	55.05
24	15.66	34.67	68.15	24	14.94	42.26	61.99
36	18.73	39.22	70.66	36	16.13	46.73	64.50

Porcine Pancreas
Potato Total Carbohydrate

Porcine Pancreas
Waxy Maize Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.68	1.64	1.67	1	0.45	3.34	9.97
2	1.02	2.30	2.36	2	1.00	4.95	19.15
4	1.79	3.33	4.11	4	2.04	8.43	26.41
8	2.31	4.92	5.89	8	2.54	13.54	40.29
16	3.45	5.50	11.15	16	4.47	21.98	48.88
24	3.72	7.09	12.59	24	6.21	29.30	57.35
36	4.28	8.98	15.61	36	8.25	33.39	61.83

Porcine Pancreas
Maize Total Carbohydrate

Porcine Pancreas
Amylomaize-7 Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.09	4.13	10.67	1	0.25	0.72	1.66
2	2.61	7.50	18.50	2	0.43	1.07	2.76
4	4.89	14.83	27.11	4	0.61	1.49	4.11
8	12.39	22.02	36.31	8	0.89	2.86	5.63
16	16.68	32.94	46.85	16	1.27	4.25	9.41
24	20.69	37.40	50.59	24	1.43	5.83	11.25
36	20.38	38.49	<mark>29.96</mark>	36	1.67	7.05	12.72

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph. Note how PPA acting upon Maize at 10.0 IU/ml on the 36 H time point is erroneous.

Porcine Pancreas
Rice Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.32	8.33	14.65
2	3.42	10.17	17.39
4	4.43	12.82	19.89
8	7.19	18.07	26.80
16	8.78	20.84	30.35
24	11.38	22.24	32.38
36	13.76	26.76	43.11

Porcine Pancreas
Wheat Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.19	1.12	5.85
2	0.28	1.77	8.50
4	0.28	2.55	11.42
8	0.36	4.28	17.02
16	0.71	5.02	26.36
24	1.05	6.10	31.47
36	2.43	8.54	34.50

Porcine Pancreas
Barley Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.25	1.46	4.96
2	0.50	2.61	7.35
4	1.12	4.13	10.51
8	1.64	5.70	16.58
16	3.12	9.51	21.63
24	4.46	12.74	26.47
36	5.77	14.67	31.74

Porcine Pancreas
Tapioca Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.30	1.47	5.07
2	0.42	2.19	7.85
4	0.85	4.11	12.69
8	1.34	6.36	18.81
16	2.13	10.52	26.00
24	2.43	13.11	26.74
36	2.98	15.58	30.68

Human Saliva

Potato Total Carbohydrate

TC	0.1 IU	1 IU	10 IU
1	0.55	1.20	1.97
2	1.20	1.95	3.49
4	1.53	3.27	5.69
8	2.37	4.67	9.33
16	3.09	6.12	12.33
24	3.99	7.85	16.54
36	4.88	9.80	18.89

Human Saliva
Waxy Maize Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.12	6.31	19.70
2	3.42	16.09	36.36
4	7.46	26.84	51.51
8	14.79	44.99	69.15
16	22.62	54.50	77.60
24	27.88	68.00	81.12
36	34.65	72.01	83.08

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph.

Hours	Human Saliva Maize Total Carbohydrate 0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.71	3.84	10.30
2	2.95	10.78	20.61
4	5.29	17.15	27.47
8	10.14	26.06	42.15
16	15.37	31.88	49.50
24	21.40	37.30	61.61
36	24.90	42.58	61.29

Hours	Human Saliva Amylomaize-7 Total Carbohydrate 0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.74	1.95	4.22
2	1.42	3.56	6.93
4	1.87	5.71	11.17
8	3.57	8.15	14.96
16	5.09	11.13	20.23
24	6.25	12.47	22.81
36	7.83	14.05	26.30

Hours	Human Saliva Rice Total Carbohydrate 0.1 IU/ml	1 IU/ml	10 IU/ml
1	9.80	23.38	38.48
2	12.57	26.68	46.10
4	16.56	30.58	52.45
8	22.94	39.21	58.05
16	29.86	46.82	70.50
24	31.87	54.55	83.80
36	36.32	58.62	91.89

Hours	Human Saliva Wheat Total Carbohydrate 0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.14	10.27	17.77
2	3.11	14.32	26.05
4	3.76	18.38	37.74
8	5.20	24.27	48.54
16	6.51	32.02	62.42
24	7.64	38.54	70.17
36	9.17	41.17	71.48

Hours	Human Saliva Barley Total Carbohydrate 0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.45	7.65	28.49
2	4.52	11.95	46.19
4	6.27	17.57	67.13
8	10.61	27.38	84.97
16	17.39	39.80	105.04
24	23.82	44.60	113.91
36	29.81	48.53	118.32

Hours	Human Saliva Tapioca Total Carbohydrate 0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.24	9.28	26.91
2	4.31	17.90	41.85
4	8.21	25.57	54.16
8	13.25	42.30	69.66
16	23.03	53.62	84.84
24	29.43	60.02	92.98
36	34.82	63.63	97.96

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph. Note how HSA acting upon barley exceeds 106 mg in the final two time points.

Bacillus amyloliquefaciens

Potato Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.50	0.98	1.29
2	0.67	1.17	1.56
4	0.96	1.47	2.08
8	1.05	1.94	2.75
16	1.21	2.26	3.33
24	1.22	2.42	3.98
36	1.32	2.66	5.18

Bacillus amyloliquefaciens
Waxy Maize Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.96	3.38	7.96
2	2.80	4.48	11.31
4	3.46	6.39	22.51
8	4.68	9.57	27.22
16	6.07	14.48	37.25
24	6.35	17.59	43.45
36	8.55	22.29	50.79

Bacillus amyloliquefaciens

Maize Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.51	2.94	5.95
2	1.96	4.06	8.09
4	2.90	6.74	11.35
8	3.97	9.27	19.82
16	5.35	13.43	24.40
24	7.36	16.72	31.63
36	8.81	20.28	37.25

Bacillus amyloliquefaciens
Amylomaize-7 Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.67	1.02	1.52
2	0.83	1.38	2.28
4	1.14	2.15	3.28
8	1.54	2.88	5.27
16	2.04	4.08	6.94
24	2.31	4.96	8.50
36	2.60	6.36	11.12

Bacillus amyloliquefaciens
Rice Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	6.71	8.99	12.48
2	7.79	10.88	14.23
4	8.59	11.76	16.59
8	9.77	15.49	21.33
16	11.80	17.30	26.18
24	14.10	20.23	32.56
36	15.60	21.97	33.99

Bacillus amyloliquefaciens
Wheat Total Carbohydrate

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.16	1.12	4.45
2	0.43	1.22	4.93
4	0.64	2.44	7.08
8	1.44	3.87	11.02
16	2.02	6.71	17.70
24	3.45	9.64	20.29
36	4.77	16.22	23.26

Total carbohydrate values of the supernatant are plotted against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each graph. Note how HSA acting upon barley exceeds 106 mg in the final two time points.
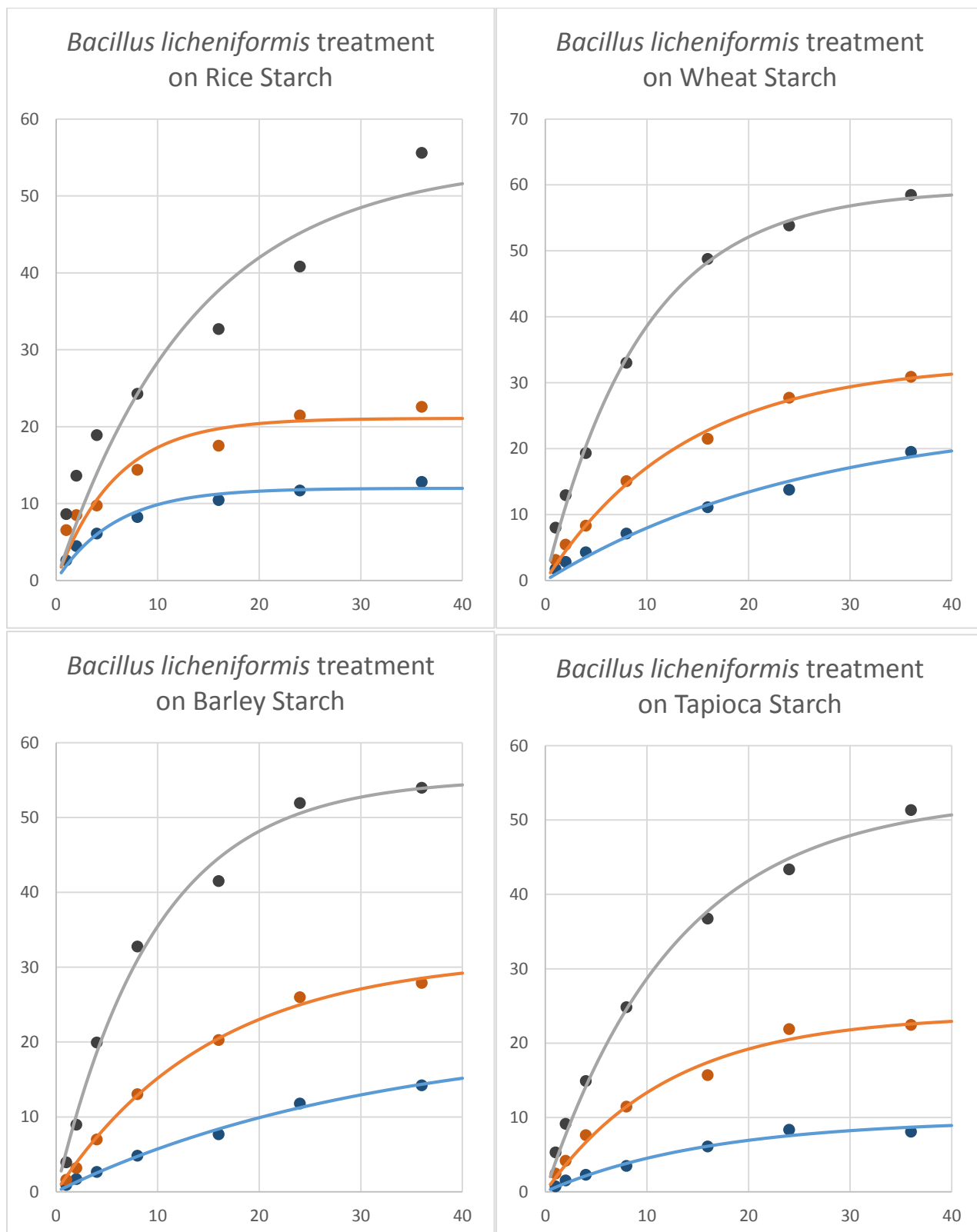
| Hours | *Bacillus amyloliquefaciens* Barley Total Carbohydrate | | | | Hours | *Bacillus amyloliquefaciens* Tapioca Total Carbohydrate | | |
	0.1 IU/ml	1 IU/ml	10 IU/ml			0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.32	0.56	1.25		1	0.52	1.27	1.72
2	0.29	1.06	3.24		2	0.66	1.71	3.49
4	0.48	1.67	5.87		4	0.98	2.98	5.75
8	0.84	3.32	9.49		8	1.40	4.55	12.03
16	1.62	5.42	14.73		16	2.04	7.28	15.86
24	2.50	9.15	18.59		24	3.17	10.33	23.72
36	3.56	11.77	21.35		36	4.05	12.07	28.87

Total carbohydrate values of the supernatant are presented against time in hours. The total carbohydrate values are in mg of maltose-equivalents that were present in the 2 ml aliquots. The three enzyme concentrations of 0.1, 1.0, and 10.0 IU/ml are presented on each table.
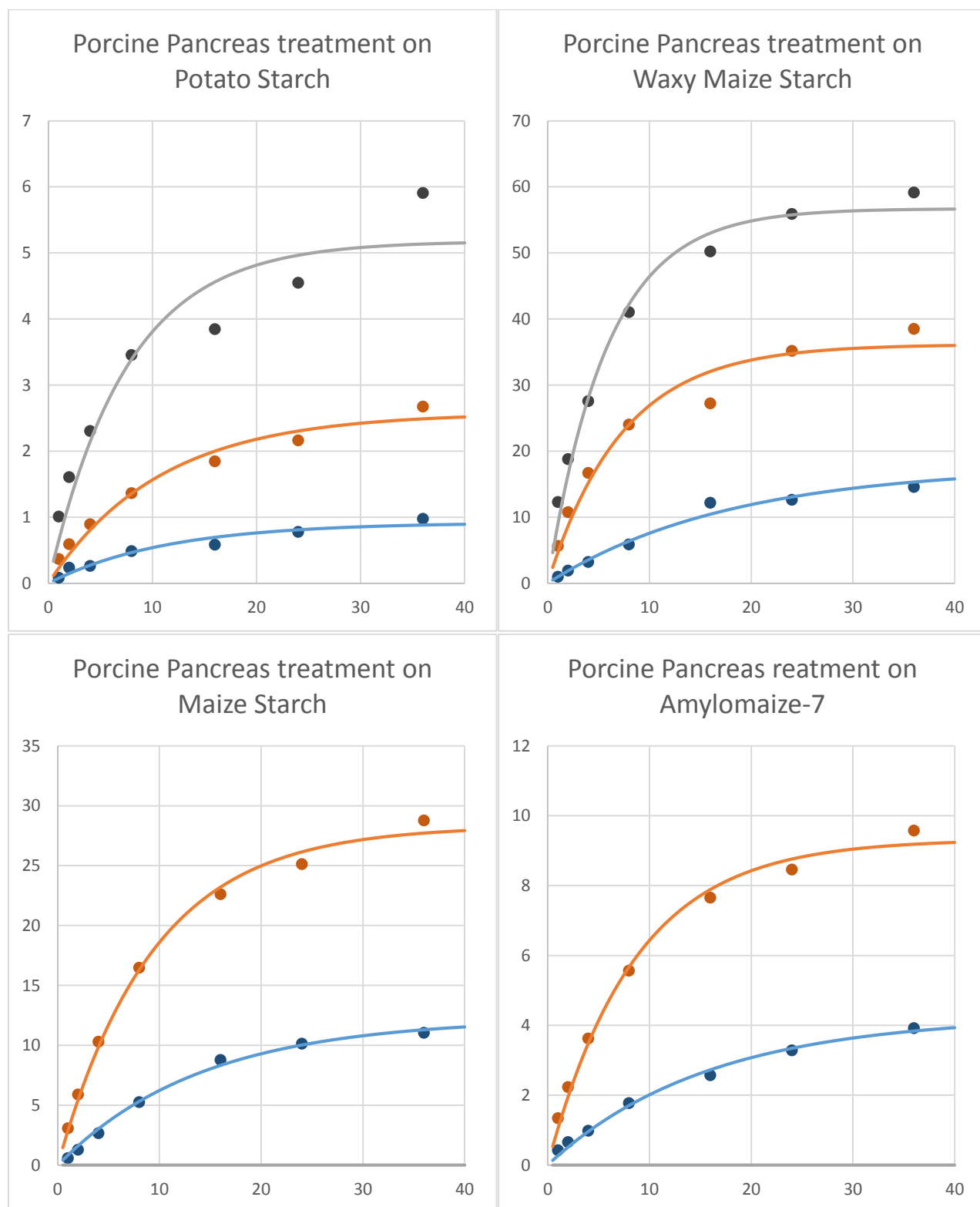
APPENDIX B: REDUCING VALUE GRAPHS

Bacillus licheniformis treatment on Potato Starch

Bacillus licheniformis treatment on Waxy Maize Starch

Bacillus licheniformis treatment on Maize Starch

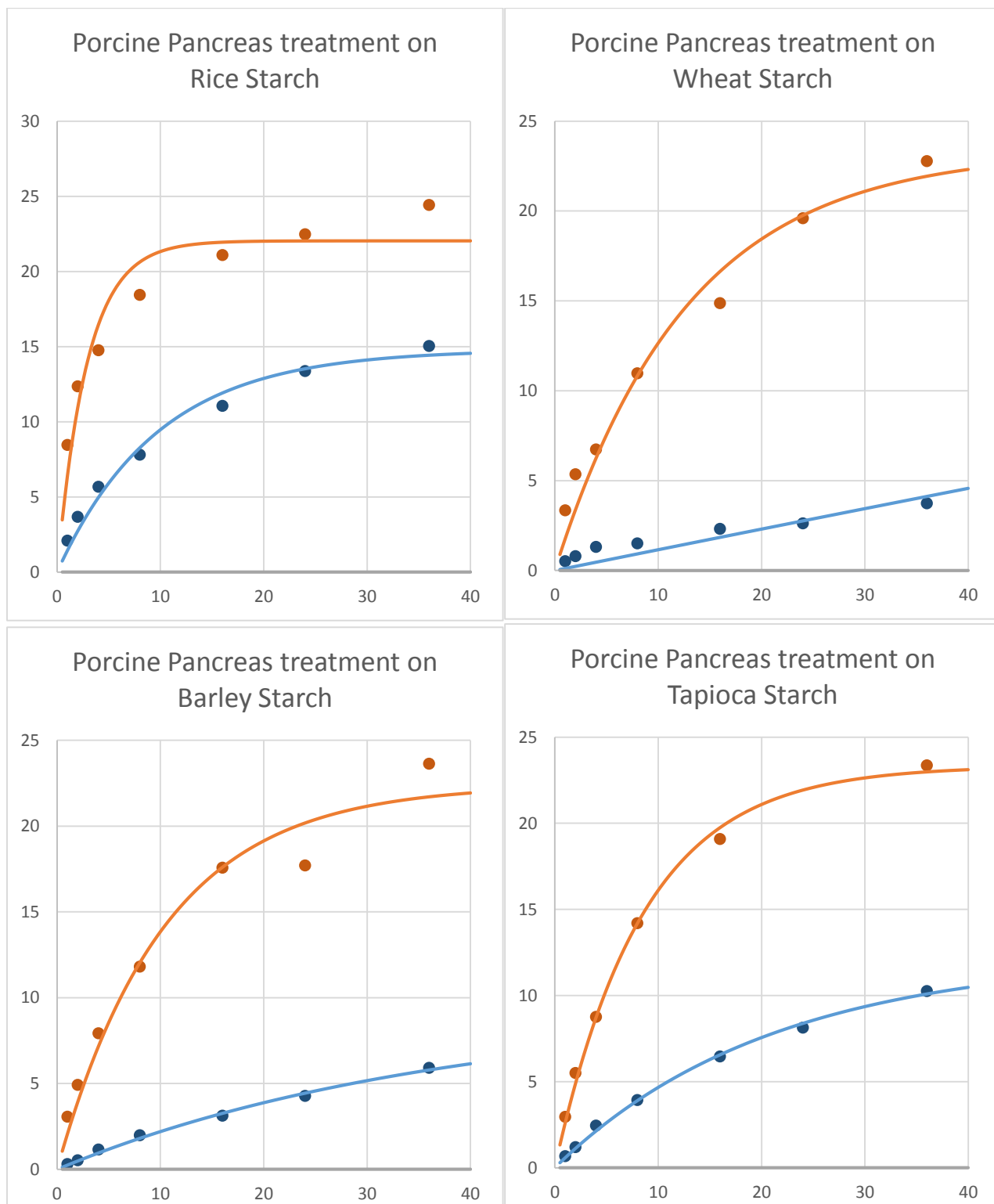### Bacillus licheniformis reatment on Amylomaize-7 Starch

These reducing value graphs plot the maltose-equivalents of reducing value in units of mg. The x-axis is in units of hours. The equations used to smooth the data is in the form $[P]_{max}(1 - e^{Arg*t})$ with $[P]_{max}$ and Arg as parameters. These parameters and their associated errors are at the end of this Appendix

Bacillus licheniformis treatment on Rice Starch

Bacillus licheniformis treatment on Wheat Starch

Bacillus licheniformis treatment on Barley Starch

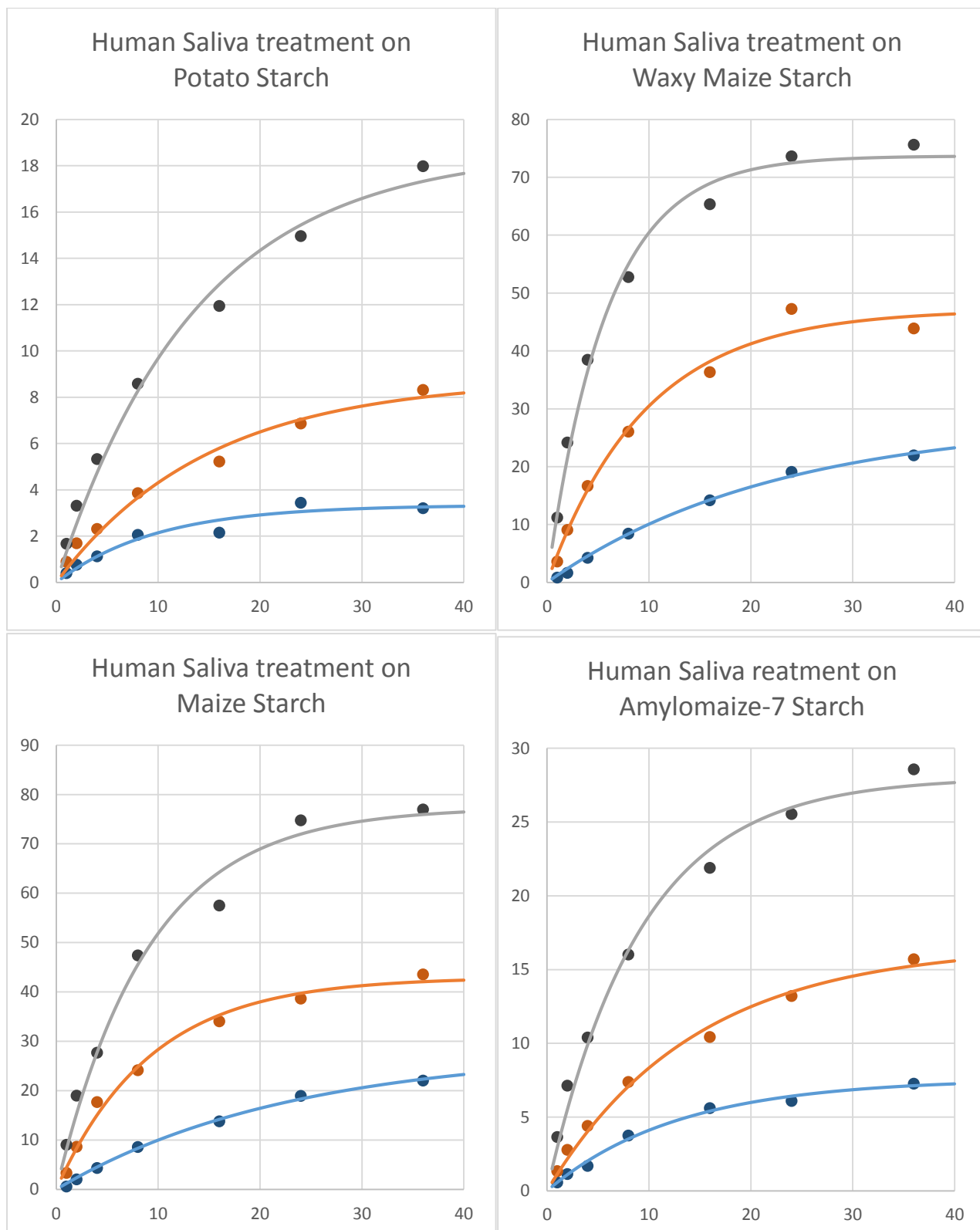*Bacillus licheniformis* treatment on Tapioca Starch

These reducing value graphs plot the maltose-equivalents of reducing value in units of mg. The x-axis is in units of hours. The equations used to smooth the data is in the form $[P]_{max}(1 - e^{Arg*t})$ with $[P]_{max}$ and Arg as parameters. These parameters and their associated errors are at the end of this Appendix

Porcine Pancreas treatment on Potato Starch

Porcine Pancreas treatment on Waxy Maize Starch

Porcine Pancreas treatment on Maize Starch

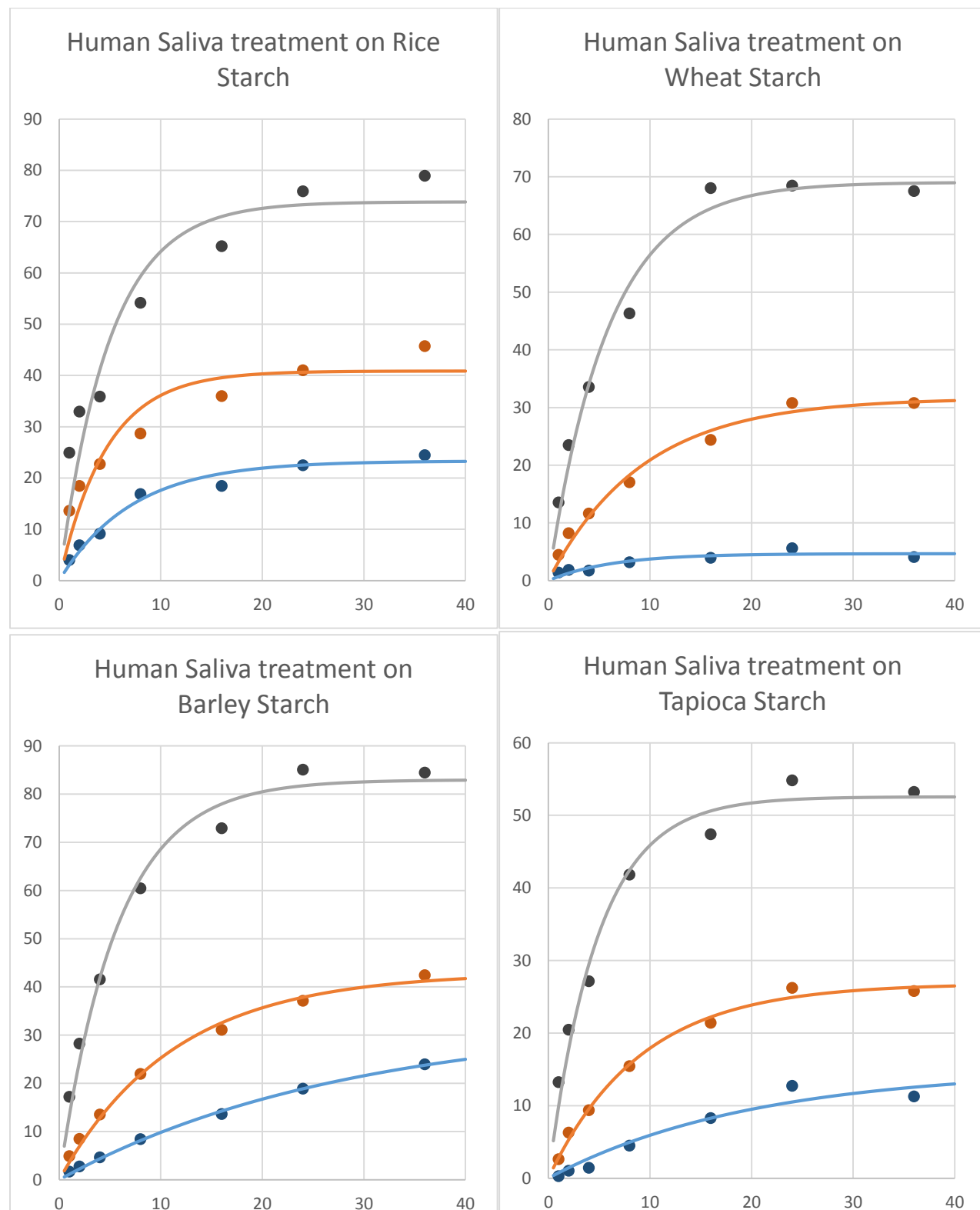Porcine Pancreas reatment on Amylomaize-7

These reducing value graphs plot the maltose-equivalents of reducing value in units of mg. The x-axis is in units of hours. The equations used to smooth the data is in the form $[P]_{max}(1 - e^{Arg*t})$ with $[P]_{max}$ and Arg as parameters. These parameters and their associated errors are at the end of this Appendix. Only 0.1 IU/ml and 1.0 IU/ml are available for maize and amylomaize.
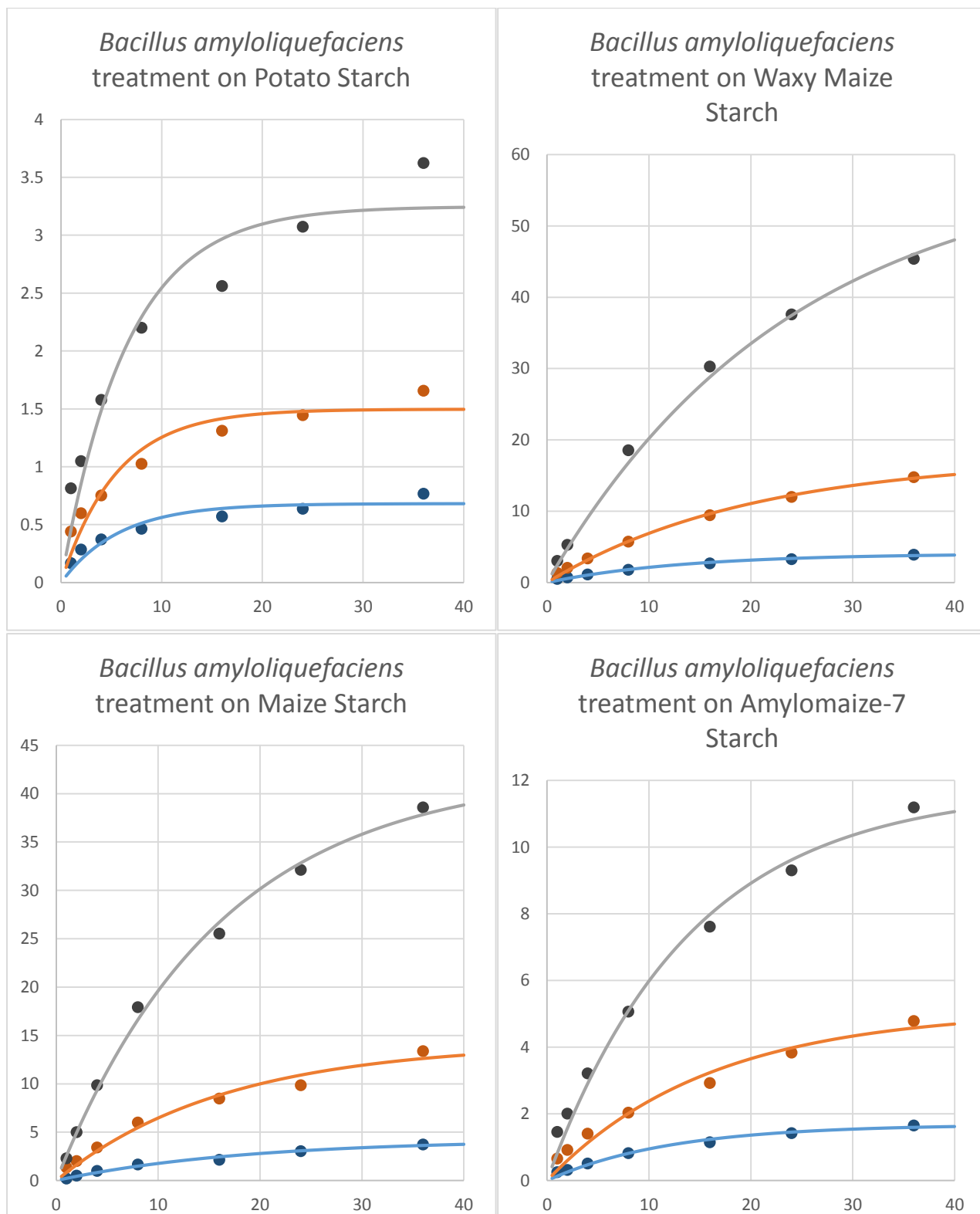
These reducing value graphs plot the maltose-equivalents of reducing value in units of mg. The x-axis is in units of hours. The equations used to smooth the data is in the form $[P]_{max}(1 - e^{Arg*t})$ with $[P]_{max}$ and Arg as parameters. These parameters and their associated errors are at the end of this Appendix. Only 0.1 IU/ml and 1.0 IU/ml are available for this specific combination.

Human Saliva treatment on Potato Starch

Human Saliva treatment on Waxy Maize Starch

Human Saliva treatment on Maize Starch

Human Saliva reatment on Amylomaize-7 Starch

These reducing value graphs plot the maltose-equivalents of reducing value in units of mg. The x-axis is in units of hours. The equations used to smooth the data is in the form $[P]_{max}(1 - e^{Arg*t})$ with $[P]_{max}$ and Arg as parameters. These parameters and their associated errors are at the end of this Appendix
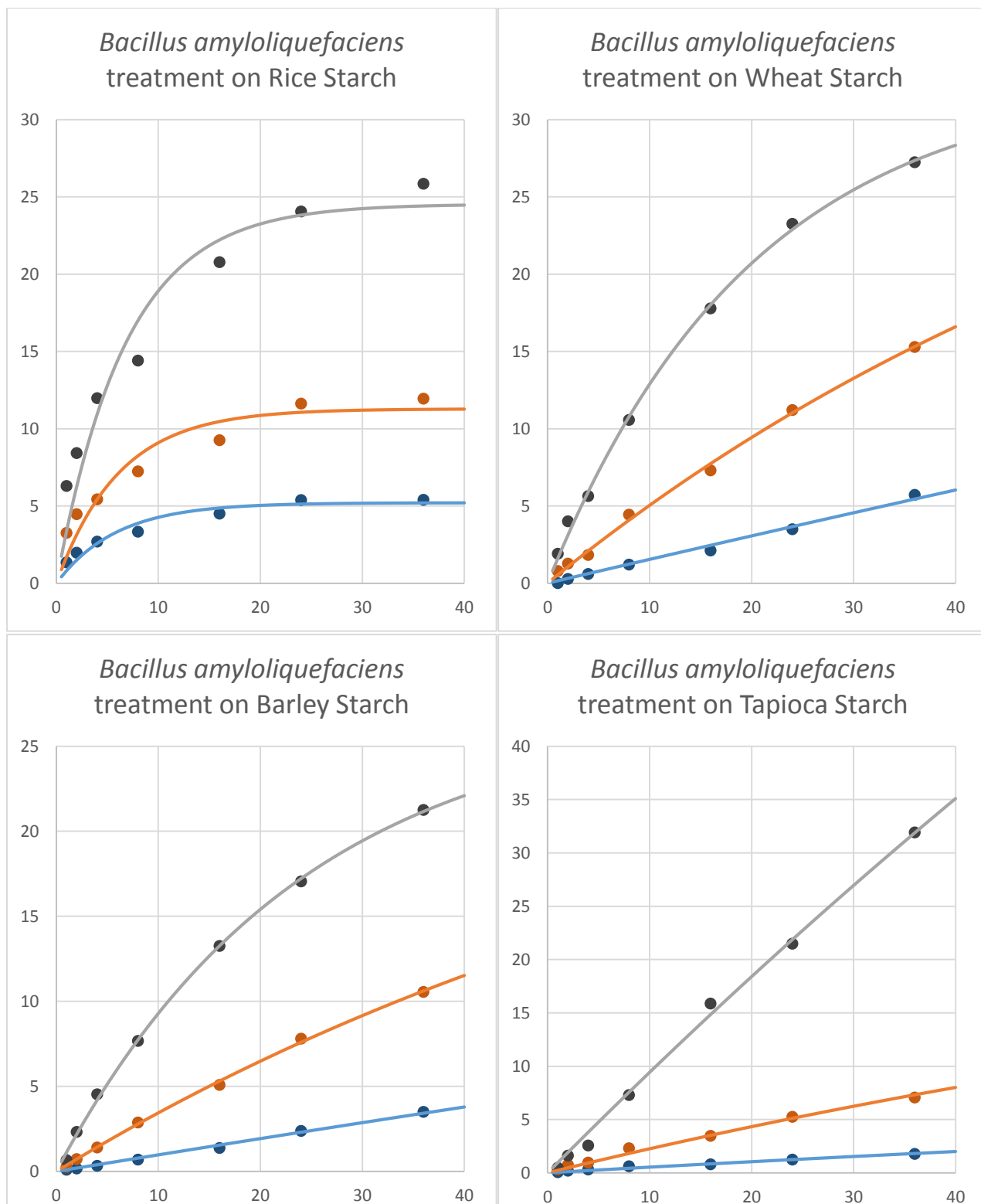
54

These reducing value graphs plot the maltose-equivalents of reducing value in units of mg. The x-axis is in units of hours. The equations used to smooth the data is in the form $[P]_{max}(1 - e^{Arg*t})$ with $[P]_{max}$ and Arg as parameters. These parameters and their associated errors are at the end of this Appendix

These reducing value graphs plot the maltose-equivalents of reducing value in units of mg. The x-axis is in units of hours. The equations used to smooth the data is in the form $[P]_{max}(1 - e^{Arg*t})$ with $[P]_{max}$ and Arg as parameters. These parameters and their associated errors are at the end of this Appendix

These reducing value graphs plot the maltose-equivalents of reducing value in units of mg. The x-axis is in units of hours. The equations used to smooth the data is in the form $[P]_{max}(1 - e^{Arg*t})$ with $[P]_{max}$ and Arg as parameters. These parameters and their associated errors are at the end of this Appendix

Bacillus licheniformis
Potato Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.44	0.83	1.59
2	0.73	1.44	2.81
4	1.10	1.72	5.02
8	1.58	2.57	6.32
16	1.92	3.63	8.64
24	2.20	4.67	13.60
36	2.53	5.87	13.64

Bacillus licheniformis
Waxy Maize Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.95	2.88	6.82
2	1.59	4.76	11.56
4	2.69	7.85	18.08
8	4.51	12.34	27.42
16	7.06	17.90	38.34
24	8.41	21.12	46.79
36	10.76	25.68	48.57

Bacillus licheniformis

Maize Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.30	3.52	10.29
2	2.16	5.62	15.15
4	3.67	8.67	23.49
8	5.38	12.66	32.70
16	7.12	15.05	49.11
24	9.99	22.02	55.25
36	11.73	24.50	57.30

Bacillus licheniformis
Amylomaize-7 Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.65	1.56	3.17
2	1.03	2.38	5.48
4	1.60	3.60	6.92
8	2.14	5.34	12.58
16	2.54	6.46	18.56
24	3.81	8.61	23.59
36	4.57	9.62	29.17

Bacillus licheniformis
Rice Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.62	6.55	8.62
2	4.46	8.50	13.61
4	6.09	9.74	18.90
8	8.24	14.39	24.27
16	10.45	17.54	32.70
24	11.71	21.48	40.82
36	12.83	22.58	55.61

Bacillus licheniformis
Wheat Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.73	3.13	8.00
2	2.84	5.45	12.94
4	4.30	8.33	19.32
8	7.13	15.08	33.02
16	11.12	21.48	48.79
24	13.78	27.74	53.87
36	19.52	30.90	58.48

Bacillus licheniformis
Barley Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.91	1.61	3.93
2	1.70	3.17	8.97
4	2.65	6.99	19.94
8	4.82	13.03	32.76
16	7.70	20.29	41.51
24	11.81	26.01	51.95
36	14.22	27.90	53.97

Bacillus licheniformis
Tapioca Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.73	2.44	5.31
2	1.53	4.16	9.13
4	2.27	7.62	14.91
8	3.49	11.45	24.81
16	6.09	15.70	36.73
24	8.33	21.89	43.35
36	8.08	22.44	51.34

Porcine Pancreas
Potato Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.08	0.37	1.01
2	0.24	0.59	1.61
4	0.27	0.90	2.31
8	0.49	1.37	3.45
16	0.59	1.85	3.85
24	0.78	2.16	4.55
36	0.98	2.68	5.91

Porcine Pancreas
Waxy Maize Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.18	0.99	5.68
2	0.30	1.94	10.78
4	0.40	3.24	16.71
8	1.01	5.87	24.03
16	1.57	12.20	27.23
24	2.12	12.63	35.20
36	2.62	14.58	38.51

Porcine Pancreas
Potato Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.08	0.37	1.01
2	0.24	0.59	1.61
4	0.27	0.90	2.31
8	0.49	1.37	3.45
16	0.59	1.85	3.85
24	0.78	2.16	4.55
36	0.98	2.68	5.91

Porcine Pancreas
Waxy Maize Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.99	5.68	12.31
2	1.94	10.78	18.79
4	3.24	16.71	27.57
8	5.87	24.03	41.06
16	12.20	27.23	50.23
24	12.63	35.20	55.90
36	14.58	38.51	59.16

Porcine Pancreas

Maize Reducing Values

Hours	0.1 IU/ml	1 IU/ml
1	0.59	3.08
2	1.29	5.90
4	2.67	10.30
8	5.27	16.48
16	8.78	22.61
24	10.15	25.12
36	11.05	28.77

Porcine Pancreas
Amylomaize-7 Reducing Values

Hours	0.1 IU/ml	1 IU/ml
1	0.43	1.35
2	0.66	2.24
4	0.99	3.63
8	1.78	5.57
16	2.58	7.66
24	3.29	8.46
36	3.92	9.58

Porcine Pancreas
Rice Reducing Values

Hours	0.1 IU/ml	1 IU/ml
1	2.10	8.46
2	3.68	12.37
4	5.68	14.76
8	7.80	18.44
16	11.06	21.09
24	13.38	22.47
36	15.04	24.43

Porcine Pancreas
Wheat Reducing Values

Hours	0.1 IU/ml	1 IU/ml
1	0.52	3.35
2	0.80	5.36
4	1.32	6.74
8	1.52	10.97
16	2.32	14.87
24	2.63	19.60
36	3.74	22.78

Porcine Pancreas
Barley Reducing Values

Hours	0.1 IU/ml	1 IU/ml
1	0.30	3.06
2	0.53	4.92
4	1.15	7.92
8	1.98	11.81
16	3.13	17.57
24	4.27	17.70
36	5.90	23.63

Porcine Pancreas
Tapioca Reducing Values

Hours	0.1 IU/ml	1 IU/ml
1	0.68	2.96
2	1.20	5.50
4	2.45	8.76
8	3.93	14.20
16	6.47	19.08
24	8.13	Err
36	10.26	23.36

Human Saliva

Maize Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.59	3.36	9.02
2	2.04	8.63	19.01
4	4.33	17.70	27.72
8	8.59	24.15	47.42
16	13.77	34.03	57.51
24	18.93	38.68	74.77
36	22.04	43.55	76.98

Human Saliva
Amylomaize-7 Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.57	1.34	3.65
2	1.13	2.78	7.12
4	1.69	4.41	10.39
8	3.75	7.38	16.01
16	5.60	10.43	21.88
24	6.09	13.21	25.54
36	7.26	15.69	28.56

Human Saliva

Rice Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	4.00	13.61	24.96
2	6.91	18.47	32.94
4	9.16	22.74	35.91
8	16.90	28.67	54.15
16	18.47	36.01	65.20
24	22.51	41.04	75.93
36	24.44	45.73	78.95

Human Saliva

Wheat Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.38	4.48	13.58
2	1.86	8.23	23.51
4	1.74	11.62	33.58
8	3.19	17.07	46.31
16	3.95	24.40	68.03
24	5.64	30.78	68.48
36	4.07	30.79	67.53

Human Saliva

Barley Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.69	4.92	17.21
2	2.74	8.48	28.22
4	4.65	13.50	41.55
8	8.40	21.95	60.44
16	13.62	31.09	72.88
24	18.86	37.10	85.03
36	23.93	42.42	84.43

Human Saliva

Tapioca Reducing Values

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.30	2.63	13.24
2	1.05	6.28	20.49
4	1.45	9.39	27.16
8	4.49	15.46	41.84
16	8.29	21.42	47.41
24	12.75	26.24	54.84
36	11.28	25.80	53.24

Bacillus amyloliquefaciens Potato Reducing Values				*Bacillus amyloliquefaciens* Waxy Maize Reducing Values		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.17	0.44	0.81	1	0.50	1.33	3.02
2	0.28	0.60	1.05	2	0.72	2.08	5.28
4	0.37	0.75	1.58	4	1.12	3.38	3.04
8	0.46	1.03	2.20	8	1.81	5.74	18.54
16	0.57	1.31	2.56	16	2.69	9.45	30.28
24	0.64	1.45	3.07	24	3.27	12.02	37.61
36	0.77	1.66	3.62	36	3.89	14.79	45.40

Bacillus amyloliquefaciens Maize Reducing Values				*Bacillus amyloliquefaciens* Amylomaize-7 Reducing Values		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.20	1.37	2.30	1	0.25	0.66	1.46
2	0.52	2.03	5.01	2	0.32	0.92	2.01
4	1.01	3.43	9.86	4	0.51	1.41	3.22
8	1.67	6.01	17.93	8	0.82	2.04	5.07
16	2.16	8.49	25.54	16	1.15	2.92	7.62
24	3.04	9.86	32.11	24	1.42	3.84	9.31
36	3.74	13.38	38.59	36	1.65	4.78	11.19

Bacillus amyloliquefaciens Rice Reducing Values				*Bacillus amyloliquefaciens* Wheat Reducing Values		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.36	3.27	6.30	1	0.01	0.79	1.92
2	1.98	4.48	8.42	2	0.29	1.27	4.02
4	2.70	5.44	11.98	4	0.60	1.84	5.64
8	3.34	7.24	14.42	8	1.21	4.45	10.58
16	4.50	9.26	20.78	16	2.12	7.31	17.78
24	5.39	11.63	24.06	24	3.51	11.21	23.26
36	5.40	11.95	25.84	36	5.72	15.30	27.24

	Bacillus amyloliquefaciens Barley Reducing Values				*Bacillus amyloliquefaciens* Tapioca Reducing Values		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.09	0.26	0.66	1	0.07	0.35	0.48
2	0.16	0.72	2.31	2	0.21	0.76	1.61
4	0.33	1.41	4.53	4	0.33	0.98	2.56
8	0.69	2.87	7.67	8	0.62	2.32	7.30
16	1.37	5.08	13.26	16	0.80	3.47	15.87
24	2.38	7.81	17.06	24	1.24	5.25	21.50
36	3.50	10.55	21.25	36	1.79	7.07	31.92

Argument

Bacillus licheniformis

Activity	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.149	0.048	0.067	0.073	0.173	0.038	0.031	0.062
1.0 IU/ml	0.069	0.078	0.086	0.105	0.172	0.073	0.066	0.082
10.0 IU/ml	0.076	0.108	0.123	0.059	0.074	0.105	0.103	0.078

Argument

Porcine Pancreas

Activity	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.088	0.056	0.071	0.064	0.102	0.001	0.027	0.048
1.0 IU/ml	0.093	0.136	0.107	0.117	0.343	0.078	0.096	0.117
10.0 IU/ml	0.133	0.171						

Argument

Human Saliva

Activity	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.103	0.045	0.044	0.078	0.141	0.161	0.035	0.050
1.0 IU/ml	0.068	0.104	0.108	0.069	0.215	0.108	0.089	0.110
10.0 IU/ml	0.073	0.171	0.111	0.109	0.203	0.170	0.175	0.206

Argument

Bacillus amyloliquefaciens

Activity	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
0.1 IU/ml	0.1744	0.0742	0.0539	0.0833	0.1711	0.0014	0.0018	0.0042
1.0 IU/ml	0.1820	0.0501	0.0606	0.0628	0.1640	0.0137	0.0126	0.0081
10.0 IU/ml	0.1532	0.0417	0.0623	0.0711	0.1475	0.0498	0.0417	0.0049

APPENDIX C: FIRST ORDER ANALYSIS

$$\frac{d[P]}{dt} = k_{cat}[ES] = k_{cat} * K[E]_{soln}[S]_{avail}$$

$$[E]_{soln} = [E]_{tot} - [ES]$$

$$[E]_{soln} = [E]_{tot} - K[E]_{soln}[S]$$

$$[E]_{soln}(1 + K[S]) = [E]_{tot}$$

$$[E]_{soln} = \frac{[E]_{tot}}{(1 + K[S])} \approx [E]_{tot}$$

$$[S]_{avail} = C[S]_{tot} - [ES]$$

$$[S]_{avail} = C[S]_{tot} - K[E]_{soln}[S]_{avail} \approx C[S]_{tot} - K[E]_{tot}[S]_{avail}$$

$$[S]_{avail} = \frac{C[S]_{tot}}{1 + K[E]_{tot}}$$

$$[S]_{avail} = \frac{C(106 - [P])}{1 + K[E]_{tot}}$$

$$\frac{d[P]}{dt} = k_{cat} * K[E]_{tot} * \frac{C(106 - [P])}{1 + K[E]_{tot}}$$

$$\frac{d[P]}{(106\,mg - [P])} = \frac{k_{cat} * C * K[E]_{tot}}{1 + K[E]_{tot}}dt$$

$$\ln(106 - [P]) = -\frac{k_{cat} * C * K[E]_{tot}}{1 + K[E]_{tot}}t + \ln(106) = \Phi t + \ln(106)$$

The above equations describe the derivation of the first order model. Graphs depicting the evaluation according to this model are shown below. Since the data was not well-linearized with this, it is safe to assume that the reaction kinetics do not follow a first-order reaction with respect to starch. Better explanation of this derivation can be seen in Chapter 4.

Values are plotted against time in hours. The y-axis represents -ln(106-[P]) + ln(106). These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B.
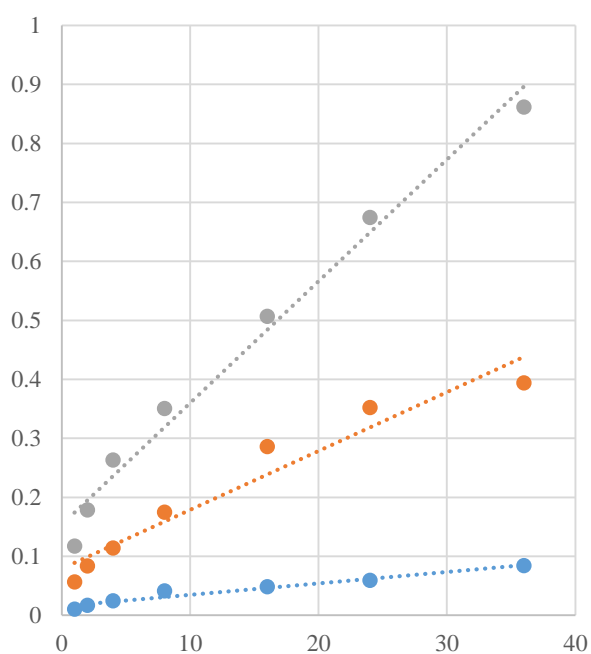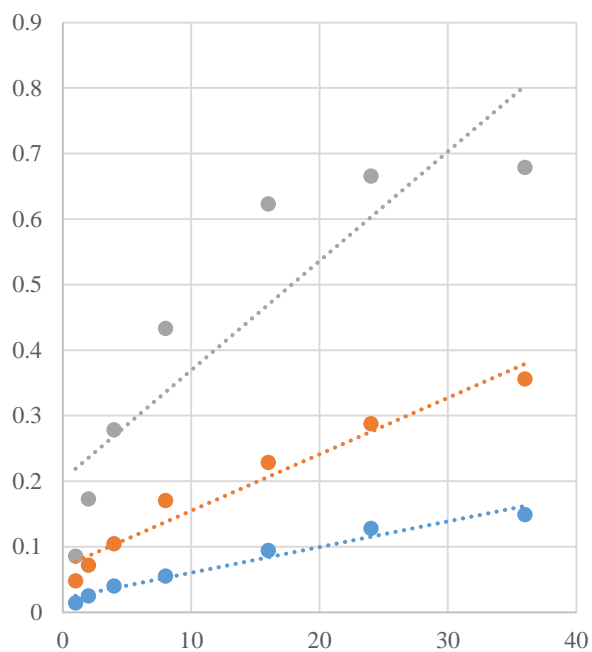
Values are plotted against time in hours. The y-axis represents $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B.
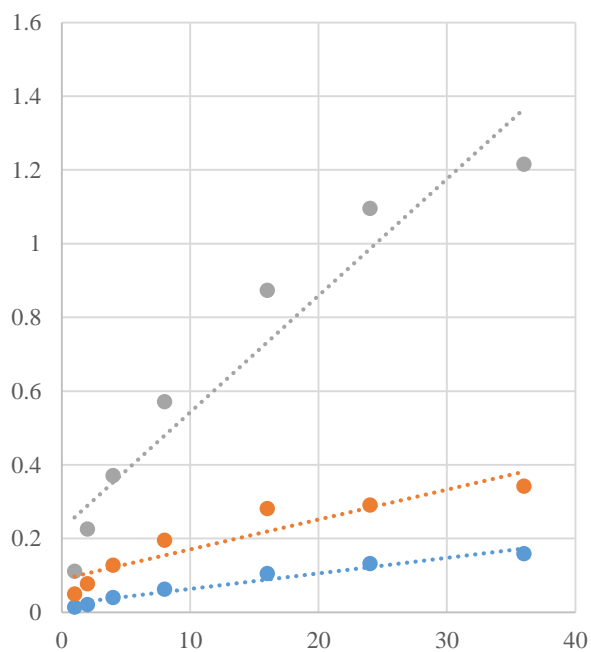
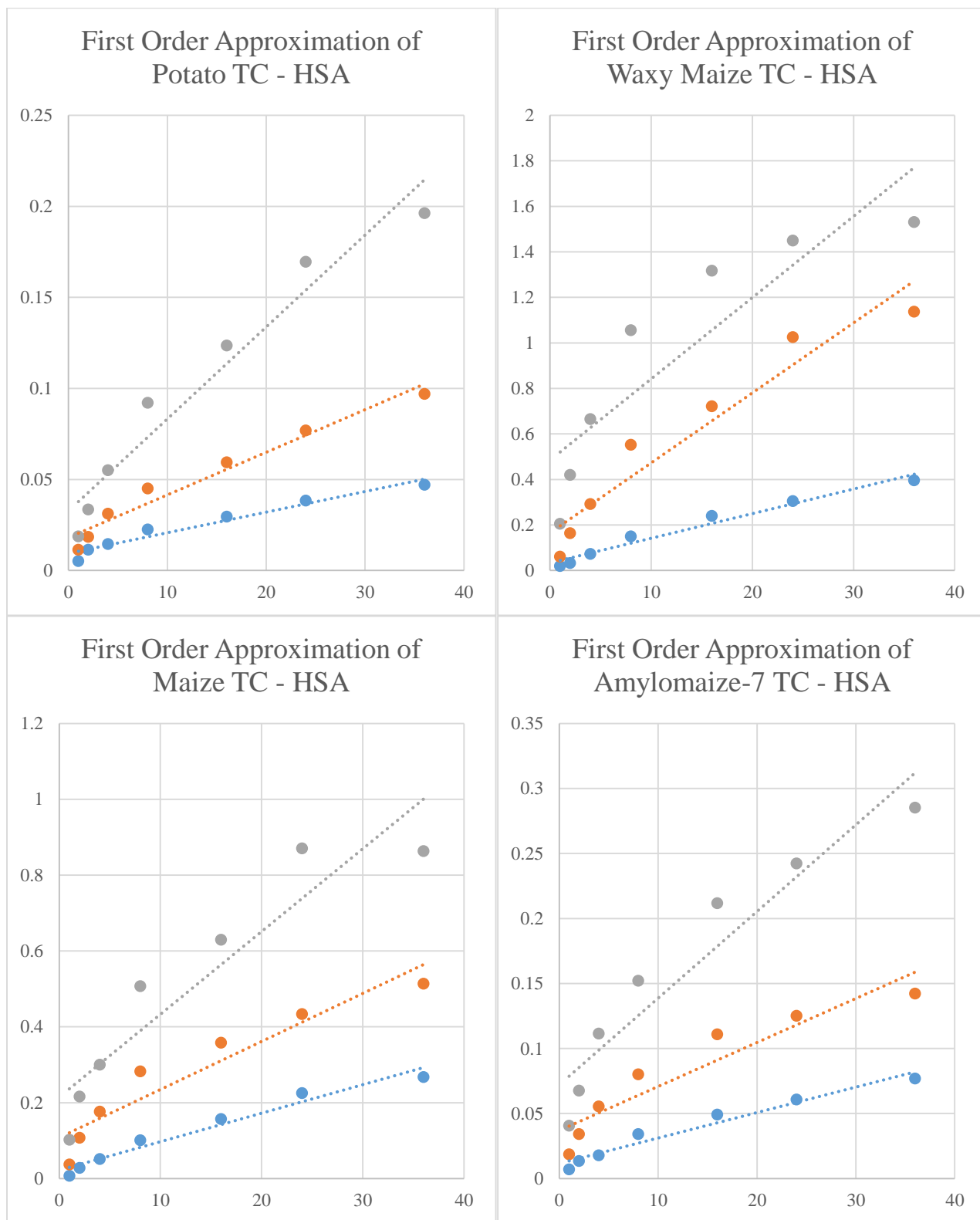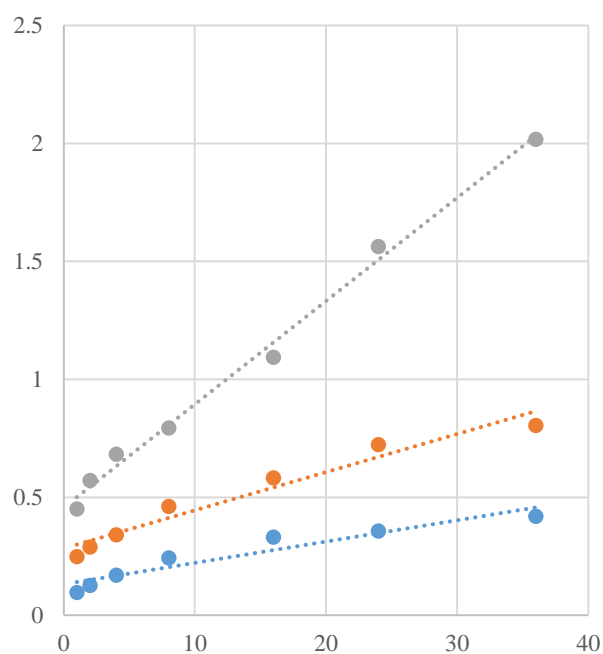First Order Approximation of Potato TC - PPA

First Order Approximation of Waxy Maize TC - PPA

First Order Approximation of Maize TC - PPA

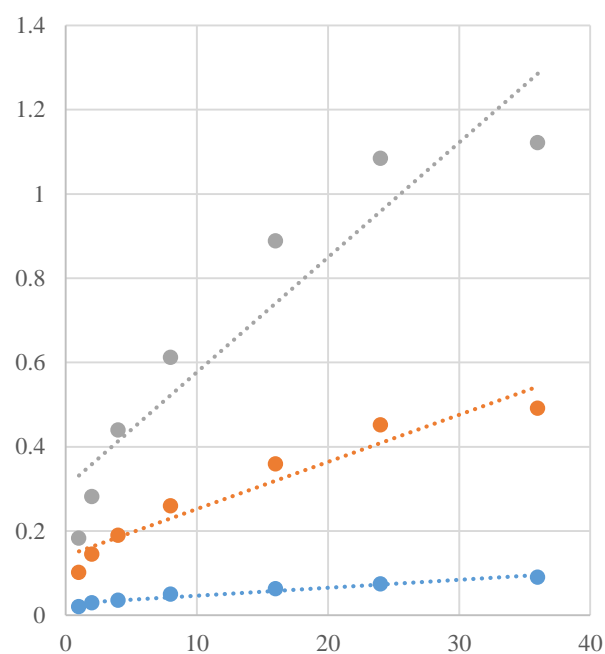First Order Approximation of Amylomaize-7 TC - PPA

Values are plotted against time in hours. The y-axis represents $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B.

First Order Approximation of Rice TC - PPA

First Order Approximation of Wheat TC - PPA

First Order Approximation of Barley TC - PPA

First Order Approximation of Tapioca TC - PPA

Values are plotted against time in hours. The y-axis represents $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B.

Values are plotted against time in hours. The y-axis represents $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B.
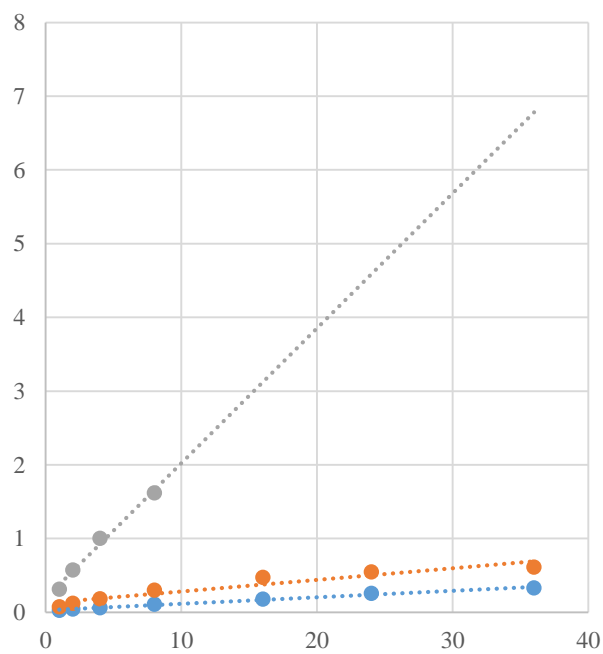
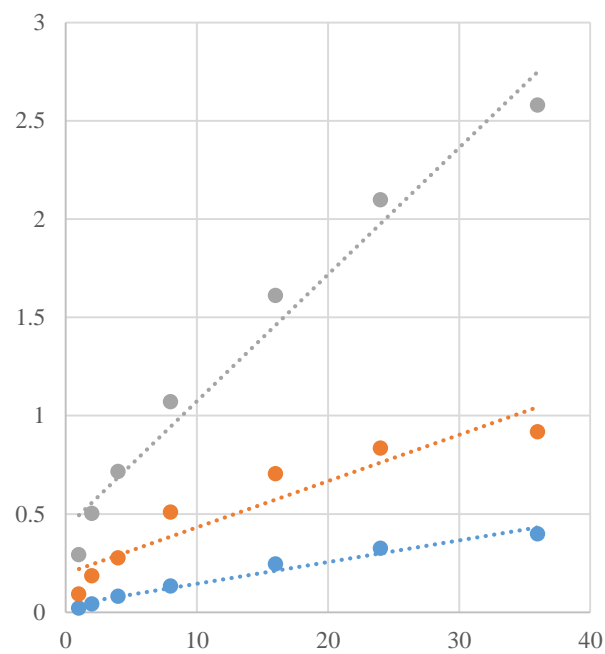First Order Approximation of Rice TC - HSA

First Order Approximation of Wheat TC - HSA
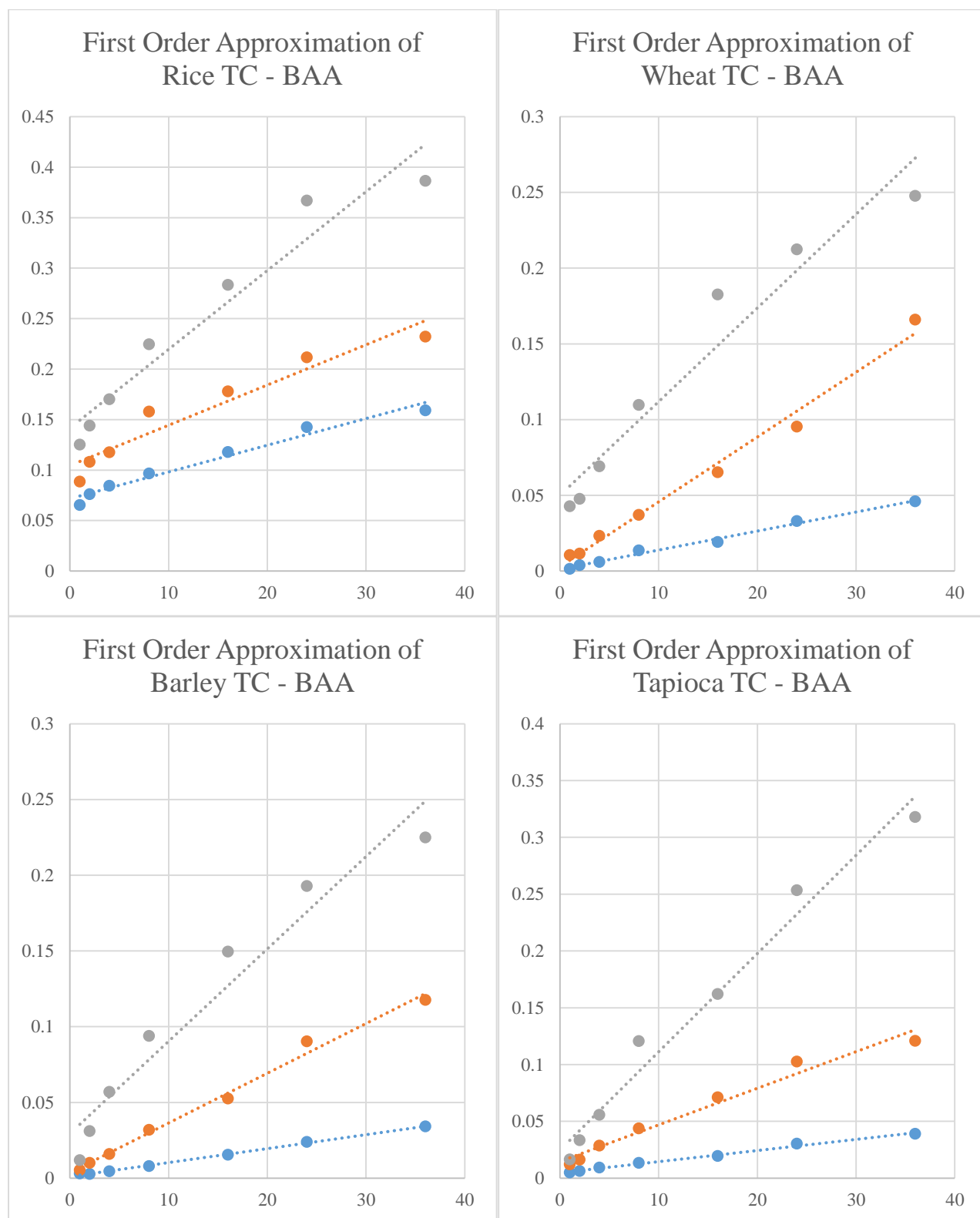
First Order Approximation of Barley TC - HSA

First Order Approximation of Tapioca TC - HSA

Values are plotted against time in hours. The y-axis represents $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B.

Values are plotted against time in hours. The y-axis represents $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B.

First Order Approximation of Rice TC - BAA

First Order Approximation of Wheat TC - BAA

First Order Approximation of Barley TC - BAA

First Order Approximation of Tapioca TC - BAA
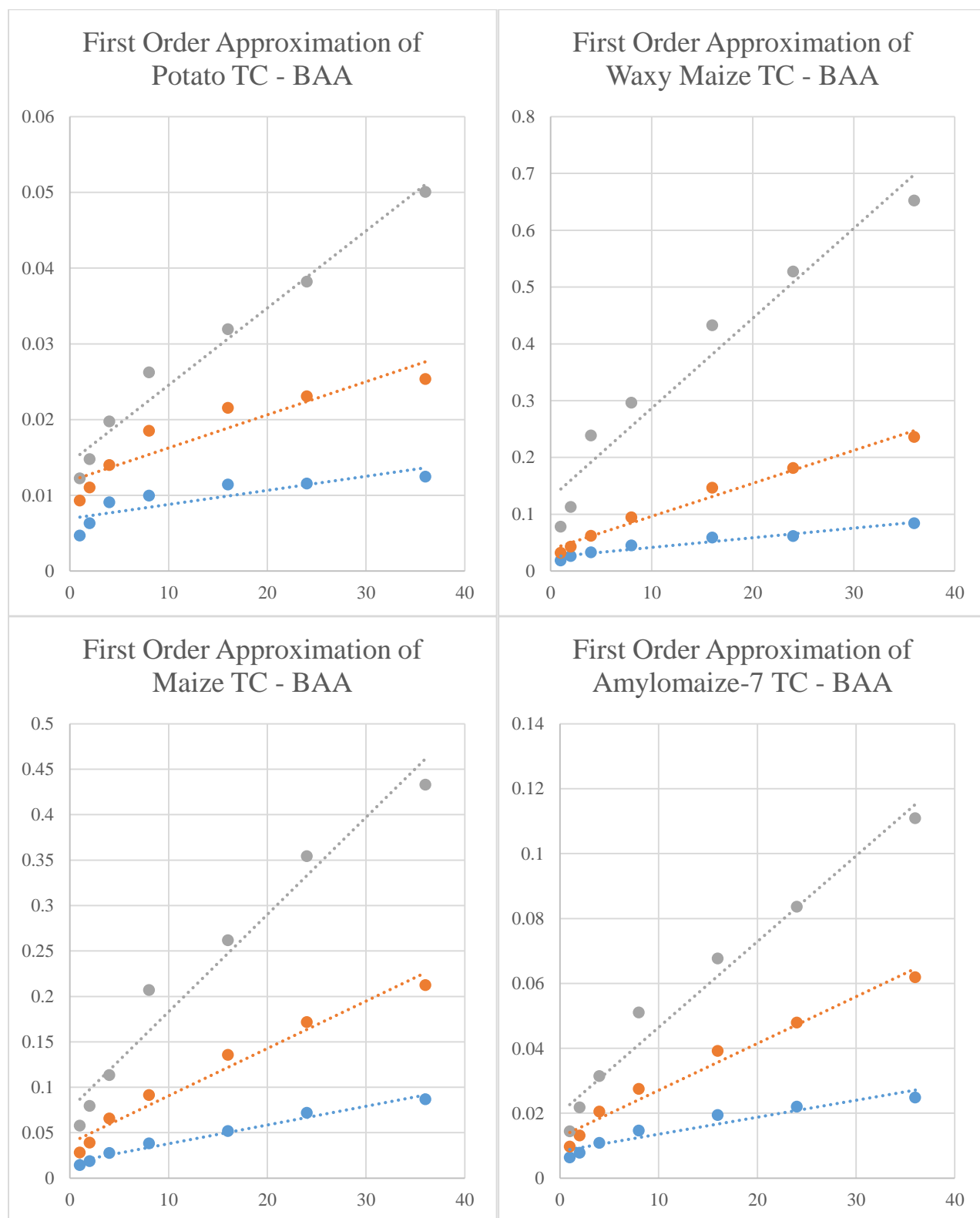
Values are plotted against time in hours. The y-axis represents $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B.

Bacillus licheniformis

Potato First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.02	0.03
2	0.02	0.02	0.04
4	0.02	0.03	0.05
8	0.03	0.05	0.09
16	0.04	0.06	0.11
24	0.05	0.08	0.16
36	0.06	0.10	0.22
slope =	0.0015	0.0023	0.0053
R^2 =	0.9520	0.9766	0.9893

Bacillus licheniformis
Waxy Maize First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.03	0.10	0.15
2	0.05	0.14	0.25
4	0.08	0.25	0.38
8	0.13	0.39	0.65
16	0.19	0.64	0.98
24	0.25	0.70	1.25
36	0.32	0.97	1.60
slope =	0.0082	0.0242	0.0412
R^2 =	0.9818	0.9610	0.9734

Bacillus licheniformis

Maize First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.03	0.07	0.16
2	0.05	0.12	0.23
4	0.08	0.17	0.34
8	0.11	0.24	0.52
16	0.14	0.30	0.71
24	0.22	0.45	0.84
36	0.27	0.53	0.99
slope =	0.0068	0.0128	0.0233
R^2 =	0.9792	0.9615	0.9295

Bacillus licheniformis
Amylomaize-7 First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.03	0.05
2	0.02	0.04	0.08
4	0.03	0.06	0.11
8	0.04	0.09	0.18
16	0.05	0.11	0.30
24	0.08	0.14	0.33
36	0.09	0.17	0.42
slope =	0.0023	0.0039	0.0105
R^2 =	0.9633	0.9386	0.9521

Values are plotted against time in hours. The tables represent $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B. Units of the slopes are in hours^{-1}.

	Bacillus licheniformis Rice First Order Analysis				*Bacillus licheniformis* Wheat First Order Analysis		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.08	0.15	0.20	1	0.02	0.04	0.09
2	0.11	0.18	0.27	2	0.03	0.07	0.16
4	0.13	0.22	0.39	4	0.05	0.11	0.25
8	0.17	0.30	0.47	8	0.08	0.17	0.51
16	0.22	0.37	0.64	16	0.12	0.27	0.75
24	0.25	0.47	0.82	24	0.15	0.35	0.90
36	0.26	0.54	1.21	36	0.18	0.42	1.10
slope =	0.0050	0.0112	0.0267	slope =	0.0047	0.0111	0.0290
R^2 =	0.8707	0.9602	0.9870	R^2 =	0.9575	0.9647	0.9371

	Bacillus licheniformis Barley First Order Analysis				*Bacillus licheniformis* Tapioca First Order Analysis		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.03	0.04	1	0.02	0.05	0.09
2	0.02	0.04	0.12	2	0.03	0.10	0.15
4	0.03	0.10	0.28	4	0.04	0.17	0.28
8	0.06	0.18	0.49	8	0.07	0.30	0.41
16	0.10	0.28	0.69	16	0.12	0.40	0.73
24	0.16	0.40	1.03	24	0.15	0.51	0.88
36	0.19	0.46	1.10	36	0.17	0.58	0.94
slope =	0.0053	0.0129	0.0309	slope =	0.0045	0.0150	0.0254
R^2 =	0.9825	0.9576	0.9217	R^2 =	0.9288	0.9190	0.9004

Values are plotted against time in hours. The tables represent $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B. Units of the slopes are in hours^{-1}.

Porcine Pancreas

Potato First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.02	0.02
2	0.01	0.02	0.02
4	0.02	0.03	0.04
8	0.02	0.05	0.06
16	0.03	0.05	0.11
24	0.04	0.07	0.13
36	0.04	0.09	0.16
slope =	0.0010	0.0019	0.0042
R^2 =	0.8853	0.9512	0.9545

Porcine Pancreas
Waxy Maize First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.03	0.10	0.15
2	0.05	0.20	0.25
4	0.08	0.29	0.39
8	0.14	0.48	0.62
16	0.23	0.62	0.84
24	0.32	0.78	1.07
36	0.38	0.88	1.33
slope =	0.0021	0.0103	0.0216
R^2 =	0.9877	0.9665	0.9100

Porcine Pancreas

Maize First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.04	0.11
2	0.02	0.07	0.19
4	0.05	0.15	0.30
8	0.12	0.23	0.42
16	0.17	0.37	0.58
24	0.22	0.44	0.65
36	0.21	0.45	0.33
slope =	0.0062	0.0121	0.0227
R^2 =	0.8369	0.8624	0.9113

Porcine Pancreas
Amylomaize-7 First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.02	0.04
2	0.01	0.03	0.07
4	0.01	0.04	0.09
8	0.03	0.05	0.14
16	0.04	0.09	0.19
24	0.06	0.11	0.22
36	0.07	0.13	0.29
slope =	0.0004	0.0018	0.0032
R^2 =	0.9122	0.9730	0.9366

Values are plotted against time in hours. The tables represent $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B. Units of the slopes are in hours^{-1}. The highlighted value was omitted as it is erroneous.

Porcine Pancreas
Rice First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.08	0.15	0.23
2	0.10	0.18	0.28
4	0.13	0.21	0.37
8	0.19	0.29	0.55
16	0.22	0.34	0.63
24	0.24	0.36	0.87
36	0.29	0.52	0.97
slope =	0.0032	0.0056	0.0097
R^2 =	0.9597	0.9070	0.9549

Porcine Pancreas
Wheat First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.06	0.12
2	0.02	0.08	0.18
4	0.02	0.11	0.26
8	0.04	0.18	0.35
16	0.05	0.29	0.51
24	0.06	0.35	0.67
36	0.08	0.39	0.86
slope =	0.0006	0.0019	0.0100
R^2 =	0.9086	0.9574	0.9370

Porcine Pancreas
Barley First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.05	0.09
2	0.02	0.07	0.17
4	0.04	0.10	0.28
8	0.06	0.17	0.43
16	0.09	0.23	0.62
24	0.13	0.29	0.67
36	0.15	0.36	0.68
slope =	0.0015	0.0039	0.0086
R^2 =	0.9888	0.9634	0.9591

Porcine Pancreas
Tapioca First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.05	0.11
2	0.02	0.08	0.23
4	0.04	0.13	0.37
8	0.06	0.20	0.57
16	0.10	0.28	0.87
24	0.13	0.29	1.10
36	0.16	0.34	1.21
slope =	0.0007	0.0042	0.0081
R^2 =	0.9359	0.9584	0.8667

Values are plotted against time in hours. The tables represent $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B. Units of the slopes are in hours^{-1}.

Human Saliva

Potato First Order Analysis

TC	0.1 IU	1 IU	10 IU
1	0.01	0.01	0.02
2	0.01	0.02	0.03
4	0.01	0.03	0.06
8	0.02	0.05	0.09
16	0.03	0.06	0.12
24	0.04	0.08	0.17
36	0.05	0.10	0.20
slope =	0.0011	0.0023	0.0050
R^2 =	0.9548	0.9603	0.9461

Human Saliva
Waxy Maize First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.06	0.21
2	0.03	0.16	0.42
4	0.07	0.29	0.67
8	0.15	0.55	1.06
16	0.24	0.72	1.32
24	0.31	1.03	1.45
36	0.40	1.14	1.53
slope =	0.0108	0.0308	0.0357
R^2 =	0.9680	0.9247	0.7952

Human Saliva

Maize First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.04	0.10
2	0.03	0.11	0.22
4	0.05	0.18	0.30
8	0.10	0.28	0.51
16	0.16	0.36	0.63
24	0.23	0.43	0.87
36	0.27	0.51	0.86
slope =	0.0075	0.0127	0.0218
R^2 =	0.9622	0.8990	0.8686

Human Saliva
Amylomaize-7 First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.02	0.04
2	0.01	0.03	0.07
4	0.02	0.06	0.11
8	0.03	0.08	0.15
16	0.05	0.11	0.21
24	0.06	0.13	0.24
36	0.08	0.14	0.29
slope =	0.0020	0.0034	0.0067
R^2 =	0.9587	0.8831	0.9095

Values are plotted against time in hours. The tables represent $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B. Units of the slopes are in hours^{-1}.

Human Saliva
Rice First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.10	0.25	0.45
2	0.13	0.29	0.57
4	0.17	0.34	0.68
8	0.24	0.46	0.79
16	0.33	0.58	1.09
24	0.36	0.72	1.56
36	0.42	0.81	2.02
slope =	0.0090	0.0162	0.0438
R^2 =	0.9074	0.9520	0.9933

Human Saliva
Wheat First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.10	0.18
2	0.03	0.15	0.28
4	0.04	0.19	0.44
8	0.05	0.26	0.61
16	0.06	0.36	0.89
24	0.07	0.45	1.08
36	0.09	0.49	1.12
slope =	0.0019	0.0112	0.0273
R^2 =	0.9473	0.9293	0.8831

Human Saliva
Barley First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.07	0.31
2	0.04	0.12	0.57
4	0.06	0.18	1.00
8	0.11	0.30	1.62
16	0.18	0.47	4.70
24	0.25	0.55	Err
36	0.33	0.61	Err
slope =	0.0088	0.0157	0.1831
R^2 =	0.9902	0.9102	0.9879

Human Saliva
Tapioca First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.09	0.29
2	0.04	0.18	0.50
4	0.08	0.28	0.72
8	0.13	0.51	1.07
16	0.25	0.70	1.61
24	0.33	0.84	2.10
36	0.40	0.92	2.58
slope =	0.0110	0.0235	0.0645
R^2 =	0.9700	0.8843	0.9713

Values are plotted against time in hours. The tables represent $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B. Units of the slopes are in hours^{-1}.

Highlighted values are omitted as they were either too close to or surpassed 106 mg and therefore, could not be evaluated.

Bacillus amyloliquefaciens

Potato First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.00	0.01	0.01
2	0.01	0.01	0.01
4	0.01	0.01	0.02
8	0.01	0.02	0.03
16	0.01	0.02	0.03
24	0.01	0.02	0.04
36	0.01	0.03	0.05
slope =	0.0002	0.0004	0.0010
R^2 =	0.7045	0.8508	0.9717

Bacillus amyloliquefaciens
Waxy Maize First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.03	0.08
2	0.03	0.04	0.11
4	0.03	0.06	0.24
8	0.05	0.09	0.30
16	0.06	0.15	0.43
24	0.06	0.18	0.53
36	0.08	0.24	0.65
slope =	0.0017	0.0058	0.0158
R^2 =	0.9430	0.9811	0.9427

Bacillus amyloliquefaciens

Maize First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.03	0.06
2	0.02	0.04	0.08
4	0.03	0.07	0.11
8	0.04	0.09	0.21
16	0.05	0.14	0.26
24	0.07	0.17	0.35
36	0.09	0.21	0.43
slope =	0.0021	0.0052	0.0107
R^2 =	0.9758	0.9672	0.9615

Bacillus amyloliquefaciens
Amylomaize-7 First Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.01	0.01
2	0.01	0.01	0.02
4	0.01	0.02	0.03
8	0.01	0.03	0.05
16	0.02	0.04	0.07
24	0.02	0.05	0.08
36	0.02	0.06	0.11
slope =	0.0005	0.0014	0.0026
R^2 =	0.9113	0.9726	0.9697

Values are plotted against time in hours. The tables represent $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B. Units of the slopes are in hours^{-1}.

	Bacillus amyloliquefaciens Rice First Order Analysis					*Bacillus amyloliquefaciens* Wheat First Order Analysis		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml		Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.07	0.09	0.13		1	0.00	0.01	0.04
2	0.08	0.11	0.14		2	0.00	0.01	0.05
4	0.08	0.12	0.17		4	0.01	0.02	0.07
8	0.10	0.16	0.22		8	0.01	0.04	0.11
16	0.12	0.18	0.28		16	0.02	0.07	0.18
24	0.14	0.21	0.37		24	0.03	0.10	0.21
36	0.16	0.23	0.39		36	0.05	0.17	0.25
slope =	0.0026	0.0040	0.0078		slope =	0.0013	0.0043	0.0062
R^2 =	0.9686	0.9228	0.9369		R^2 =	0.9915	0.9871	0.9408

	Bacillus amyloliquefaciens Barley First Order Analysis					*Bacillus amyloliquefaciens* Tapioca First Order Analysis		
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml		Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.00	0.01	0.01		1	0.00	0.01	0.02
2	0.00	0.01	0.03		2	0.01	0.02	0.03
4	0.00	0.02	0.06		4	0.01	0.03	0.06
8	0.01	0.03	0.09		8	0.01	0.04	0.12
16	0.02	0.05	0.15		16	0.02	0.07	0.16
24	0.02	0.09	0.19		24	0.03	0.10	0.25
36	0.03	0.12	0.22		36	0.04	0.12	0.32
slope =	0.0009	0.0033	0.0061		slope =	0.0010	0.0032	0.0087
R^2 =	0.9977	0.9912	0.9432		R^2 =	0.9916	0.9726	0.9765

Values are plotted against time in hours. The tables represent $-\ln(106-[P]) + \ln(106)$. These values are unit-less. Tables of values and R^2 values are listed after the graphs in Appendix B. Units of the slopes are in hours^{-1}.

APPENDIX D: SECOND ORDER ANALYSIS

$$\frac{d[P]}{dt} = k_{cat}[ES]_{bound}$$

$$[ES]_{bound} = K_2[ES]_{gran}[S]_{susc(unbound)}$$

$$[S]_{susc(unbound)} = C[S]_{tot} - [ES]_{bound}$$

$$[ES]_{bound} = K_2[ES]_{gran}(C[S]_{tot} - [ES]_{bound})$$

$$[ES]_{bound} = \frac{K_2 C[ES]_{gran}[S]_{tot}}{1 + K_2[ES]_{gran}}$$

$$\frac{d[P]}{dt} = k_{cat}\frac{K_2 C[ES]_{gran}[S]_{tot}}{1 + K_2[ES]_{gran}} \approx k_{cat}K_2 C[ES]_{gran}[S]_{tot}$$

$$[ES]_{gran} = K_1[E]_{soln}[S] \approx K_1[E]_{tot}[S]$$

$$[S] = [S]_{tot} - [ES]_{gran}$$

$$[S] = [S]_{tot} - [ES]_{gran} \approx [S]_{tot} - K_1[E]_{tot}[S]$$

$$[S] = \frac{[S]_{tot}}{1 + K_1[E]_{tot}}$$

$$[ES]_{gran} = \frac{K_1[E]_{tot}[S]_{tot}}{1 + K_1[E]_{tot}}$$

$$\frac{d[P]}{dt} = k_{cat}K_2 C\frac{K_1[E]_{tot}[S]_{tot}}{1 + K_1[E]_{tot}}[S]_{tot}$$

$$\frac{d[P]}{dt} = \frac{k_{cat}K_2 K_1 C[E]_{tot}[S]_{tot}^2}{1 + K_1[E]_{tot}}$$

$$\frac{d[P]}{[S]_{tot}^2} = \frac{k_{cat}K_2 K_1 C[E]_{tot}}{1 + K_1[E]_{tot}}dt$$

$$\frac{d[P]}{(106 - [P])^2} = \frac{k_{cat}K_2 K_1 C[E]_{tot}}{1 + K_1[E]_{tot}}dt$$

$$-\frac{1}{106 - [P]} + \frac{1}{106} = \frac{k_{cat}K_2 K_1 C[E]_{tot}}{1 + K_1[E]_{tot}}t = \Phi * t$$

The above equations describe the derivation of the second-order model. Graphs depicting the evaluation according to this model are shown below. A better explanation of this derivation can be seen in Chapter 4.
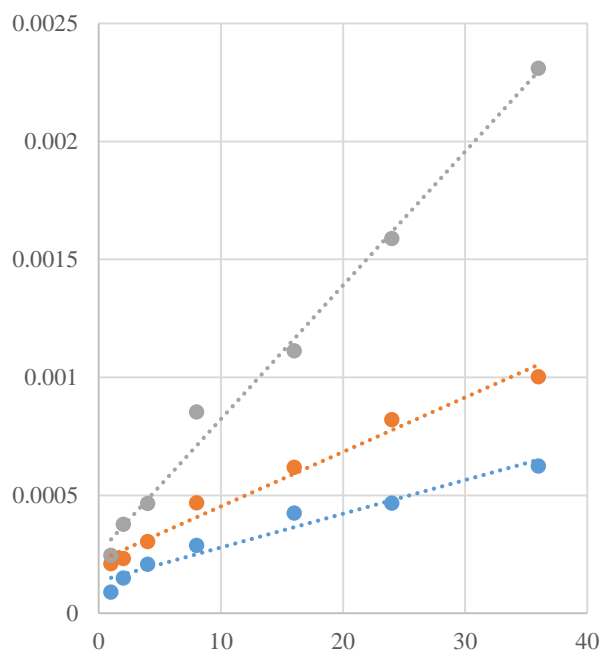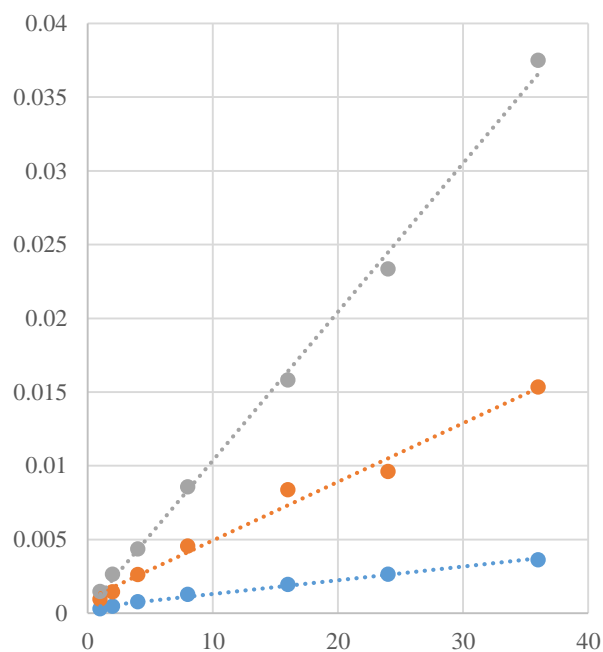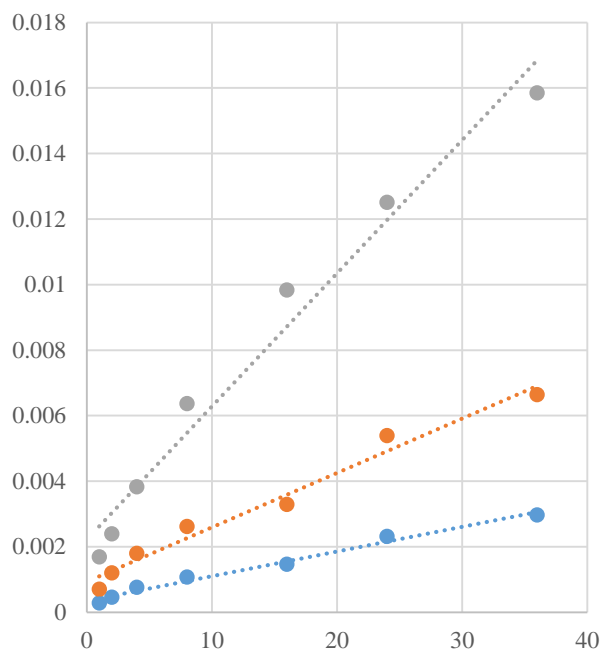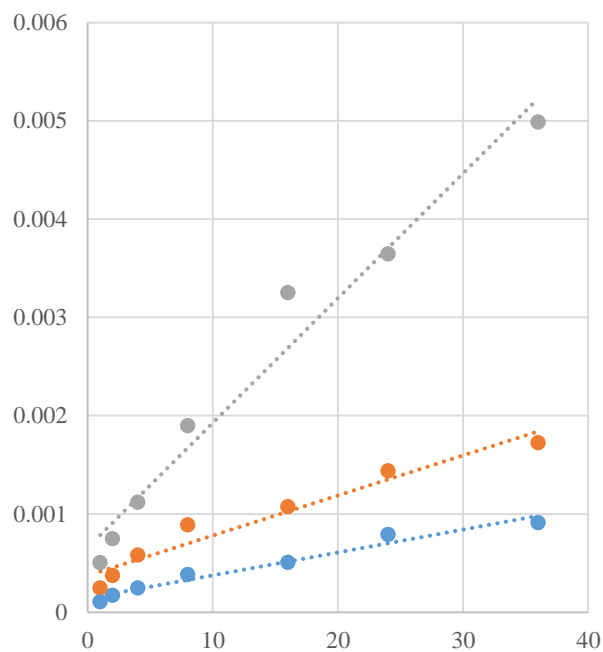
Values are plotted against time in hours. The y-axis represents 1/106+1/(106-[P]). Values are in units of mg^{-1}. Tables of values and R^2 values are listed following the graphs.
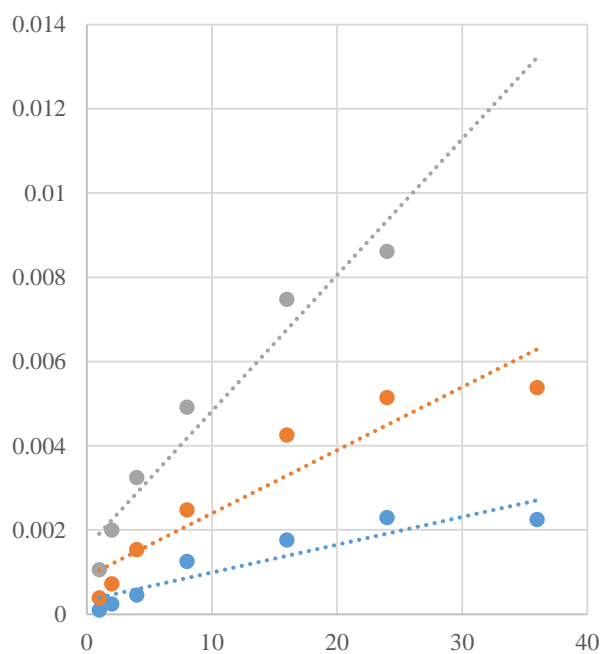
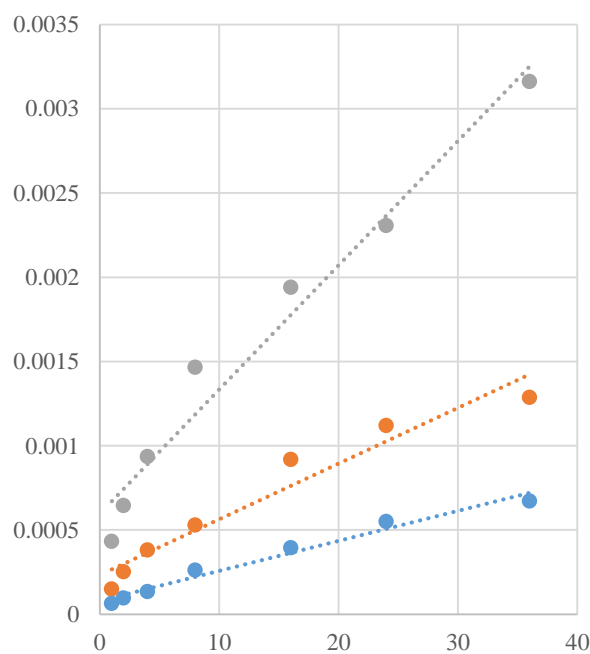Second Order Approximation of Rice TC - BLA
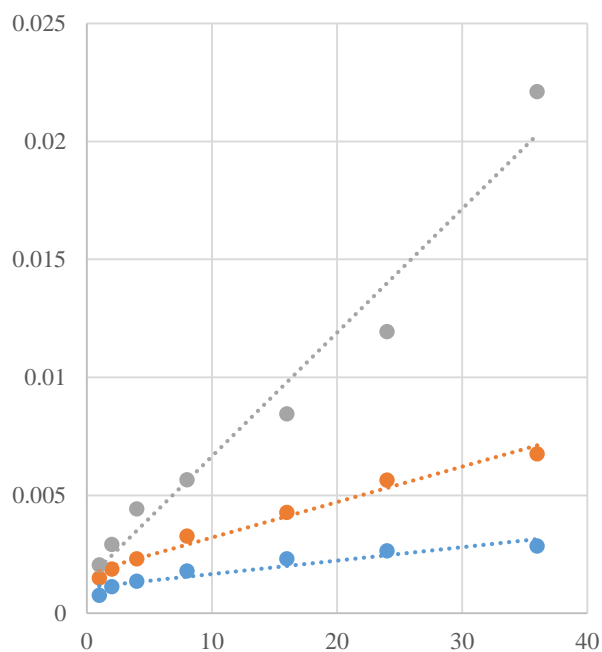
Second Order Approximation of Wheat TC - BLA
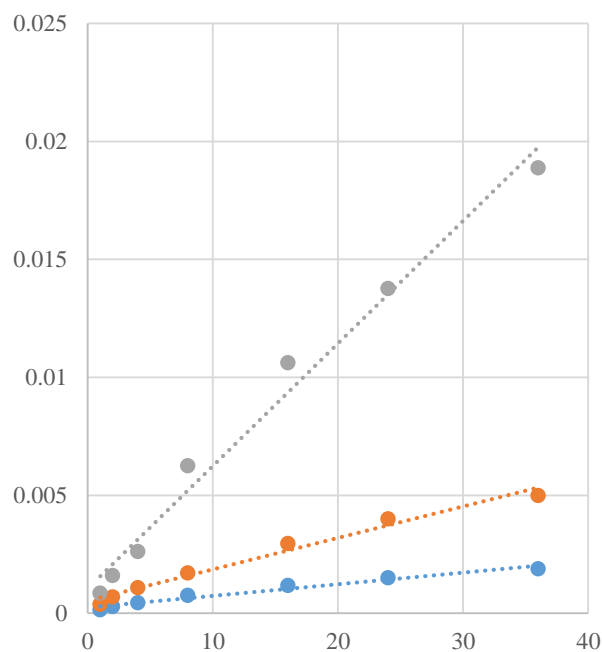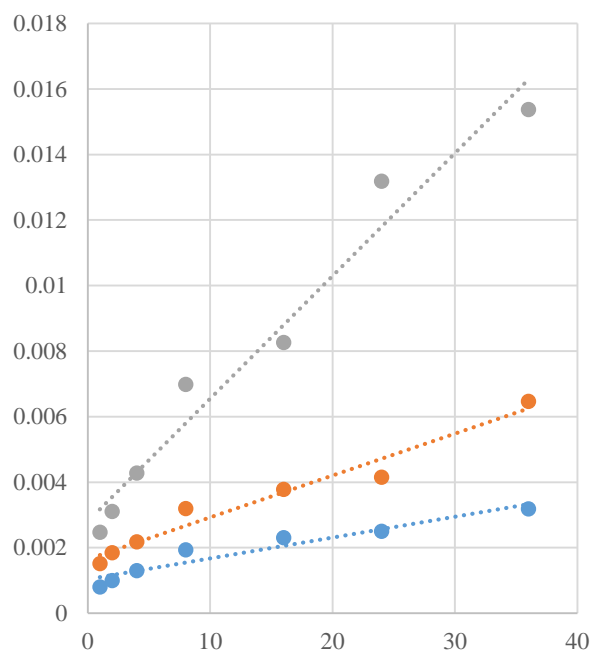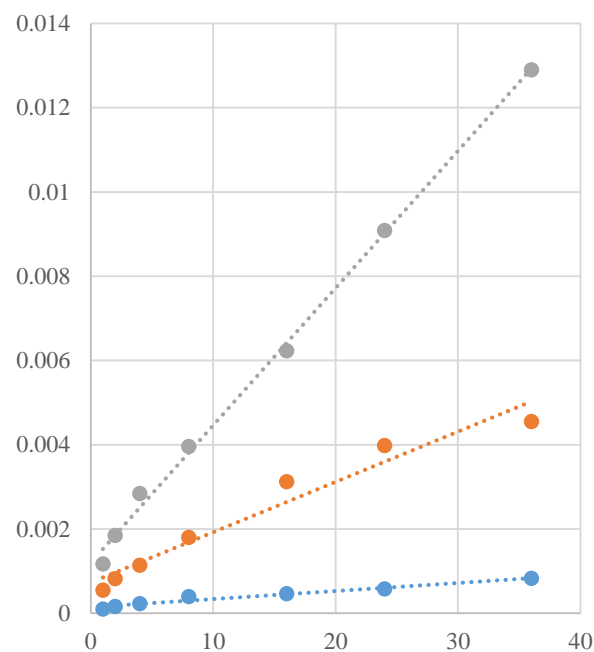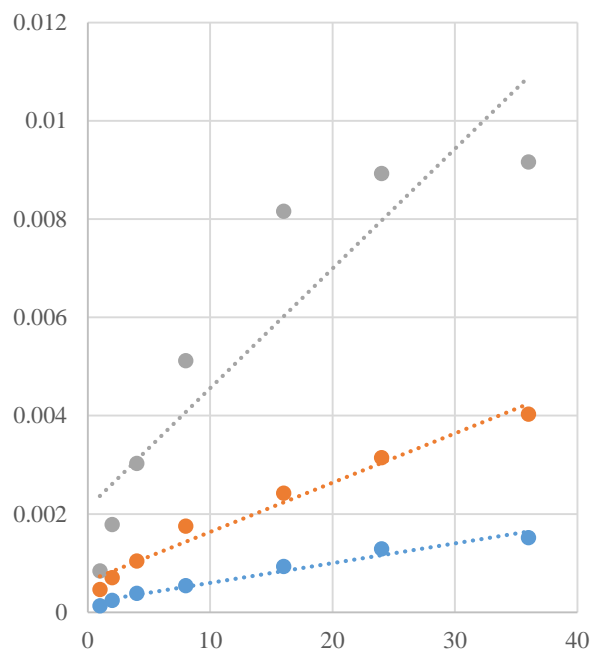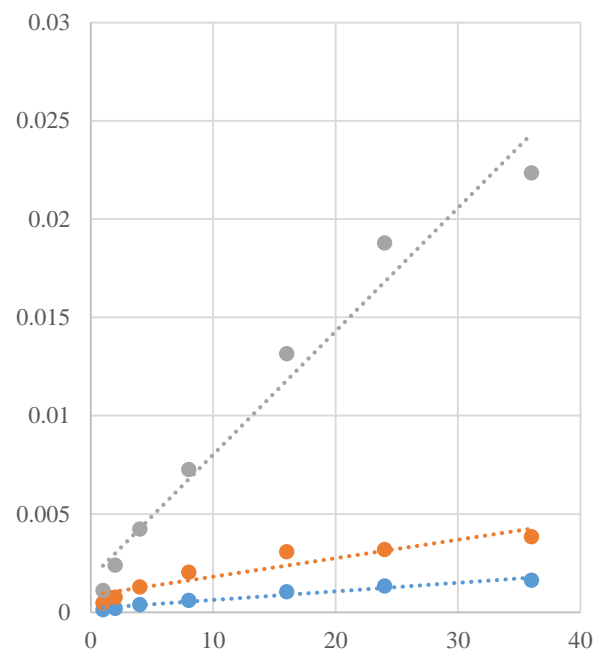
Second Order Approximation of Barley TC - BLA
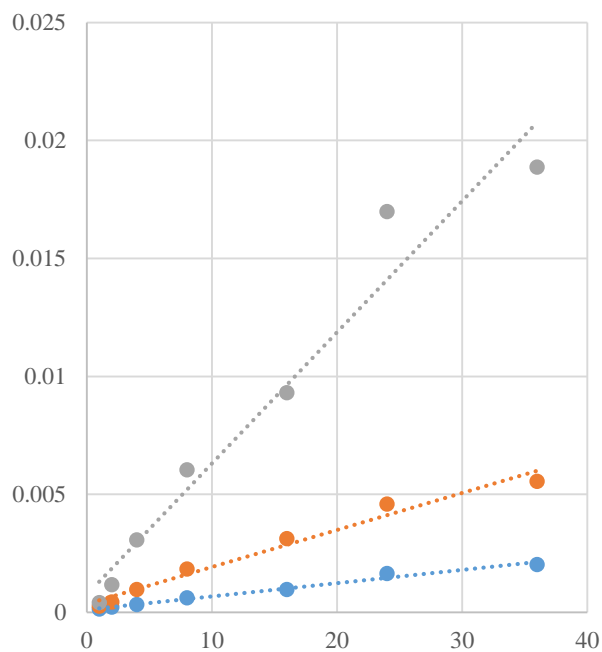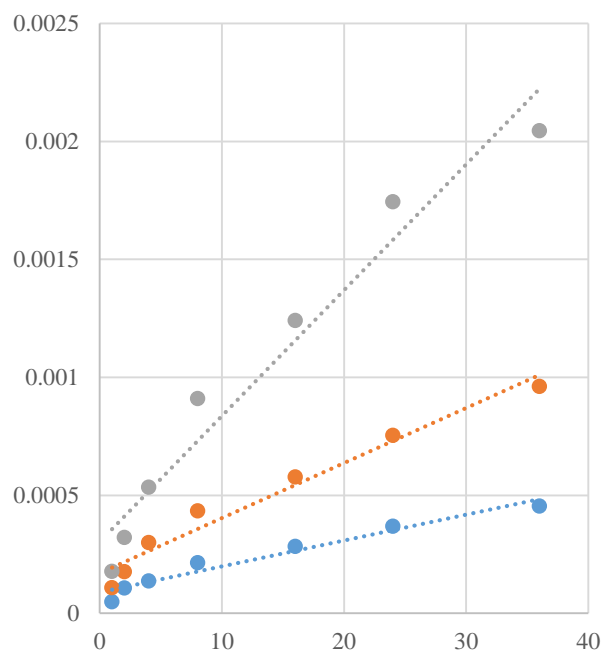
Second Order Approximation of Tapioca TC - BLA
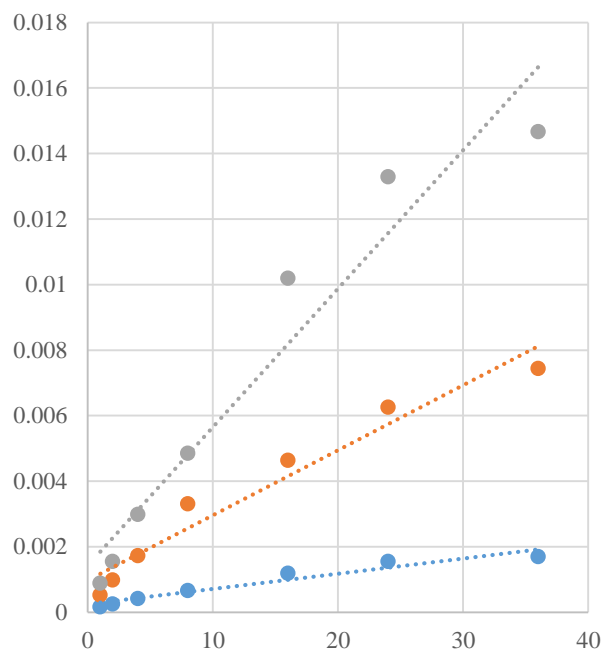
Values are plotted against time in hours. The y-axis represents $1/106+1/(106-[P])$. Values are in units of mg^{-1}. Tables of values and R^2 values are listed following the graphs.

Second Order Approximation of Potato TC - PPA
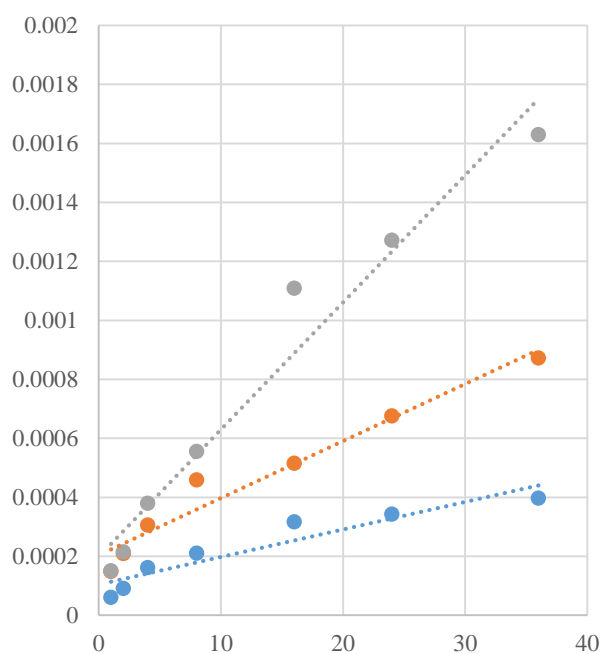
Second Order Approximation of Waxy Maize TC - PPA
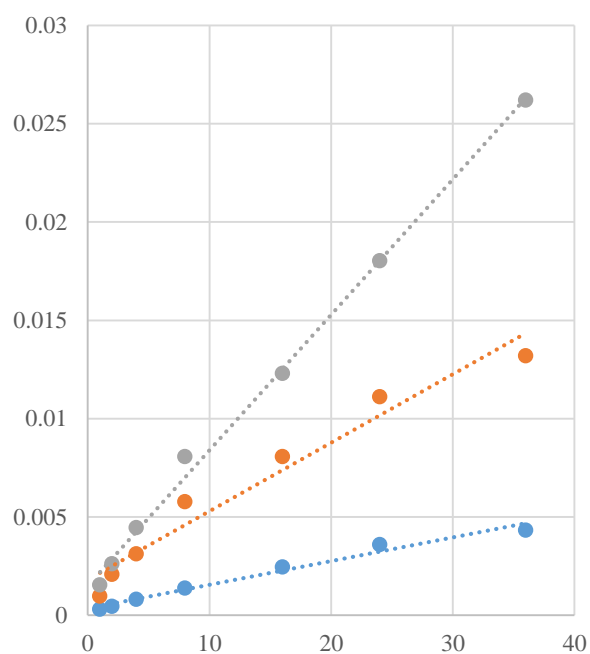
Second Order Approximation of Maize TC - PPA

Second Order Approximation of Amylomaize-7 TC - PPA

Values are plotted against time in hours. The y-axis represents $1/106 + 1/(106-[P])$. Values are in units of mg^{-1}. Tables of values and R^2 values are listed following the graphs.

Second Order Approximation of Rice TC - PPA

Second Order Approximation of Wheat TC - PPA

Second Order Approximation of Barley TC - PPA
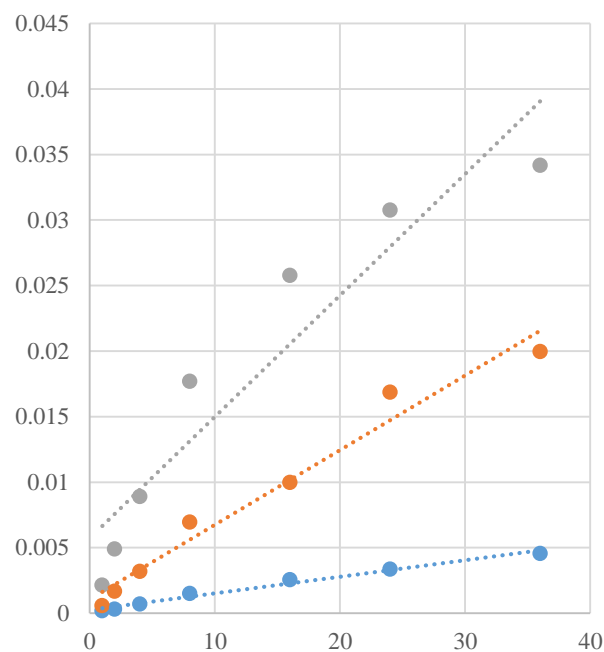
Second Order Approximation of Tapioca TC - PPA

Values are plotted against time in hours. The y-axis represents $1/106+1/(106-[P])$. Values are in units of mg^{-1}. Tables of values and R^2 values are listed following the graphs.
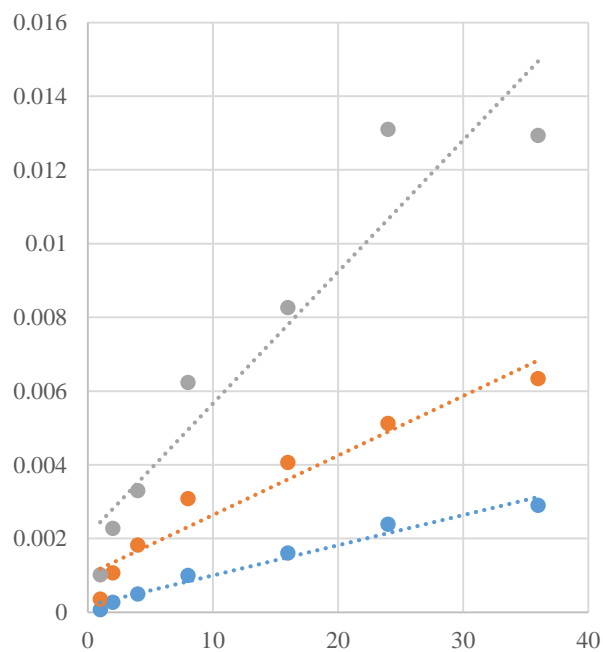
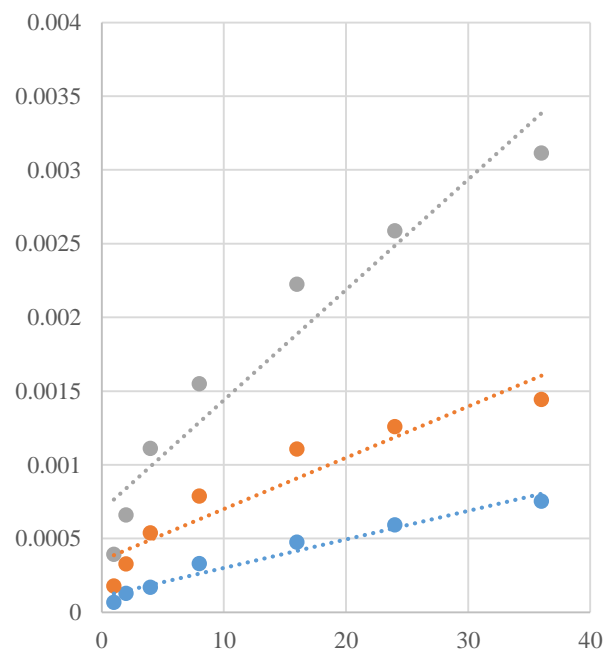## Second Order Approximation of Potato TC - HSA

Second Order Approximation of Waxy Maize TC - HSA
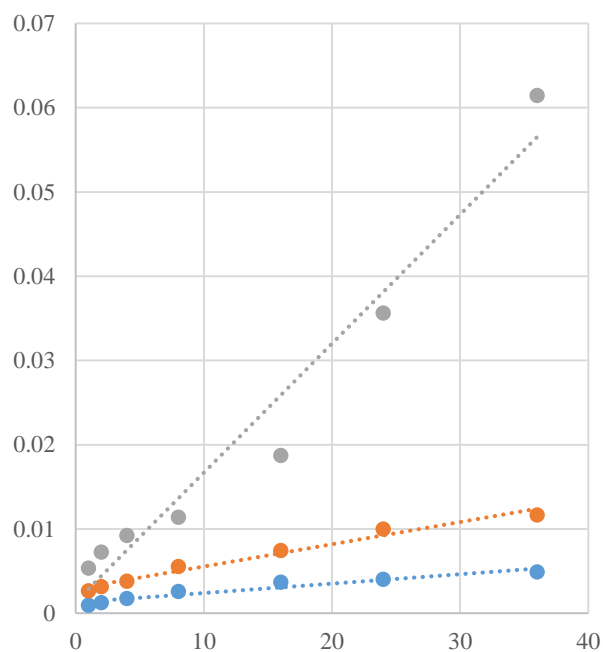
Second Order Approximation of Maize TC - HSA

Second Order Approximation of Amylomaize-7 TC - HSA

Values are plotted against time in hours. The y-axis represents $1/106 + 1/(106-[P])$. Values are in units of mg^{-1}. Tables of values and R^2 values are listed following the graphs.

Second Order Approximation of Rice TC - HSA

Second Order Approximation of Wheat TC - HSA

Second Order Approximation of Barley TC - HSA

Second Order Approximation of Tapioca TC - HSA
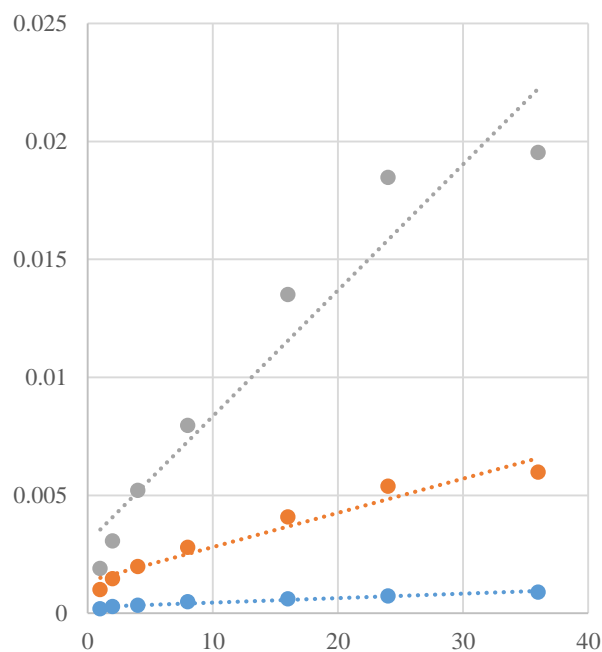
Values are plotted against time in hours. The y-axis represents $1/106+1/(106-[P])$. Values are in units of mg^{-1}. Tables of values and R^2 values are listed following the graphs.

Second Order Approximation of Potato TC - BAA

Second Order Approximation of Waxy Maize TC - BAA

Second Order Approximation of Maize TC - BAA
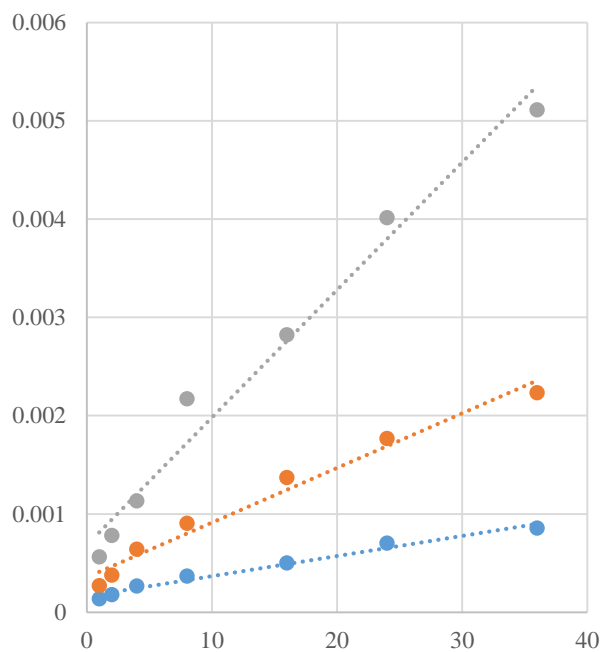
Second Order Approximation of Amylomaize-7 TC - BAA
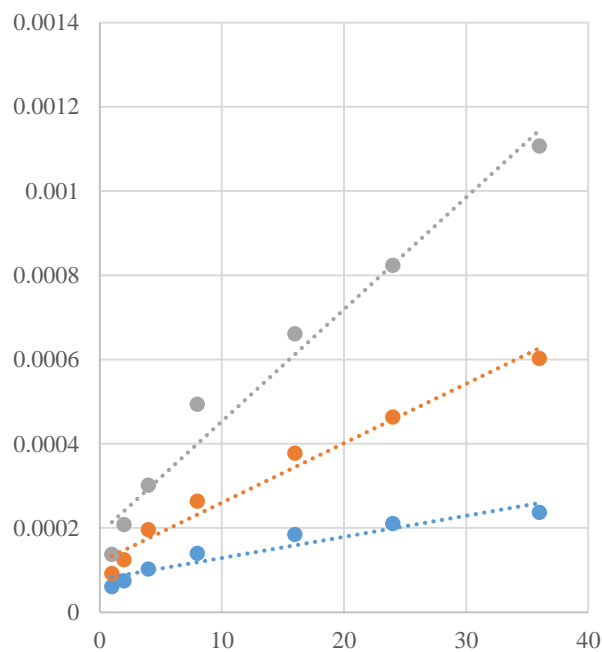
Values are plotted against time in hours. The y-axis represents 1/106+1/(106-[P]). Values are in units of mg^{-1}. Tables of values and R^2 values are listed following the graphs.
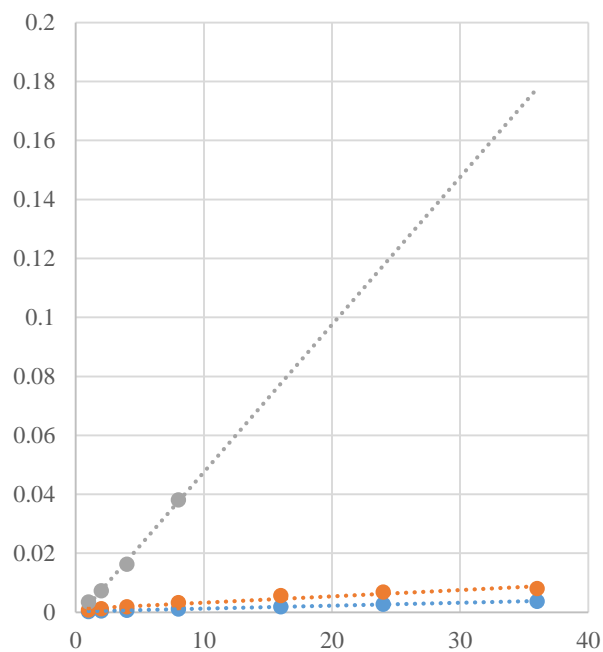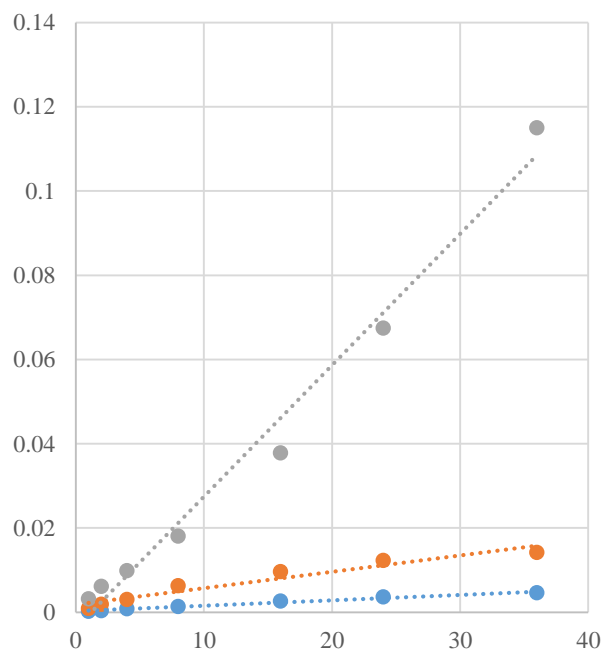
Second Order Approximation of Rice TC - BAA

Second Order Approximation of Wheat TC - BAA

Second Order Approximation of Barley TC - BAA
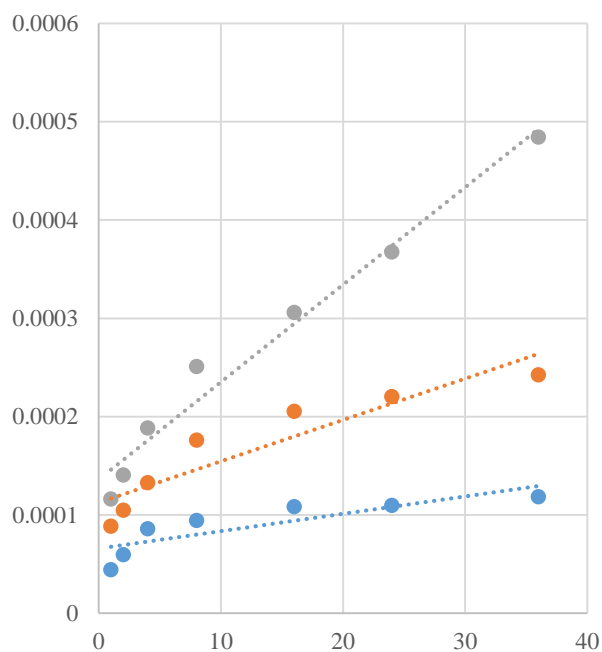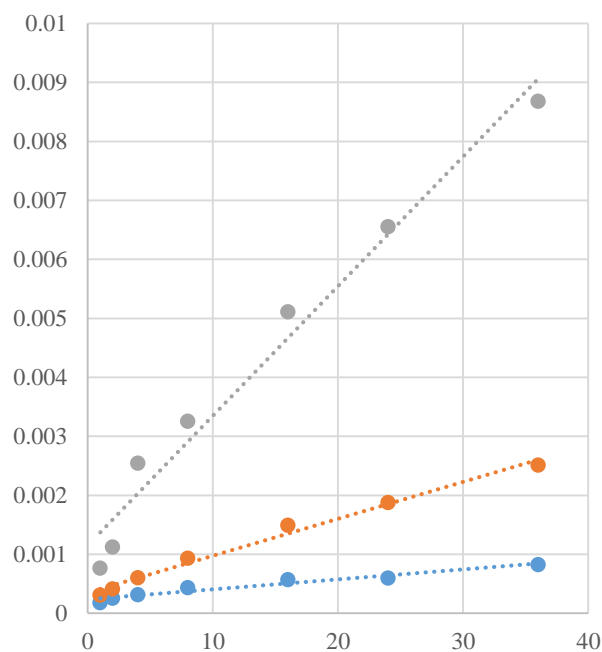
Second Order Approximation of Tapioca TC - BAA

Values are plotted against time in hours. The y-axis represents $1/106+1/(106-[P])$. Values are in units of mg^{-1}. Tables of values and R^2 values are listed following the graphs.
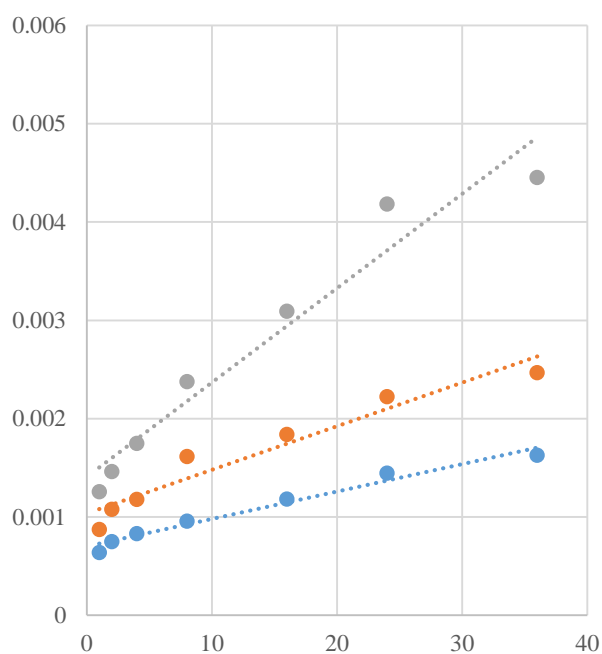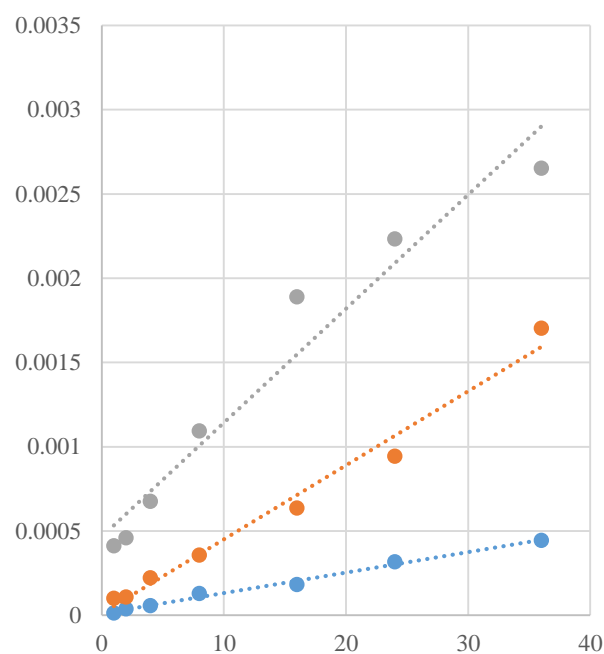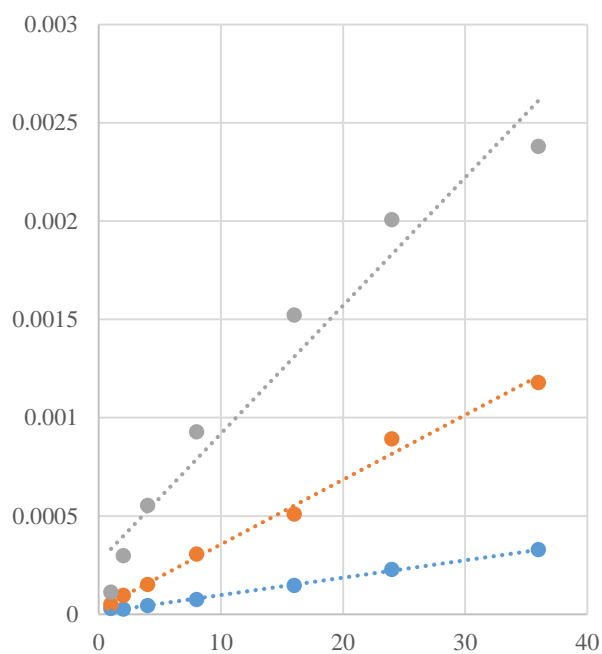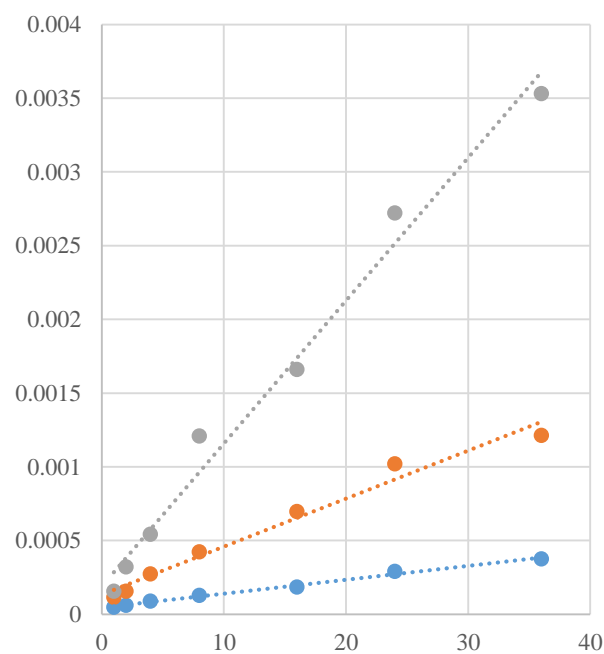
Bacillus licheniformis

Potato Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	9.0E-05	2.1E-04	2.5E-04
2	1.5E-04	2.3E-04	3.8E-04
4	2.1E-04	3.0E-04	4.7E-04
8	2.9E-04	4.7E-04	8.5E-04
16	4.2E-04	6.2E-04	1.1E-03
24	4.7E-04	8.2E-04	1.6E-03
36	6.2E-04	1.0E-03	2.3E-03
slope =	1.4E-05	2.3E-05	5.7E-05
R^2 =	0.9549	0.9795	0.9912

Bacillus licheniformis

Waxy Maize Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	3.2E-04	9.5E-04	1.5E-03
2	4.8E-04	1.5E-03	2.7E-03
4	7.9E-04	2.6E-03	4.4E-03
8	1.3E-03	4.6E-03	8.6E-03
16	2.0E-03	8.4E-03	1.6E-02
24	2.7E-03	9.6E-03	2.3E-02
36	3.6E-03	1.5E-02	3.8E-02
slope =	9.3E-05	4.0E-04	1.0E-03
R^2 =	0.9907	0.9859	0.9974

Bacillus licheniformis

Maize Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.8E-04	7.0E-04	1.7E-03
2	4.5E-04	1.2E-03	2.4E-03
4	7.6E-04	1.8E-03	3.8E-03
8	1.1E-03	2.6E-03	6.4E-03
16	1.5E-03	3.3E-03	9.8E-03
24	2.3E-03	5.4E-03	1.3E-02
36	3.0E-03	6.6E-03	1.6E-02
slope =	7.5E-05	1.7E-04	4.1E-04
R^2 =	0.9851	0.9756	0.9735

Bacillus licheniformis

Amylomaize-7 Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.1E-04	2.5E-04	5.0E-04
2	1.7E-04	3.7E-04	7.5E-04
4	2.5E-04	5.8E-04	1.1E-03
8	3.8E-04	8.9E-04	1.9E-03
16	5.1E-04	1.1E-03	3.3E-03
24	7.9E-04	1.4E-03	3.6E-03
36	9.1E-04	1.7E-03	5.0E-03
slope =	2.3E-05	4.1E-05	1.3E-04
R^2 =	0.9659	0.9473	0.9687

Values are plotted against time in hours. Values represent -1/106+1/(106-[P]). For the sake of clarity, the values have been multiplied by -1 in relation to the derivation of the second order kinetic model. Values are in units of mg^{-1}. The slope is in units of $mg^{-1}hours^{-1}$.

Bacillus licheniformis
Rice Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	7.6E-04	1.5E-03	2.1E-03
2	1.1E-03	1.9E-03	2.9E-03
4	1.4E-03	2.3E-03	4.4E-03
8	1.8E-03	3.3E-03	5.7E-03
16	2.3E-03	4.3E-03	8.5E-03
24	2.7E-03	5.6E-03	1.2E-02
36	2.8E-03	6.8E-03	2.2E-02
slope =	5.7E-05	1.5E-04	5.2E-04
R^2 =	0.8850	0.9770	0.9645

Bacillus licheniformis
Wheat Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.5E-04	3.8E-04	8.6E-04
2	2.8E-04	7.0E-04	1.6E-03
4	4.6E-04	1.1E-03	2.6E-03
8	7.7E-04	1.7E-03	6.3E-03
16	1.2E-03	3.0E-03	1.1E-02
24	1.5E-03	4.0E-03	1.4E-02
36	1.9E-03	5.0E-03	1.9E-02
slope =	4.9E-05	1.3E-04	5.2E-04
R^2 =	0.9659	0.9799	0.9842

Bacillus licheniformis
Barley Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.3E-04	2.6E-04	4.0E-04
2	2.1E-04	4.3E-04	1.2E-03
4	3.2E-04	9.7E-04	3.1E-03
8	6.0E-04	1.8E-03	6.0E-03
16	9.6E-04	3.1E-03	9.3E-03
24	1.6E-03	4.6E-03	1.7E-02
36	2.0E-03	5.5E-03	1.9E-02
slope =	5.6E-05	1.6E-04	5.6E-04
R^2 =	0.9853	0.9747	0.9577

Bacillus licheniformis
Tapioca Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.6E-04	5.2E-04	8.8E-04
2	2.5E-04	9.9E-04	1.6E-03
4	4.2E-04	1.7E-03	3.0E-03
8	6.6E-04	3.3E-03	4.8E-03
16	1.2E-03	4.6E-03	1.0E-02
24	1.5E-03	6.3E-03	1.3E-02
36	1.7E-03	7.4E-03	1.5E-02
slope =	4.6E-05	2.0E-04	4.2E-04
R^2 =	0.9352	0.9508	0.9370

Values are plotted against time in hours. Values represent $-1/106+1/(106-[P])$. For the sake of clarity, the values have been multiplied by -1 in relation to the derivation of the second order kinetic model. Values are in units of mg^{-1}. The slope is in units of $mg^{-1}hours^{-1}$.

Porcine Pancreas

Potato Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	6.1E-05	1.5E-04	1.5E-04
2	9.1E-05	2.1E-04	2.1E-04
4	1.6E-04	3.1E-04	3.8E-04
8	2.1E-04	4.6E-04	5.6E-04
16	3.2E-04	5.2E-04	1.1E-03
24	3.4E-04	6.8E-04	1.3E-03
36	4.0E-04	8.7E-04	1.6E-03
slope =	9.3E-06	1.9E-05	4.3E-05
R^2 =	0.8878	0.9550	0.9607

Porcine Pancreas
Waxy Maize Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	3.1E-04	9.8E-04	1.5E-03
2	4.6E-04	2.1E-03	2.6E-03
4	8.2E-04	3.1E-03	4.5E-03
8	1.4E-03	5.8E-03	8.1E-03
16	2.5E-03	8.1E-03	1.2E-02
24	3.6E-03	1.1E-02	1.8E-02
36	4.3E-03	1.3E-02	2.6E-02
slope =	2.1E-05	1.2E-04	3.5E-04
R^2 =	0.9896	0.9779	0.9565

Porcine Pancreas

Maize Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	9.8E-05	3.8E-04	1.1E-03
2	2.4E-04	7.2E-04	2.0E-03
4	4.6E-04	1.5E-03	3.2E-03
8	1.2E-03	2.5E-03	4.9E-03
16	1.8E-03	4.3E-03	7.5E-03
24	2.3E-03	5.1E-03	8.6E-03
36	2.2E-03	5.4E-03	3.7E-03
slope =	6.6E-05	1.5E-04	3.2E-04
R^2 =	0.8480	0.8856	0.9450

Porcine Pancreas
Amylomaize-7 Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	6.4E-05	1.5E-04	4.3E-04
2	9.6E-05	2.5E-04	6.5E-04
4	1.3E-04	3.8E-04	9.4E-04
8	2.6E-04	5.3E-04	1.5E-03
16	3.9E-04	9.2E-04	1.9E-03
24	5.5E-04	1.1E-03	2.3E-03
36	6.7E-04	1.3E-03	3.2E-03
slope =	3.5E-06	1.8E-05	3.3E-05
R^2 =	0.9132	0.9754	0.9422

Values are plotted against time in hours. Values represent $-1/106+1/(106-[P])$. For the sake of clarity, the values have been multiplied by -1 in relation to the derivation of the second order kinetic model. Values are in units of mg^{-1}. The slope is in units of $mg^{-1}hours^{-1}$.

The highlighted value was omitted since it is erroneous. The centrifuge tube leaked.

Porcine Pancreas
Rice Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	8.0E-04	1.5E-03	2.5E-03
2	1.0E-03	1.9E-03	3.1E-03
4	1.3E-03	2.2E-03	4.3E-03
8	1.9E-03	3.2E-03	7.0E-03
16	2.3E-03	3.8E-03	8.3E-03
24	2.5E-03	4.1E-03	1.3E-02
36	3.2E-03	6.5E-03	1.5E-02
slope =	3.3E-05	6.4E-05	1.3E-04
R^2 =	0.9654	0.9216	0.9590

Porcine Pancreas
Wheat Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.0E-04	5.5E-04	1.2E-03
2	1.6E-04	8.2E-04	1.8E-03
4	2.3E-04	1.1E-03	2.8E-03
8	4.0E-04	1.8E-03	4.0E-03
16	4.7E-04	3.1E-03	6.2E-03
24	5.8E-04	4.0E-03	9.1E-03
36	8.3E-04	4.6E-03	1.3E-02
slope =	5.3E-06	1.9E-05	1.2E-04
R^2 =	0.9071	0.9603	0.9521

Porcine Pancreas
Barley Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.3E-04	4.6E-04	8.4E-04
2	2.4E-04	7.0E-04	1.8E-03
4	3.8E-04	1.0E-03	3.0E-03
8	5.4E-04	1.7E-03	5.1E-03
16	9.3E-04	2.4E-03	8.2E-03
24	1.3E-03	3.1E-03	8.9E-03
36	1.5E-03	4.0E-03	9.2E-03
slope =	1.5E-05	4.0E-05	1.0E-04
R^2 =	0.9901	0.9685	0.9739

Porcine Pancreas
Tapioca Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.3E-04	4.7E-04	1.1E-03
2	2.0E-04	7.5E-04	2.4E-03
4	3.8E-04	1.3E-03	4.2E-03
8	6.0E-04	2.0E-03	7.3E-03
16	1.0E-03	3.1E-03	1.3E-02
24	1.3E-03	3.2E-03	1.9E-02
36	1.6E-03	3.8E-03	2.2E-02
slope =	7.1E-06	4.4E-05	9.4E-05
R^2 =	0.9375	0.9652	0.8874

Values are plotted against time in hours. Values represent $-1/106+1/(106-[P])$. Values are in units of mg^{-1}. The slope is in units of $mg^{-1}hours^{-1}$.

Human Saliva

Potato Second Order Analysis

TC	0.1 IU	1 IU	10 IU
1	5.0E-05	1.1E-04	1.8E-04
2	1.1E-04	1.8E-04	3.2E-04
4	1.4E-04	3.0E-04	5.4E-04
8	2.2E-04	4.3E-04	9.1E-04
16	2.8E-04	5.8E-04	1.2E-03
24	3.7E-04	7.5E-04	1.7E-03
36	4.6E-04	9.6E-04	2.0E-03
slope =	1.1E-05	2.3E-05	5.3E-05
R^2 =	0.9571	0.9648	0.9553

Human Saliva
Waxy Maize Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.9E-04	6.0E-04	2.2E-03
2	3.1E-04	1.7E-03	4.9E-03
4	7.1E-04	3.2E-03	8.9E-03
8	1.5E-03	7.0E-03	1.8E-02
16	2.6E-03	1.0E-02	2.6E-02
24	3.4E-03	1.7E-02	3.1E-02
36	4.6E-03	2.0E-02	3.4E-02
slope =	1.3E-04	5.7E-04	9.3E-04
R^2 =	0.9833	0.9700	0.8914

Human Saliva

Maize Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	6.4E-05	3.5E-04	1.0E-03
2	2.7E-04	1.1E-03	2.3E-03
4	5.0E-04	1.8E-03	3.3E-03
8	1.0E-03	3.1E-03	6.2E-03
16	1.6E-03	4.1E-03	8.3E-03
24	2.4E-03	5.1E-03	1.3E-02
36	2.9E-03	6.3E-03	1.3E-02
slope =	8.2E-05	1.6E-04	3.6E-04
R^2 =	0.9726	0.9349	0.9025

Human Saliva
Amylomaize-7 Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	6.6E-05	1.8E-04	3.9E-04
2	1.3E-04	3.3E-04	6.6E-04
4	1.7E-04	5.4E-04	1.1E-03
8	3.3E-04	7.9E-04	1.5E-03
16	4.8E-04	1.1E-03	2.2E-03
24	5.9E-04	1.3E-03	2.6E-03
36	7.5E-04	1.4E-03	3.1E-03
slope =	1.9E-05	3.5E-05	7.5E-05
R^2 =	0.9623	0.8927	0.9273

Values are plotted against time in hours. Values represent $-1/106 + 1/(106-[P])$. Values are in units of mg^{-1}. The slope is in units of $mg^{-1} hours^{-1}$.

Human Saliva
Rice Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	9.6E-04	2.7E-03	5.4E-03
2	1.3E-03	3.2E-03	7.3E-03
4	1.7E-03	3.8E-03	9.2E-03
8	2.6E-03	5.5E-03	1.1E-02
16	3.7E-03	7.5E-03	1.9E-02
24	4.1E-03	1.0E-02	3.6E-02
36	4.9E-03	1.2E-02	6.1E-02
slope =	1.1E-04	2.6E-04	1.5E-03
R^2 =	0.9285	0.9750	0.9588

Human Saliva
Wheat Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.9E-04	1.0E-03	1.9E-03
2	2.9E-04	1.5E-03	3.1E-03
4	3.5E-04	2.0E-03	5.2E-03
8	4.9E-04	2.8E-03	8.0E-03
16	6.2E-04	4.1E-03	1.4E-02
24	7.3E-04	5.4E-03	1.8E-02
36	8.9E-04	6.0E-03	2.0E-02
slope =	1.9E-05	1.4E-04	5.3E-04
R^2 =	0.9515	0.9489	0.9294

Human Saliva
Barley Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.2E-04	7.3E-04	3.5E-03
2	4.2E-04	1.2E-03	7.3E-03
4	5.9E-04	1.9E-03	1.6E-02
8	1.0E-03	3.3E-03	3.8E-02
16	1.9E-03	5.7E-03	1.0E+00
24	2.7E-03	6.9E-03	-1.4E-01
36	3.7E-03	8.0E-03	-9.1E-02
slope =	1.0E-04	2.1E-04	5.0E-03
R^2 =	0.9960	0.9392	0.9961

Human Saliva
Tapioca Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.0E-04	9.1E-04	3.2E-03
2	4.0E-04	1.9E-03	6.2E-03
4	7.9E-04	3.0E-03	9.9E-03
8	1.3E-03	6.3E-03	1.8E-02
16	2.6E-03	9.7E-03	3.8E-02
24	3.6E-03	1.2E-02	6.7E-02
36	4.6E-03	1.4E-02	1.1E-01
slope =	1.3E-04	3.9E-04	3.1E-03
R^2 =	0.9832	0.9351	0.9840

Values are plotted against time in hours. Values represent $-1/106+1/(106-[P])$. Values are in units of mg^{-1}. The slope is in units of $mg^{-1}hours^{-1}$.

Highlighted values are omitted from analysis as their values were too close to or surpassed 106 mg to be evaluated accurately.

Bacillus amyloliquefaciens

Potato Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	4.4E-05	8.8E-05	1.2E-04
2	6.0E-05	1.0E-04	1.4E-04
4	8.6E-05	1.3E-04	1.9E-04
8	9.5E-05	1.8E-04	2.5E-04
16	1.1E-04	2.1E-04	3.1E-04
24	1.1E-04	2.2E-04	3.7E-04
36	1.2E-04	2.4E-04	4.8E-04
slope =	1.8E-06	4.2E-06	9.9E-06
R^2 =	0.7053	0.8522	0.9732

Bacillus amyloliquefaciens

Waxy Maize Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.8E-04	3.1E-04	7.7E-04
2	2.6E-04	4.2E-04	1.1E-03
4	3.2E-04	6.0E-04	2.5E-03
8	4.4E-04	9.4E-04	3.3E-03
16	5.7E-04	1.5E-03	5.1E-03
24	6.0E-04	1.9E-03	6.6E-03
36	8.3E-04	2.5E-03	8.7E-03
slope =	1.7E-05	6.3E-05	2.2E-04
R^2 =	0.9462	0.9878	0.9740

Bacillus amyloliquefaciens

Maize Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.4E-04	2.7E-04	5.6E-04
2	1.8E-04	3.8E-04	7.8E-04
4	2.7E-04	6.4E-04	1.1E-03
8	3.7E-04	9.0E-04	2.2E-03
16	5.0E-04	1.4E-03	2.8E-03
24	7.0E-04	1.8E-03	4.0E-03
36	8.5E-04	2.2E-03	5.1E-03
slope =	2.0E-05	5.6E-05	1.3E-04
R^2 =	0.9784	0.9755	0.9772

Bacillus amyloliquefaciens

Amylomaize-7 Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	6.0E-05	9.2E-05	1.4E-04
2	7.4E-05	1.2E-04	2.1E-04
4	1.0E-04	2.0E-04	3.0E-04
8	1.4E-04	2.6E-04	4.9E-04
16	1.8E-04	3.8E-04	6.6E-04
24	2.1E-04	4.6E-04	8.2E-04
36	2.4E-04	6.0E-04	1.1E-03
slope =	5.0E-06	1.4E-05	2.7E-05
R^2 =	0.9125	0.9749	0.9738

Values are plotted against time in hours. Values represent $-1/106+1/(106-[P])$. Values are in units of mg^{-1}. The slope is in units of $mg^{-1}hours^{-1}$.

Bacillus amyloliquefaciens
Rice Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	6.4E-04	8.7E-04	1.3E-03
2	7.5E-04	1.1E-03	1.5E-03
4	8.3E-04	1.2E-03	1.7E-03
8	9.6E-04	1.6E-03	2.4E-03
16	1.2E-03	1.8E-03	3.1E-03
24	1.4E-03	2.2E-03	4.2E-03
36	1.6E-03	2.5E-03	4.5E-03
slope =	2.8E-05	4.4E-05	9.6E-05
R^2 =	0.9723	0.9319	0.9467

Bacillus amyloliquefaciens
Wheat Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	1.4E-05	1.0E-04	4.1E-04
2	3.8E-05	1.1E-04	4.6E-04
4	5.7E-05	2.2E-04	6.8E-04
8	1.3E-04	3.6E-04	1.1E-03
16	1.8E-04	6.4E-04	1.9E-03
24	3.2E-04	9.4E-04	2.2E-03
36	4.4E-04	1.7E-03	2.7E-03
slope =	1.2E-05	4.4E-05	6.8E-05
R^2 =	0.9917	0.9819	0.9505

Bacillus amyloliquefaciens
Barley Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	2.9E-05	5.0E-05	1.1E-04
2	2.5E-05	9.5E-05	3.0E-04
4	4.3E-05	1.5E-04	5.5E-04
8	7.5E-05	3.0E-04	9.3E-04
16	1.5E-04	5.1E-04	1.5E-03
24	2.3E-04	8.9E-04	2.0E-03
36	3.3E-04	1.2E-03	2.4E-03
slope =	8.8E-06	3.3E-05	6.5E-05
R^2 =	0.9976	0.9920	0.9545

Bacillus amyloliquefaciens
Tapioca Second Order Analysis

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	4.6E-05	1.1E-04	1.6E-04
2	5.9E-05	1.5E-04	3.2E-04
4	8.8E-05	2.7E-04	5.4E-04
8	1.3E-04	4.2E-04	1.2E-03
16	1.8E-04	7.0E-04	1.7E-03
24	2.9E-04	1.0E-03	2.7E-03
36	3.7E-04	1.2E-03	3.5E-03
slope =	9.4E-06	3.3E-05	9.7E-05
R^2 =	0.9919	0.9760	0.9846

Values are plotted against time in hours. Values represent $-1/106 + 1/(106-[P])$. Values are in units of mg^{-1}. The slope is in units of $mg^{-1}hours^{-1}$.

APPENDIX E: EXPONENTIAL INTEGRAL ANALYSIS

$$[ES] = K[E]_{soln}[S]_{avail} \approx K[E]_{tot}[S]_{avail}$$

$$[S]_{avail} = C([S]_{tot})[S]_{tot} - [ES]$$

$$C([S]_{tot}) = C^0 * \left(\frac{C_{min}}{C^0}\right)^{[P]/106}$$

$$\frac{d[P]}{dt} = k_{cat}[ES] = K[E]_{tot}[S]_{avail}$$

$$[S]_{avail} = C^0[S]_{tot}\left(\frac{C_{min}}{C^0}\right)^{[P]/106} - [ES]$$

$$[S]_{avail} = C^0[S]_{tot}\left(\frac{C_{min}}{C^0}\right)^{[P]/106} - K[E]_{tot}[S]_{avail}$$

$$[S]_{avail} = \frac{C^0[S]_{tot}\left(\frac{C_{min}}{C^0}\right)^{[P]/106}}{1 + K[E]_{tot}}$$

$$\frac{d[P]}{dt} = k_{cat}K[E]_{tot}[S]_{avail}$$

$$\frac{d[P]}{dt} = \frac{k_{cat}K[E]_{tot}C^0[S]_{tot}\left(\frac{C_{min}}{C^0}\right)^{[P]/106}}{1 + K[E]_{tot}}$$

$$\frac{d[P]}{[S]_{tot}}\left(\frac{C_{min}}{C^0}\right)^{-[P]/106} = \frac{k_{cat}K[E]_{tot}C^0}{1 + K[E]_{tot}}dt$$

$$\frac{d[P]}{(106 - [P])}\left(\frac{C_{min}}{C^0}\right)^{-[P]/106} = \frac{k_{cat}K[E]_{tot}C^0}{1 + K[E]_{tot}}dt$$

$$-\left(\frac{C^0}{C_{min}}\right)E_i\left(\frac{([P] - 106) * \ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right)E_i\left(-106 * \ln\left(\frac{C^0}{C_{min}}\right)\right) = \frac{k_{cat}K[E]_{tot}C^0}{1 + K[E]_{tot}}t$$
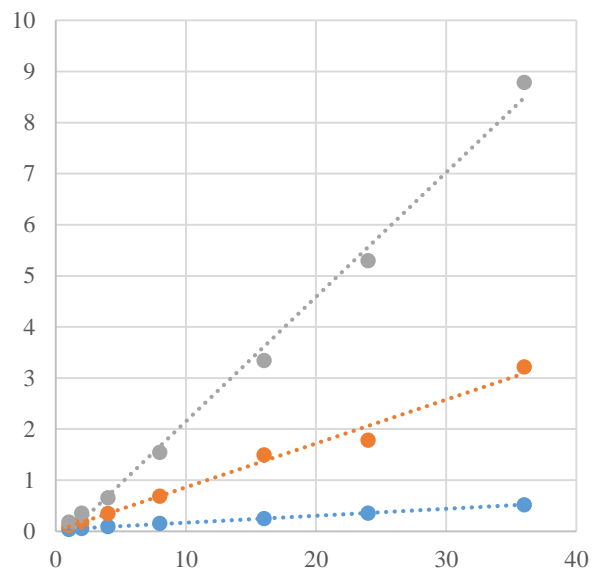
$$= \Phi t$$

The above equations describe the derivation of the exponential integral model. Graphs depicting the evaluation according to this model are shown below. A better explanation of this derivation can be seen in Chapter 4.
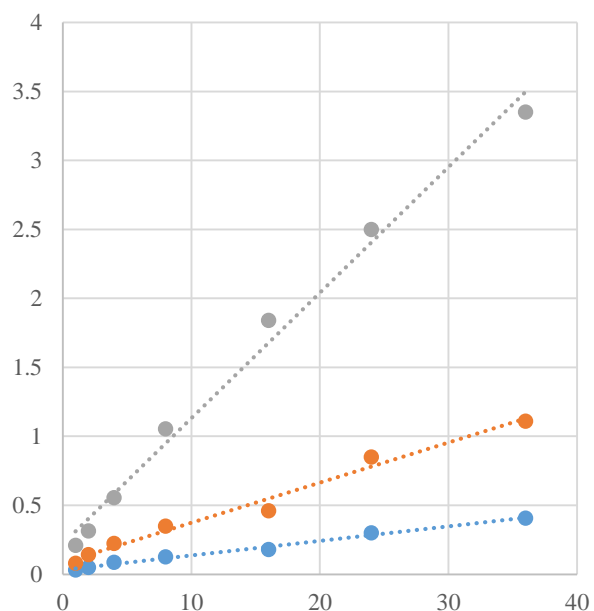
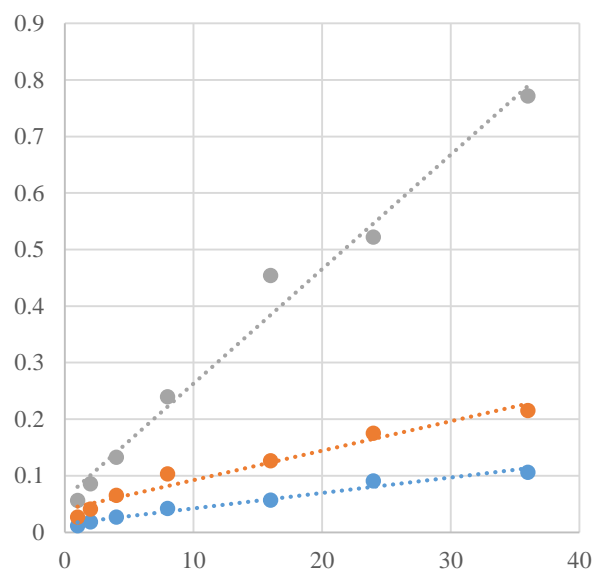**Potato TC Exponential Integral BLA**
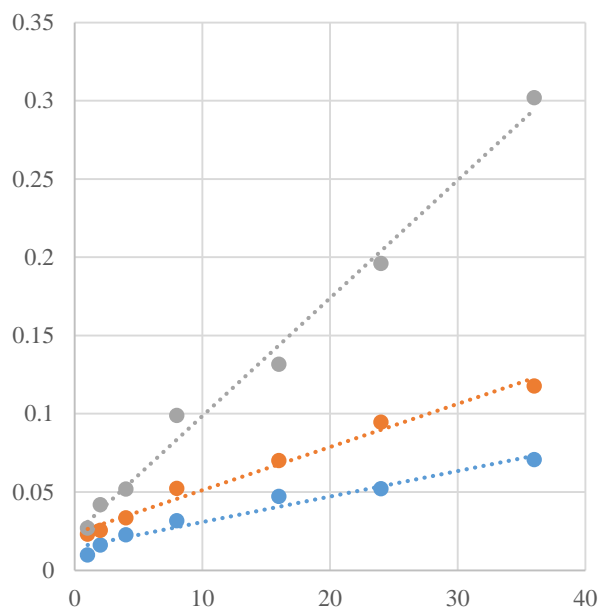
Waxy Maize TC Exponential Integral BLA

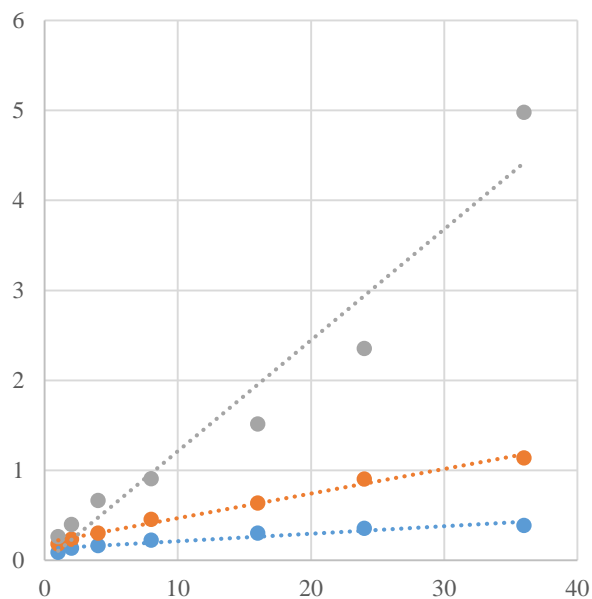**Maize TC Exponential Integral BLA**
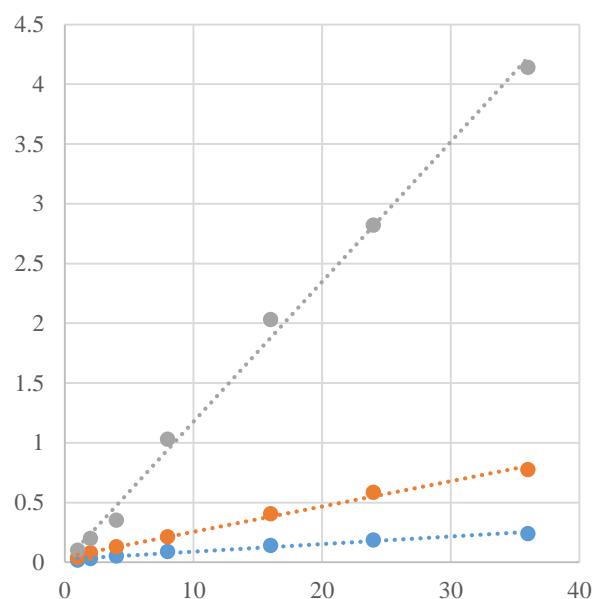
Amylomaize-7 TC Exponential Integral BLA

Values are plotted against time in hours. Values represent $-\left(\frac{c^0}{c_{min}}\right) E_i \left(\frac{(106-[P])*\ln\left(\frac{c^0}{c_{min}}\right)}{106}\right) +$ $\left(\frac{c^0}{c_{min}}\right) E_i \left(-106 * \ln\left(\frac{c^0}{c_{min}}\right)\right)$. For this particular set, a $\frac{c^0}{c_{min}}$ value was assigned to 20. Y-values are unitless.
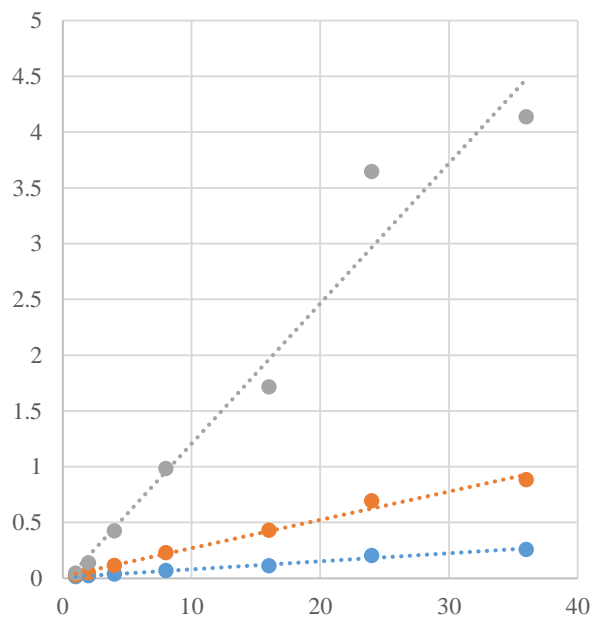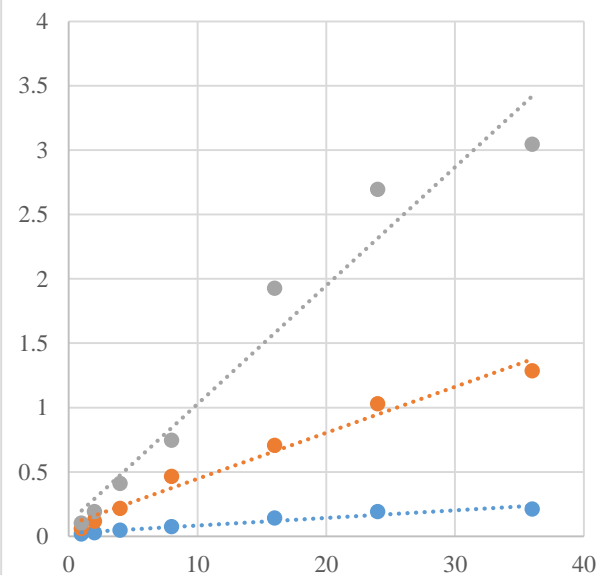
Rice TC Exponential Integral BLA
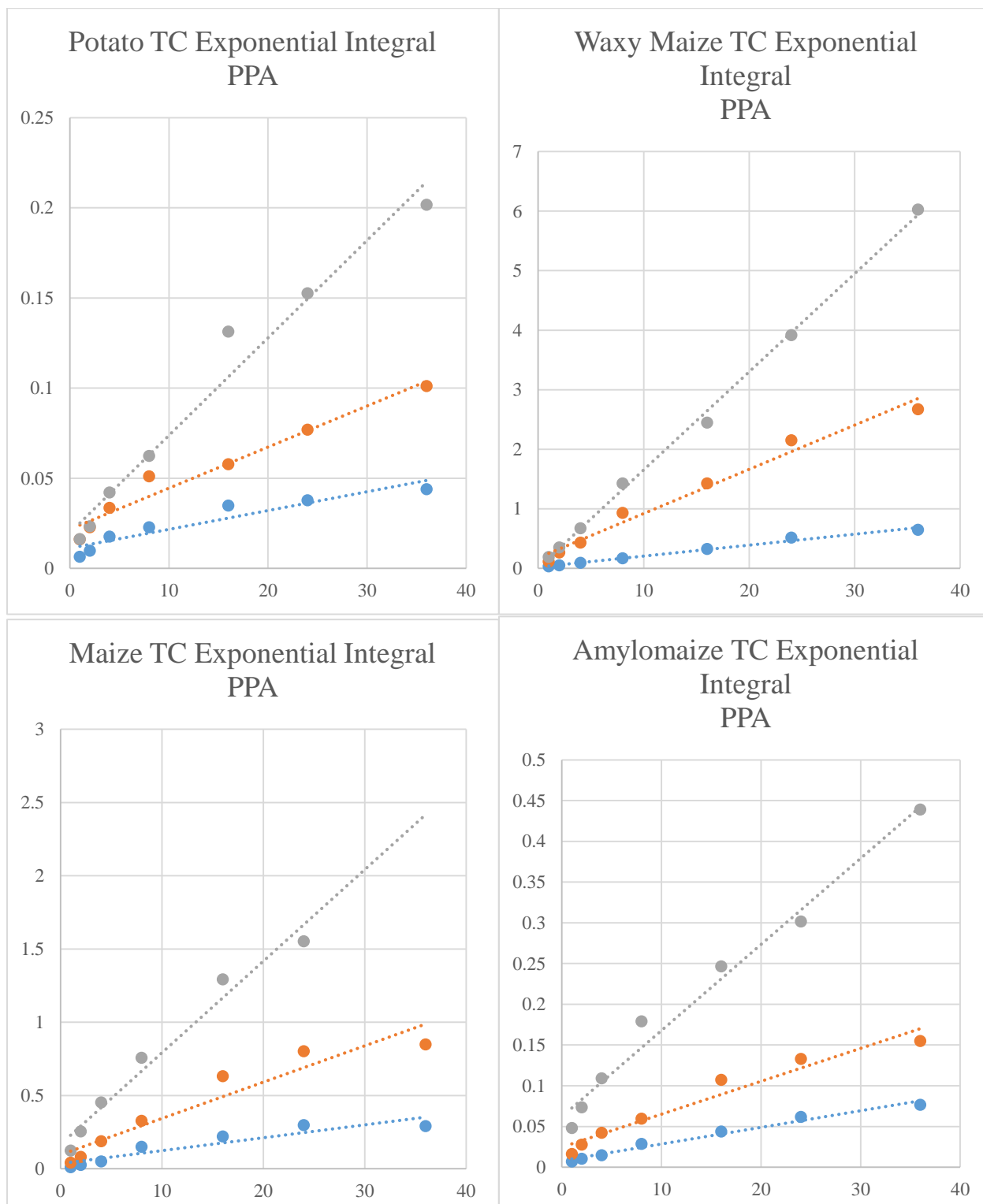
Wheat TC Exponential Integral BLA

Barley TC Exponential Integral BLA

Tapioca TC Exponential Integral BLA

Values are plotted against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i \left(\frac{(106-[P]) * \ln\left(\frac{C^0}{C_{min}}\right)}{106} \right) + \left(\frac{C^0}{C_{min}}\right) E_i \left(-106 * \ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Y-values are unitless.

Potato TC Exponential Integral PPA

Waxy Maize TC Exponential Integral PPA

Maize TC Exponential Integral PPA
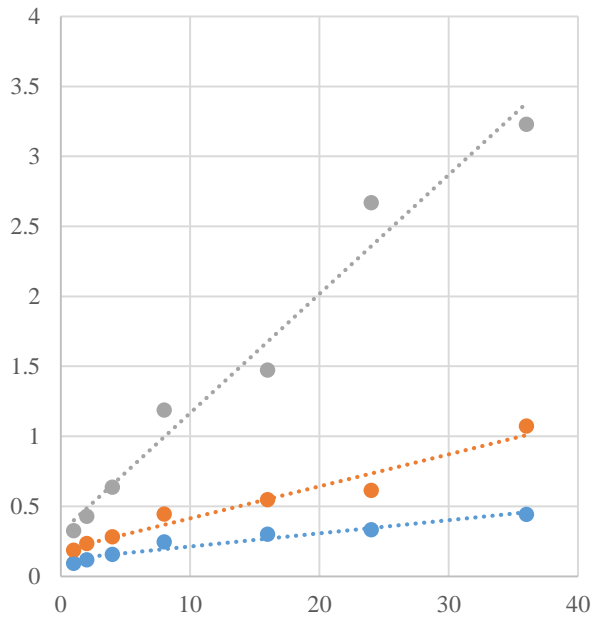
Amylomaize TC Exponential Integral PPA

Values are plotted against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i \left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) +$

$\left(\frac{C^0}{C_{min}}\right) E_i \left(-106 * \ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Y-values are unitless.

Rice TC Exponential Integral PPA

Wheat TC Exponential Integral PPA

Barley TC Exponential Integral PPA

Tapioca TC Exponential Integral PPA

Values are plotted against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i \left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right) E_i \left(-106 * \ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Y-values are unitless.
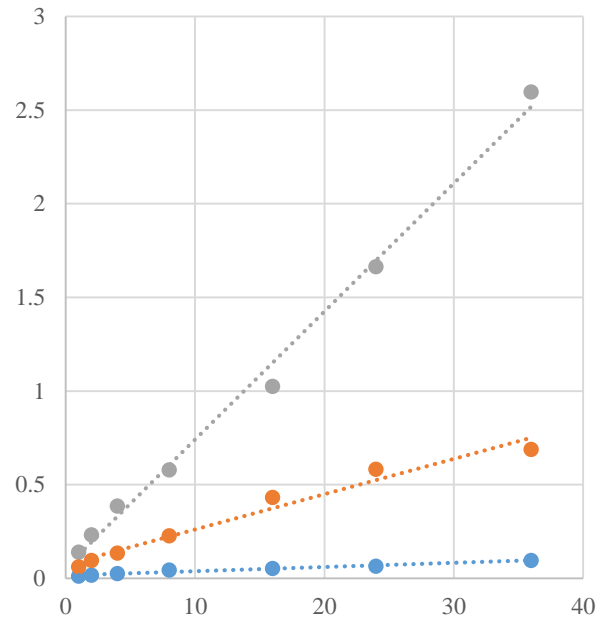
Potato TC Exponential Integral HSA

Waxy Maize TC Exponential Integral HSA

Maize TC Exponential Integral HSA

Amylomaize-7 TC Exponential Integral HSA

Values are plotted against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) +$

$\left(\frac{C^0}{C_{min}}\right) E_i\left(-106 * \ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Y-values are unitless.
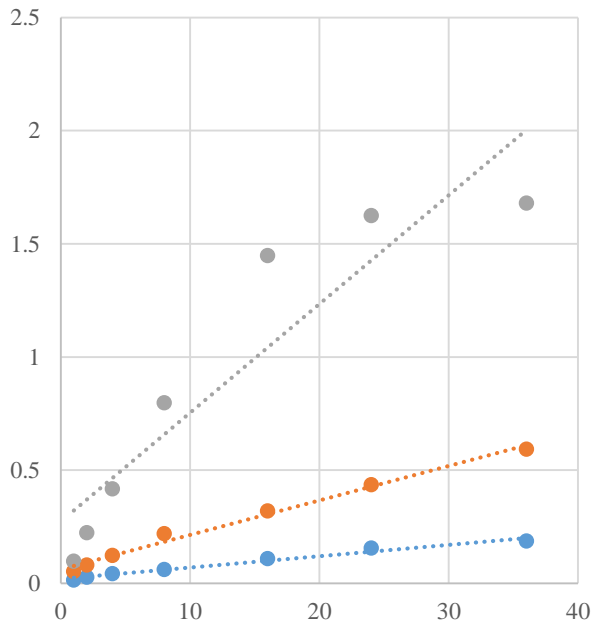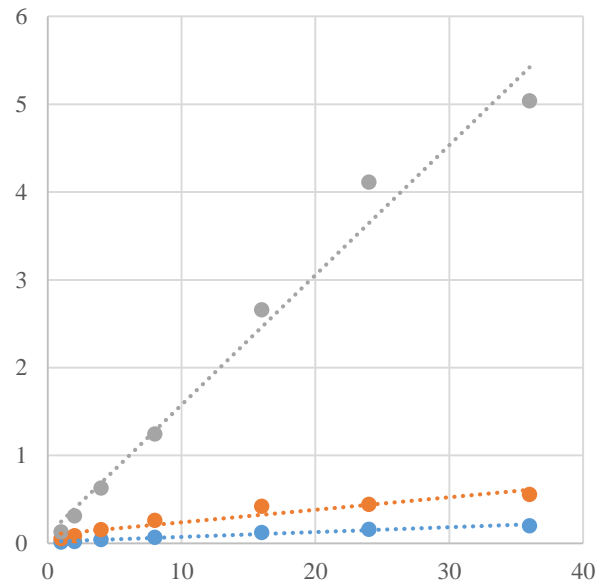
Rice TC Exponential Integral HSA
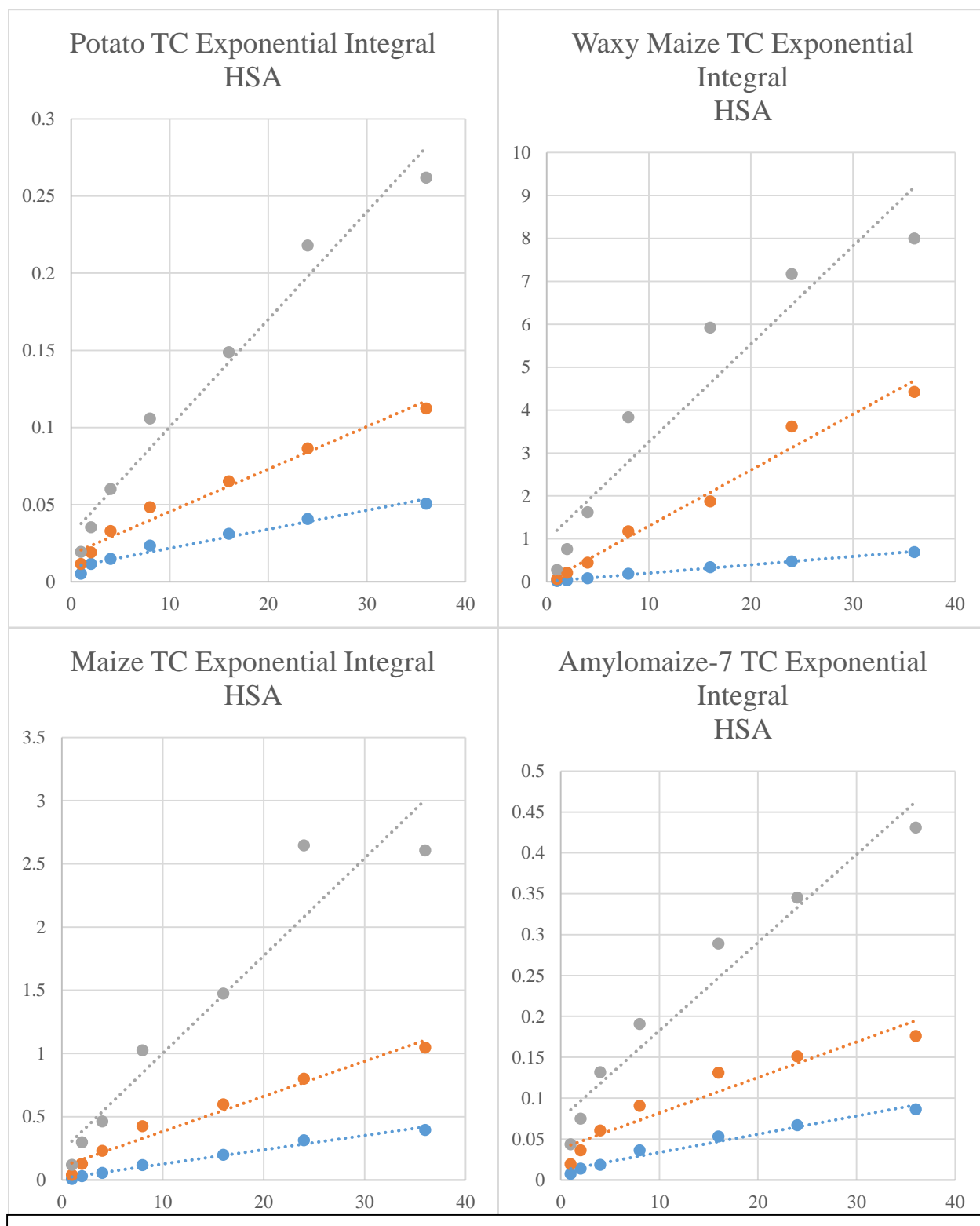
Wheat TC Exponential Integral HSA

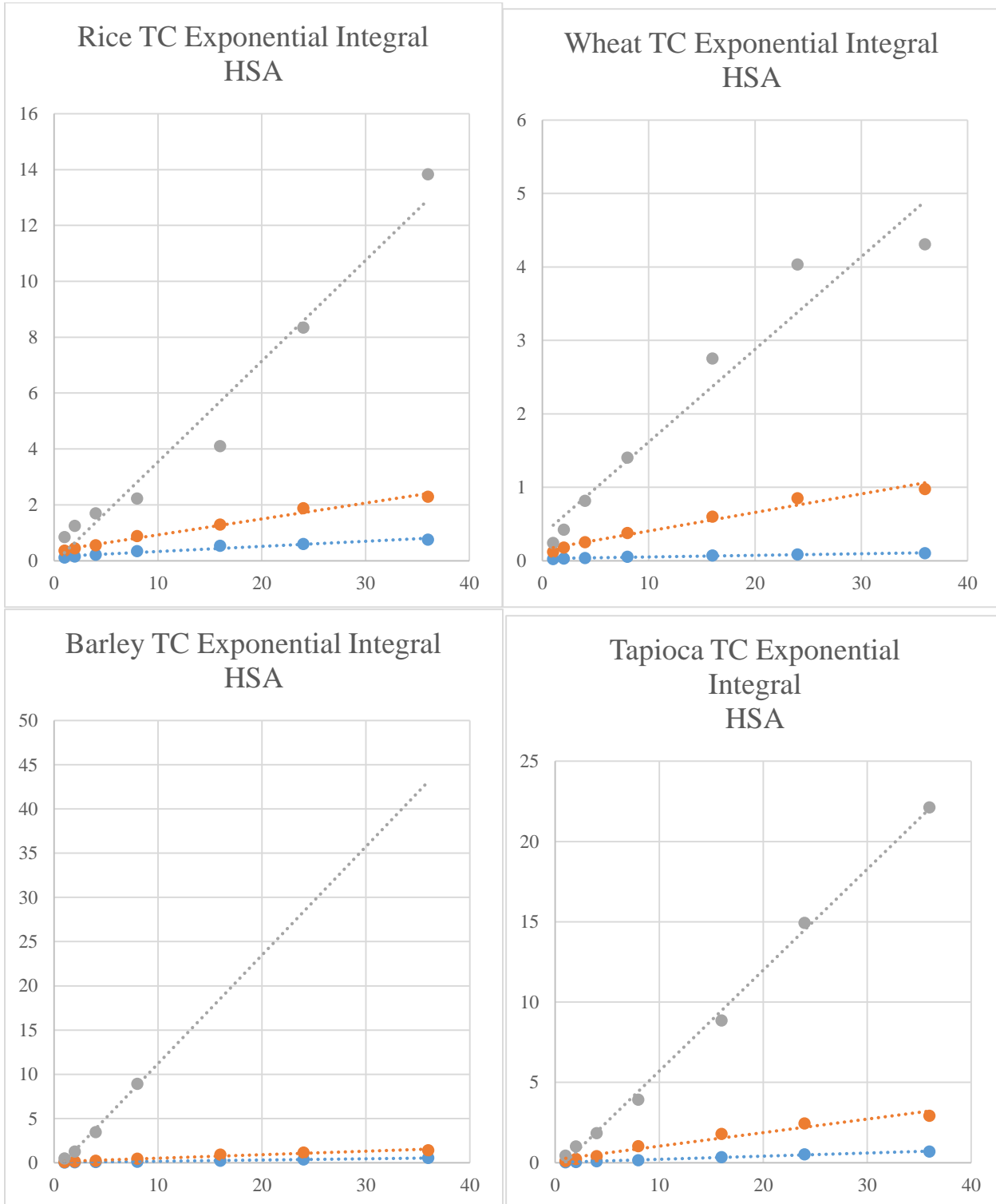**Barley TC Exponential Integral HSA**

Tapioca TC Exponential Integral HSA

Values are plotted against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right) E_i\left(-106*\ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Y-values are unitless. The last 3 datapoints for barley are omitted due to their proximity to 106 mg.

Potato TC Exponential Integral BAA

Waxy Maize TC Exponential Integral BAA

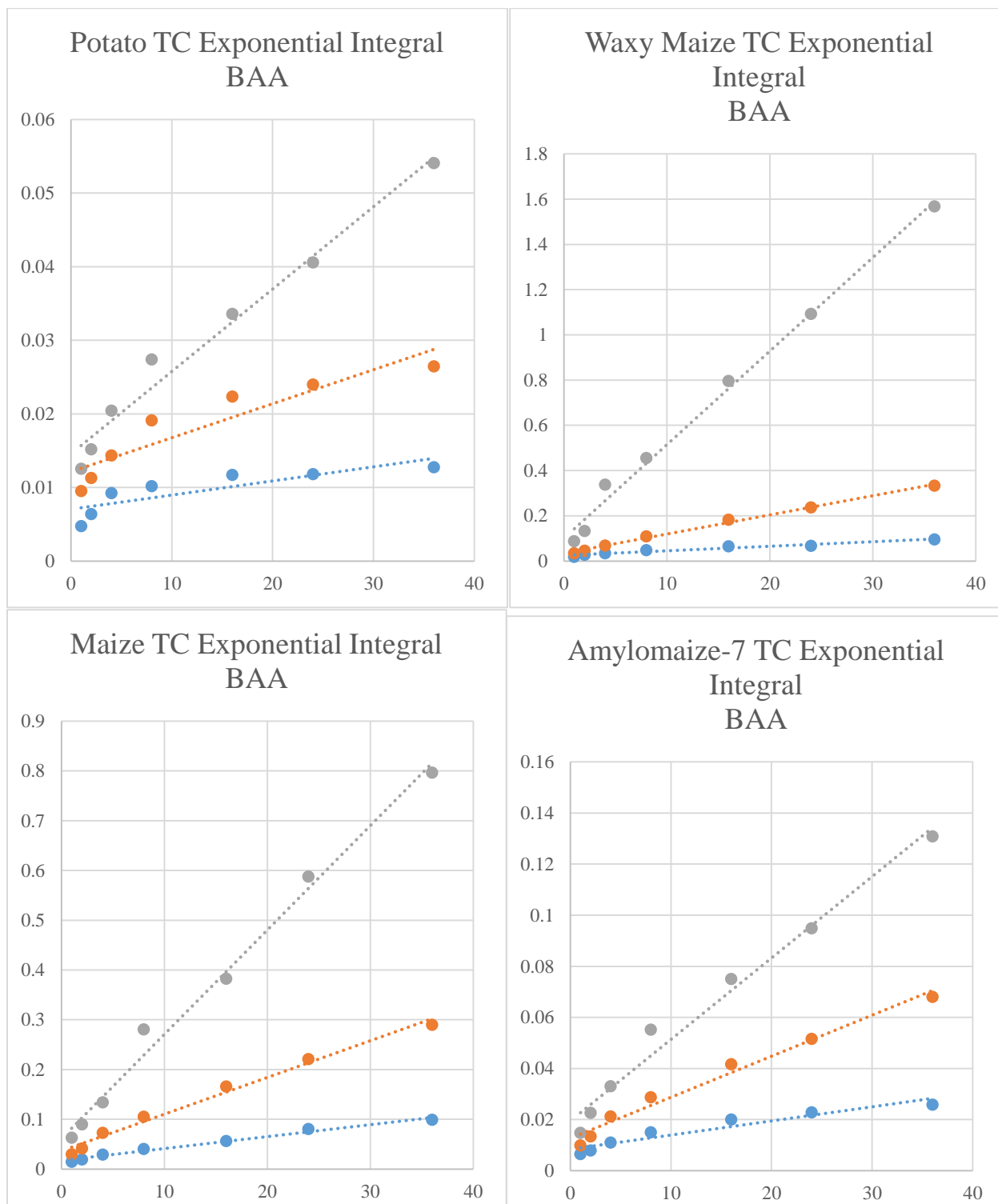## Maize TC Exponential Integral BAA

Amylomaize-7 TC Exponential Integral BAA

Values are plotted against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right) E_i\left(-106*\ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Y-values are unitless.

Values are plotted against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i \left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right) E_i \left(-106 * \ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Y-values are unitless.
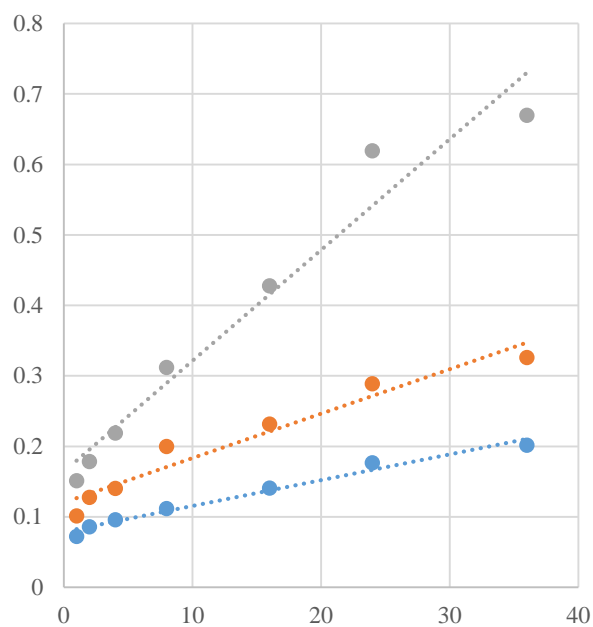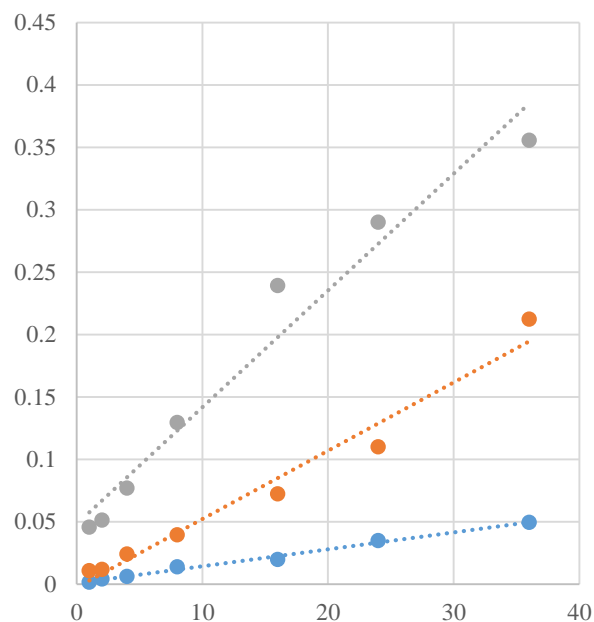
Bacillus licheniformis
Potato Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.02	0.03
2	0.02	0.03	0.04
4	0.02	0.03	0.05
8	0.03	0.05	0.10
16	0.05	0.07	0.13
24	0.05	0.09	0.20
36	0.07	0.12	0.30
slope =	0.0016	0.0028	0.0075
R^2 =	0.9599	0.9842	0.9910

Bacillus licheniformis
Waxy Maize Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.03	0.11	0.18
2	0.05	0.18	0.36
4	0.09	0.35	0.65
8	0.16	0.69	1.55
16	0.25	1.50	3.34
24	0.36	1.79	5.30
36	0.52	3.22	8.79
slope =	0.0136	0.0857	0.2434
R^2 =	0.9979	0.9859	0.9948

Bacillus licheniformis
Maize Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.03	0.08	0.21
2	0.05	0.14	0.31
4	0.09	0.22	0.55
8	0.13	0.35	1.05
16	0.18	0.46	1.84
24	0.30	0.85	2.50
36	0.41	1.11	3.35
slope =	0.0106	0.0291	0.0911
R^2 =	0.9899	0.9823	0.9900

Bacillus licheniformis
Amylomaize-7 Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.03	0.06
2	0.02	0.04	0.09
4	0.03	0.07	0.13
8	0.04	0.10	0.24
16	0.06	0.13	0.45
24	0.09	0.18	0.52
36	0.11	0.22	0.77
slope =	0.0027	0.0052	0.0202
R^2 =	0.9700	0.9603	0.9837

Values are presented against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right) E_i\left(-106*\ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Values are unitless. The slope is in units of H^{-1}.
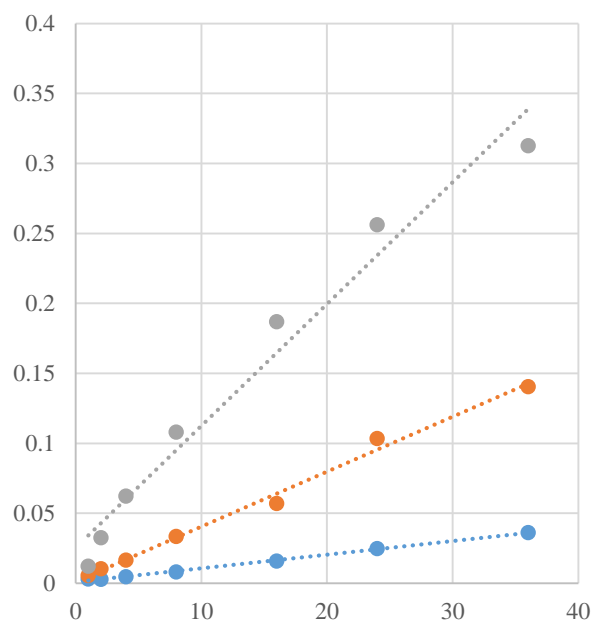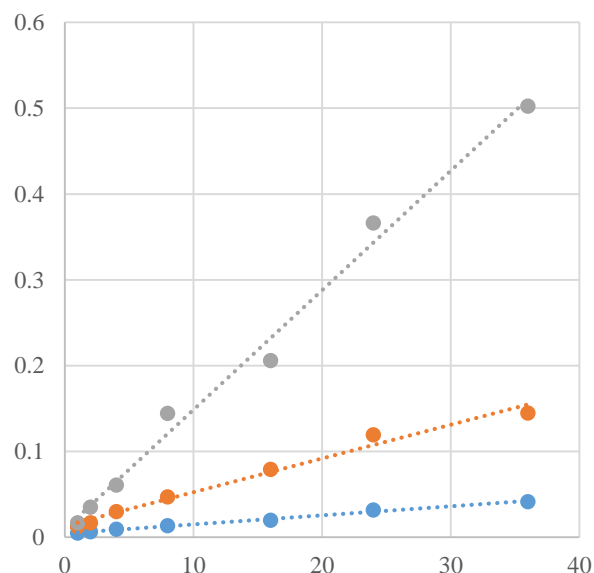
Bacillus licheniformis
Rice Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.09	0.18	0.26
2	0.13	0.24	0.40
4	0.16	0.30	0.67
8	0.23	0.46	0.91
16	0.30	0.64	1.52
24	0.36	0.90	2.36
36	0.39	1.14	4.98
slope =	0.0083	0.0274	0.1232
R^2 =	0.9048	0.9894	0.9432

Bacillus licheniformis
Wheat Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.04	0.10
2	0.03	0.08	0.20
4	0.05	0.13	0.35
8	0.09	0.21	1.03
16	0.14	0.41	2.03
24	0.18	0.59	2.82
36	0.24	0.77	4.14
slope =	0.0063	0.0212	0.1171
R^2 =	0.9782	0.9930	0.9962

Bacillus licheniformis
Barley Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.03	0.04
2	0.02	0.05	0.14
4	0.04	0.11	0.42
8	0.07	0.23	0.98
16	0.11	0.43	1.71
24	0.20	0.69	3.65
36	0.26	0.88	4.13
slope =	0.0072	0.0254	0.1258
R^2 =	0.9877	0.9884	0.9620

Bacillus licheniformis
Tapioca Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.06	0.10
2	0.03	0.12	0.19
4	0.05	0.22	0.41
8	0.08	0.46	0.74
16	0.14	0.70	1.93
24	0.19	1.03	2.69
36	0.21	1.29	3.05
slope =	0.0059	0.0357	0.0920
R^2 =	0.9452	0.9766	0.9522

Values are presented against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right) E_i\left(-106*\ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Values are unitless. The slope is in units of H^{-1}.

Porcine Pancreas

Potato Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.27	0.28	0.28
2	0.27	0.28	0.29
4	0.28	0.30	0.30
8	0.28	0.31	0.32
16	0.30	0.32	0.39
24	0.30	0.34	0.41
36	0.31	0.36	0.46
slope =	0.0010	0.0023	0.0054
R^2 =	0.8926	0.9612	0.9700

Porcine Pancreas
Waxy Maize Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.03	0.11	0.19
2	0.05	0.27	0.35
4	0.09	0.43	0.67
8	0.17	0.93	1.43
16	0.33	1.43	2.45
24	0.51	2.15	3.92
36	0.65	2.67	6.03
slope =	0.0024	0.0184	0.0743
R^2 =	0.9924	0.9882	0.9790

Porcine Pancreas

Maize Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.04	0.12
2	0.03	0.08	0.25
4	0.05	0.19	0.45
8	0.15	0.33	0.76
16	0.22	0.63	1.29
24	0.30	0.80	1.55
36	0.29	0.85	0.54
slope =	0.0088	0.0248	0.0625
R^2 =	0.8643	0.9100	0.9693

Porcine Pancreas
Amylomaize-7 Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.02	0.05
2	0.01	0.03	0.07
4	0.01	0.04	0.11
8	0.03	0.06	0.18
16	0.04	0.11	0.25
24	0.06	0.13	0.30
36	0.08	0.15	0.44
slope =	0.0004	0.0020	0.0040
R^2 =	0.9150	0.9794	0.9514

Values are presented against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right) E_i\left(-106*\ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Values are unitless. The slope is in units of H^{-1}.

Porcine Pancreas Rice Exponential Integral			
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.09	0.19	0.33
2	0.12	0.23	0.43
4	0.16	0.28	0.64
8	0.25	0.44	1.19
16	0.30	0.55	1.47
24	0.33	0.61	2.67
36	0.44	1.07	3.23
slope =	0.0041	0.0094	0.0229
R^2 =	0.9742	0.9404	0.9551

Porcine Pancreas Wheat Exponential Integral			
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.06	0.14
2	0.02	0.09	0.23
4	0.03	0.14	0.39
8	0.04	0.23	0.58
16	0.05	0.43	1.02
24	0.06	0.58	1.66
36	0.10	0.69	2.60
slope =	0.0006	0.0022	0.0188
R^2 =	0.9041	0.9650	0.9683

Porcine Pancreas Barley Exponential Integral			
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.05	0.10
2	0.03	0.08	0.22
4	0.04	0.12	0.42
8	0.06	0.22	0.80
16	0.11	0.32	1.45
24	0.15	0.44	1.62
36	0.19	0.59	1.68
slope =	0.0017	0.0050	0.0152
R^2 =	0.9922	0.9762	0.9892

Porcine Pancreas Tapioca Exponential Integral			
Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.05	0.13
2	0.02	0.09	0.31
4	0.04	0.15	0.63
8	0.07	0.26	1.25
16	0.12	0.42	2.66
24	0.16	0.44	4.11
36	0.20	0.56	5.04
slope =	0.0008	0.0055	0.0143
R^2 =	0.9404	0.9754	0.9137

Values are presented against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right) E_i\left(-106 * \ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Values are unitless. The slope is in units of H^{-1}.

	Human Saliva Potato Exponential Integral				Human Saliva Waxy Maize Exponential Integral		
						1	10
TC	0.1 IU	1 IU	10 IU	Hours	0.1 IU/ml	IU/ml	IU/ml
1	0.01	0.01	0.02	1	0.02	0.07	0.28
2	0.01	0.02	0.04	2	0.03	0.21	0.76
4	0.01	0.03	0.06	4	0.08	0.45	1.62
8	0.02	0.05	0.11	8	0.19	1.18	3.83
16	0.03	0.07	0.15	16	0.34	1.88	5.92
24	0.04	0.09	0.22	24	0.47	3.62	7.17
36	0.05	0.11	0.26	36	0.69	4.43	8.00
slope =	0.0012	0.0028	0.0070	slope =	0.0193	0.1303	0.2284
R^2 =	0.9614	0.9722	0.9685	R^2 =	0.9963	0.9784	0.8989

	Human Saliva Maize Exponential Integral				Human Saliva Amylomaize-7 Exponential Integral		
		1	10				
Hours	0.1 IU/ml	IU/ml	IU/ml	Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.04	0.12	1	0.01	0.02	0.04
2	0.03	0.13	0.30	2	0.01	0.04	0.07
4	0.06	0.23	0.46	4	0.02	0.06	0.13
8	0.12	0.42	1.02	8	0.04	0.09	0.19
16	0.20	0.60	1.47	16	0.05	0.13	0.29
24	0.31	0.80	2.65	24	0.07	0.15	0.35
36	0.39	1.05	2.60	36	0.09	0.18	0.43
slope =	0.0113	0.0278	0.0771	slope =	0.0022	0.0043	0.0108
R^2 =	0.9848	0.9683	0.9161	R^2 =	0.9684	0.9085	0.9508

Values are presented against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right)E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right)E_i\left(-106*\ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Values are unitless. The slope is in units of H^{-1}.

Human Saliva
Rice Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.11	0.36	0.85
2	0.15	0.44	1.25
4	0.22	0.55	1.70
8	0.35	0.88	2.23
16	0.53	1.29	4.10
24	0.60	1.88	8.34
36	0.76	2.29	13.83
slope =	0.0183	0.0570	0.3610
R^2 =	0.9517	0.9856	0.9670

Human Saliva
Wheat Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.12	0.24
2	0.03	0.18	0.42
4	0.04	0.25	0.82
8	0.05	0.38	1.40
16	0.07	0.60	2.75
24	0.08	0.85	4.03
36	0.10	0.97	4.31
slope =	0.0022	0.0253	0.1261
R^2 =	0.9588	0.9665	0.9422

Human Saliva
Barley Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.08	0.49
2	0.05	0.14	1.25
4	0.07	0.24	3.47
8	0.12	0.46	8.93
16	0.23	0.91	60.83
24	0.37	1.16	13.43
36	0.53	1.40	1.68
slope =	0.0145	0.0396	1.2270
R^2 =	0.9988	0.9627	0.9931

Human Saliva
Tapioca Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.11	0.45
2	0.04	0.24	1.01
4	0.09	0.41	1.85
8	0.16	1.03	3.93
16	0.35	1.80	8.86
24	0.52	2.45	14.93
36	0.70	2.92	22.13
slope =	0.0199	0.0841	0.6282
R^2 =	0.9945	0.9606	0.9973

Values are presented against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i \left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106} \right) + \left(\frac{C^0}{C_{min}}\right) E_i \left(-106 * \ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Values are unitless. The slope is in units of H^{-1}.

Bacillus amyloliquefaciens

Potato Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.00	0.01	0.01
2	0.01	0.01	0.02
4	0.01	0.01	0.02
8	0.01	0.02	0.03
16	0.01	0.02	0.03
24	0.01	0.02	0.04
36	0.01	0.03	0.05
slope =	0.0002	0.0005	0.0011
R^2 =	0.7069	0.8547	0.9757

Bacillus amyloliquefaciens
Waxy Maize Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.02	0.03	0.09
2	0.03	0.05	0.13
4	0.03	0.07	0.34
8	0.05	0.11	0.46
16	0.06	0.18	0.80
24	0.07	0.24	1.09
36	0.10	0.33	1.57
slope =	0.0020	0.0085	0.0414
R^2 =	0.9515	0.9953	0.9928

Bacillus amyloliquefaciens

Maize Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.03	0.06
2	0.02	0.04	0.09
4	0.03	0.07	0.13
8	0.04	0.11	0.28
16	0.06	0.17	0.38
24	0.08	0.22	0.59
36	0.10	0.29	0.80
slope =	0.0024	0.0074	0.0210
R^2 =	0.9825	0.9865	0.9902

Bacillus amyloliquefaciens
Amylomaize-7 Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.01	0.01	0.01
2	0.01	0.01	0.02
4	0.01	0.02	0.03
8	0.02	0.03	0.06
16	0.02	0.04	0.08
24	0.02	0.05	0.09
36	0.03	0.07	0.13
slope =	0.0005	0.0016	0.0032
R^2 =	0.9150	0.9788	0.9803

Values are presented against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right)E_i\left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106}\right) + \left(\frac{C^0}{C_{min}}\right)E_i\left(-106*\ln\left(\frac{C^0}{C_{min}}\right)\right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Values are unitless. The slope is in units of H^{-1}.

Bacillus amyloliquefaciens
Rice Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.07	0.10	0.15
2	0.09	0.13	0.18
4	0.10	0.14	0.22
8	0.11	0.20	0.31
16	0.14	0.23	0.43
24	0.18	0.29	0.62
36	0.20	0.33	0.67
slope =	0.0037	0.0063	0.0157
R^2 =	0.9777	0.9448	0.9565

Bacillus amyloliquefaciens
Wheat Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.00	0.01	0.05
2	0.00	0.01	0.05
4	0.01	0.02	0.08
8	0.01	0.04	0.13
16	0.02	0.07	0.24
24	0.03	0.11	0.29
36	0.05	0.21	0.36
slope =	0.0014	0.0055	0.0094
R^2 =	0.9919	0.9706	0.9640

Bacillus amyloliquefaciens
Barley Exponential Integral

Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.00	0.01	0.01
2	0.00	0.01	0.03
4	0.00	0.02	0.06
8	0.01	0.03	0.11
16	0.02	0.06	0.19
24	0.02	0.10	0.26
36	0.04	0.14	0.31
slope =	0.0010	0.0039	0.0087
R^2 =	0.9974	0.9924	0.9700

Bacillus amyloliquefaciens
Tapioca Exponential Integral

| Hours	0.1 IU/ml	1 IU/ml	10 IU/ml
1	0.00	0.01	0.02
2	0.01	0.02	0.04
4	0.01	0.03	0.06
8	0.01	0.05	0.14
16	0.02	0.08	0.21
24	0.03	0.12	0.37
36	0.04	0.14	0.50
slope =	0.0010	0.0039	0.0139
R^2 =	0.9925	0.9813	0.9906

Values are presented against time in hours. Values represent $-\left(\frac{C^0}{C_{min}}\right) E_i \left(\frac{(106-[P])*\ln\left(\frac{C^0}{C_{min}}\right)}{106} \right) + \left(\frac{C^0}{C_{min}}\right) E_i \left(-106 * \ln \left(\frac{C^0}{C_{min}} \right) \right)$. For this particular set, a $\frac{C^0}{C_{min}}$ value was assigned to 20. Values are unitless. The slope is in units of H^{-1}.

APPENDIX F: INVERSE MICHAELIS-MENTEN GRAPHS
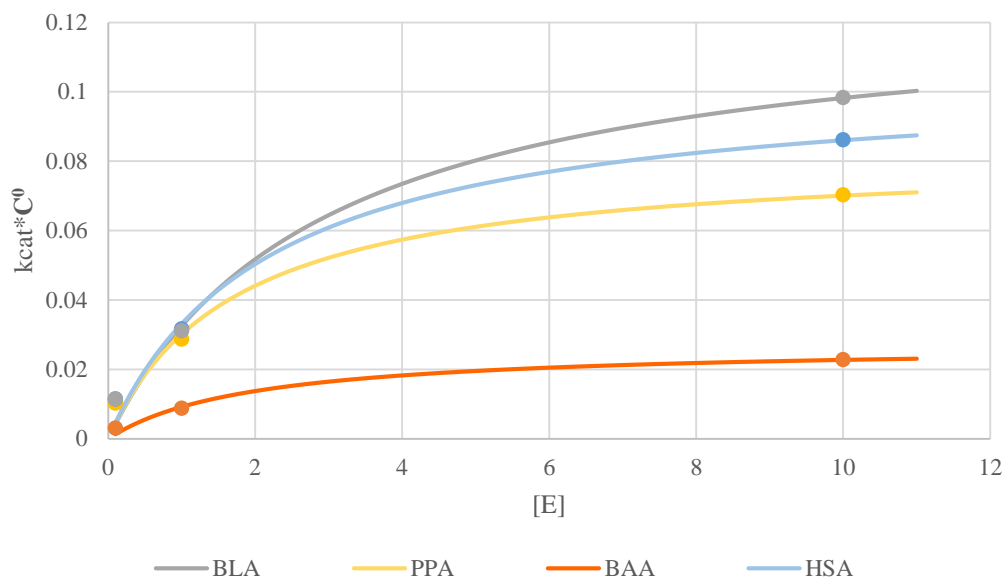

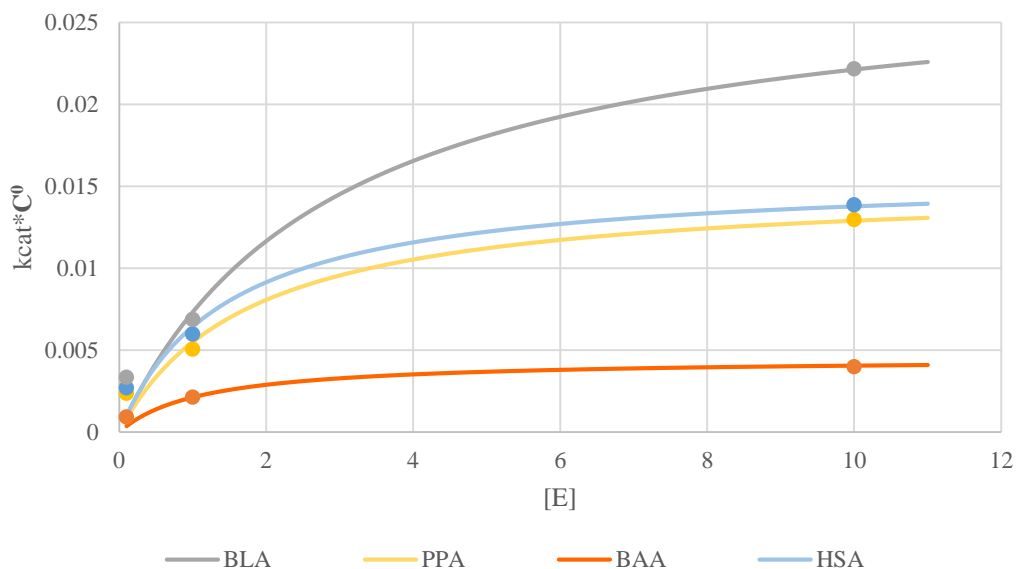Potato Reaction Rate Curves vs [E]
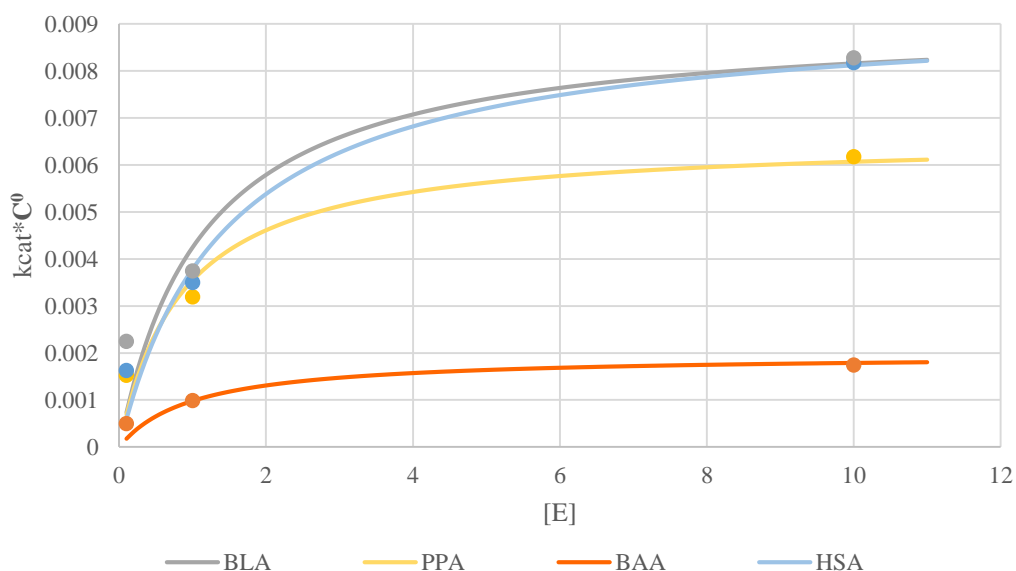
Waxy Maize Reaction Rate Curves vs [E]
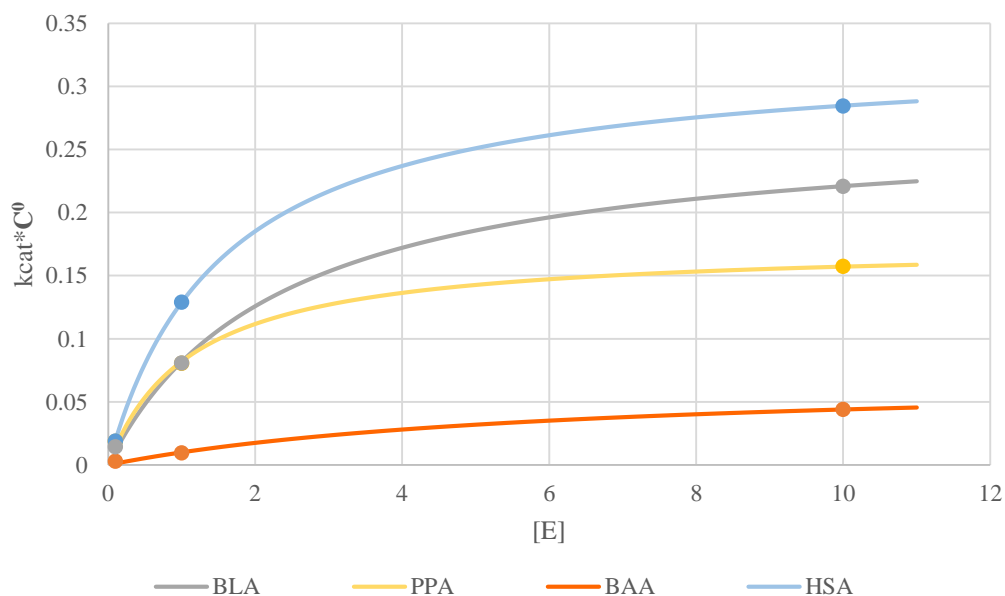
Using a least squares method and adjusting K and $k_{cat} * C^0$ as a parameter, models fitting the three slopes for the three concentrations of [E] are plotted. The slope was calculated by taking the 24H time point from the smoothened curves, running the exponential integral, and dividing by 24H. The equation used to fit the data points is $\frac{k_{cat}K[E]_{tot}C^0}{1+K[E]_{tot}}$. Values and errors are in the tables at the end of this appendix.

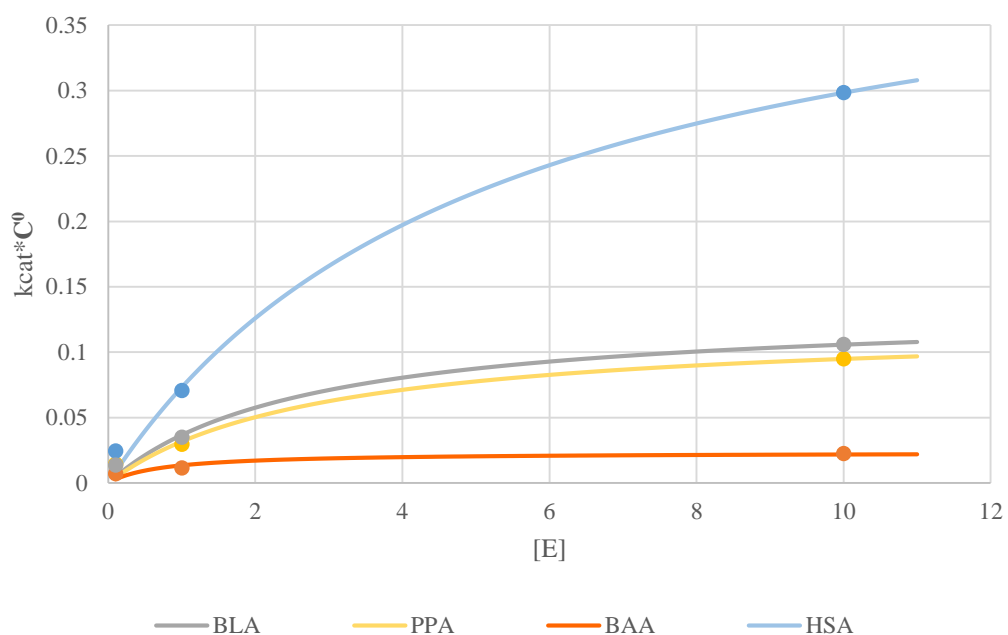## Maize Reaction Rate Curves vs [E]

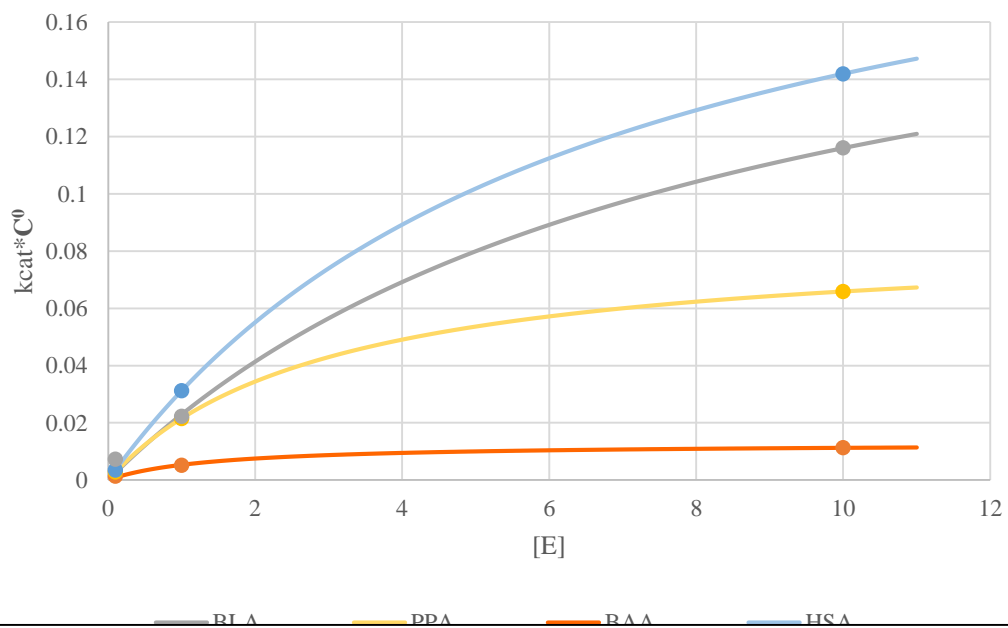## Amylomaize-7 Reaction Rate Curves vs [E]

Using a least squares method and adjusting K and $k_{cat} * C^0$ as a parameter, models fitting the three slopes for the three concentrations of [E] are plotted. The slope was calculated by taking the 24H time point from the smoothened curves, running the exponential integral, and dividing by 24H. The equation used to fit the data points is $\frac{k_{cat}K[E]_{tot}C^0}{1+K[E]_{tot}}$. Values and errors are in the tables at the end of this appendix.
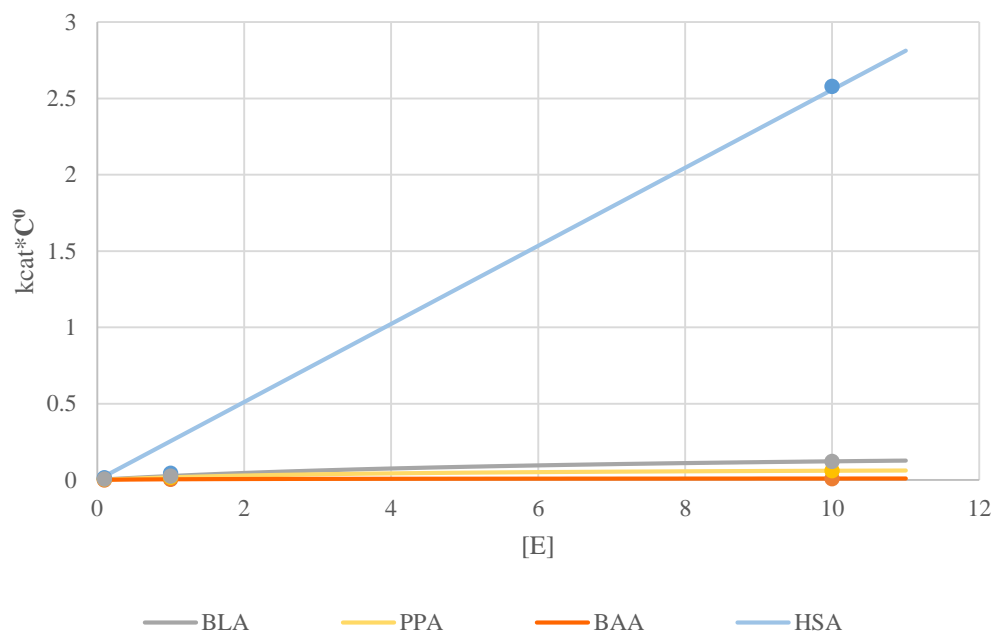
Rice Reaction Rate Curves vs [E]
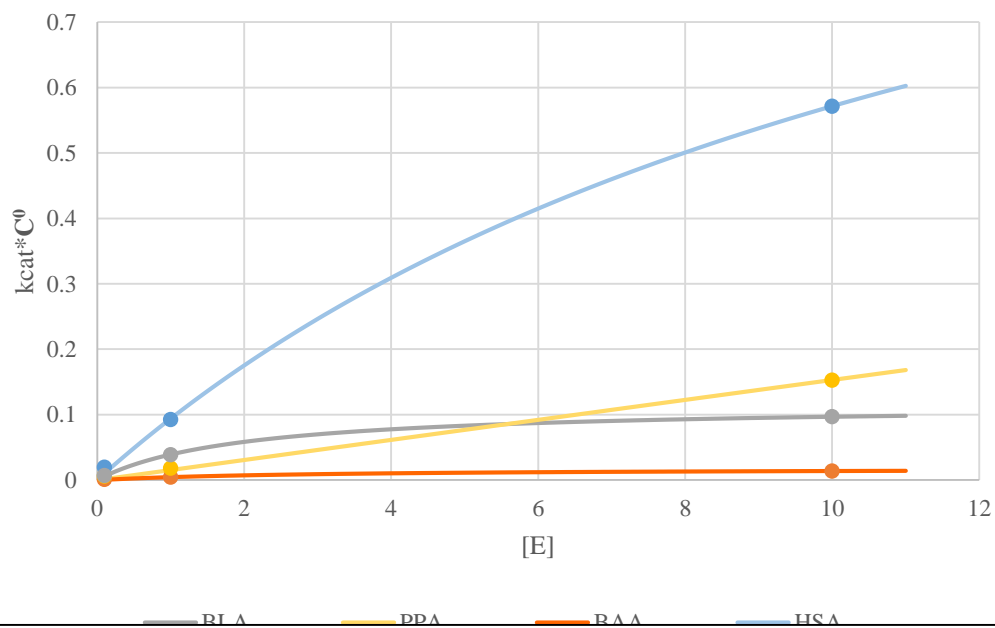

Wheat Reaction Rate Curves vs [E]

Using a least squares method and adjusting K and $k_{cat} * C^0$ as a parameter, models fitting the three slopes for the three concentrations of [E] are plotted. The equation used to fit the datapoints is $\frac{k_{cat}K[E]_{tot}C^0}{1+K[E]_{tot}}$. Values and errors are in the tables at the end of this appendix.

Barley Reaction Rate Curves vs [E]

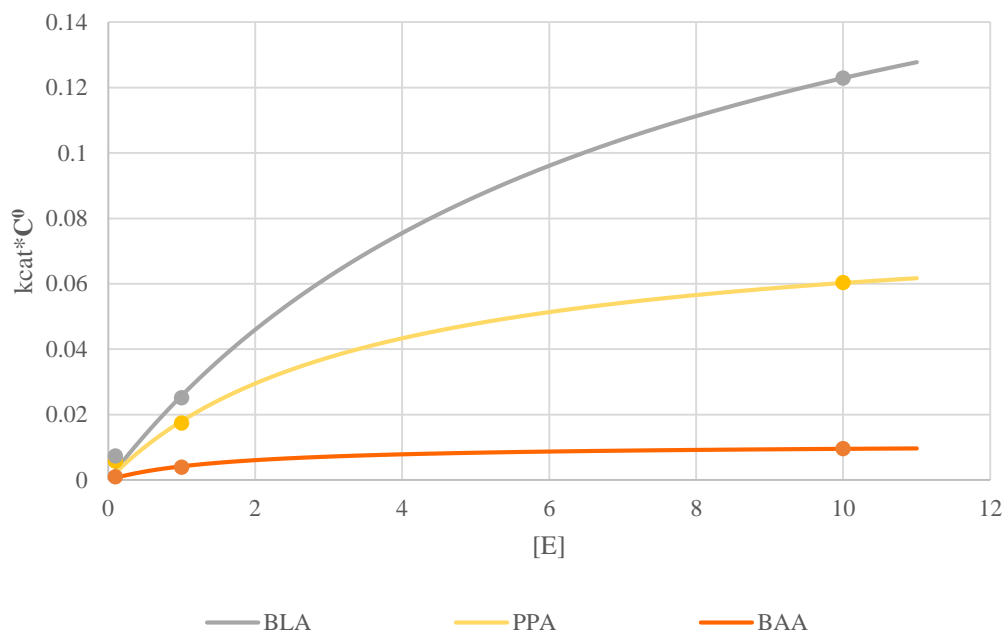Tapioca Reaction Rate Curves vs [E]

Using a least squares method and adjusting K and $k_{cat} * C^0$ as a parameter, models fitting the three slopes for the three concentrations of [E] are plotted. The equation used to fit the data points is $\frac{k_{cat}K[E]_{tot}C^0}{1+K[E]_{tot}}$. Values and errors are in the tables at the end of this appendix. Note how barley and HSA do not fit the model well.

Barley Reaction Rate Curves vs [E]

Axis label: kcat*C^0 (vertical), [E] (horizontal)

Legend: BLA — PPA — BAA

Tapioca Reaction Rate Curves vs [E]
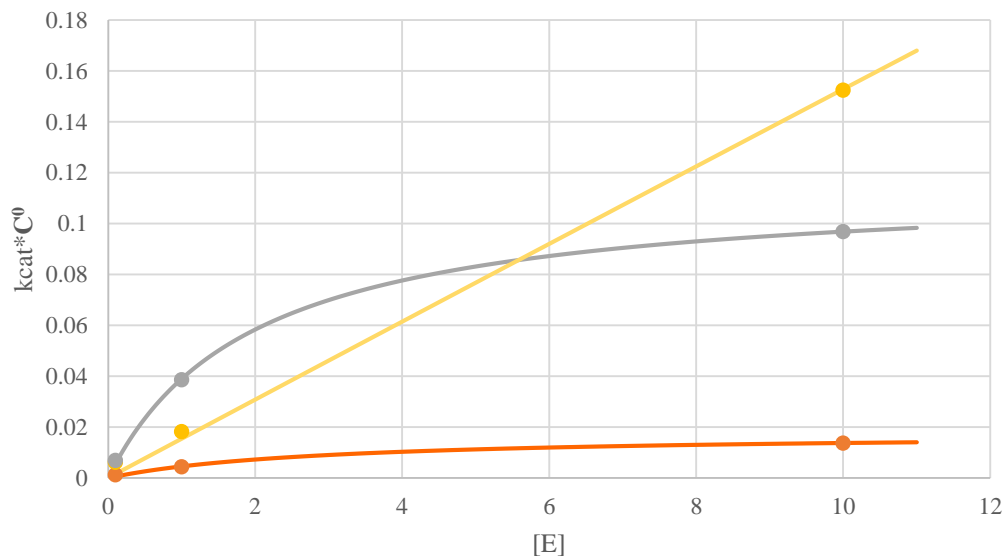

Axis label: kcat*C^0 (vertical), [E] (horizontal)

Using a least squares method and adjusting K and $k_{cat} * C^0$ as a parameter, models fitting the three slopes for the three concentrations of [E] are plotted. The equation used to fit the data points is $\frac{k_{cat}K[E]_{tot}C^0}{1+K[E]_{tot}}$. Values and errors are in the tables at the end of this appendix. These graphs have HSA data omitted so that the other enzyme values can be seen.

Bacillus licheniformis

Starch	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
K	0.877	0.428	0.345	0.345	0.379	0.122	0.139	0.506
$k_{cat}*C^0$	0.009	0.272	0.127	0.029	0.134	0.211	0.211	0.116
Error	0.002	0.003	0.007	0.002	0.009	0.005	0.005	0.001

Porcine Pancreas

Starch	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
K	1.165	0.884	0.578	0.568	0.352	0.338	0.284	0.001
$k_{cat}*C^0$	0.007	0.175	0.082	0.015	0.122	0.085	0.082	19.19
Error	0.0007	0.0003	0.0051	0.0001	0.0027	0.0007	0.0010	0.00001

Human Saliva

Starch	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
K	0.686	0.644	0.464	0.690	0.192	0.154	0.0001	0.077
$k_{cat}*C^0$	0.009	0.329	0.105	0.016	0.453	0.234	2732.28	1.317
Error	0.0008	0.0049	0.0074	0.0016	0.0122	0.0006	0.0828	0.0111

Bacillus amyloliquefaciens

Starch	Potato	Waxy Maize	Maize	Amylomaize-7	Rice	Wheat	Barley	Tapioca
K	0.994	0.165	0.513	0.890	1.353	0.700	0.600	0.348
$k_{cat}*C^0$	0.002	0.071	0.027	0.005	0.023	0.013	0.011	0.018
Error	0.0003	0.0010	0.0014	0.0003	0.0027	0.0004	0.0004	0.0006

Errors are defined as the square root of the sum of the squared deviations. The deviation is defined as the difference between the calculated reaction rate versus the measured reaction rate. Values of K are in IU^{-1}. Values of $k_{cat}*C^0$ are in $hour^{-1}$. Errors were minimized with K and $k_{cat}*C^0$ using solver on excel. A scale for the errors is listed below.

0	0.0005	0.001	0.0015	0.002	0.003	0.005	0.01	0.02	0.03	0.05	0.08	0.1

Note that HSA acting upon barley and PPA acting upon tapioca does not fit the data well, and therefore, has values that are not feasible with this model.